INFLUENCE OF FARMING SYSTEMS AND CROP HOST VARIETIES ON *PYTHIUM* ROOT ROT EPIDEMICS IN A HIGHLAND AGROECOLOGY OF SOUTH WESTERN UGANDA

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DECLARATION

This thesis is entirely my own work and has not been presented for a degree award in any University

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DEDICATION

To Josemaria whom I owe the later part of my life. From whom I have learn't many of the things that I am today and his inspiration has spurred me on!

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SUMMARY

The bean crop is one of East African's principal crops. It is grown primarily by small-scale farmers who are mainly women, for home consumption and any excess is sold (Wortmann *et al.,* 1998). The increase in severity and incidence of bean root rots has been associated with recent changes in farming systems, especially under high demographic pressure and decline in soil fertility (Rusuku *et al.,* 1997). The importance of root rots in causing bean crop failures was recognised in Rwanda in 1988 and subsequently in Burundi, the Democratic Republic of Congo, Kenya and Uganda (CIAT, 1992; Otsyula *et al.,* 1998; Opio, 1998). Although bean root rot is caused by a number of soil borne pathogens depending on environmental conditions, *Pythium spp.* are the fungal pathogens most frequently associated with severe epidemics in eastern Africa (Rusuku *et al.,* 1997).

In south western Uganda, root rot is caused by a number of pathogens, which occur either singly or as complexes. These include *Fusarium spp, Rhizoctonia solanii* and *Pythium spp.* with the latter being the major pathogen (Opio, 1998). Studies on root rots have indicated that continuous cropping of beans, a common practice in eastern Africa exacerbates the problem (Rusuku *et al.,* 1997). Due to population and land pressure in these high productive areas, beans are commonly cultivated with other crop plants. Yet *Pythium* species attack a number of crop species and other plants (Ampaire, 2003). There is a need therefore to investigate whether root rots occur in other crops in the bean based system and in addition, to characterise *Pythium* species responsible for these root rots.

This information will provide evidence on whether novel *Pythium* species are implicated in the bean root rot epidemics and whether other crop species are influencing the root rot epidemics in south western Uganda.

In the first part of the study, surveys were done in Kabale district so as to characterise root rots of non bean crops grown in association with beans. Molecular characteristation using the ITS-DNA sequences was also carried out on these crop species. Non bean crops in bean pathosystem of south western Uganda were found to be affected by root rots. The crops included Irish potato, sorghum and peas. This implys that beans are not the only crops in the pathosystem to be attacked by the disease. Using ITS-DNA sequences, 142 *Pythium* species were characterised from non bean crops. The most abundant of the *Pythium* species on these crops was *Pythium* ultimum. Also, a complex of pathogens were isolated from non bean crops and these included *Pythium*, Fusarium species and Veriticillium. The implication of this is that there is a host-pathogen selectivity as some *Pythium* species were found to affect leguminous crops and other solanaceous crops.

In the second part of the study cross pathogenicity was done in the screen house. Bean derived and non bean derived *Pythium* species were use to test their pathogenicity on resistant and susceptible bean variety, cereals and legumes. Sorghum and peas were found to be susceptible to both bean derived and non bean derived *Pythium* species. Maize and millet were found to be resistant. These resistant crops may be able to produce biochemical reactions in their cells and tissues which are toxic to pathogen. Cereal crops

having fibrous roots could counteract infection better than legumes which have tap roots. Hence cereals had a higher root mass compared to legumes. Symptoms charactersistic of *Pythium* infection such as wilting, stunting and chlorosis were observed. This arises due to *Pythium* species reducing water uptake to leaves therefore resulting in wilting.

The third part of the study involved the use of light and electron microscopy techniques to investigate the pattern of infection of bean pathogenic *Pythium* species on sorghum and maize. Sorghum was found to be susceptible to bean pathogenic *Pythium* species. The infection pattern in sorghum was similar to susceptible bean variety (CAL 96). Maize was resistant to bean pathogenic *Pythium* species and the infection pattern was similar to resistant bean variety (AND 1062). This confirms that sorghum is an alternative host of *Pythium*. *Pythium* infection in crop species was mediated by the formation of appressoria-bearing hyphae. In the study, there was also evidence of hemibiotrophic infection found with *Pythium* ultimum possessing two kinds of hyphae. This suggests that virulence of *P.ultimum* is affected by these two hyphae.

This study has therefore found evidence that th cultivation of beans in mixed cropping systems with non bean crop species may partly contribute to bean root rot epidemics. Sorghum and peas which are popular intercrops were found to be alternative hosts of pathogenic *Pythium* species implying that they contribute to pathogen inoculum load in the soil hence increased disease outbreaks. 2. Maize and millet were found to be resistant to *Pythium* species . This implies that these crops are poor hosts of pathogenic *Pythium*

species therefore these crops could be included in bean rotations in south western Uganda so as to reduce *Pythium* soil inoculum load. 3. Differences in pathogenicity were found to occur within the pathogenic *Pythium* species.This phenomenon suggests the possibility for directional selection leading to increase in species or even pathotype abundances among *Pythium* pathogenic species. 4. Of the *Pythium* species isolated from bean and non bean hosts some were pathogenic others were not. Given the mulit- pathogenicity capacity of this genus, evolution of novel *Pythium* strains/ pathogens on both beans and non-bean hosts cannot be precluded. 5. Resistant bean varieties (RWR 719 & AND 1062) and non bean crops such as maize had similar disease reaction to bean pathogenic *Pythium* infection ..

CHAPTER ONE

INTRODUCTION

1.1 Importance of beans

The common bean (*Phaseolus vulgaris* L.) is the second most important source of human dietary protein and the third most important source of calories of all agricultural commodities produced in Eastern and Southern Africa (Pachico, 1993). The bean crop originated from Latin America and was introduced into Eastern Africa by Spanish and Portuguese travelers to the East African Coast (Purseglove, 1988). Beans are important to many low-income urban and rural households in eastern Africa because animal protein is often rare or completely absent from the diet. For example in Uganda, in the 1960's, beans were used to respond to a high incidence of malnutrition among children (David and Sperling, 1999). Beans also have other uses; the grain can be eaten fresh or dried, the leaves can be used as vegetables and the stalks can make soda ash. The bean crop is subsistently produced by women farmers who market approximately 40% of their produce estimated at 452 $ million per annum, while the rest of the crop is for home consumption (Wortmann *et al.,* 1998). The reason beans are a coveted cash crop for small-scale farmers, is due to their short maturity duration (three months on average), ease of handling and storability. Thus beans play an essential role in sustaining livelihoods of smallholder farmers.

1.2 Bean production in East Africa

Eastern Africa has the highest bean production in sub-Saharan Africa at 75, 369 metric tonnes per annum (FAO, 2004). In the region, the largest bean producing

countries are Kenya, Uganda, Democratic Republic of Congo, Burundi, Tanzania, Rwanda and Ethiopia. Production occurs in two main environments, that is, the cool highlands of east and central African countries (i.e. Kenya, Uganda, Tanzania, Rwanda and Burundi) and the warmer mid-elevation areas of the Democratic Republic of Congo, Ethiopia and some countries of southern Africa. In Uganda, beans are grown throughout the country but especially in the cool highlands of south western Uganda (Mukalazi, 12004). These areas lie at an altitude of 800-2,300 metres above sea level. Mostly, in Africa, the bean crop grows well in fertile volcanic soils with a pH of 4.2 or more. The mean temperatures range from 16-24^{0}C. In addition, the bean crop needs an annual precipitation of 500-2000 mm. These environments have a high potential, hence beans compete with a lot of other crops for land and labour (Nderitu *et al.,* 1997).

In Uganda, bean research began in the 1960's lead to the release in 1968 of the bean bush variety K 20. This variety is currently widely grown in Uganda, Kenya and Tanzania because of its marketability attributes and yield stability (Grisely, 1994). In 1994, two CIAT bred lines were released; K 132 and K 131. K132 is a determinate bush type (Type I) characterised by dark red mottled, large seeds. Although it is highly marketable it is susceptible to many production constraints. K 131 is an indeterminate bush type (Type II), which is characterised by small beige seeds, and is resistant to bean common mosaic virus (BCMV), a moderately important production constraint (Grisely, 1994).

1.3 Bean production constraints

In most parts of Africa, common beans are grown as an intercrop with other food crops, especially with maize. Such cropping methods with little or no added inputs such as fertilisers or pesticides, do not allow beans to realise their maximum yield potential (Nderitu *et al.,* 1997). Consequently, under such production systems, the biotic and abiotic constraints seriously reduce yields. The common bean is affected by several biotic and abiotic production constraints. The major abiotic constraints include nitrogen and phosphorous deficiency, low pH and drought (CIAT, 1992). Low potassium, aluminum and manganese toxicities are of intermediate importance; sodium toxicity is important in some localities (Wortmann, 1994). Low phosphorus is a major factor limiting bean production in western Kenya (FURP, 1994; Rachier *et al.*, 1998). The biotic constraints in order of descending importance in Africa; include angular leaf spot (*Phaeoisariopsis griseola*), anthracnose (*Colletotrichum lindemuthianum*), bean stem maggot (*Ophiomyia spencerella*) bean bruchids (*Acanthoscelides obtectus*) and root rots (Buruchara, undated). Bean root rots occur in most bean-producing regions of Africa, their importance is limited to certain locations, especially in the high-potential areas of eastern, central and southern Africa (Buruchara and Scheidegger, 1993). In eastern, central and southern Africa bean root rot pathogens and bean stem maggot occur in a complex and are synergistic where there is low soil fertility (CIAT, 1992; Ampofo, 1994). Thus, an overall integrated crop management approach that incorporates various components could be a useful strategy for increasing bean production. The occurrence and severity of root rot

disease is associated with high intensity of bean growing attributed to lack of crop rotation.

Root rots are currently a major problem in Uganda causing crop failures in some seasons. In south western Uganda where this study was based, bean root rot epidemics are frequent. Root rots are caused by one or more soil-borne pathogens acting either alone or as a complex of two or more pathogens depending on environmental conditions (Rusuku *et al.,* 1997). These pathogens include *Fusarium oxysporum*, *Fusarium solani*, *Rhizoctonia solani* and *Pythium spp.* In Rwanda, western Kenya and south western Uganda, *Pythium spp.* are the fungal pathogens most frequently associated with severe root rot epidemics thus the focus of this thesis (Rusuku *et al.,* 1997).

Plate 1: A *Pythium* root rot infested bean field in south western Uganda.

1.4 Management options for the control of root rot

Various management options can be used to control root rots of beans (Abawi and Pastor –Corrales, 1990). They include resistant cultivars, cultural methods (crop rotation, organic mulches, ploughing and seedbed preparation, adjustment of the time, depth and density of planting, cultivation, influence of fertiliser, avoidance of fields infested with root rot), chemical methods (seed and soil treatment) and biological control.

The use of resistant cultivars is a relatively effective and preferable strategy for small-scale farmers to control root rots (Otsyula and Ajanga, 1995; CIAT, 2004). Certain cultural practices have been observed to reduce severity and incidence of root rot diseases. For example, the incidence of *Fusarium spp, Pythium spp.* and *Rhizoctonia solani* reduced when maize was included in rotations with beans (Rosado *et al.,* 1985). Also, sowing dates in relation to rains, affect the incidence and severity of *Fusarium* because bean plants are most susceptible to root rot diseases during the first 2 to 3 weeks of crop growth (Salih and Agreeb, 1997). Therefore, adjusting the planting time to avoid excess rainfall during that period can help the plants escape disease attack at the most vulnerable stage.

Deep ploughing and use of raised ridges to grow beans has been found to reduce root rots favoured by high moisture, such as *Rhizoctonia* root rot, southern blight, *Fusarium* root rots and *Pythium* root rot (CIAT, 1992). This is because ridging and deep tillage increase aeration and drainage, creating less favourable conditions for disease development. Applications of organic soil amendments are also known to reduce root rot diseases (Voland and Epstein, 1994). Buruchara (1991) and Buruchara

and Scheidegger (1993), showed that incorporating *Leucaena spp.* leaves and twigs of *Calliandra magrantha* and *Sesbania* as green manure two weeks before planting reduces plant mortality and increases bean grain yield. These amendments enhance soil microbial activity and plant nutrition thus enhancing tolerance to root rot (CIAT, 1992).

It appears therefore that the approach most likely to be effective in the management of root rots of beans is an integrated control. This is due to the fact that the effectiveness of single control measures varies from season to season or over different locations and production conditions (Abawi and Pastor-Corrales, 1990). In Africa, the integration of organic amendments, raised beds and resistant varieties has been shown to be advantageous over single components in controlling the severity of root rots and yield. (Buruchara and Scheidegger, 1993; CIAT, 1993).

1.5 Justification of the study

The bean crop is one of East Africa's principal crops. It is grown primarily by small-scale farmers who are mainly women, for home consumption and any excess is sold (Wortmann *et al.,* 1998). The increase in severity and incidence of bean root rots has been associated with recent changes in farming systems, especially under high demographic pressure and decline in soil fertility (Rusuku *et al.,* 1997). The importance of root rots in causing bean crop failures was recognised in Rwanda in 1988 and subsequently in Burundi, the Democratic Republic of Congo, Kenya and Uganda (CIAT, 1992; Otsyula *et al.,* 1998; Opio, 1998). Although bean root rot is caused by a number of soil borne pathogens depending on environmental conditions,

Pythium spp. are the fungal pathogens most frequently associated with severe epidemics in eastern Africa (Rusuku *et al.,* 1997).

In south western Uganda, root rot is caused by a number of pathogens, which occur either singly or as complexes. These include *Fusarium spp, Rhizoctonia solani* and *Pythium spp.* with the latter being the major pathogen (Opio, 1998). Mukalazi (2004) reported on various *Pythium species* associated with root rot. He was able to isolate eleven *Pythium spp.* from bean plants with typical root rot symptoms which included yellowing of leaves and rotting of stems and roots. The *Pythium* species identified were *Pythium ultimum, Pythium spinosum, Pythium torulosum, Pythium salpingophorum, Pythium vexans, Pythium dissotocum, Pythium nodosum*, *Pythium echinulatum, Pythium pachycaule, Pythium oligandrum* and *Pythium deliense* (Mukalazi, 2004). The most abundant *Pythium* species was *Pythium ultimum var.ultimum* (Mukalazi, 2004).

Studies on root rots have indicated that continuous cropping of beans, a common practice in eastern Africa exacerbates the problem (Rusuku *et al.,* 1997). Due to population and land pressure in these high productive areas, beans are commonly cultivated with other crop plants. In south western Uganda beans are often intercropped with other crops (Ampaire, 2003). In addition in studies elsewhere, Pythium species have been found to cause root rot in a number of crop species (Andandonon, 2004). There is a need therefore to investigate whether root rots occur in other crops in the bean based system and in addition, to characterise *Pythium* species responsible for these root rots on those crops. This information will provide evidence on whether novel *Pythium* species are implicated in the bean root rot

epidemics and whether other crop species are influencing the root rot epidemics in south western Uganda.

Moreover in this region, there have been many concerted efforts by bean breeders to develop and deploy varieties resistant to various diseases including root rot. This implies that the bean-*Pythium* pathosystem, has been subjected to selection pressure which could likely result in parasite population modification. Farmers can also influence the bean pathosystem through ploughing, sowing, weeding, fertiliser and pesticide use. All these effects could result in the bean-*Pythium* pathosystem being an unstable system and consequently resulting in increased parasitism and crop losses. Since *Pythium* variability on beans is known, Koch's postulate will be used to investigate the cross pathogenicity properties of *Pythium* species on other crops in the bean based system of south western Uganda. This study will help generate information critical for formulating integrated management options for bean root rot epiphytotics

1.6 Aim of the study

The goal of this study was to investigate the role of mixed crop species on root rot epidemics in a bean-*Pythium* pathosystem of south western Uganda to generate information that will guide design of disease management strategies.

1.7 Specific objectives

1. To study the possible role of mixed cropping systems on bean root rot epidemics in south western Uganda.

2. To study species diversity of *Pythium* pathogens associated with bean root rot in a mixed cropping system of south western Uganda.
3. To elucidate the mode of infection of *Pythium* species in crop hosts commonly included in bean mixed cropping systems.

1.8 Hypotheses tested

1. The practice of cultivating beans in crop mixtures influences bean root rot epiphytotics.
2. Mixed cropping systems provide alternative hosts to *Pythium* species and thereby influence inoculum build-up.
3. Pathogenesis of *Pythium* species is influenced by host genotype and biology attributes.

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CHAPTER TWO

OCCURRENCE OF ROOT ROT ON OTHER CROP SPECIES IN A BEAN BASED CROPPING SYSTEM IN SOUTH WESTERN UGANDA

2 Introduction

The common bean (*Phaseolus vulgaris*) is a diverse species showing considerable variations in growth habit, vegetative characters, flower colour, size and shape of both seeds and pods (Ocitti p'Obwoya, 1996). In Africa, beans are largely intercropped. For instance in Rwanda, beans are seasonally rotated with sorghum (*Sorghum bicolor*) or sweet potato (*Ipomea batatas*) (Rusuku *et al.,* 1997). In south western Uganda, beans are grown in an intensive agricultural system together with sorghum (*Sorghum bicolor*), maize (*Zea mays*), sweet potato (*Ipomoea batatas*), potatoes (*Solanum tuberosum*), bananas (*Musa spp*.) and garden peas (*Pisum satium*) (Ampaire, 2003). Intercropping sweet potato with beans is the most popular sweet potato based intercropping system in Uganda (Bashaasha *et al.,* 1995). Intercropping is important because of land pressure. Moreover, intercropping beans is an important strategy for food security, improves labor use efficiency enhances soil nutrients, light and rainfall because of the different demands made by the mixture of crops (Ocitti p'Obwoya, 1996). Intercropping beans is nonetheless not without impediments. Most of the root rot pathogens of beans in fact attack many crop species (Agrios, 1997). Indeed continuous intercropping of beans which is common in Kabale district and other areas in Africa may have exacerbated the root rot problem (Rusuku *et al.,* 1997).

The sudden increase in root rots in common beans is relatively recent and could be linked to changes in farming systems (ISAR, 1990). Root rots are known to occur in maize, wheat (*Triticum aestivum)*, garden peas (*Pisum satium*), potato (*Solanum tuberosum*) and cowpea (*Vigna unguiculata*.) (Adandonon, 2004). Given that intercropping is a common practice among resource constrained farmers, it is critical to know what role such crop species have in the current bean epiphytotics in south western Uganda. The objective of this study is to investigate the role of crops intercropped with beans on the bean root rot epidemics in south western Uganda.

2.1 Literature review

2.1.1 Crop mixtures and disease epiphytotics

Modern agricultural practices have resulted in a decline in crop diversity and an increase in genetically and spatially uniform plant populations. This lack of host diversity can lead to an escalation in the frequency and severity of epidemics. However in south western Uganda, where beans are intercropped, it is expected that there should be less severe epidemics possibly due to the diversity of host genotypes (Andrivon *et al.,* 2003). Spatial diversification of host resistance therefore appears to be an important approach for achievement of successful and sustainable management of crop pathogens. Applications of this approach have included inter- and intra specific diversity both within fields (e.g. alternate strips or rows) and between fields (e.g. cultivar rotation and mosaic cropping) (Wolfe, 1985). The potential of genotype mixtures to slow down or prevent epidemic development has been established by computer simulation (Mundt and Leornard, 1996), and by experimentation (Andrivon *et al.,* 2003).

There are three factors, which account for disease reduction in genotype mixtures. Firstly, the dilution of inoculum due to the presence of resistant plants; secondly, the physical barrier created by resistant plants affects successful deposition, thirdly there is potential for induced host resistance when propagules are deposited on neighbouring plants (Garret and Mundt, 1999). With regard to the spatial arrangement of genotypes within a mixture, the ground area of individual genotype units (genotype unit area) is an important configuration variable influencing epidemic development (Mundt and Leornard, 1996). An ideal mixture can therefore often be approximated in the field through random seed mixing prior to planting, provided that the pathogen spreads its propagules over distances well exceeding the plant and row distances of the crop. For example, in the potato late-blight pathosystem (*Phytophthora infestans-Solanum tuberosum*), the relatively large size of potato plants, as the smallest genotype unit possible results in high levels of autoinfection (Robinson, 1987). This study seeks to investigate the effect of crop mixtures a common practice in south western Uganda on bean root rot epidemics.

Plate 2: A farm with mixed cropping system of beans, cassava and banana in south western Uganda.

2.1.2 Root rot pathogens and symptoms

Bean root rots are characterised by above ground symptoms such as poor seedling establishment, uneven growth, chlorosis, and premature defoliation of severely infected plants (Abawi *et al*., 1985; CIAT, 2005). Poor seedling establishment and reduced plant density are the result of seed rot and damping off. Severity of seedling damping off is highest when germinating seeds and seedlings are attacked during the first two to three weeks after planting. Root rot infection of older plants usually results in reduced vigor, discoloration, and slow rotting of stem and root tissues (Abawi *et al*., 1985). In both adult and young plants, roots that are severely infected are reduced in size and may exhibit different degrees of decay. Tap roots of severely infected plants often die, although coarse adventitious roots may develop from the hypocotyls areas above infected tissues. These roots also become infected later but their production continues during moist soil conditions and helps the plant to survive (Abawi *et al.,* 1985). The shape and colour of lesions on stem and root tissues are very specific and characteristic of the pathogen.

For example a study done on farmer fields in Kabale district, south western Uganda, found differing root rot lesions on maize, sorghum, peas and beans (CIAT, 2005). Sorghum plants exhibited stunted growth, purpling of leaves, shoot death, extensive tillering, prop root development, dark-red to black root lesions and ultimately a poor plant establishment (CIAT, 2005). The pathogens associated with root rots in beans and other crops are presented below.

2.1.2.1 *Phytopthora* species

Most species of *Phytopthora* cause disease primarily on the lower stem. In the cowpea, plants are susceptible to infection by *Phytopthora spp.* at all stages of growth (Andandonon, 2004). Infected plants show wilting symptoms when leaves are still green and a brownish lesion starts at or near the soil surface, extending upwards. The lesion girdles the stem and results in permanent wilting and death of the plant.

2.1.2.2 *Pythium* species

Pythium pathogens cause root rot or seedling rot in a number of legumes and other crops (Harman, 2001; Harvey, 2004). *Pythium* reduces crop emergence through 'damping off', reducing plant population and yield potential. The pathogen infects germinating seeds and seedlings of all major grain crops and pastures. For example in cowpea, *Pythium* root rot is characterised by grey-green, water soaked girdle of the seedling stem (Singh and Allen, 1979). This stage is referred to as pre-emergence damping off. *Pythium* also causes a reduction and discoloration of the root system and a complete rotting and decay of fibrous rootlets (Abawi *et al*., 1985). Other symptoms include elongated, water-soaked areas on the stem, the cortical region of both root and stem tissues of severely infected plants also become very soft, brownish, and somewhat sunken and eventually collapse (Abawi *et al*., 1985). During continual wet weather the pathogen spreads upward, infecting stem branches, petioles, leaves, and at times may reach the growing tip resulting in wilt and plant death. (Abawi *et al.,* 1985).

2.1.2.3 *Rhizoctonia* species

Rhizoctonia solani may induce seed rot, damping-off, stem canker, root rot and pod rot. This pathogen can also infect seeds before germination resulting in seed decay. Lesions on young seedling expand rapidly and result in damping-off (Abawi *et al.,* 1985; Adandonon, 2004). Typical symptoms are small, sunken lesions that are light to dark-brown. As infection progresses, sunken cankers enlarge and those that are close together may coalesce and girdle the stem, retarding growth and eventually killing the plant. In garden peas, the pathogen causes seed and seedling rot (Harman, 2001).

2.1.2.4 *Fusarium* species

Initial symptoms of *Fusarium* root rot appear as longitudinal, narrow, reddish lesions or streaks on the hypocotyls and primary root about one to two weeks after seedling emergence. The most common causative pathogen is *Fusarium solani*. As infection progresses, lesions become numerous, coalesce and the entire underground stem and root systems become covered with reddish brown superficial lesions. The discolorations may extend to the soil surface, but rarely beyond. The lesions have no definite margins and may be accompanied by longitudinal fissures. The primary and lateral roots are frequently killed by the fungus and may remain attached as decomposed and dried debris. When the primary root is killed, the lower stem may become pithy or hollow. In addition, severely infected plants are stunted, chlorotic, and exhibit premature defoliation. *Fusarium* is able to infect several susceptible crops (Lager, 2002).

This plant pathogen is often divided into three groups mainly i) those commonly attacking cereals such as F. *avenaceum* (Fr) Sacc. *F. graminearum* Schwabe and *F. culmorum* (Smith) Sacc. ii) causing root rots on multi hosts such as *F. solani* (Mart) Sacc. and iii) wilts *F. oxysporum* Schl. group (Levenfors, 2003). Many *Fusarium spp.* also attack a wide range of legume crops such as clovers (Lager and Gerhardson, 2002), snap and French beans (Hall, 1994) and broad bean (Majchrzak *et al.,* 1996). Legume root rot is often associated with infections caused by *F. oxysporum, F. solani* and *F. avenaceum* (Salt, 1983). Symptoms in wheat include pre-emergence and post-emergence damping-off, necrosis of the roots, sub crown internodes, culms, and lower leaf sheaths, wilting, stunting and chlorosis and eventually plant death (Gonzales and Trevathan, 2000).

Previous studies in south western Uganda have implicated *Fusarium* species in bean root rot especially where *Fusarium solani f. sp. phaseoli* occurs concurrently with *Pythium* species (Sippel and Hall, 1982). *Fusarium oxysporum* (Schlecht.) *f. sp. phaseoli* Kendrick & Snyder, causal agent of *Fusarium* wilt is another important opportunistic pathogen which takes advantage of damage caused by other root rot pathogens to enter the vascular system of plants (Rusuku *et al*., 1997; Buruchara *et al*., 1999). Therefore it is necessary to understand bean root rot epidemics with specific focus on *Pythium* species which appear to be the most abundant and destructive compared to *Fusarium*. Due to the diversity of root rot pathogens associated with beans, correct identification is needed to design and implement appropriate management options.

2.1.3 Molecular characterisation

The major disease of beans causing high production constraints in south western Uganda is bean root rot. This disease is caused by *Fusarium* species, *Pythium* species and *Rhizoctonia solani* which are soil borne pathogens and may act singly or in a complex (Rusuku *et al*., 1997). *Pythium* species have been demonstrated to be the most abundant pathogen responsible for the increased bean root rot epidemics in south western Uganda (Mukalazi, 2004). In addition, molecular analyses have revealed that there is a wide diversity within *Pythium* populations in south western Uganda (Mukalazi, 2004). Previously the taxonomy of oomycetes was based exclusively on morphological characteristics that are highly variable and make identification of species within this group difficult (Vander Plaats-Niterink, 1981). The historical lack of consensus on the most important morphological characteristics for identification, the high variability within the most important structures and considerable overlap among species, and the absence of diagnostic morphological structures for many isolates or species, all contribute to potential errors in identification (Matsumoto *et al.,* 1999). Today better techniques are being used to elucidate the relationship and evolution of organisms.

A number of DNA – based techniques have been assessed for diagnostic potential of *Pythium*. Sequence analysis of selected DNA fragments to determine species relationships is direct and theoretically more accurate than any other technique (White *et al*., 1990). The ribosomal internal transcribed spacer (ITS) DNA (rDNA) regions have been the major tool used. To-date the ITS sequences of over 80 *Pythium spp.* are deposited in GenBank with some having been analysed extensively for phylogeny

and differentiation of multiple *Pythium* species (Paul, 2003). Additional examples of DNA sequences assessed include the 28S rDNA (Briad *et al.,* 1995), the *cox II* gene (Martin, 2000), and the ras-related protein gene (Moorman, 2002). Restriction fragment polymorphism (RFLP) of ribosomal DNA or the Internal transcribed spacer (ITS) has been characterised for nearly 40 species providing important evidence on phylogenic relationships within the genus (Kageyama, 1998). Species-specific probes have been developed for many *Pythium spp* allowing the use of PCR-ELISA (Bailey *et al*., 2002) and the reverse dot blot assay for identification of multiple species(Levesque, 1998). Similarly, species-specific PCR primers have been designed for detection of multiple *Pythium* species (Wang *et al.,* 2003).

A variety of both neutral and selective genetic markers have been used to study *Pythium* genetic diversity. These include Amplified Fragment Length Polymorphism (AFLP's) (Majer *et al*., 1998; Arenal *et al*., 1999) and RFLPs detected with numerous single-locus probes (Paul, 1999). Whereas species-specific primers and other diagnostic tools can be used to distinguish between members of the same species, they too have their limits especially when more detailed genetic analyses are required. It is in that line that analysing sequences especially the ITS region is a main focus of oomycetes (Levesque *et al.,* 2004). In the recent past, the ITS region especially based on RFLP of amplicons, have been used to identify *Pythium s*pecies from south western Uganda (Mukalazi, 2004). This approach could however not be used to study intraspecies variation. It nevertheless identified five species of *Pythium* namely (*Pythium salpingophorum*, *Pythium torulosum*, *Pythium spinosum*, *Pythium nodosum* and *Pythium ultimum*). These *Pythium* species are associated with root rots of beans

(Mukalazi, 2004).The objective of this study was to characterise *Pythium* species associated with root rots in a bean-pathosystems.

2.1.4 Sectional Conclusion

Due to modern agricultural practices there has been an increase in genetically and spatially uniform plant populations. Lack of host diversity therefore leads to an increase in frequency and severity of epidemics. In south western Uganda when beans are intercropped, it is expected that there should be less severe epidemics of bean root rot albeit the disease is on the increase. Bean root rots are caused by a complex of pathogens which include: *Phytopthora, Pythium, Fusarium* and *Rhizoctonia* species (Rusuku *et al*., 1997). The symptoms of bean root rot due to these pathogens have already been described in studies elsewhere. The gap therefore revealed in this literature review is the lack of root rot symptom in crops which are usually intercropped with beans in south western Uganda. *Pythium* root rot is the most abundant bean root rot disease in south western Uganda. Molecular analyses using various DNA-based techniques have revealed the diversity of *Pythium* species in beans. The gap revealed in this literature review is that the diversity of *Pythium* species in crops which are intercropped with beans has not been investigated. This chapter sets out therefore to investigate the role of mixed cropping systems on bean root rot epidemics and to characterise *Pythium* species responsible for root rots in crops intercropped with beans in south western Uganda.

2.2 Materials and Methods

2.2.1 Study Area

Samples of crops intercropped with beans with or without typical symptoms of root rot were collected from infected bean fields from Kabale district in south western Uganda. The survey was carried out in the 15 of the 16 sub counties of Kabale district. These included Ikumba, Muko, Bufundi, Rubaya, Hamurwa, Kashambya, Bubaale, Kitumba, Kamuganguzi, Buhara, Maziba, Kamwezi, Bukinda, Rwamucucu, Kaharo and Kyanamira. Samples were not collected from Kabale sub county (Figure 1). In each sub county, samples were picked from a pre-determined position using a grid distance of 5 to 10 km. The sampling strategy was meant to maximise collection of disease isolates. It is based on the premise that bean root rot is spread to other fields by slow water, which may flow over long distances in hilly places (Martinez and Williams- Woodward, undated). In each of the sampled fields, at least 5 plants per crop were randomly selected following a "W" pattern. Information was collected on: (a) Physical location : village, parish and sub-county were noted (b) Geographic Positioning System (GPS) recording (c) The host or variety sampled (d) Disease symptoms on roots, stems and leaves e.g. lesions, root rot etc. (e) The cropping systems found in each field. At each sampling point a 2 m by 2 m quadrat was used. Ten plants were sampled for tall crop species and twenty for short crop species. For the purpose of studying the seasonal variability and distribution of *Pythium* species, three collections were done in April 2004, May 2005 and May 2006.

2.3 Isolation of *Pythium* species from plant tissues

Infected root pieces from the field samples (approximately 0.5- 2 cm long) were cut from expanding lesions and plated onto selective medium of Corn Meal agar (CMA) (Oxoid Ltd, Basingtoke and Hampshire, England) amended with 100 µg/ml and 45µg/ml of the antibiotics pimaricin and rifamycin (Sigma-Aldirch, Louis, Mo, USA) respectively, to prevent and kill bacterial growth (White, 1988). The selective medium was prepared by combining 20 g CMA, 1000 ml of deionised water and autoclaving at 15 psi for 20 minutes. Rifamycin and pimaricin were added on cooling the media to 40^0C. Cultures were incubated at room temperature (20-25^0C) for 2-5 days. *Pythium* mycelia from tissue were later transferred onto CMA containing rifamycin and later maintained on slants of Potato Dextrose Agar (PDA) (Becton, Dickinson and company, Sparks, MD 21152 USA).

2.4 Molecular characterisation of *Pythium* species

2.4.1 Cultural conditions

The *Pythium* field isolates were grown on PDA at 18- 22^0C to obtain an active culture of each isolate relatively free of bacteria. After two to three days, a plug of pure culture from PDA was cut from the growing margin of cultures and placed in sterilised 20% V8 juice broth (King's Lynn Norfolk, USA) containing 2.5 g of $CaCO_3$ and incubated in darkness at room temperature for four days to allow the pathogen to form mycelia. Mycelia was harvested using sterilised forceps, filtered through a layer of 85- mm filter paper and blot dried of excess juice with paper towels. Subsequently

the mycelia was placed in sterile micro-centrifuge tubes and kept at -20ºC until ready for DNA extraction.

2.4.2 DNA extraction

DNA was extracted from harvested mycelia according to Mahuku (2004). Mycelia were ground to a fine paste in a mortar containing Tris- EDTA- SDS (TES) extraction buffer (Sigma- Aldrich, Louis, Mo, USA) and sterilised acid-washed sea sand (BDH Laboratory Supplies, Poole, BH 15, ITD, England). Additional TES buffer containing Proteinase K (Sigma- Aldrich, Louis, Mo, USA) was added and the mixture incubated at 65ºC for 30 min. DNA was precipitated using ice-cold isopropanol (Sigma- Aldrich, Louis, Mo, USA) and the pellet was washed twice with 70% ethanol, dried and dissolved in Tris- EDTA (TE) buffer (Sigma- Aldrich, Louis, Mo, USA).

2.4.3 Polymerase Chain Reaction

The PCR analysis was performed using oomycete Internal transcribed spacer (ITS) specific primers (White *et al.,* 1990). PCR reaction was performed in 50 µl- reaction volumes containing 5µl of 10X PCR buffer, 8 µl of 25 mM $MgCl_2$, 2.5 µl of 1.25 mM dNTP, 0.2 µl of each primer (20µM), 18S (5'-TCC GTA GGT GAA CCT GCG G-3') and 28S (5'-TCC TCC GCT TAT TGA TAT GC-3'), 20 ng of DNA, and 0.2µl Taq DNA polymerase (CIAT Virology Laboratory, Cali, Colombia) (5U/µl). Amplification was performed in a Primus 96 Plus Thermal Cycler (MWG-Biotech,

Germany) programmed for 35 cycles of denaturation at 94ºC for 1 min, annealing at 68ºC for 2 min, and extension at 72ºC for 1 min, followed by a final extension for 8 min at 72ºC. Efficiency of amplification was monitored by running 12 µl of each reaction through 2% agarose gels containing 5 mg/ml of ethidium bromide (Sigma-Aldrich, Louis, Mo, USA). This was done using 1 X TBE (Tris Borate EDTA buffer) as the running buffer at a voltage of 100 Volts for 2 hours. At the end of electrophoresis, the agarose was placed on a UV light table and documented using a Polaroid camera (Ep-H7 0.7 x, electrophoresis hood, Polaroid, GelCam, UK) fitted with B/W 667 Polaroid films (Sigma- Aldrich, Louis, Mo, USA). Samples with amplicons below the expected size of 800 base pairs were eliminated from further analysis. PCR amplifications containing no DNA template were included as controls. A 100-bp molecular weight ladder was used as the size standard marker stained with ethidium bromide, visualised and photographed under UV light.

2.4.4 Analysis of ribosomal DNA sequences of *Pythium* isolates and identification of species

2.4.4.1 Sequencing of amplified DNA

Residual primers and dNTPS in the PCR products were removed using QIAquick™ PCR purification spin columns following the manufacturer's protocol (QIAGEN, Crawley, UK). Direct sequencing of the PCR amplified products was carried out using ITS 2 primer (White *et al.,* 1990). Sequencing reactions of the double stranded DNA templates were carried out using the ABI Prism ™ Dye terminator cycle sequencing ready reaction kit (Applied Biosystems, CA-USA). The products were purified by ethanol precipitation following the manufacturer's protocol and nucleotide

sequences were determined by the ABI 377 automated sequencing technology (Applied Biosystems, CA-USA).

2.4.4.2 DNA sequences analysis and identification

Sequences from ITS 1 region of the ribosomal gene were edited using the BioEdit programme (DNASTAR Inc., Madison, Wis, USA). The ITS1 sequences were aligned to sequences deposited at the National Center for Biotechnology Information (NCBI Gene Bank) using blast N (National Center for Biotechnology Information U.S National Library of Medicine 8600 Rockville Pike, Bethesda, MD 20894). *Pythium* sequences obtained were aligned with Clustal X (Thompson *et al.,* 1994). Consequently they were saved in Phylip format and used for phylogenetic analysis. A neighbour-joining tree was drawn using Clustal X and the boot strapping done to generate trees using 1000 replications. The Tree View software was used to view the consensus trees (Page, 1996).

2.5 Data analysis

Data from the formal surveys was analysed using Statistic Programme for Social Scientists version 11.0 (SPSS) for Windows (SPSS Inc, Chertsey, England). The data was summarised using means, frequencies and percentages. Cross tabulations were used to establish the strength of the association between variables. The data were analysed by SPSS analyses included a one-way ANOVA with no replication and subsequently differences among crop species were compared using Fishers' Protected least significant differences (LSD) at $P \leq 0.05$ (Steel *et al.,* 1997).

2.6 Results

2.6.1 Incidence of root rot in other crops in a bean based cropping system

Root rot incidence in each field for each crop species was determined by taking the number of crops infected with root rot over the total number (healthy and infected) crops. A disease category of less than 59 % indicates low disease in the crop. For the bean crop, the highest numbers of plants (45 %) were in the root rot disease incidence category of 40-59 (Table 1). In addition, potato had the most number of plants (49 %) sampled in the root rot incidence category of 40-59. However, maize and peas displayed 69 % and 77.5 % of their plants respectively in the disease category of 20-39. In contrast maize and green pepper had 50 % of their plants in the root rot incidence disease category of 80-100.

The surveys were carried out for three years from 2004 –2006 during the same season i.e. short rains (three months) (Figure 1). The number of sub counties sampled in 2006 was only 8 compared to the other two years (Table 2). The reason was mainly because interest was in increasing the number of *Pythium* isolates obtained for purposes of having enough samples for DNA sequencing.

Table 1: Mean frequencies of root rot disease incidence found on diseased crop species in Kabale district

	[a] Root rot disease incidence categories (%)					
Crop species	0-19	20-39	40-59	60-79	80-100	LSD ($p \leq 0.05$)
Beans	15.0	19.5	45.0	17.0	34.0	56.12
Cabbage	37.5	109.5	28.0	133.5	16.5	116.15
Cauliflower	7.5	45.0	21.0	59.5	38.5	51.53
Green pepper	0.0	0.0	0.0	100.0	50.0	136.02
Potato	9.5	36.0	49.0	29.0	19.5	13.30
Maize	25.0	69.0	6.5	0.0	50.0	106.94
Peas	16.5	77.5	46.0	0.0	31.5	65.66
Sorghum	28.5	28.5	33.5	18.0	18.0	52.20

LSD = Least Significance Difference test computed at $P \leq 0.05$.
a = Root rot incidence categories is based on the number of crops infected with root rot divided by the total number of healthy and infected crops.

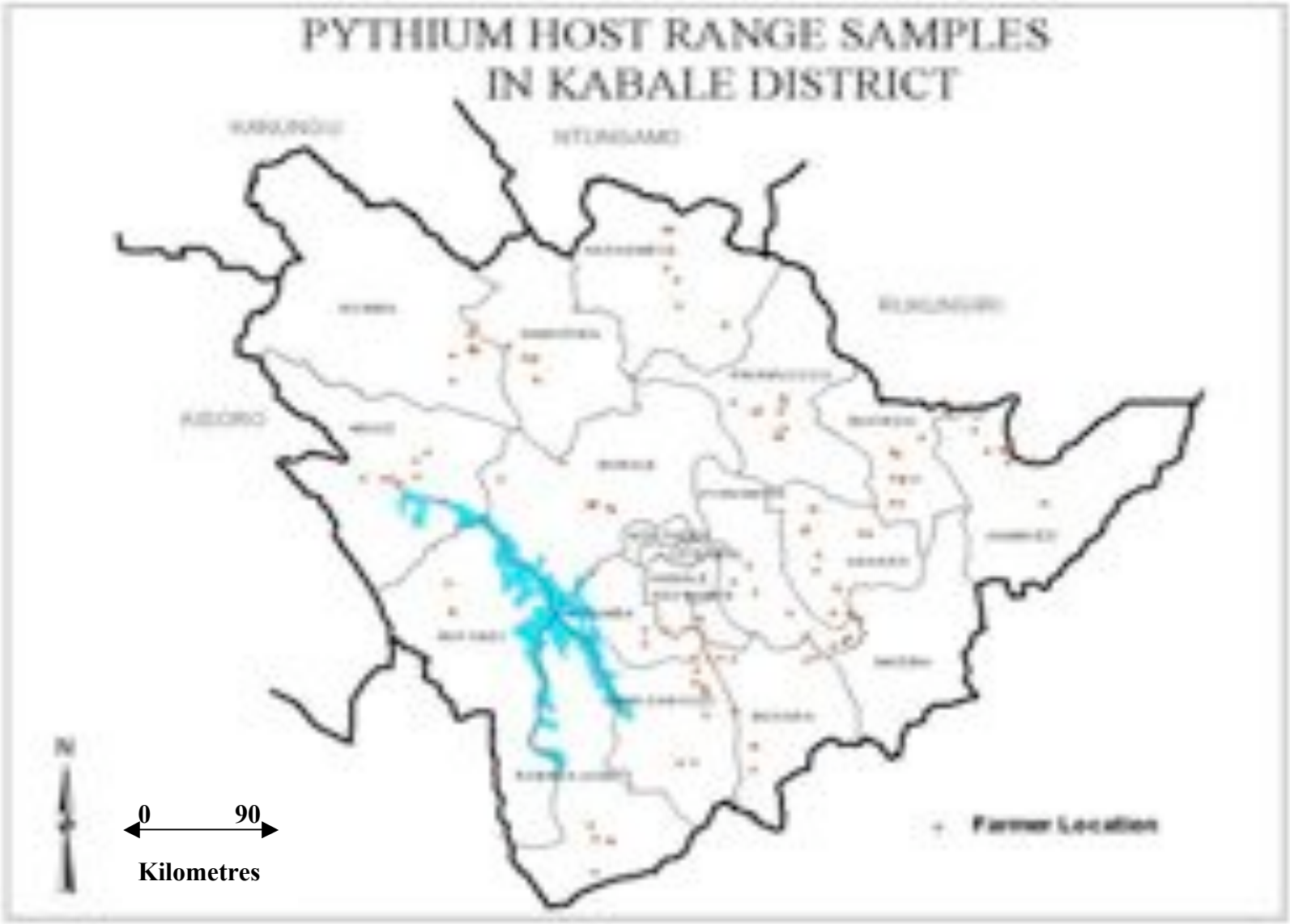

Figure1: A map of south western Uganda where samples were obtained for the study.

Table 2: Distribution of counties, sub counties, parishes, villages and farms sampled during the surveys in 2004, 2005 and 2006

Areas sampled	Years when surveys took place		
	2004	2005	2006
Sub counties	15	18	8
Villages	46	56	21
Parishes	68	-	47

2.6.2 Crops grown in Kabale district

In the 2004 survey the order of abundance of crops found growing in descending order were beans (*Phaseolus vulgaris*) (87.1 %), potato (*Solanum tuberosum*) (63.8%), and maize (*Zea mays*) (25.9 %) (Table 3). On the other hand in the survey conducted in 2005, the abundance of major crops grown in descending order was beans (89.4 %), potato (66.4 %) and sorghum (*Sorghum bicolor*) (29.2 %). However, the order was slightly different in 2006: beans (21.9 %), sorghum (17.5 %) and Potato (14.6 %) (Table 3). The abundance of crops found growing in 2006 were less because fewer sub counties were surveyed compared to the previous two years. In general, from the three surveys, it was found that there were four major crops intercropped with beans in south western Uganda mainly; potato, maize, sorghum and sweet potato (Table 3).

Table 3: Frequencies of the major crops grown in Kabale district in 2004, 2005 and 2006 cropping seasons.

	No. of farms where crops were growing (%)		
Crops	2004	2005	2006
Beans	87.1	89.4	21.9
Maize	25.9	23.0	9.5
Sorghum	16.4	29.2	17.5
Peas	23.3	14.2	6.6
Potato	63.8	66.4	14.6
Sweet potato	14.7	10.6	9.5

2.6.3 Cropping patterns

Various cropping systems were evident throughout the survey with farmers growing a number of crops in different combinations. For example, sweet potato was intercropped with potato only (Plate 3). Also, beans were intercropped with bananas (Plate 4).

Plate 3: The picture was taken in a field where sweet potatoes were intercropped with potatoes.

Plate 4: The picture was taken in a field where beans were mixed cropped with bananas. The beans were infected with root rot as seen by yellowing of leaves.

There were 20 different crops found in mixed cropping systems in this study. In addition, each farm was found to have 1 to 6 different crop species planted (Table 4). Most of the potatoes (*Solanum tuberosum*) in 2005 and 2006 surveys were grown in association with two other crops by 33.0 % and 43.7 % of the farmers respectively (Table 4). A similar cropping pattern was observed with sorghum in 41.2 % and 47.7 % of the farms sampled respectively (Table 4). Maize was also grown with two other crops in 44.4 % and 50 % of the farms sampled. In addition, sweet potato was intercropped with two other crops in 2005 and 2006 in 33.3 % and 44.4 % of the sampled farms respectively. Beans were never cultivated as mono crops but were always grown in association with other crops.

2.6.4 Root rot symptoms in other crops

During the survey, various crop species in Kabale district were found to display symptoms characteristic of root rot (Table 5). Beans displayed 100 % root rot symptoms in 2004 and 2005 respectively. However in 2006, 11.5 % of the bean crops had root rot, while 22.5 % had yellowing of leaves. Maize had 16 % of the sampled plants with symptoms of stem rotting in 2004 but in 2005 and 2006 surveys no symptoms characteristic of root rot were observed. Moreover, sorghum had a number of symptoms which could be attributed to root rot.

Table 4: Mean frequencies of crop species cultivated as intercrops in Kabale district

	[a] Frequencies of crop species in 2005 (%)					[a] Frequencies of crops species in 2006 (%)					
Number of crop species cultivated	1	2	3	4	5	1	2	3	4	5	6
Potato	1.3	29.5	33.0	24.4	11.5	10.3	20.0	43.7	18.4	4.6	2.3
Climbing beans	-	25.0	-	25.0	50.0	-	-	-	-	-	-
Sorghum	11.8	14.7	41.2	17.6	14.7	3.1	13.0	47.7	24.6	7.7	3.1
Beans	-	35.7	31.6	20.4	12.2	-	-	-	-	-	-
Bananas	-	27.8	11.1	33.3	27.8	7.1	28.0	42.9	21.4	-	-
Pumpkins	-	-	-	71.4	28.6	-	-	-	-	-	-
Peas	-	7.1	21.4	35.7	35.7	-	3.7	48.1	29.6	11.1	7.4
Maize	-	3.7	44.4	25.9	25.9	3.8	-	50.0	30.8	7.7	7.7
Cassava	-	-	-	-	100.0	-	-	-	-	-	-
Sweet potato	-	25.0	33.3	25.0	16.0	-	-	44.4	44.4	-	11.1
Yams	-	-	45.5	27.3	27.3	-	-	-	-	-	-
Cabbage	-	-	42.9	14.3	42.9	-	-	-	-	-	-
Egg plants	-	-	33.3	33.3	33.3	-	-	-	100.0	-	-
Green pepper	-	-	-	-	100.0	-	-	-	-	-	-
Tomatoes	-	-	-	33.3	66.7	-	40.0	-	40.0	20.0	-
Tobacco	-	-	50.0	25.0	25.0	-	-	-	-	-	-
Millet	-	-	33.3	-	66.7	-	-	-	-	-	-
Wheat	-	-	50.0	-	50.0	-	-	37.5	-	50.0	12.5
Sugarcane	-	-	-	-	100.0	-	-	-	-	-	-

a= Frequencies of crop species are based on observation and scores taken during the study period.

For example in the survey of 2004, five different root rot symptoms were observed: rotten stem, red lesions on stem, leaves with black spots, black lesions on roots and rotting roots (Table 5) . Majority of the sorghum plants in the 2004 survey (46.2 %) had red lesions on the stem characteristic of stem anthracnose (*Colletotrichum sublineolum*). In the survey of 2005, majority of the sorghum plants (66.7 %) were not diseased while in 2006, albeit the sorghum plants (28.6 %) had wilted leaves. Stunting was a common feature in both surveys of 2005 and 2006. For peas in 2004, only a few plants were found to have typical root rot symptoms such as rotting of roots (5 %), and reduced root system (3%). However, in 2005 there was no root rot disease symptoms observed in 90.9 % of the sampled plants. In case of potato, the major symptoms of root rot disease was wilting of leaves found occurring in 51.3 %, 65.4 % and 53 % of sampled plants in 2004, 2005 and 2006.

2.6.5 ITS analysis and DNA sequencing of *Pythium* species

Gel electrophoresis of PCR products yielded bands ranging from 810 to 1030 base pairs for *Pythium* isolates. PCR products below this range were identified as *Mortirella* species. Using DNA sequences, twenty-one *Pythium* species were identified from the samples (Table 6). In addition, *Pythium* species were found occurring on 13 different crops. Some of these crops were potato (*Solanum tuberosum*), sorghum (*Sorghum bicolor*), maize (*Zea mays*) and sweet potato (*Ipomoea batatas*). Some *Pythium* species were also isolated from beans for comparison purpose. Different clades and sub groups were inferred from the phylogenetic analysis, yielding diverse associations (Figure 2).

Table 5: Frequencies of symptoms found on diseased crop species in Kabale

	2004 [a]		2005 [b]		2006 [c]	
Crops	Symptoms observed on crops	Percentage number of crops with symptoms	Root rot symptoms observed on crops	Percentage number of crops with symptoms	Symptoms observed on crops	Percentage number of crops with symptoms
Beans	Root rot	100.0	Root rot	100.0	Root rot	11.5
	Yellowing of leaves	100.0	Yellowing of leaves	100.0	Yellowing of leaves	22.5
Maize	Yellow and red spots on leaves	83.3	No disease symptom	100.0	No disease symptoms	100.0
	Stem rotting	16.0				
Sorghum	Rotten stem	15.4	Stunting	25.0	Stunted plants	14.3
	Red lesions on stem	46.2	Not diseased	66.7	Black roots	14.3
	Leaves with black spots	15.4	Black purple leaves	8.3	Drying of leaves	28.6
	Black lesions on roots	7.7				
	Rotting roots	15.4				
Peas	Reduced root system	3.0	Not diseased	90.9	Drying of roots	26.7
	Root rot				Yellowing stem	20.0
	Wilted leaves	5.0				
		1.0				
Potato	Wilted leaves	51.3	Not diseased	65.4	Wilting leaves	55.0
	Rotten stems	7.7	Tubers rotting	3.8	Rotting roots	10.0
	Rotten roots	7.7	Wilting leaves	30.8	Yellowing of leaves	3.3
	Black spots on leaves	17.9				
Sweet potato	Reduced root system	100.0				
Cabbage	Yellow leaves	100.0	Rotting roots	100.0	Drying of leaves	11.1
					Dry roots	11.1
					Shrunken leaves	33.3
					Rotten roots	11.1

[a] = The study covered 15 sub counties
[b] = The study covered 18 sub counties
[c] The study covered 8 sub counties

The sub groups were supported by a bootstrap value of 100 %. There was a sub-group with various *Pythium* species clustering together irrespective of location and crop species. For example *Pythium folliculosum* isolated from peas in Rwamucucu sub-county clustered together with *Pythium ultimum* isolated from sorghum in Kaharo sub-county. Also *Pythium* from the same genus were isolated from different crops. For instance, *Pythium irregulare* was found to occur on two different crops i.e. potato and tomato which are from the same genus (*Solanaceae*). Other pathogens were also isolated together with *Pythium* and these included *Fusarium oxysporum, Fusarium equiseti and Verticillium coccosporum* which were isolated from millet, cabbage, potato, sorghum and bean. In this study it was also found that *Pythium* of different species were isolated from the same crop. For example *Pythium ultimum* and *Pythium acanthicum* were both isolated from potato, sorghum, bean and weeds. Also similar *Pythium* species from diverse crop species were found to be isolated from neighbouring sub-counties. For example *Pythium ultimum* was isolated from Rubaya, Bufundi, and Muko and Ikumba sub-counties (Figure 2).

2.6.6 Distribution of *Pythium* species isolated from other crops in a bean based cropping system

Pythium species isolated from other crops were found to be distributed in all the sub counties of Kabale district (Figure 1). Of the *Pythium* species isolated , 39.0 % were *Pythium ultimum* followed by *Pythium folliculosum* (11.0 %), *Pythium acanthicum* (7.0 %) and *Pythium spinosum* (7.0 %). The other *Pythium* species occurred at less than 5.0 % frequency. Out of the 142 isolates characterised, 21 *Pythium* species were

identified (Table 6). The crops which yielded the highest number of *Pythium* species in descending order were potato (20 %), sorghum (16 %) and maize (8 %). However, the lowest occurrence of *Pythium* species (1.4 %) was found on tomatoes, millet, wheat and bananas.

Table 6: *Pythium spp*. isolated from other crops in a bean based cropping system of south western Uganda identified by ITS sequencing

	Crops sampled													
Pythium spp.	Potato	Sorghum	Maize	Bean	Sweet potato	Cabbage	Peas	Tomatoes	Millet	Wheat	Bananas	Weed	Yams	Total
P. ultimum	15	15	11	2	1	5	2	-	-	2	1	-	1	55
P. acanthicum	5	3	-	1	-	-	-	-	-	-	-	1	-	10
P. spinosum	3	4	1	-	-	-	1	1	-	-	-	-	-	10
P. torulosum	2	-	2	1	-	-	1	-	-	-	-	-	-	6
P. folliculosum	1	5	3	5	-	-	1	-	-	-	-	-	-	15
P. oligandrum	1	4	2	-	-	-	-	-	-	-	-	-	-	7
P. parvum	-	2	-	-	-	2	-	-	2	-	-	-	-	6
P.irregulare	2	-	-	-	-	-	-	1	-	-	-	-	-	3
P. glomeratum	2	-	-	-	-	-	-	-	-	-	-	-	-	2
P. heterothallicum	1	1	-	1	2	-	-	-	-	-	-	-	-	5
P. rostratum	1	-	-	-	1	-	-	-	-	-	-	-	-	2
P. arrhenomanes	-	1	-	-	-	-	-	-	-	-	-	-	-	1

Table 6 continued: *Pythium spp*. isolated from other crops in a bean based cropping system of south western Uganda identified by ITS sequencing

	Crops sampled													
Pythium spp.	Potato	Sorghum	Maize	Bean	Sweet potato	Cabbage	Peas	Tomatoes	Millet	Wheat	Bananas	Weed	Yams	Total
P. macrosporum	1	1	-	-	-	-	-	-	-	-	-	-	-	2
P. mamillatum	1	-	-	-	-	-	-	-	-	-	-	-	-	1
P. orthogonon	-	-	-	1	-	-	-	-	-	-	-	-	-	1
P.arrhenomanes	-	-	4	-	-	-	-	-	2	-	-	-	-	6
P. conodiophorum	-	-	-	5	-	-	-	-	-	-	-	-	-	5
P. erinaceum	-	1	-	-	-	-	-	-	-	-	-	-	-	1
P. periplocum	-	-	-	-	1	-	-	-	-	-	-	-	-	1
P. vexans	-	1	-	-	-	-	-	-	-	-	-	-	-	1
P.pachycaule		2											-	2
Total	29	23	12	7	5	5	4	2	4	2	1	1	1	142

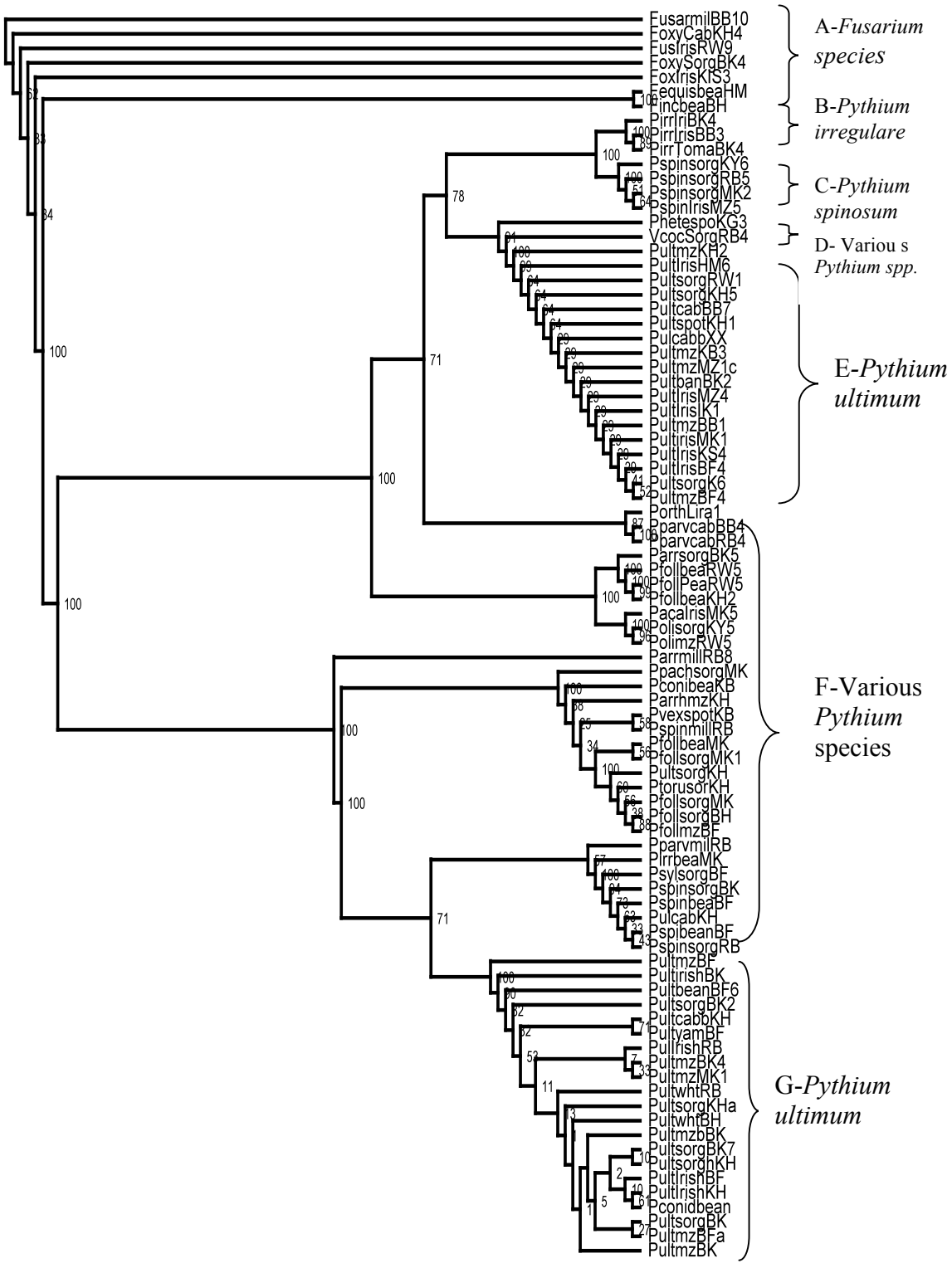

Figure 2: *Pythium* species isolated from various crops in south western Uganda. The phylogenetic tree was rooted using *Fusarium* species sequences collected during this study.The dendogram was generated using Clustal X program (Thompson, 1994) with bootstrapping of 1000 replications. Details of the isolates used are provided as Appendix 2.

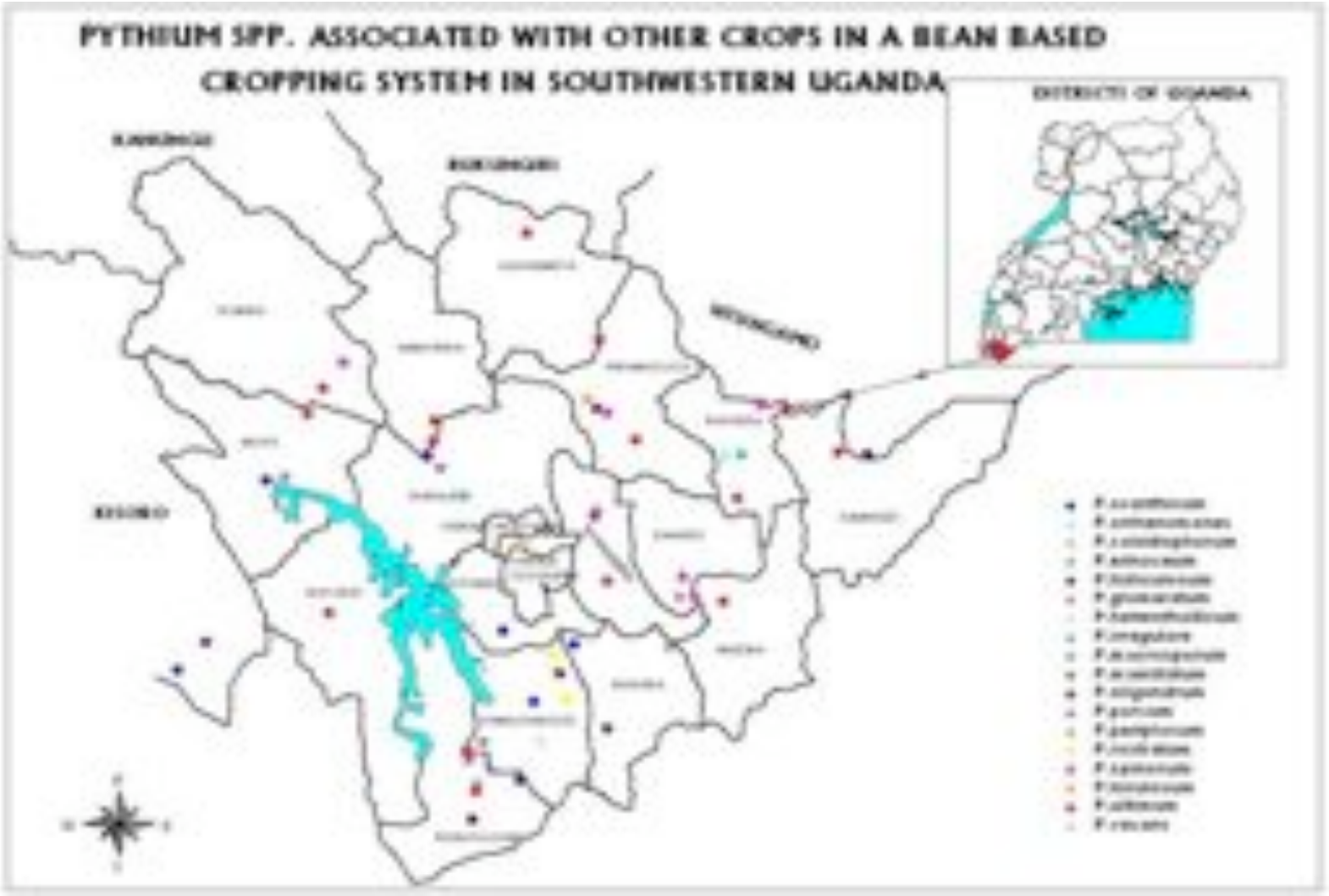

Figure 3: Distribution of *Pythium* species in various subcounties of Kabale district in south western Uganda. The *Pythium* species were identified using species specific primers and sequences. This map was generated using Geographic Information Systems (GIS) software Arc View (Environmental Systems Research Institute, Inc. Seattle, WA, and U.S.A).

2.7 Discussion

The aim of this study was to elucidate the role of intercropping or mixed cropping in the current bean root rot epiphytotics. Beans and potato in this study had high number of plants in the high disease incidence in the category of 40- 59. This suggests that these two crops are affected by root rot disease. In contrast maize had a low root rot disease incidence category suggesting that it is not affected by root rot disease. Whereas peas had characteristic root rot symptoms, the majority of the pea plants were found in low root rot disease category. In general, disease incidence is affected by root damage from other soil-borne pathogens (Pieczarka and Abawi, 1978). Thus the patchy appearance of root rots especially among peas suggests that indeed, crop mixtures especially with beans have lower incidence of the disease. This may be due to the fact that beans are the preferred host compared to peas creating patchy environments in the field, with higher incidence of root rots around bean plants. Mixed plant genotypes from a theoretical standpoint, influence diversity in host populations and ultimately disease epidemics (Burdon, 1987; Burdon *et al.,* 1989).

In the surveys carried out in this study, beans were found to be the main crops grown by farmers followed by potato (*Solanum tuberosum*), maize (*Zea mays*), sorghum (*Sorghum bicolor*) and sweet potato (*Ipomoea ba*tatas). Previous studies indicated that beans are a traditional crop grown by the Bakiga and Bafumbira people in Kabale district because they form a major source of protein in the diets. In addition, the previous study established that the main crops grown in Kabale were beans, sorghum, maize and sweet potato (Ampaire, 2003). The study found that cropping patterns (intercropping) were popular, consisting of two or more crops grown together.

Intercropping in Kabale district is a traditional practice done to satisfy the need for other food requirements (Ampaire, 2003). Intercropping systems also provide for deliberate design and manipulation of land and plant populations to optimise the use of spatial, temporal and physical resources both above- and below ground. This is achieved by maximizing positive interactions and minimizing negative ones (Silwana and Lucas, 2002).

This study also shows that other crops in bean based cropping systems are affected by root rots for example, sorghum, peas and potato. Symptoms observed on sorghum in this study included rotten stem, red lesions on stem, black lesions on roots and rotting roots. In previous studies, symptoms on sorghum have included stunting and black roots, characteristic of *Pythium* root rot (Vincelli and Hershmann, 2002). In another field study carried out in Kabale district, sorghum plants were found to display symptoms of stunted growth, purple leaves and dark-red to black root lesions (CIAT, 2004).

Other symptoms of root rot observed in this study were yellowing of leaves in beans and cabbage. These symptoms found in this study on other crops are characteristic of root rot pathogens infection. Root rots are characterised by above ground symptoms such as poor seedling establishment, uneven growth, chlorosis and premature defoliation of severely infected plants (CIAT, 2005). In Eastern Washington State, USA *Pythium* root rot of wheat was characterised by a reduction in seedling emergence, plant stunting, reduced tillering and loss of fine feeder roots (Paulitz and Adams, 2003). Moreover the problem of poor re-establishment and poor seasonal

production in long term sub terranean clover pastures has been recognised for some time (Paulitz and Adams, 2003). These independent studies provide support for the hypothesis that other crop species may provide alternative hosts to *Pythium* root rot pathogens.

In this study, sorghum did not display any root rot disease symptoms in the survey of 2005, while in the previous survey the symptoms were present. This suggests that root rot disease is seasonal and may be dependent on presence of suitable environmental conditions as well as differences in time of sowing and plant population, which in some instances may aid escape from disease. Also it is known that growing crops in mixtures is important to overcome disease epidemics (Skelsey *et al.,* 2005). Studies elsewhere indicate that increased host diversity can contribute to a reduction of disease progress (Garret *et al*., 2001; Andrivon *et al*., 2003). There is evidence of potential benefits of host diversity in reducing potato late blight in temperate regions such as France and the United States where late blight epidemics tend to develop from obvious foci (Garret and Mundt, 1999). Part of the mixture effect might be due to differences in the spatio temporal pattern of disease spread, with disease progressing mainly along the rows in mixed-cultivar plots (lines of least resistance) as opposed to spatial spread in direct correspondence with the mean wind direction.

In this study, using ribosomal DNA sequences of the ITS region, 21 *Pythium* species were found to be associated with root rot in other crops commonly included in intercrops of beans. Of these, six had been reported previously to be associated with bean root rot (Mukalazi, 2004) but fifteen were new additions identified for the first time. These species are *Pythium spinosum*, *Pythium oligandrum*, *Pythium parvum,*

*Pythium arrhenomane*s and *Pythium conidiophorum*. Previous work made use of restriction fragment length polymorphisms of the Internal transcribed spacer (ITS) regions of ribosomal DNA, 36 *Pythium* species were distinguished (Wang and White, 1997). *Pythium ultimum* was found to be the most abundant of all the *Pythium* species in this study. It was isolated from potato, sorghum, maize, bean, sweet potato, cabbages, peas, wheat, bananas and yams. Previous work in Kabale district identified *Pythium ultimum* as the most abundant *Pythium* species where beans are grown (Mukalazi, 2004). *Pythium ultimum* is also mainly pathogenic to dicotyledons (Bruno and Griffith, 2004). In addition in this study *Pythium ultimum* was found associated with wheat. In other studies *Pythium* root rot of wheat has been found to be associated with *Pythium irregulare*, *Pythium aristosporum* and *Pythium ultimum* var. *sporangiiferum (*Mazzola and Cook, 2001). This *Pythium* species also form a pathogen complex causing spinach root rot (Larsson *et al.,* 1994).

Other *Pythium* species isolated included *Pythium torulosum* from potato, maize, bean and peas, *Pythium folliculosum* isolated from potato, sorghum, maize and peas. In other studies elsewhere, *Pythium torulosum* and *Pythium folliculosum* have been isolated from monocotyledons, bryophytes, green algae, soil and occasionally from dicotyledons and conifers (Levesque *et al*., 2004).

This study also found *Pythium arrhenomanes* occurring in Kabale where temperatures are (optimum 11 ^{0}C, maximum 24 ^{0}C). It was isolated from sorghum. In other studies, *Pythium arrhenomanes* occurs in the Northern Hemisphere at cardinal temperatures of (optimum 25 ^{0}C, maximum 30^{0}C) and mostly isolated from monocotyledons, predominantly grasses. *Pythium arrhenomanes* has also been known to cause root rot

in banana (Musa *spp*.), common wheat (*Triticum aesitivum*), maize (*Zea mays*), sweet potato *(Ipomoea batatas*) where it causes rootlet rot and in potato (*Solanum tuberosum*) (Raabe *et al.,* 1981). Root rot caused by *Pythium* species including *Pythium arrhenomanes* has been recognised as a potentially important disease of graminaceous crops such as wheat, corn and sorghum (Chen *et al*., 1999).

The study also found *Pythium irregulare* on potato and tomato. In previous work this pathogen had also been isolated from the same hosts in Florida (Alferi *et al*., 1994). In addition *Pythium irregulare* was amongst 10 species of *Pythium* isolated from roots of pepper plants from various fields in Florida (Chellemi *et al*., 2000).

In this study *Pythium acanthicum* was obtained from potato, sorghum, bean and weed; *Pythium periplocum* from sweet potato and *Pythium oligandrum* from potato, sorghum and maize. In previous studies, *Pythium acanthicum*, *Pythium periplocum* and *Pythium oligandrum* were found to be pathogens of mainly dicotyledons while also being mycoparasites (Chellemi *et al*., 2000). Moreover, *Pythium oligandrum* and *Pythium periplocum* were found to be active biocontrol agents against *Pythium ultimum,* the damping –off organism of cucumber (Mohammed and Amjed, 1999).

Another, new *Pythium* species found was *Pythium rostratum* isolated from potato and sweet potato. In other studies, this *Pythium* species has been isolated from soil and has a worldwide distribution (Raabe *et al.,* 1981). It causes root rot in pigeon pea (*Cajanus cajan*), potato, maize, sweet potato (Raabe *et al.,* 1981).

Interestingly in this study, *Pythium parvum* was found on sorghum and cabbage. In Kabale district, cabbages are grown in the valleys, in the swamps and wetlands and this could explain the occurrence of this species. *Pythium* species require a moist environment to be able to infect plants. In work done elsewhere, *Pythium parvum* was mostly isolated from water, debris or soil (Raabe *et al*., 1981). Other studies carried out in Japan found that damping off in cabbage seedlings can also be caused by *Pythium ultimum* var. *ultimum* (Masaharu *et al.,* 2006). In this study, *Pythium orthogonon* was isolated from beans. Other studies in Kabale district, did not find this pathogen to be a causative agent of bean root rot (Mukalazi, 2004). However, Levesque *et al*. (2004) identified *Pythium orthogonon* as a new *Pythium* species.

Pythium spinosum in this study was isolated from potato, sorghum and maize; *Pythium irregulare* from potato and peas and *Pythium mamillatum* from potato. This suggests that *Pythium spinosum* affects cereal crops. It is known that *Pythium* species can cause seedling blights and root rots of a number of field crops including soybean and corn (Chen *et al*., 1999). Also, *Pythium spinosum, Pythium irregulare* and *Pythium mamillatum* are important plant pathogens with a worldwide distribution. In studies carried out in Kabale district, *Pythium spinosum* was found to cause severe root rot in beans (Mukalazi, 2004). On the other hand *Pythium irregulare* is a causative agent of root rot on pigeon pea (*Cajanus cajan*) and sweet potato (*Ipomoea batatas*) (Raabe *et al*., 1981). *Pythium mamillatum* has also been found to be the causative agent of root rot in pineapple (*Ananas comosus*). In this study, the *Pythium heterothallicum* was found associated with sweet potatoes. Little is known about the pathogenicity of *Pythium heterothallicum*.

In this study *Pythium* from the same genus were isolated from different crops. For example damping-off of cabbage plug seedlings was caused by *Pythium megalacanthum* de Bary, *Pythium aphanidermatum* (Edson) Fitzpatrick, *Pythium zingiberi* and *Pythium ultimum var. ultimum* (Masaharu *et al*., 2006). Members of the genus *Pythium* are common inhabitants of agricultural soils and form diverse associations with a wide range of plant species. The genus *Pythium* includes a wide range of plant species (Dick, 1990), with many crop plants being susceptible to multiple species (Van der Plaats-Niterink, 1981). Important individual crops appear to support particular strains of *Pythium* (Harvey, 2004). In this study other pathogens apart from *Pythium* were also isolated from crop samples. These included *Fusarium spp.* and *Verticilium*. In eastern Africa, bean root rots are caused by a number of soil borne pathogens, namely; *Fusarium solani f.sp. phaseoli, Sclerotium rolfsii, Rhizoctonia solani* and *Pythium spp*. The pathogens occur either individually or as a complex of two or more pathogens depending on suitable environmental conditions (CIAT, 1992; Rusuku *et al*., 1997). Other pathogens that are frequent include *Fusarium* spp. and *Rhizoctonia solani*, which may occur singly or in complex, with *Pythium* spp. (Opio, 1998).

The fact that similar *Pythium* species in this study were found occurring on different crops in sub- counties neighbouring each other may have resulted due to spread by air and water. Kabale is a hilly place where long distance transport via slow water could be a critical factor in long distance disease spread. The spores of *Pythium* may be transported by insects, wind or farm equipment (David and Dina, 1997). Water from rain and irrigation also facilitates movement, especially when zoospores are produced (David and Dina, 1997).

DNA sequence analyses showed diverse clades and sub groups that did not correspond to host or origin of occurrence of the *Pythium* isolates. These results are similar to those of Cilliers *et al*. (2000) and Harlton *et al*. (1995). Cilliers *et al*. (2000) compared rDNA of *Sclerotium rolfsii* isolates and reported that there was no apparent clustering according to host or geographic origin. Similarly, Harlton *et al.* (1995) found that unique individuals were not necessarily correlated to the host nor restricted in geographical range. Results in this study however show that there is a close affinity of the *Pythium* species. In addition diversity exists among the species but there is no grouping based on host or geographic origin.

Taken together, this study for the first time finds evidence suggesting that *Pythium* species are found on diverse crop species which are usually grown with beans. This may play a role in affecting inoculum level and hence the increase of bean root rot epidemics in south western Uganda. Potato, sorghum, maize and peas were found to have typical root rot symptoms. Also, fifteen new *Pythium* species were identified from diverse crop species commonly included in intercrops with beans. In addition, *Fusarium* species and *Verticillium coccosporum* were also concomitantly isolated though in low numbers compared to *Pythium*. The characterisation of epidemics among these hosts as well as *Pythium* pathogenesis in these crops species is presented in the next chapters.

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CHAPTER THREE

PATHOGENICITY WITHIN *PYTHIUM* PATHOSYSTEMS OF SOUTH WESTERN UGANDA

3 Introduction

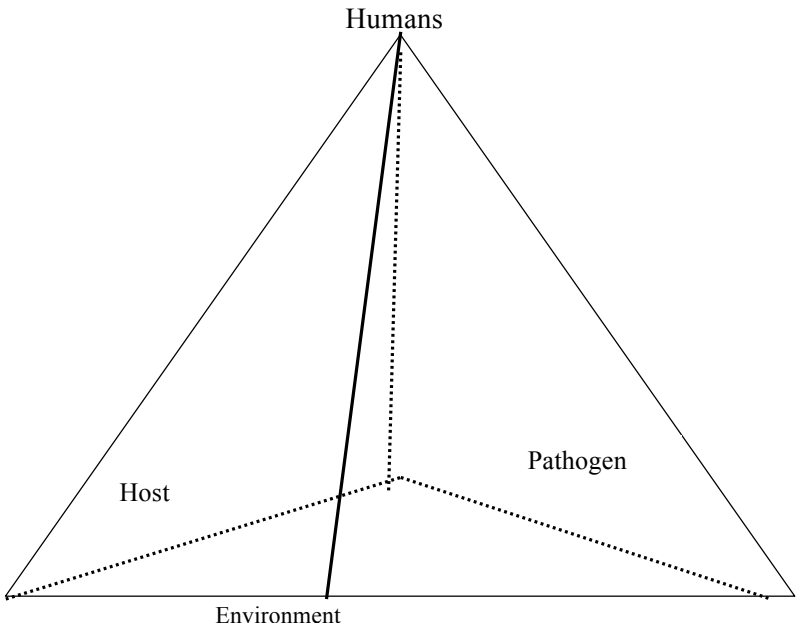
3.1 The pathosystem concept

A pathosystem is a subsystem of an ecosystem and is characterised by the phenomenon of parasitism (Robinson, 1987). Any host-pathogen combination with its multitudes of host and pathogen genotypes can be called a pathosystem (Zadoks and Schein, 1979). A plant pathosystem is one in which the host species is a plant. The parasite is any species that spends a significant part of its lifespan inhabiting one host individual and obtaining nutrients from it. The parasite may thus be an insect, mite, nematode, parasitic angiosperm, fungus, bacterium, mycoplasma, virus or viroid. In a plant pathosystem, a host has the property of resistance to a parasite while the parasite has the property of parasitism on a host. Another feature of a pathosystem concept is that the parasitism is studied in terms of populations at the higher levels and in the ecologic aspects of the system (Robinson, 1987).

Within the pathosystem, the host-pathogen relations are governed by the laws of population genetics and population dynamics; the pathosystem responds to its environment and to the influence of man. There are three categories of plant pathosystems: wild plant pathosystem, crop pathosystem and weed pathosystem (Robinson, 1987). A wild pathosystem is one in which people are not involved. It is in a state of natural dynamic equilibrium and it is also autonomous. There are three

primary components in a wild pathosystem: the host population, the parasite population and the environment. This pathosystem is represented by a two-dimensional pyramid as indicated below:

In addition, the crop pathosystem is derived from a wild pathosystem but it differs fundamentally because of a fourth component, people. It is represented by a three dimensional tetrahedron and it is a deterministic system (Zadoks and Schein, 1979; Agrios, 1997).



3.2 Bean-*Pythium* pathosystems

The common bean (*Phaseolus vulgaris*, L.) is an important component of mixed cropping systems, which are typical of small-scale subsistence farming especially in Eastern Africa (Gitu and Ngalyuka, 1989). In this region, beans are mainly intercropped with potato (*Solanum tuberosum*), banana (*Musa spp.*), groundnut (*Arachis hypogea*) and even coffee (*Coffea robusat*) (Muigai, 1990). The bean and

other crops in the system differ greatly from their wild ancestors. Diseases especially are also part of the bean pathosystem and in this case *Pythium* root rots are considered as one of the most important production constraints especially in south western Uganda. The environment also plays a crucial role in bean root rot epidemics (CIAT, 1992). For example in Rwanda, seasonal variation influences patterns of bean root rots. Furthermore, high soil moisture content also create favourable environment to *Pythium spp.*

3.3 Eco physiology of Plant-Rhizosphere

The rhizosphere is a soil zone in which the soil microflora is influenced by the plant root (Rovira and Davey 1971). It is the site of organic deposition and the generator of habitat and resource heterogeneity for soil organisms (Stanton, 1988). An important property of plants is their ability to change or modify the rhizosphere during their growth. Root exudates have a profound qualitative and quantitative effect on the rhizosphere micro-flora (Schenck, 1976). A plant can modify its rhizosphere through ion uptake, production of root exudates, rhizo-deposition (carbon loss from roots) and changes in the acidity and alkalinity of the rhizosphere (Darrah, 1991). Plant root exudates can enhance growth of both beneficial micro organisms and pathogens and can oxidize many nutrients such as iron, sulphur and manganese. Changes in rhizosphere pH can occur in response to adverse nutritional conditions (Mugwira *et al.,* 1978). A decrease in rhizosphere pH may be induced by plants growing under phosphorous (P) and iron (Fe) (Marschner *et al.,* 1983) deficiencies. These root exudates are involved in the interactions between plants.

3.4 Literature review

3.4.1 Bean root rot pathogens

Root rot diseases have been associated with five or more fungal pathogens, which occur singly or in a complex (Rusuku *et al*., 1997). Four *Pythium spp* namely *P. ultimum* Trow, *P. irregulare* Buismann, *P. aphanidermatum* (Edson) Fitz, and *P. myriotylum* Drechs. are pathogenic to beans (Abawi *et al.,* 1985). Pieczarka and Abawi (1978) reported severe bean root rot in soil infested with *Pythium ultimum* and *Fusarium solani f. sp*. *phaseoli*, which suggested a synergistic relationship between the pathogens. In the same study, *Rhizoctonia solani* reduced the severity of root rot incited by *Pythium ultimum* suggesting an antagonistic relationship of the two pathogens. There were no interactions between *Fusarium solani* f. sp. *phaseoli* and *Rhizoctonia solani.*

In Eastern Africa, bean root rots are caused by a number of soil borne pathogens, namely; *Fusarium solani f.sp*. *phaseoli, Sclerotium rolfsii, Rhizoctonia solani* and *Pythium spp*. (Opio, 1998). In south western Uganda, the major pathogen causing severe root rot has been identified as *Pythium spp* (Mukalazi, 2004). Eleven *Pythium* species were found to be associated with bean root rots in Uganda. Apart from the traditional species, *Pythium ultimum* var. *ultimum* known to infect beans, other *Pythium* species recovered from infected beans were *Pythium spinosum, Pythium torulosum, Pythium salpingophorum, Pythium vexans, Pythium dissotocum, Pythium nodosum*, *Pythium echninulatum, Pythium pachycaule, Pythium oligandrum* and *Pythium deliense*. Other pathogens that were frequent in the area include *Fusarium spp.* and *Rhizoctonia solani* (Tusiime, 2004). *Pythium ultimum var. ultimum, Pythium*

spinosum, Pythium torulosum, Pythium nodosum and *Pythium pachycaule* were found to be pathogenic to the bean varieties CAL 96 and K20 (Mukalazi, 2004). Morphological, molecular and pathogenicity analyses also revealed that there is wide diversity within the *Pythium* population. However the diversity of these *Pythium* species in other crops is largely unknown thus the focus of this study. This study sought to establish whether crops grown as intercrops with beans are likely alternative hosts of *Pythium* and whether this could account for increased *Pythium* epidemics in beans despite efforts to develop integrated cultural management options for the control of root rot. Some of the methods which have been used singly or in combination to control root rots in the region over the last two decades include growing of beans on raised beds or ridges (Abawi and Pastor-Corrales, 1990), use of *leucaena* green manure (Buruchara, 1991), farmyard manure and *Calliandra* green manure (Buruchara and Scheidegger, 1993; Otsyula *et al.,* 1998; Opio *et al.,* 2000; Mukalazi, 2004), use of resistant bean cultivars (CIAT, 1992). This study will therefore seek to formulate recommendations on the use of intercrops for reducing incidence of bean root rot on the basis of them not being likely alternative host.

3.4.2 *Pythium* host range

The genus *Pythium* belongs to the kingdom Chromista, family Pythiaceae, order Perenosporales and class Oomycetes (Martin, 1995). More than 200 species of this genus have been described (Dick, 1990). In addition, the members of this genus have coenocytic branched mycelium with phylogenetic comparisons, placing them close to algae and higher plants (Baldauf and Palmer, 1993). Furthermore, *Pythium* species can either produce asexually or sexually. In the former case they generate zoospores

while in the latter case, the sexual processes result in the formation of oospores, which are able to survive in plant tissue and soil for many years (Martin, 1995).

Pythium species have a wide host range and mainly cause seed rot, damping off and root rot of seedlings (Matsumoto *et al.,* 2000). While phytopathogenic species of *Pythium* are capable of infecting a wide range of plants, members of the genus are not uniform with regard to pathogenicity; individual species vary in the extent of their host range as well as virulence on specific hosts. *Pythium aphanidermatum* and *Pythium ultimum* are capable of causing serious losses of a number of economic crops in a variety of plant families (Van der Platts-Niterink, 1981). The diploid oomycete *Pythium irregulare* is highly pathogenic to a wide range of cereal and legume hosts, causing pre-emergent blight and post-emergent stunting of crops and pastures (Pankhust *et al.,* 1995). Infected tissues become spongy, wet, discoloured with many cavities. Other symptoms of this disease include lower leaf yellowing (similar to nitrogen deficiency), stunting, leaf browning and death of the plant (Pankhust *et al.,* 1995). This pathogen may also cause pre- or post-emergent seedling blight. This pathogen is known to increase disease severity in cereals infected with other soil borne plant-pathogenic fungi. Poor yield performance of crops in cereal-legume rotations is thought to be due to persistence of this pathogen (Ingram and Cook, 1990). *Pythium myriotylum* is considered to be the main causal agent of pod rot in groundnuts and is also pathogenic to tomato, rye, wheat, oats, cucumber, soybean, sorghum, tobacco, cabbage and maize. *Pythium ultimum* causes a watery wound rot of potatoes upon invasion of wounded sites and has been reported to cause root rot of sugar beet in Iran (Ahary-Babai *et al*., 2004). *Pythium oligandrum* is a potential biocontrol agent of a number of soil borne plant pathogens (Takenaka *et al.,* 2003). It

has been reported to have an antagonistic activity via mycoparasitism and antibiosis (Berry *et al.,* 1993), enzyme production and competition (Martin and Hancock, 1987). Also, *Pythium oligandrum* has been shown to colonise the rhizosphere of a wide range of agriculturally important crops (Al-Rawahi and Hancock, 1997). *Pythium acanthicum, Pythium irregulare, Pythium mamillatum*, *Pythium oligandrum* and *Pythium spinosum* have been associated with disease of sugar beet seedlings (Ahary-Babai *et al*., 2004). Also, *Pythium splendens, Pythium irregulare* and *Pythium vexans* are sometimes associated with dysfunctional plants in Florida (Kucharek, 2000). Root rot in peas is associated *with Pythium ultimum* as well as complexes of other pathogens such as *Aphanomyces euteiches, Fusarium solani f. sp. pisi,* and *Rhizoctonia solani*. *Pythium* root rot of wheat (*Triticum aestivum* L) is caused by at least nineteen *Pythium* species in North America (Farr *et al.,* 1989). From a sample of 80 wheat fields in eastern Washington, Paulitz and Adams(2003), found 46 combinations of 13 different *Pythium spp*, and nine of these were virulent on wheat (Higginbotham *et al*., 2004).

In East Africa the following *Pythium* species have been found to be associated with beans resulting in root rot *Pythium spinosum, Pythium torulosum, Pythium salpingophorum, Pythium vexans*, *Pythium dissotocum, Pythium nodosum, Pythium echninulatum, Pythium pachycaule, Pythium oligandrum* and *Pythium deliense*. However no study has been done on the pathogenicity of *Pythium* species that could be occurring on other crops especially in a bean based cropping system.

3.4.3 Bean cropping systems in East Africa

The cultivation of beans in association with other crops species is a primary characteristic of bean production in Eastern Africa (Muigai, 1990) and Africa as a whole (Allen *et al.,* 1989). In general, a cropping system refers to the distribution of plants and animals in space and time and the combination of agro-inputs. Cropping systems may vary from one farmer to another and across location. For example at high altitudes between 2000 and 2300 m, monoculture of beans predominates but relay cropping and associated cropping with maize are also practiced (Pachico, 1989). At lower altitudes, 1200-2000 m, complex associations become more common. In Rwanda and Burundi, the most common associations of beans are with bananas, maize and sweet potatoes. In north Kivu, Democratic Republic of Congo, staked climbing beans are most often grown in monoculture, perhaps because of their more market-oriented production. However associations with maize, bananas and coffee are also practiced. (Allen *et al.,* 1989). Each farm household formulates its own cropping system based on resource endowment, experiences and priorities (Bashaasha *et al.,* 1995). For example, intercropping maize and beans is the most common cropping system in *Striga* endemic regions of Kenya (Odhiambo and Ariga, 2001).

In Uganda, 75-90% of beans are grown in varied crop associations in small farms (Osiru, 1982; Allen *et al.,* 1989) and mainly with sweet potato (Bashaasha *et al.,* 1995). Similar complex cropping systems are found in Kenya, the southern highlands of Tanzania, northern Zambia and Malawi (Edje, 1979). The crop most commonly associated in mixed cropping bean system includes maize, although the bean-banana-coffee association predominates in some areas. Other companion crops include sweet potatoes, peas, cassava, yams, cocoyams, potatoes and peanuts (groundnuts). These

associations play an important role in reducing drought stress for the associated bean crop and thus improve stability of the system. Associated cropping (intercropping and mixed cropping) is more common in areas where land is scarce (because of high population density) and less common in areas where production is market-oriented (Allen *et al.,* 1989).

A number of reasons have been given for the popularity of intercropping. Firstly, due to land defragmentation people have less land and therefore prefer to intercrop beans rather than carry out crop rotation (Ocitti p'Obwoya, 1996). Secondly, crop species used as intercrops have different harvesting periods, ensuring food security throughout the year. Sweet potato has a short –medium growth cycle therefore it is suitable for intercropping with crops, which have a shorter growing period of 3-4 months like beans (Wolfe, 1992). Their different growth patterns mean that their resource use differs and this complementarity can give better temporal use of resources (Willey, 1990). Moreover, sweet potato and beans differ morphologically and architecturally which may influence their competitive abilities for growth resources (Ocitti p'Obwoya, 1996). Sweet potato requires much radiation while beans are relatively shade tolerant (Wortmann *et al.,* 1991). These significant genotypic differences contribute to the difference in both above and below ground competition (Ocitti p'Obwoya, 1996). Thirdly, some of the crop species in the cropping system act as cover crops. When beans are intercropped with maize (*Zea mays*), cassava (*Manihot esculenta*), coffee (*Coffea Arabica*) or banana (*Musa spp.*), they are primarily planted as cover crops (Kisakye, 1991). Fourthly, cropping mixtures also provide better disease control than those of sole cropping and the rate of development of disease epidemic is reduced if a crop susceptible to a disease is intercropped with

another that is resistant to it (Ocitti p'Obwoya, 1996). In other studies intercropping sweet potato and bean reduces the incidence of common bacterial blight and angular leaf spot by 21.9% and 27.5% respectively of the sole crop (Ocitti p'Obwoya, 1996). The reduction is higher in beans grown with erect sweet potato probably because the two grow intimately in mixtures and therefore create a microclimate for the bean component which is not conducive for the development and spread of the diseases (Ocitti p'Obwoya, 1996).

In south western Uganda, beans are grown either as sole crops (91%) or in association with other crops (94%) depending on growth habit or season (Ampaire, 2003). Climbing varieties of beans are normally grown as pure stands while bush varieties are intercropped. During the first rains of every year in Kabale district, beans are planted as pure stands because the short rainy season is not suitable for long term crops like maize (Ampaire, 2003). The second rain season of each year, that is the long rainy season, beans are commonly intercropped because this is a suitable time for other intercrop components (Ampaire, 2003). The major crops intercropped with beans in this district include maize (79%), sorghum (52%), peas (46%), potatoes (4%), sweet potatoes (1%) and yam (1%) (Ampaire, 2003). At least three crops are planted in the same field at the same time, resulting into continuous bean cultivation on the same field during both seasons of a year.

3.4.4 Pathogenesis and epidemiology of root rots

3.4.4.1 Pathogenesis of *Pythium* root rots

Infection of crop plant species by *Pythium spp.* usually occurs via motile zoospores, which form spore germ tubes or saprophytic mycelium on contact with seeds or seedling tissues of host plants. This happens either by chance or because the exudates of these crops serve as nutrients and chemotropic stimulants. Initial infection occurs at or slightly below the surface of the soil depending on moisture level and depth of planting (Agrios, 1997). When the mycelium contacts the host tissue, it penetrates and begins to absorb nutrients using haustoria (Whitney and Duffus, 1995). In seedlings, the fungus penetrates the embryo or emerging seedling tissue either by mechanical pressure or by pectinolytic enzymes, which dissolve the middle lamella of cells and result in the maceration of the tissues. The products of the plant cell breakdown are then used by the pathogen for its metabolic activity (Agrios, 1997).

When vascular tissues are invaded by the *Pythium* mycelium, they become discoloured (Agrios, 1997). The infested seedlings also die. However, well-developed mature tissues present considerable resistance to the invasion of the fungus because of the increased thickness and modified cell wall structure (Agrios, 1997). Apart from invading embryos and seedlings, the *Pythium* attacks the rootlets of most plants. This happens when the pathogen enters root tips and proliferates in the young cells causing rapid collapse and death of the rootlet. When *Pythium* root rot occurs, the outer portion (cortex) of the roots is easily removed from the root core. If *Pythium* root rot occurs early in the life of the crop, it may cause an infected root to produce multiple roots (Kucharek, 2000). It also results in wilting and older leaves of infected plants

become yellow. This symptom is used as a positive diagnosis of *Pythium* root rot (Chase, 1999). In addition, field examination of roots infected with *Pythium* may reveal an abbreviated root system (Kucharek, 2000). Other symptoms related to *Pythium* infection include reductions in grain and fruit yield as well as wilting of numerous crops (Kucharek, 2000).

Pythium is able to attack a wide range of crop species. For example sorghum seed may be attacked by *Pythium* species prior to emergence and germination (Vincelli and Hershmann, 2002). This usually occurs in poorly drained, cold-wet soils or in very dry crusted soils. In the case of wheat, *Pythium* root rot is characterised by a reduction in seedling emergence, smaller and distorted first leaves, plant stunting, reduced tillering, loss of fine feeder roots and lower yields (Paulitz and Adams, 2003). The symptoms associated with *Pythium* infection in peas include the roots being destroyed and pale yellow leaves (Malvic and Babadoost, 2002). Monocrops also experience *Pythium* attack. The seedlings undergo pre-emergent or post emergent damping off (Kucharek, 2000). Beans suffer *Pythium* root rot attack at all growth stages but especially while the crop is young (2 to 3 weeks after planting) (Rusuku *et al.,* 1997). The symptoms in beans include seedling damping off, yellowing of leaves, stunted growth and rotting of roots (Otsyula *et al.,* 1998; Mukalazi, 2004).

In conclusion, symptoms due to *Pythium* infection in beans have been well determined in East Africa. However, the same cannot be said of other crops species in the bean based system.

3.4.4.2 Epidemiology of *Pythium* root rots in mixed cropping populations

A greater understanding of epidemiology in populations of mixed plant genotypes is appealing from several perspectives. Firstly from a theoretical standpoint, it offers insight into how pathogens may use a patchy environment (Burdon *et al.,* 1989) and the costs and benefits of diversity in host populations (Burdon, 1987). Secondly from an applied point of view, understanding epidemics in plant genotype mixtures offers attractive possibilities of deployment of disease resistance in agricultural crops (Wolfe, 1985). Epidemiology therefore seeks to study disease in populations. In the case of *Pythium* root rot, the disease complex is affected by environmental, host and cultural factors (Zadoks and Schein, 1979). The factors, which enhance root rot include; high soil moisture, high soil temperature, poor soil compaction, high organic matter, high levels of herbicide use, high plant density, poor seed quality and irrigation run off (Zadoks and Schein, 1979). The critical environmental factors may influence *Pythium* root rot spatial and temporal disease patterns.

Indeed two soil factors namely; high clay content and low soil pH enhance *Pythium* infection especially in wet soil (Paulitz *et al.,* 2002). High clay content enhances water retention at a given matrix potential. More water in the soil means greater diffusivity of seed and root exudates to far distances stimulating more germination of *Pythium* spores resting dormant in the soil and thus spreading disease over distance. On the other hand, low pH is suppressive to microorganisms that are competitors of *Pythium* in the soil (Paulitz *et al.,* 2002). Also improved soil drainage in response to structural changes in soil following the transition from conventional to no tillage can greatly limit *Pythium* damage (Paulitz *et al.,* 2002).

Another important factor in the *Pythium* root rot epidemics is the role of other hosts in maintenance or reduction of inoculum load. *Pythium* species attack several crop species and therefore this key feature cannot be ignored (Paulitz *et al.,* 2002). There is evidence that different crops favour different *Pythium* species. Thus rotation of crops may also rotate the species of *Pythium* available for infection of the next crop (Paulitz *et al.,* 2002). *Pythium irregulare* thrives on barley, while *Pythium ultimum* thrives on peas and both of these species are pathogenic on wheat (Ingram and Cook, 1990). Thus, a two-year wheat-pea rotation could be expected to select for *Pythium ultimum* while a wheat-barley-pea rotation could be expected to select for both *Pythium ultimum* and *Pythium irregulare*. Unfortunately crop rotation is rarely practised in many parts of Africa mainly due to unavailability of land in areas where population is high. Elsewhere, crop rotation reportedly suppresses *Pythium* populations and reduces disease on many hosts (Pankhust *et al.,* 1995). Severity and incidence of disease has also been known to be influenced by availability of carbon (Agrios, 1997). In many farming systems, soil carbon availability is usually enhanced by manure and plant residue. Organic amendments including animal manure, composts and green manures are commonly used in agricultural systems to recycle nutrients and energy as well as improve soil conditions for plant growth (Trankner, 1992; Muchovey and Pacovsky, 1997). Thus amendments provide energy and nutrients to soil, drastically changing the environment for the growth and survival of crops and microorganisms (Drinkwater *et al.,* 1995). Some organic amendments suppress certain soil borne plant pathogens or cause diseases (Akhtar and Malik, 2000). In the case of bean root rots organic amendments alter the carbon: nitrogen (C: N) ratio in the soil and regulate microbial activity in the soil (Hall, 1996). In Eastern Africa, use of green manures

particularly *calliandra* and farmyard manure in root rot infested soils reportedly reduces disease severity and increases bean yield (Buruchara and Scheidegger, 1993; Otsyula *et al.,* 1998; Opio *et al.,* 2000). Taken together, a number of factors influence bean root rot epidemics. These factors influence temporal aspects of epidemics by alternating survival and pathogenesis of *Pythium* and spatial aspects in terms of spread over distance. In this thesis the effects of other crops on the bean root rot epidemics in south western Uganda will be studied.

3.4.5 Rhizosphere modification

Rhizosphere processes are driven primarily by the plant root (Stanton, 1988). The plant root affects the rhizosphere mainly through release of organic and inorganic material into the soil-rooting zone. The organic material arises from sloughing of root material and from direct root exudation (Hale and Moore, 1979). Consequently, microbial activity in the rhizosphere is associated with plant metabolism. Plant growing conditions that affect shoot photosynthesis; photosynthate translocation to the root and root metabolic activity affect the density and activity of rhizosphere microflora (Rovira and Davey, 1971). Rhizosphere microorganisms such as *Pythium spp., Fusarium spp., and Rhizoctonia solani* can affect plant nutrition through their influence on availability of nutrients, the growth and morphology of roots, nutrient uptake processes and the physiology and development of the roots (Harari *et al.,* 1988).

3.4.6 Influence of plant exudates on root infecting fungi

Plant exudates play a role in pathogenesis by root infecting fungi and other plant pathogens. Techniques such as paper chromatography and other methods of biochemical analysis for studying microbial populations in a soil environment has shown the importance of plant exudates in initiating and sustaining pathogenesis of these rhizosphere inhabiting pathogens (Schroth and Hildebrand, 1964). These techniques have indicated that exudates both directly and indirectly affect pathogenesis by root pathogens. Pathogens are affected indirectly by competition and antibiosis by the root micro-flora whose activities are also mediated by exudates. Given that root exudation is a phenomenon common to all higher plants, it can be said without question that the interactions between the abiotic and biotic environments in the rhizosphere are mediated to a large extent by the occurrence of plant exudation. Pearson and Parkinson (1961) investigated the location of root exudation with broad bean (*Vicia faba*) and found that exudation was predominantly from root tips. The areas where exudation is the greatest appear to coincide with the regions of highest enzymatic activity in the root. Other major sites of exudation appear to be associated with breaks in the epidermis. The occurrence in exudates of substances involved in cellular metabolism is not surprising, since, in nature, part of the exudates found in soil is from sloughed-off root cap cells, injured cells, root hairs, and the autolysis of epidermal cells. These exudates would consequently include the diverse substances in cell vacuoles, such as salts, sugars, organic acids, glycosides, fatty substances and other soluble substances. These, in addition to the contents of the cytoplasm, provide the soil solution with a wide spectrum of plant substances. Another source of exudates, although seldom considered, is nutrient loss from above ground plant parts by the leaching action of water.

3.4.7 Role of exudates in pathogenesis

Root exudates are known to affect pathogenesis of fungi in various ways. Primarily the exudates affect the germination of fungi, which exist in soil as resting spores in absence of the host. The spores of *Fusarium solani f.sp. phaseoli* germinate when in susceptible plant variety rhizospheres or in the presence of susceptible crop varieties exudates. Germination occurs in the rhizosphere of hosts because, exudates from the plant root and hypocotyls provide a source of carbon and nitrogen (Schroth and Hilderbrand, 1964). Root exudates affect chemotaxis of zoospores, due to presence of inorganic ions (proteins, amino acids and sugars) in exudates (Nelson, 1990). In *Phytophthora cinnamoni,* zoospore attraction is greatest in a susceptible host, whereas roots of other plants exhibit little or no attraction for zoospores (Hinch and Weste, 1979). The reason is that since zoospores are positively charged they are attracted to the root surface by a weak electric current through electrostatic forces. In addition, some of the substances in the exudates may have a dual role in the attraction of zoospores. Nutritive substances such as amino acids, which have amino groups, may provide anion exchange centers in the mucigel around root tips (Jenny and Grossenbacher, 1964). Thirdly root exudates may also support growth of pathogens in the rhizosphere. Unless a fungus is situated in the rhizoplane, it must reach the host by vegetative growth with an accompanying expenditure of energy. After reaching the host, some pathogens may have the ability to penetrate with a minimum of ectotrophic growth (Sewell, 1959), whereas others produce a mycelial mat or thallus on the host before penetration (Whitney, 1954). The establishment of a pathogen on the host is therefore partly a function of the nutritional environment as influenced by root exudates, and the interactions between associated microorganisms whose activities also are mediated by the exudation of energy sources or growth substances.

The amount of exudation has been found to influence disease incidence. For instance it was shown that exudation of sucrose is a major factor governing susceptibility of peas to pre-emergence damping-off caused by *Pythium debaryanum* (Flentje, 1957). Temperature also appears to influence the amount of exudation and disease development. At temperatures of 17° C and lower, the rate of bean seed germination and emergence of bean cotyledons from soil are greatly retarded, yet exudation from the seed and developing plant occurred continuously. The net effect is an increase in the amount of exudates available to stimulate pathogens around the germinating seed and developing seedling as compared with seeds which germinated at higher temperatures and whose cotyledons rapidly emerged from the soil (Flentje, 1957). The incidence of pre-emergence damping-off by *Pythium* species is also greater at lower temperatures (Flentje, 1957). Thus, temperatures of 17° C and lower retard the development of the seedling and result in a greater concentration of exudates in the rhizosphere about the seedling and therefore favor increased activity of damping-off fungi. Root exudates also affect the establishment of infection structures, which affect morphogenesis, and establishment of the pathogens. For example exudates from radish stems and cotyledons stimulate cushion formation in the absence of host tissue (Kerr and Flenteje, 1956).

3.4.8 Sectional Conclusion

The bean pathosystem of south western Uganda consists of the bean crop, and other crops grown in association with it, as well as any pathogens which affect the system. Environment and the influence of man also affect the pathosystem (CIAT, 1992). The cultivation of beans in association with other crop species is a primary characteristic

of bean production in Eastern Africa (Muigai, 1990). In south western Uganda, the major pathogen causing severe root rot has been identified as *Pythium spp.* (Mukalazi, 2004). Five of these species were found to be pathogenic to beans namely: *Pythium ultimum* var. *ultimum*, *Pythium spinosum*, *Pythium torulosum, Pythium nodosum* and *Pythium pachycaule. Pythium* species are known to have a wide host range infecting a wide range of plants (Matsumoto *et al,* 2000) and resulting in yellowing of leaves, stunting and finally death of the plant (Ingram and Cook, 1990). The occurrence of intercropping in south western Uganda may thus be influencing bean root rot epidemics. In this chapter I investigate the contribution of various crop species to the bean root rot epiphytotics in south western Uganda.

3.5 Materials and methods

3.5.1 Cross -pathogenicity of bean pathogenic *Pythium* species

The objective of this study was to carry out cross pathogenicity tests of some crop species with bean pathogenic *Pythium* species. It is based on the hypothesis that mixed cropping systems provide alternative hosts to *Pythium* species. To test this hypothesis experiments were conducted under controlled conditions in a screen house.

Experiments to study the cross pathogenicity properties of bean derived *Pythium spp.* were initiated in a screen house at the National Agricultural Research Laboratories-Kawanda (N.A.L.I). Four *Pythium* species previously found to be pathogenic on beans were used (Mukalazi, 2004). These isolates were MS 61 (*Pythium ultimum var.ultimum*), KAK 5 B (*Pythium macrosporum*), VIH 2A (*Pythium chamaehyphon*) and JM 29A *(Pythium pachycaule*). The *Pythium* isolates were grown on corn meal

agar (CMA). After 2-3 days *Pythium* inoculum was raised on autoclaved millet (100 g) mixed with 200 ml of water in 500 ml bottles. After two weeks, pre sterilised soil was mixed with the infested millet at a ratio of 1:10 v/v in wooden trays of 42 cm x 72 cm. The trays were set up following a Randomised Complete Block Design (RCBD). The inoculum was applied to maize, millet and sorghum, peas, susceptible bean varieties (CAL 96) and resistant bean varieties (RWR 719).

After germination, the seedlings were watered every day to provide a favourable environment for the pathogen establishment and development. Emergence data was collected one week after planting. Three weeks after emergence of the seedlings, the surviving plants were harvested and washed with water to remove soil. Severity of root rots was then estimated based on CIAT scale of 1-9 (Abawi and Pastor Corrales, 1990), where 1 = no root symptoms; 3 =a maximum of 10 % of the hypocotyls and root tissues have lesions; 5 = approximately 25 % of the hypocotyls and root tissues have lesions and the root system suffers a considerable decay; 9 =75% or more of the hypocotyls and root tissues have lesions and the root system suffers advanced stages of decay and considerable reduction. *Pythium* isolates which gave a mean disease score of 1-2, were considered non pathogenic; those which gave a score of 3-5 were considered mildly pathogenic and those that gave a score of 6-9 were highly pathogenic and virulent. After scoring, the root tips of the various crops were destructively sampled for dry matter determination. Since the crops' roots varied in size, 5 plants of maize, peas and beans (CAL 96 and RWR 719) were used, while 10 plants were used for sorghum and millet, to minimise between host variations and enhance estimation of the error level.

3.5.2 Cross -pathogenicity of *Pythium* species derived from other crop species

In this study seven *Pythium* species from crop species intercropped with beans were obtained during the surveys carried out in south western Uganda were used. The *Pythium spp*. included *Pythium macrosporum, Pythium oligandrum, Pythium spinosum* isolated from sorghum*, Pythium glomeratum* isolated from potato, *Pythium arrhenomanes* isolated from maize and *Pythium heterothallicum* isolated from sweet potato. Inoculum was prepared and used as described in section 3.5.1. Pathogenicity of these *Pythium* species derived from other crop species were tested on cereals (maize ad millet) and legume crops (peas and beans) as described in section 3.5.1. Data were collected as described before in section 3.5.1. Emergence, disease severity scores and dry matter data were subjected to analysis of variance (ANOVA) using Genstat software package (Lawes Agricultural Trust Rothamsted Experimental Station, 1995). Means were compared using Fisher's protected least significant difference test at 5 % probability level (Steel *et al.,* 1997).

3.6 Results

3.6.1 Emergence of crops species after inoculation with bean pathogenic *Pythium* species

The data shows that *Pythium* species varied significantly ($P \leq 0.05$) in the pathogenicity quantified as emergence of seedlings when compared with the control on crop species. The highest seedling emergence was in the non inoculated control with all the crop species (Figure 3). Millet had a similar trend of high germination like the resistant bean variety (RWR 719). The lowest germination of seedlings was with

peas which had a similar trend of germination like the susceptible bean variety (CAL 96) (Figure 3). The data also revealed that *Pythium pachycaule* was the most pathogenic *Pythium* species on most of the crops while *Pythium chamaehyphon* was the least pathogenic. In general, within host reaction to *Pythium* infection was not significantly different (Figure 3).

3.6.2 Post emergence damage of crops species after inoculation with bean pathogenic *Pythium* species

3.6.2.1 Root rot disease severity on selected crop species

Various root symptoms were observed in the trays inoculated with *Pythium* species, which were absent in the noninoculated control trays. Susceptible bean variety (CAL 96) displayed typical root rot symptoms which included waterish stem and reduced root system (Plate 5). Sorghum was observed to have red-black lesions on the roots (Plate 6). Millet displayed the formation of prop roots while peas had brownish watery stems and root. Furthermore there were also above ground symptoms commonly associated with *Pythium* infection. Stunting was visible in sorghum and millet. Maize had leaf chlorosis while the leaves of millet dried up at the shoot tips. Also, sorghum leaves exhibited anthocyanescence in the inoculated trays. The data shows that *Pythium* species varied significantly ($P \leq 0.05$) from the control in their disease scores on cereal crops.

(4a)

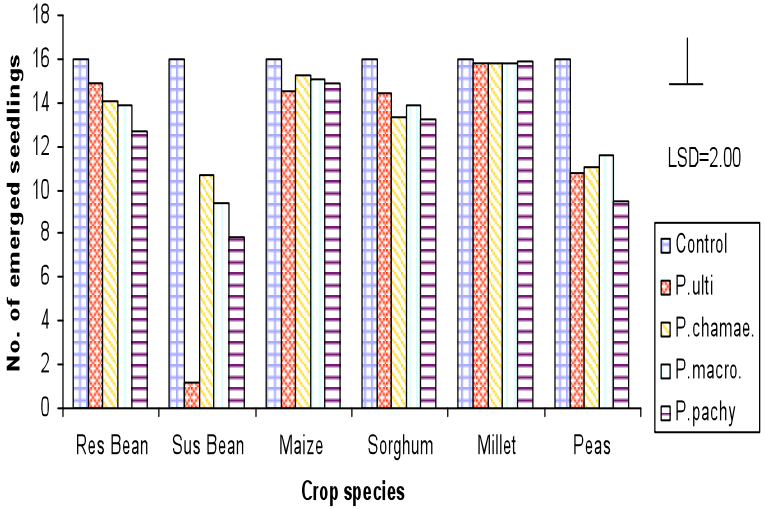


Figure 4 a: Emergence scores of selected crop species after inoculation with bean pathogenic *Pythium* species.

(4b)

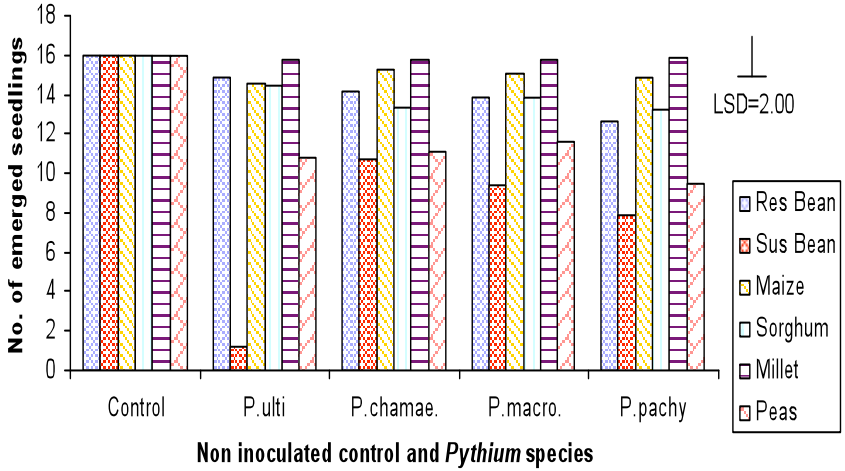


Figure 4 b: Damage by bean pathogenic *Pythium* species on selected crop species.

From trial one to trial three, the disease scores increased for each of the crop species with the various *Pythium* species (Table 7). Sorghum appeared to be the most affected with similar reactions to the susceptible bean variety (CAL 96). For example, in trial three, sorghum had a score of 8.61 with *Pythium pachycaule* while susceptible bean variety (CAL 96) had a score of 8.44 with the same pathogen (Table 7). This suggests that sorghum is susceptible to bean derived pathogenic *Pythium* species. Maize and millet inoculated with *Pythium pachycaule* had a score of 4.75 and 4.78 respectively. The reaction was similar to the resistant bean variety (RWR 719) which had a disease score of 3.03 with *Pythium pachycaule.* This suggests that maize and millet were resistant to this pathogen. From this study, it was found that sorghum and peas were the most susceptible crops to bean pathogenic *Pythium* species. Maize and millet were resistant to most of the *Pythium* species. Also it was observed that each of the *Pythium* species had a different effect on each of the crop species.

3.6.2.2 Dry matter of crop species

There was no significant difference in the root dry matter yield of crop species inoculated with the different *Pythium* species. Interestingly, the root dry matter yield of the cereals where *Pythium* species had been inoculated was greater than in the plants from non inoculated control. For instance maize inoculated with *Pythium ultimum* var. *ulitmum* had a root dry matter of 0.11 grams compared with the control which was 0.09 grams (Table 8). The root dry matter of peas where *Pythium* species had been inoculated was smaller than the plants in the non inoculated control. For example peas inoculated with *Pythium ultimum var. ultimum* had a root dry matter of

0.06 grams compared with the non inoculated control where the root dry matter was 0.07 grams.

3.6.3 Emergence of selected crop species after inoculation with *Pythium macrosporum*, *Pythium glomeratum* and *Pythium ultimum* derived from other crop species

The data shows that the diverse *Pythium* species used to inoculate crop species did not induce significant differences in amount of root rot. Crop emergence was not significantly affected by the *Pythium* species. Sorghum and millet were resistant to the *Pythium* species and they had a high emergence of seedlings (Figure 4). Peas and maize were moderately resistant to the *Pythium* species since they did not have a high emergence of seedlings. Also the effect of the *Pythium* species on the germination of the seedlings was not significantly different ($P \leq 0.05$).

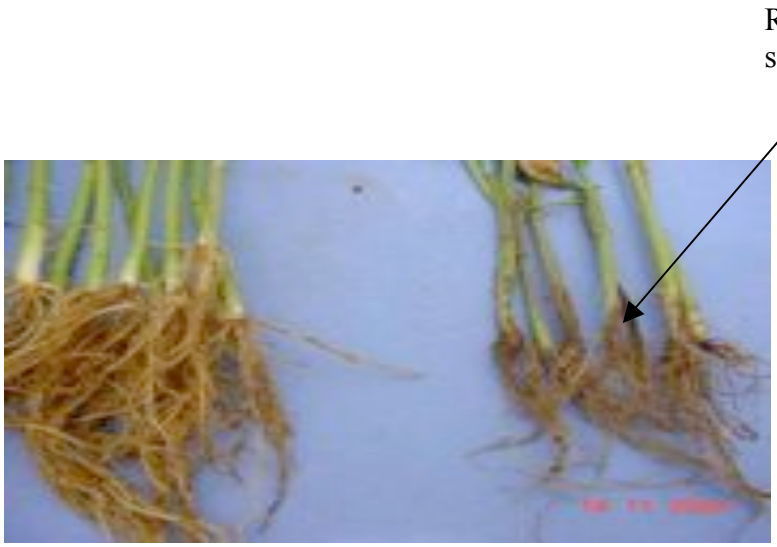


Plate 5: Root damage on the susceptible bean variety (CAL 96) right hand panel, and roots from non inoculated control (CAL 96). This root damage was used to assess severity of disease on a 1-9 scale (Abawi and Pastor-Corrales, 1990).

Plate 6: Root damage on sorghum (right hand panel), and roots from non inoculated control(left hand panel). This root damage was used to assess severity of disease on a 1-9 scale (Abawi and Pastor-Corrales, 1990).

Table 7: Mean disease scores of crop species after inoculation with bean derived pathogenic *Pythium* species

	Crop Host Plants								Crop Host Plants							
	Trial One								Trial Two							
Pythium species	[a]Resistant bean variety	[b]Susceptible bean variety	Maize	Sorghum	Millet	Peas	LSD (P≤ 0.05) [d]	CV (%) [e]	[a]Resistant bean variety	[b]Susceptible bean variety	Maize	Sorghum	Millet	Peas	LSD (P≤ 0.05) [d]	CV (%) [e]
Non inoculated control [c]	1.00	1.00	1.00	1.00	1.00	1.00	0.00	0.00	1.00	1.33	1.00	1.00	1.00	1.81	0.23	47.0
P.ultimum var. *ultimum*	1.00	0.89	1.03	1.11	1.00	5.14	0.17	36.9	1.14	5.25	2.17	5.61	1.78	7.50	0.72	48.6
P.chamaehyphon	1.00	3.42	2.81	3.17	1.00	4.61	0.50	47.6	1.97	5.58	4.22	6.53	3.03	6.89	0.58	29.2
P.macrosporum	0.94	2.56	1.58	3.61	0.94	2.92	0.51	57.4	1.11	4.08	2.75	6.64	1.64	4.69	0.45	30.0
P.pachycaule	1.03	3.03	1.58	4.36	1.00	4.06	0.79	76.8	1.67	6.00	3.00	6.83	2.53	6.97	0.65	35.1
LSD(P≤ 0.05) [d]	0.06	0.66	0.36	0.78	0.05	0.84			0.24	0.68	0.47	0.70	0.58	0.62		
CV (%) [e]	12.9	64.8	48.2	62.8	10.5	50.9			36.7	32.6	38.1	28.1	62.1	23.8		

a= RWR 719 (resistant bean variety) (Mukalazi, 2004)
b= CAL 96 (susceptible bean variety) (Mukalazi, 2004)
c= Non inoculated control
d= Least Significance Difference (Steel *et al.,* 1997)
e= % Coefficient of Variation

Table 7 continued: Mean disease scores of crop species after inoculation with bean derived pathogenic *Pythium* species

	Crop Host Plants							
	Trial Three							
Pythium species	[a] Resistant bean variety	[b] Susceptible bean variety	Maize	Sorghum	Millet	Peas	LSD (P≤ 0.05) [d]	CV (%) [e]
Non inoculated control [c]	1.33	1.50	1.53	1.39	1.06	1.56	0.21	33.5
P.ultimum var. ultimum	2.86	7.56	4.31	8.19	4.44	8.67	0.54	21.3
P.chamaehyphon	3.14	7.89	5.26	8.08	5.42	8.28	0.56	19.9
P.macrosporum	2.81	8.92	3.73	7.92	3.89	7.03	0.44	17.2
P.pachycaule	3.03	8.44	4.75	8.61	4.78	8.33	0.49	17.7
LSD(P≤ 0.05) [d]	0.30	0.44	0.51	0.33	0.65	0.48		
CV (%) [e]	24.2	13.9	27.9	10.3	35.7	15.2		

a= RWR 719 (resistant bean variety) (Mukalazi, 2004)
b= CAL 96 (susceptible bean variety) (Mukalazi, 2004)
c= Non inoculated control
d= Least Significance Difference (Steel *et al.,* 1997)
e= % Coefficient of Variation

Table 8: Mean root dry matter scores of crop species inoculated with bean derived pathogenic *Pythium* species

Pythium species	[a]Resistant bean variety	[b]Susceptible bean variety	Maize	Sorghum	Millet	Peas	LSD ($P \leq 0.05$) [d]	CV(%) [e]
Non inoculated control [c]	0.04	0.05	0.09	0.01	0.01	0.07	0.03	45.8
Pythium ultimum var. *ultimum*	0.05	0.06	0.11	0.01	0.02	0.06	0.03	64.6
Pythium chamaehyphon	0.05	0.06	0.14	0.02	0.02	0.07	0.02	37.0
Pythium macrosporum	0.05	0.06	0.13	0.02	0.01	0.08	0.02	35.3
Pythium pachycaule	0.06	0.09	0.13	0.02	0.02	0.07	0.02	34.4
LSD($P \leq 0.05$) [d]	0.02	0.02	0.02	0.01	0.00	0.02		
CV(%) [e]	37.6	36.9	21.7	36.8	55.7	27.5		

a= RWR 719 (resistant bean variety) (Mukalazi, 2004)
b= CAL 96 (susceptible bean variety) (Mukalazi, 2004)
c= Non inoculated control
d= Least Significance Difference (Steel *et al.,* 1997)
e= % Coefficient of Variation

3.6.4 Post emergence damage of selected crops species after inoculation with *Pythium macrosporum*, *Pythium glomeratum* and *Pythium ultimum* derived from other crop species

3.6.4.1 Root rot disease severity on crop species

The data shows that there was a significant difference ($P \leq 0.05$) in the disease scores of the crop species inoculated with the different *Pythium* species. Sorghum was susceptible to all three *Pythium* species in Trial 3 resulting in a disease score of 6.39, 6.19 and 6.53 with *Pythium macrosporum*, *Pythium glomeratum* and *Pythium ultimum* respectively (Table 9). Peas was also susceptible mainly to *Pythium ultimum* resulting in a score of 8.03 in trial one. Maize and millet were resistant giving a score of less than 2.5 from trial one to trial three.

3.6.4.2 Root dry matter of crop species

The data shows that there was no significant difference ($P \leq 0.05$) in the root dry matter of the crop species with the different *Pythium* species. Moreover, the root dry matter of the crop species where *Pythium* species had been inoculated was smaller than in the control which had no *Pythium* (Table 10). For instance maize and peas inoculated with *Pythium macrosporum* had a root dry matter of 0.13 grams and 0.03 grams respectively while the control in both cases was 0.17 grams and 0.04 grams respectively (Table 10).

(5 a)

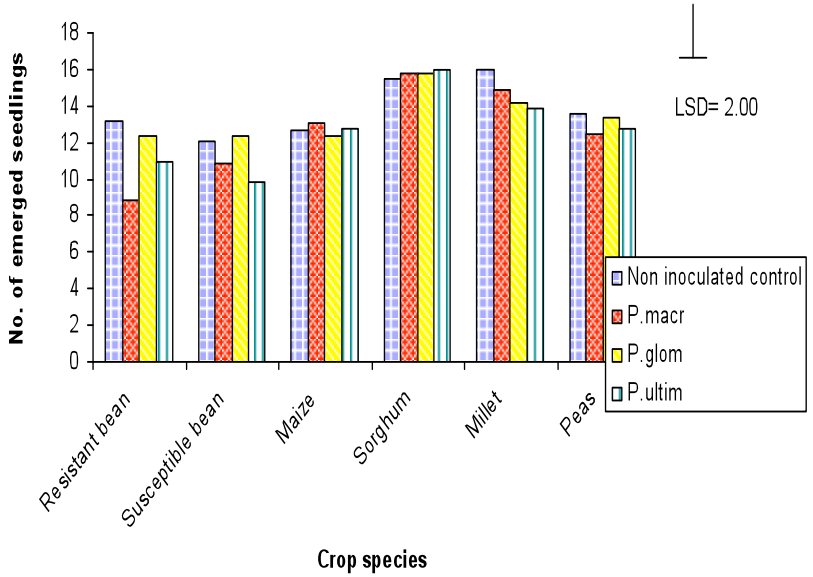


Figure 5 a: Emergence scores of selected crop species after inoculation with three *Pythium* species from crops intercropped with beans.

(5 b)

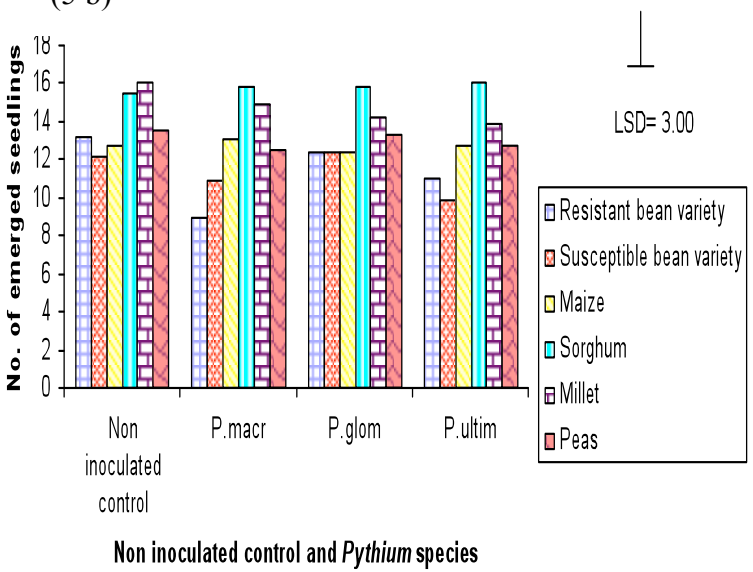


Figure 5 b: Damage on selected crop species by P*ythium* species isolated from crops intercropped with beans.

Table 9: Mean disease scores of crop species inoculated with *Pythium* species isolated from other crops intercropped with beans

	Crop Host Plants								Crop Host Plants							
	Trial One								Trial Two							
Pythium species	[a] Resistant bean variety	[b]Susceptible bean variety	Maize	Sorghum	Millet	Peas	LSD (P≤ 0.05)	CV. (%) [e]	[a]Resistant bean variety	[b]Susceptible bean variety	Maize	Sorghum	Millet	Peas	LSD(P≤ 0.05)	CV. (%) [e]
No *Pythium* species [c]	1.00	1.14	1.39	1.00	1.00	1.50	0.21	38.1	1.53	1.33	1.17	1.64	1.00	1.42	0.25	40.0
P.macrosporum	2.08	4.53	1.81	3.11	1.00	8.03	0.80	50.3	1.39	4.33	1.92	2.22	1.00	3.08	0.78	71.8
P.glomeratum	1.64	2.97	1.25	2.42	1.00	4.14	0.84	80.5	1.33	5.00	1.89	2.39	1.00	2.28	0.47	43.9
P.ultimum	1.86	2.67	1.75	2.81	1.00	5.11	0.75	63.6	2.08	4.03	1.81	3.33	1.00	2.36	0.61	54.3
LSD (P≤ 0.05) [d]	0.38	0.82	0.37	0.81	0.0	1.15			0.33	10.6	0.25	0.46	0.0	0.64		
CV. (%) [e]	50.1	62.5	51.7	74.2	0.0	52.5			44.7	61.9	31.3	41.3	0.0	60.4		

a= RWR 719 (resistant bean variety) (Mukalazi, 2004)
b= CAL 96 (susceptible bean variety) (Mukalazi, 2004)
c= Non inoculated control
d= Least Significance Difference (Steel *et al.,* 1997)
e= % Coefficient of Variation

Table 9 continued: Mean disease scores of crop species inoculated with *Pythium* species isolated from other crops intercropped with beans

	Crop Host Plants							
	Trial Three							
	[a]Resistant bean variety	[b]Susceptible bean variety	Maize	Sorghum	Millet	Peas	LSD(P≤ 0.05)	CV. (%) [e]
Pythium species								
No *Pythium* species [c]	1.00	1.42	1.00	1.14	1.00	1.00	0.14	27.6
P.macrosporum	3.78	2.08	2.28	6.39	1.00	5.14	0.89	55.7
P.glomeratum	2.39	1.73	3.17	6.19	1.00	4.14	1.03	70.4
P.ultimum	2.61	2.06	2.89	6.53	1.00	4.67	0.94	61.7
LSD (P≤ 0.05) [d]	1.67	0.39	0.45	0.34	0.00	0.94		
CV. (%) [e]	146.2	45.9	41.6	14.3	0.00	53.9		

a= RWR 719 (resistant bean variety) (Mukalazi, 2004)
b= CAL 96 (susceptible bean variety) (Mukalazi, 2004)
c= Non inoculated control
d= Least Significance Difference (Steel *et al.,* 1997)
e= % Coefficient of Variation

Table 10: Mean root dry matter scores of crop species inoculated with *Pythium* species isolated from other crops intercropped with beans

	Crop host plants							
Pythium species	[a]Resistant bean variety	[b]Susceptible bean variety	Maize	Sorghum	Millet	Peas	LSD (P≤ 0.05) [d]	CV (%) [e]
Non inoculated control [c]	0.05	0.12	0.17	0.02	0.01	0.04	0.04	67.4
Pythium macrosporum	0.05	0.08	0.13	0.02	0.01	0.03	0.02	39.6
Pythium glomeratum	0.06	0.11	0.12	0.01	0.01	0.04	0.02	34.7
Pythium ultimum	0.06	0.11	0.13	0.01	0.01	0.04	0.03	42.8
LSD(P≤ 0.05) [d]	0.01	0.01	0.04	0.01	0.01	0.01		
CV (%) [e]	22.0	26.1	26.2	38.7	46.0	22.2		

a= RWR 719 (resistant bean variety) (Mukalazi, 2004)
b= CAL 96 (susceptible bean variety) (Mukalazi, 2004)
c= Non inoculated control
d= Least Significance Difference (Steel *et al.,* 1997)
e= % Coefficient of Variation

3.6.5 Emergence of selected crop species after inoculation with *Pythium macrosporum*, *Pythium arrhenomanes*, *Pythium spinosum*, *Pythium oligandrum* and *Pythium heterothallicum* obtained from crops intercropped with beans

The data shows that *Pythium* species varied significantly (P≤ 0.05) from the control in the emergence of seedlings on crop species (Figure 5). Sorghum and millet were not affected at this stage since they had a high emergence of seedlings. Peas and maize

were moderately susceptible. *Pythium oligandrum* was the most pathogenic of the *Pythium* species on sorghum and millet. *Pythium spinosum* was most pathogenic on maize and peas.

3.6.6 Post emergence damage of crops species after inoculation with *Pythium macrosporum*, *Pythium arrhenomanes*, *Pythium spinosum*, *Pythium oligandrum* and *Pythium heterothallicum* derived from crop species intercropped with beans

3.6.6.1 Root rot disease severity of crop species after inoculation with five *Pythium* species derived from crop species intercropped with beans

The data shows that *Pythium* species varied significantly ($P \leq 0.05$) from the control in the disease scores on crop species. From trial one to trial three, the disease scores on the crop species increased. Sorghum and peas were the most susceptible of all the crop species resulting in a disease score of 7.06 and 7.69 with *Pythium heterothallicum* respectively in trial three (Table 11).

(6 a)

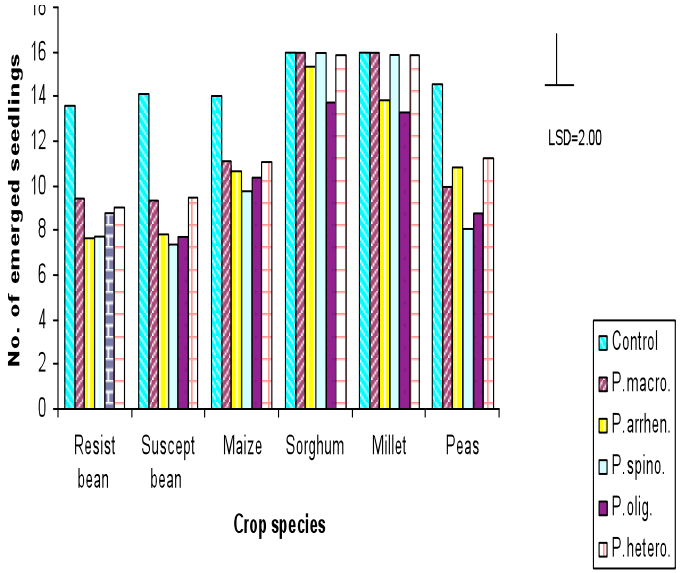


Figure 6 a: Emergence scores of selected crop species after inoculation with *Pythium* species derived from crops intercropped with beans.

(6 b)

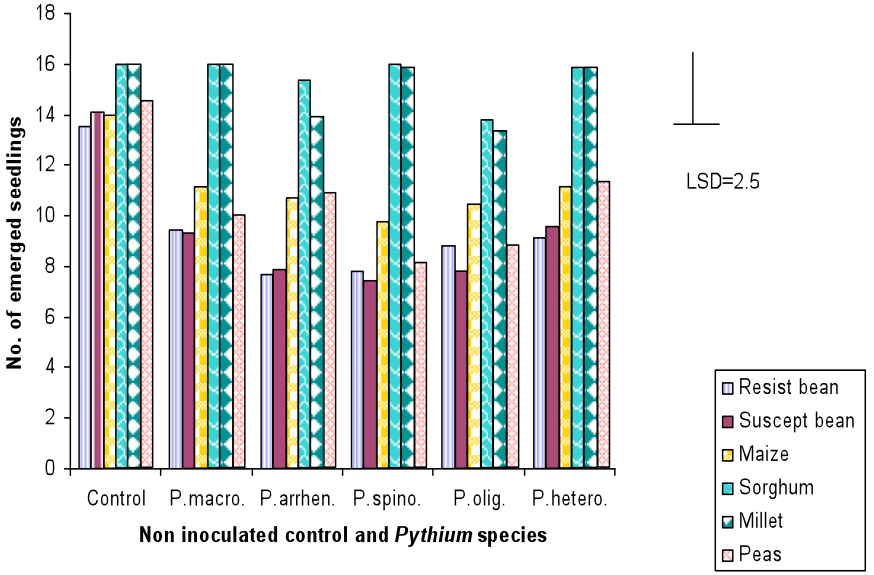


Figure 6 b: Damage on selected crop species by *Pythium* species isolated from crops intercropped with beans.

Table 11: Mean disease scores of crop species after inoculation with five *Pythium* species from crops intercropped with beans

	Crop Host Plants								Crop Host Plants							
	Trial One								Trial Two							
Pythium species	[a]Resistant bean variety	[b]Susceptible bean variety	Maize	Sorghum	Millet	Peas	LSD (P≤ 0.05) [d]	CV (%) [e]	[a]Resistant bean variety	[b]Susceptible bean variety	Maize	Sorghum	Millet	Peas	LSD (P≤ 0.05) [d]	CV (%) [e]
Non inoculated control	1.00	1.00	1.00	1.00	1.00	1.00	0.00	0.00	1.00	1.42	1.00	1.00	1.42	1.11	0.40	70.8
Pythium macrosporum	1.19	2.56	1.64	2.69	1.00	4.25	0.44	42.9	1.44	4.14	3.17	3.67	1.00	5.17	0.66	46.2
Pythium arrhenomanes	1.42	3.11	1.64	3.56	1.03	4.56	0.49	41.5	2.06	5.43	2.61	5.70	1.00	5.92	0.66	39.8
Pythium spinosum	1.00	2.56	1.25	2.83	1.00	2.58	0.43	49.1	1.42	4.25	2.17	5.28	1.00	4.75	0.64	47.0
Pythium oligandrum	1.47	3.17	1.69	3.03	1.00	3.69	0.56	51.2	2.00	4.41	2.47	5.89	1.03	5.03	0.67	44.2
Pythium heterothalicum	1.08	2.86	1.69	3.25	1.00	2.94	0.56	56.4	1.83	5.14	2.31	4.89	1.00	5.25	0.69	44.2
LSD (P≤ 0.05) [d]	0.20	0.54	0.30	0.56	0.03	0.73			0.27	0.89	0.41	0.50	0.26	0.71		
CV (%) [e]	35.7	45.5	39.6	43.9	6.8	49.5			35.1	46.3	38.3	24.3	51.6	34.4		

a= RWR 719 (resistant bean variety) (Mukalazi, 2004)
b= CAL 96 (susceptible bean variety) (Mukalazi, 2004)
c= Non inoculated control
d= Least Significance Difference (Steel *et al.,* 1997)
e= % Coefficient of Variation

Table 11: Mean disease scores of crop species after inoculation with five *Pythium* species from crops intercropped with beans

	Crop Host Plants							
	Trial Three							
Pythium species	[a] Resistant bean variety	[b] Susceptible bean variety	Maize	Sorghum	Millet	Peas	LSD(P≤ 0.05) [d]	CV (%) [e]
Pythium species								
Non inoculated control [c]	1.03	1.11	1.08	1.00	1.00	1.61	0.29	54.8
Pythium macrosporum	3.28	3.19	4.31	6.36	1.06	6.17	1.00	52.4
Pythium arrhenomanes	2.03	1.78	2.42	6.69	2.11	7.61	0.91	51.7
Pythium spinosum	1.75	2.39	2.89	6.69	1.94	1.75	0.72	42.1
Pythium oligandrum	3.47	0.94	3.31	6.72	1.44	3.94	1.01	69.4
Pythium heterothallicum	2.17	4.53	3.44	7.06	1.25	7.69	0.93	45.9
LSD(P≤ 0.05) [d]	0.95	0.47	0.42	0.41	0.61	0.72		
CV (%) [e]	36.9	32.9	15.8	60.4	49.3	24.7		

a= RWR 719 (resistant bean variety) (Mukalazi, 2004)
b= CAL 96 (susceptible bean variety) (Mukalazi, 2004)
c= Non inoculated control
d= Least Significance Difference (Steel *et al.,* 1997)
e= % Coefficient of Variation

3.6.6.2 Root dry matter of selected crop species after inoculation with five *Pythium* species derived from crops intercropped with beans

The data revealed that there was no significant difference (P≤ 0.05) in the root dry matter of the crop species when inoculated with the five *Pythium* species isolated from crops intercropped with beans. (Table 12). The root dry matter of the crop species where *Pythium* species had been inoculated was greater than in the non inoculated control. For instance maize and peas inoculated with *Pythium macrosporum* had a root dry matter of 0.11 grams and 0.09 grams respectively while the control in both cases was 0.06 grams and 0.04 grams respectively (Table 12).

Table 12: Mean root dry matter scores of crop species after inoculation with five *Pythium* species isolated from crops intercropped with beans

	Crop Host Plant							
Pythium species	[a] Resistant bean variety	[b]Susceptible bean variety	Maize	Sorghum	Millet	Peas	LSD(P≤ 0.05) [d]	CV. (%) [e]
Non inoculated control [c]	0.03	0.07	0.06	0.01	0.02	0.04	0.01	29.0
Pythium macrosporum	0.07	0.09	0.11	0.05	0.01	0.09	0.08	115.3
Pythium arrhenomanes	0.05	0.13	0.16	0.16	0.01	0.04	0.05	81.2
Pythium spinosum	0.07	0.12	0.09	0.02	0.01	0.04	0.02	37.4
Pythium oligandrum	0.07	0.12	0.12	0.02	0.01	0.04	0.03	44.0
Pythium heterothallicum	0.07	0.11	0.09	0.02	0.01	0.05	0.02	39.7
LSD(P≤ 0.05) [d]	0.02	0.04	0.06	0.05	0.01	0.05		
CV (%) [e]	30.1	35.6	61.8	223.5	60.9	113.6		

a= RWR 719 (resistant bean variety) (Mukalazi, 2004)
b= CAL 96 (susceptible bean variety) (Mukalazi, 2004)
c= Non inoculated control
d= Least Significance Difference (Steel *et al.,* 1997)
e= % Coefficient of Variation

3.6.7 Pathogenicity of bean derived *Pythium* species and *Pythium* species from crops intercropped with beans on selected hosts commonly used as intercrops in south western Uganda

This data revealed that the bean derived *Pythium ultimum* and *Pythium macrosporum* respectively were most pathogenic on peas and sorghum compared to the *Pythium* species from crops intercropped with beans (Figure 6). This is because with the former *Pythium* species, fewer seeds germinated.

Pythium ultimum and *Pythium macrosporum* derived from the bean crop gave higher disease scores on all the crop species compared to the *Pythium* species derived from crops intercropped with beans (Figure 7). Peas and sorghum were most susceptible having disease scores greater than 6.0. Maize and millet were resistant to these *Pythium* species with a disease score less than 4.0.

(7a)

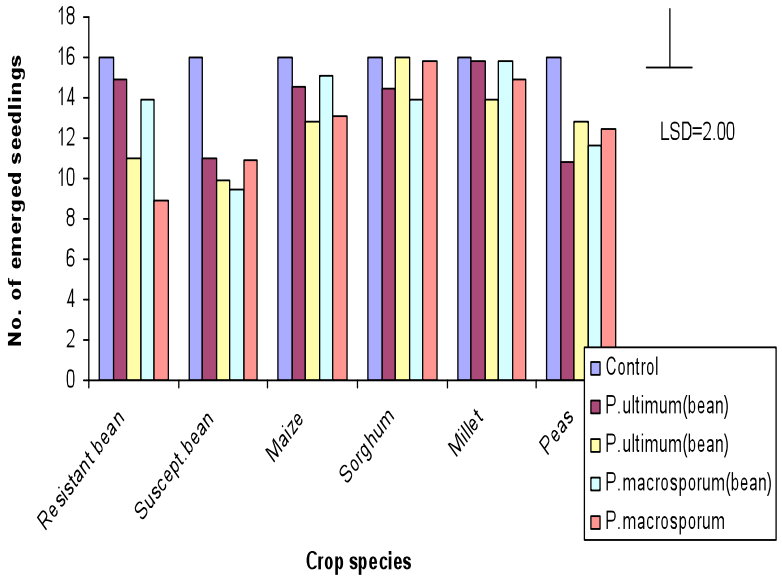


Figure 7 a: Comparison of emergence scores of selected crop species inoculated with bean pathogenic *Pythium* species and *Pythium* species derived from crops intercropped with beans.

(7b)

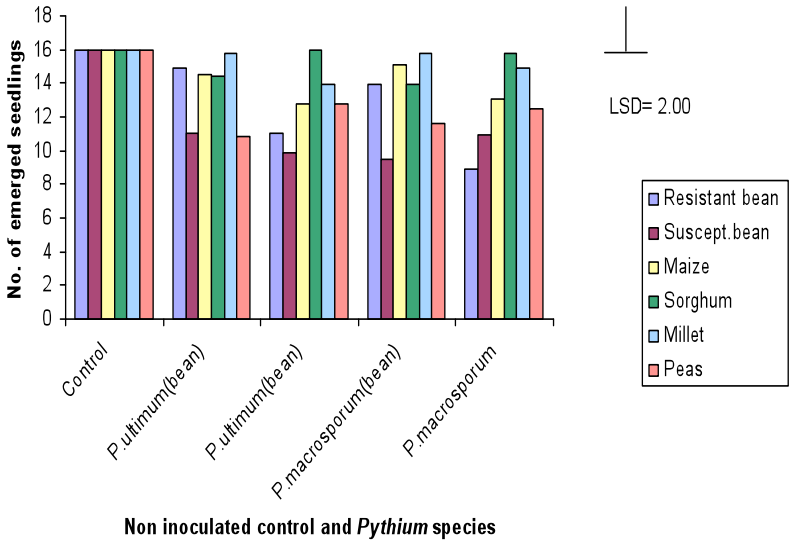


Figure 7 b: Comparison of effect of bean pathogenic *Pythium* species and *Pythium* species derived from crops intercropped with beans.

(8a)

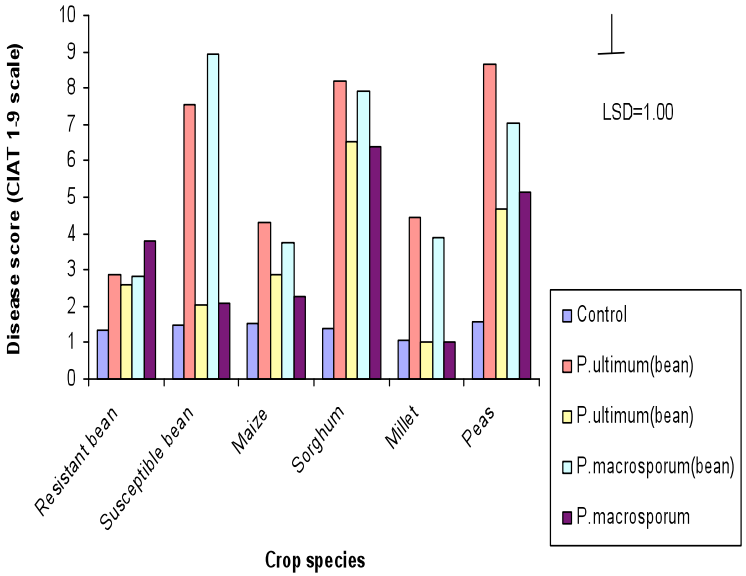


Figure 8 a: Comparison of disease scores of selected crop species after inoculation with bean pathogenic *Pythium* species and *Pythium* species derived from other crops intercropped with beans.

(8b)

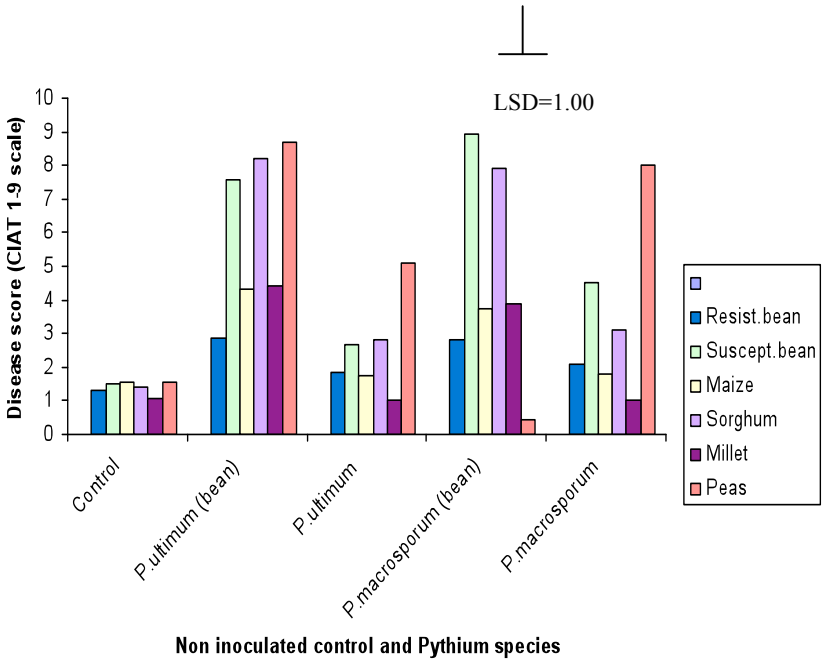


Figure 8 b: Comparison of damage by bean derived *Pythium* species and *Pythium* species derived from other crops intercropped with beans.

3.7 Discussion

The objective of this study was to investigate cross-pathogenicity properties of *Pythium* species and their potential role in influencing bean root rot epidemics. The study showed that indeed *Pythium* species are pathogenic on crop species commonly included as intercrops with beans in south western Uganda. From this study, the results confirm that sorghum and peas are susceptible to *Pythium* species derived both from beans and other crops. *Pythium* species have been recognised as potentially important pathogens of graminaceous crops such as sorghum (Hsiang, 1995). In studies, elsewhere, peas have also been found to suffer from root rot thus making them easier to uproot from the ground when they are in this state (Malvic and Babadoost 2002). Maize and millet were found to be resistant to *Pythium* species because seed emergence levels were not affected and the disease scores on CIAT 1-9 scale was low. This implies that these crops may be able to inhibit the pathogen in two ways. Firstly, they could inhibit the pathogen from entering the plant through physical barriers in their structure. Secondly, these resistant crops may produce biochemicals in their cells and tissues which are either toxic to the pathogen or inhibit the growth of the pathogen (Pearson and Parkinson, 1961). The two crops could thus easily be included as intercrops with beans to control root rots.

Interestingly it was noted that the root dry matter of the selected crop species used in this study was higher where bean derived *Pythium* species had been inoculated, compared with the control for cereals. This suggests a mechanism of counteracting infection by producing additional prop roots to aid the plant in nutrition due to it being compromised by infection (CIAT, 2005). The formation of prop roots was

observed during this study on millet and sorghum when inoculated with bean derived *Pythium* species which was not observed with *Pythium* species isolated other crops.

Observations were made of root and shoot symptoms on the crop species in this study when infected with *Pythium* species. Examples included stunting of sorghum, millet and peas. A similar report has been made in wheat fields, infested with *Pythium* (Higginbotham, 2004). Peas and susceptible bean variety (CAL 96) were observed in this study to have brown watery stems and roots which are typical signs of root rot. This water soaked appearance in the plant tissue arises due to the leakage of the moist cellular contents from plant cells that occur from the enzymatic activity of the *Pythium* species which then breaks down the soft parenchymous tissue (Kucharek, 2000). Another above ground symptom observed in this study was chlorosis of leaves. This is often associated with seedling blights of many grass and broad leaf crops, which may be infected prior to (pre-emergent damping off) or after (post emergence damping off) emergence from the soil (Kucharek, 2000). Also, wilting of millet leaves was also observed. *Pythium* species reduce water uptake and may cause leaves and shoots to wilt during warm temperatures, effects that are similar to those of osmotic stress (Couteaudier and Lemenceau, 1989). Another external symptom observed was on sorghum leaves which were observed to turn purple with *Pythium* infection as well as formation of red-black lesions on the roots. Studies elsewhere have found similar such symptoms. The purple coloration of leaves has been attributed to the fact that the pathogen blocks phosphorus metabolism leading to deficiency symptoms in the plant (Vincelli and Hershmann, 2002; CIAT, 2004).

This study also showed that *Pythium* species can attack a wide range of crops which include sorghum and peas. This corroborates earlier reports that show that sorghum seed can be attacked by *Pythium* species prior to emergence and germination (Vincelli and Hershmann, 2002).

In south western Uganda, beans are intercropped with sorghum, potato and peas amongst other crops (Ampaire, 2003). This study by showing that sorghum and peas suffer from *Pythium* root rot suggest that these crops may play a role in *Pythium* bean root rots epidemics in Kabale district. These crops may maintain or increase inoculum load. They may also favour the same *Pythium* species which attack beans hence there might be need to practice crop rotation of these crops. Such alternative hosts may thus play a crucial role in influencing pathogen selection in the pathosystem. Unfortunately crop rotation is rarely practiced in some parts of Africa due to unavailability of land in areas where population is high. Elsewhere, crop rotation reportedly suppresses *Pythium* populations and reduces disease on many hosts (Pankhust *et al*., 1995). In Australia, different crops have been used to reduce the amount and type of *Pythium* present in the soil (Harvey, 2004).

In addition, it was also noted in this study that the *Pythium* species differed in the disease reaction on the crop species. This could imply that there was variation in the aggressiveness of the *Pythium* species on host crops. This phenomenon can arise from differences in aggressiveness among *Pythium* species and/or differences in aggressiveness among isolates within a species (Zhang and Yang, 2000). McCarter and Littrel (1970) tested pathogenicity of 14 isolates of *Pythium aphanidermatum* and *Pythium myriotylum* on 12 crops. Their results indicated that the two species caused

different levels of disease on any one crop. Moreover, genetic analysis of *Pythium* species indicates that their geneotypes shift in response to different crops. This could mean that individual strains of *Pythium* are better adapted at infecting some crops than others and those different crops could be selecting for crop-adapted strains (Harvey, 2004).

Overall, this study confirms that there is cross pathogenicity amongst *Pythium* species isolated from beans and other crops. It also reveals that these *Pythium* species do not significantly affect the emergence of seedlings of cereals and legumes hence do not contribute to the pre-emergence damping off of these crops. However assessment of post-emergence damping off revealed that sorghum and peas were the most susceptible crop species to *Pythium* species. This is an indication that these crops which are usually part of the bean based farming system in south western Uganda contribute to continuous root rot epidemics since they too are also affected. However other crops such as maize and millet were found to be resistant.

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CHAPTER FOUR

PATHOGENESIS OF *PYTHIUM* SPECIES ISOLATED FROM BEANS AND OTHER CROPS USING LIGHT AND ELECTRON MICROSCOPY

4 Introduction

The ability of highly pathogenic *Pythium* species including *Pythium ultimum, Pythium aphanidermatum* and *Pythium irregulare* to induce plant wilting and root rotting has been abundantly documented (Blanchard *et al*., 1992). The colonisation of plant roots by *Pythium* species causes damping-off, severe necrosis and rots on the roots and stems of mature plants (Rey *et al.,* 2001). *Pythium* species have been isolated from several plant species and including in this thesis, evidence of disease development shown. In section 3.6.2.1, Pythium species were found to cause visible symptoms of infection on roots and shoots. Some of these species did not show any visible symptoms of infection.

In that context, the occurrence of various *Pythium* species in necrotic as well as asymptomatic roots raises a question as to the extent to which such fungi are involved in disease expression (Hodges and Coleman, 1985). Other studies have shown that *Pythium* infections may affect plant growth and yield without causing any visible symptoms on roots (Stanghellini and Rasmussen, 1994; Rey *et al.,* 1998). Only a few reports have described this phenomenon on soil-grown plants (Salt, 1979). This study was set up to investigate the potential mode of infection of *Pythium ultimum* and *Pythium irregulare* in maize, sorghum and bean crops.

4.1 Literature review

4.1.1 Ultra structural studies of *Pythium* species infection in plants

Histopathological studies of *Pythium* species have been done elsewhere. Histopathological studies of *Pythium aphanidermatum* infections on bent grass and cotton (Spencer and Cooper, 1967) and aerial parts of bean plants (Kim *et al.,* 1974); and infection of *Pythium ultimum* on roots of peach (Miller *et al.,* 1966); strawberry (Nemec, 1972), snapdragon (Mellano *et al.,* 1970) and cotton (Spencer and Cooper, 1967) have been reported. Asymptomatic infections by *Pythium* group F on tomato roots have been studied through ultra structural and cytochemical investigations, which demonstrated that *Pythium* group F behaves as necrotrophs in the outer root tissues. This pathogen is a potential inducer of plant defense reactions in the inner tissues (Rey *et al.,* 2001). Other studies have been done using light and scanning electron microscopy to investigate the interaction between *Pythium oligandrum* and various soil borne oomycete and fungal plant pathogens (Benhamou *et al.,* 1999). These investigations of the interaction show that structural alterations of all pathogenic fungi and oomycetes occur soon after contact with the antagonist.

During infection *Pythium* species are known to produce appressoria that penetrate the cuticle and epidermal cell walls mechanically (Adegbola and Hagedorn, 1969). The numbers of appressoria may aid the pathogens in aggressively invading host tissue (Kraft *et al.,* 1967). However infection can occur without the formation of distinct appressoria, but with only a slight swelling of the hyphae at the points of penetration

(Nemec, 1971). Other studies based on ultra structural investigation of asymptomatic infections by *Pythium* species have been done (Rey *et al.,* 1998).

4.1.2 Mechanism of *Pythium* colonisation in plants

The colonisation of plants by Pythium species can be studied using light and electron microscopy techniques. Using electron microscopy techniques, the early colonisation of bean tissue by *Pythium myriotylum, Pythium aphanidermatum* and *Pythium ultimum* have been characterised and found to involve the development of infection hyphae in the epidermal cell layer and the underlying cortex (Dow and Lumsden, 1975). The hyphae of these pathogens move through the tissue primarily in an intracellular manner. In later stages of pathogenesis, the hyphae in the cortex grows both intracellulary and intercellularly. In addition, oospores and sporangia of some *Pythium spp.* have been found unevenly distributed in the various tissues of infected bean plants (Dow and Lumsden, 1975). Similar results have been reported in other *Pythium* pathosystems (Nemec, 1972).

Recent video-microscopial investigations have revealed that host susceptibility to *Pythium oligandrum* correlates with growth inhibition and host hyphal coagulation, vacuolation, or both (Laing and Deacon, 1990). Furthermore, evidence from a number of histological studies have shown that *Pythium violae* has the ability to colonise the outermost root tissues in susceptible cultivars (Groom and Perry, 1985; Briard, 1990). Also it has been shown that ingress of *Pythium violae* towards the vascular stele coincided with host cell wall degradation (Campion, 1997).

4.2 Sectional conclusion

Pythium a soil borne oomycete has a wide host range and many pathogenic species have been found and the symptoms they induce documented (Blanchard *et al.,* 1992; Martin, 1995). Histopathological studies using light and scanning electron microscopy have been done to investigate *Pythium* infection. Electron microscopy techniques have been used to investigate the early colonisation of bean tissue by *Pythium myriotylum, Pythium aphanidermatum* and *Pythium ultimum* (Dow and Lumsden, 1975). The mode of infection of *Pythium* species in sorghum and maize has not been studied. The specific objective of this study was to elucidate the mode of infection of *Pythium* species in crop hosts that are commonly included in bean mixed cropping systems.

4.3 Materials and Methods

4.3.1 Screen house experiments

Experiments to investigate infection of crops commonly found in bean-intercrops were studied using bean derived pathogenic *Pythium* species (Mukalazi, 2004). The *Pythium* species were *Pythium ultimum* (MS 21) and *Pythium irregulare* (DFD 47). Autoclaved millet (100g) was mixed with 200ml of water in 500 ml bottle and used to raise bean pathogenic *Pythium* species. After two weeks, pre sterilised soil was mixed with infested millet in a ratio of 1:10 v/v in wooden trays of 42 cm x 72 cm. The trays were set up in a Completely Randomised Block Design (CRBD), with three replications.

Maize, sorghum, susceptible bean variety (CAL 96) and resistant bean variety (AND 1062) were planted in the trays. Sorghum had been shown to be affected by *Pythium* species while maize only exhibited very mild symptoms of stress (Chapter 3 of this thesis). For comparative purposes, a resistant bean variety (AND 1062) and the susceptible bean variety (CAL 96) were included. The trays were maintained in the screen house. After germination, the seedlings were watered everyday to provide a favourable environment for the pathogen establishment and development. Every week until three weeks after inoculation the various crops hosts were harvested for light and electron microscopy assays of infection. The experiment was repeated once.

4.3.2 Tissue processing for light microscopy

Samples from inoculated and non inoculated roots of each crop were collected 7, 8 and 9 days after germination of seed respectively. This was based on the earliest infection time of *Pythium* in the crops. The root samples were cut using a sharp blade and placed in acetic acid for 24 hours so as to clear and fix the tissue. Afterwards the roots were stained in lactophenol-Tryphan blue (25% w/v phenol crystals, 50% lactic acid, and 2.5 mg/ml tryphan blue) for 4 minutes, then mounted on a glass slide and fixed in 80% glycerol. The roots were viewed using a light microscope Laborlux D (Leitz Wetzlar, Germany). Pictures were taken with a digital camera at x 40 magnification.

4.3.3 Tissue processing for electron microscopy

Samples from inoculated and control roots of each crop were collected 7, 8 and 9 days after germination of seed. Root samples were then immediately fixed in 2.5 % (vol/vol) phosphate buffer-glutaraldehyde (0.1M, pH 7.2) for 24 hours. All samples were post fixed for 1 hour in 1 % osmium tetroxide in water. The samples were then dehydrated in graded series of ethanol i.e. 25%, 50%, 70%, 90% and three times in 100% for 20 minutes each time (Arroaye, personal communication). Then the samples were dried at the critical point with carbon dioxide using a drier (SAMDRI-780A). Gold plating of the samples at 30 nanometers was done (HummerVII Sputtering System). The samples were then observed in a Scanning electron microscope (Marca Jeol JSM-820, Cambridge Instruments).

4.4 Results

Light microscopy of *Pythium irregulare* infection in the resistant bean variety (AND 1062), revealed a steady process of infection starting with attachment to root epidermis and eventual entry into the tissues 9 days after germination of seed (Plate 7). With electron microscopy, 7 days after germination of seed, oomycete hypha was observed to penetrate into the epidermis. Eight days after germination of seed however, few oomycete hypha was observed to be growing intracelluarly in the epidermis. In addition, the epidermal cells appeared to be fortified. Electron microscopy confirmed light microscopy results revealing few oomycete hyphae in epidermis of root tissue by 9 days after germination of seed (Plate 7).

Light microscopy of *Pythium ultimum* infection on the resistant bean variety (AND 1062) revealed similar results to those observed with *Pythium irregulare.* However electron microscopy revealed more details (Plate 8). For instance, 7 days after germination of seed, oomycete hyphae were observed to be undergoing necrosis on the epidermal layer of the root tissue; 8 days after germination, two types of oomycete hypae were found attached on the external surface of the root tissue. One group of hyphae was short and thick while the second was long and thin. The epidermal cells were also observed to be greatly enlargened. Finally 9 days after germination, the thin oomycete hyphae had penetrated into the upper layer of the epidermis.

Light microscopy of *Pythium irregulare* infection on the susceptible bean variety (CAL 96) revealed that seven days after germination the oomycete hypha had made contact with the root surface and was penetrating the epidermis. Eight days after germination, the oomycete hypha was attached on the root surface and in both the epidermal and endodermal tissue which had stained blue (Plate 9).

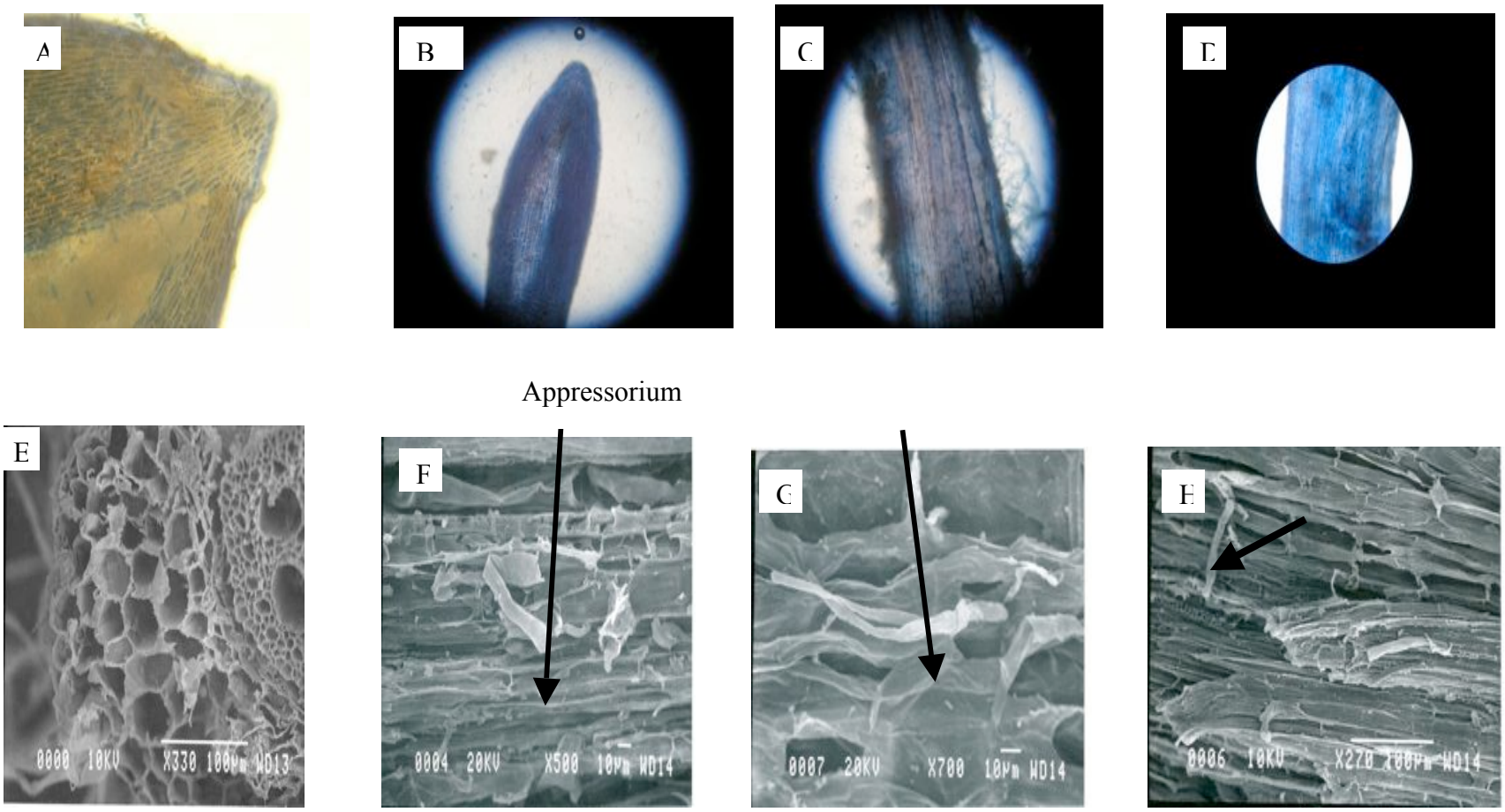

Plate 7: The panels marked A-D are pictures of light micrographs of resistant bean variety (AND 1062) tissue inoculated with *Pythium irregulare*. A: non-inoculated sample. B: tissue collected 7 days after germination of seed; stained blue indicating presence of pathogen hyphae. C: tissue taken 8 days after germination of seed; similarly staining blue and hyphae observed to be attached to the root surface. D: tissue collected 9 days after germination of seed; epidermis stained blue indicating colonisation of the epidermis. The panels marked E-H are Scanning electron micrographs (SEM) of resistant bean variety (AND 1062) tissue inoculated with *P.irregulare* E: a non inoculated control. F: infected root tissue taken 7 days after germination of seed showing appressorium on hyphae as indicated by the arrow.G: infected root sample taken 8 dyas after germination of seed showing hyphae growing intracellularly. H: infected tissue taken 9 days after germination of seed showing hyphae restricted in growth within the bean tissue.

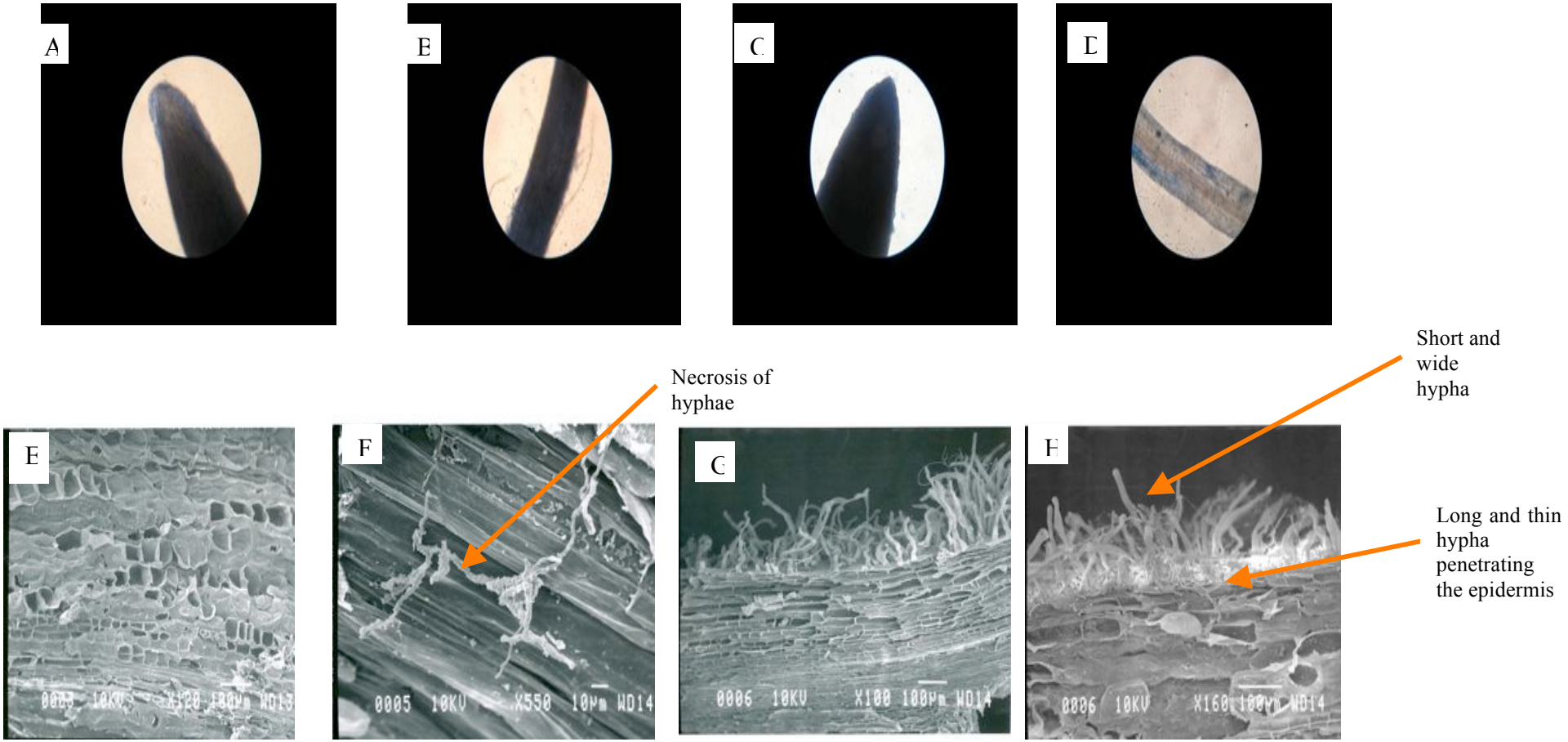

Plate 8: The panels marked A-D are pictures of light micrographs of resistant bean variety (AND 1062) tissue inoculated with *Pythium ultimum*. A: non-inoculated sample. B: tissue collected 7 days after germination of seed; hyphae were attached on root surface. C: tissue collected 8 days after germination of seed; epidermal surface stained blue indicating colonisation of surface. D: tissue collected 9 days after germination of seed; epidermis stained blue indicating colonisation of the epidermis. The panels marked E-H are Scanning electron micrographs (SEM) of resistant bean variety (AND 1062) tissue inoculated with *P.ultimum*. E: a non-inoculated control. F: infected root tissue taken 7 days after germination of seed showing hyphae undergoing necrosis in epidermal tissue. G: infected root sample taken 8 days after germination of seed showing two types of hyphae: - short and wide, long and thin hyphae attached on the root surface. H: infected tissue taken 9 dyas after germination of seed showing thin hyphae penetrating the epidermis.

Electron microscopy of *Pythium irregulare* infection on susceptible bean host (CAL 96), 7 days after germination of seed revealed appressorium that was bending towards the epidermis; 8 days after germination, the hyphae was inside the endodermis but without appressoria (Plate 9). The hyphae at this stage was approaching the vascular system in the stele part of the root tissue; 9 days after planting , the oomycete possessed paddle-like structures as well as appressorium on the hyphae. Light microscopy of *Pythium ultimum* infection revealed that in susceptible bean variety (CAL 96), oomycete hyphae attached to the surface of the root tissue (Plate 10). With electron microscopy, seven days after germination, extensive colonisation of epidermal tissue occurred with the oomycete hypha forming a network. The hyphae were short and thick; 8 days after germination of seed, the thin sized hypha was observed to penetrate the epidermal tissue (Plate 10).

Light microscopy of *Pythium irregulare* infection revealed that in maize 8 days after germination, the epidermal cells were enlargened and had stained blue (Plate 11). However, the endodermis had not taken up the dye. This suggests that the oomycete was not able to penetrate beyond the epidermis. Electron microscopy revealed 8 days after germination, the oomycete hypha growing intracellularly in the epidermis and moving towards the endodermis. Hyphae were observed to be long and to bear an appressorium. Nine days after germination, the oomycete hypha was shorter and some of the hypha was observed to have a paddle-like structure.

Light microscopy of *Pythium ultimum* infection on maize, revealed 8 days after germination that the oomycete hyphae had attached to the root tissue (Plate 12). However with electron microscopy, the oomycete hypha was attached to root

surface and was also observed in the epidermis and endodermis. Nine days after germination, the oomycete hypha in the epidermis was observed to undergo necrosis.

Light microscopy of *Pythium irregulare* infection on sorghum revealed 8 days after germination that oomycete hyphae was attached to the external surface of the root and penetrating into the epidermal tissue (Plate 13). With electron microscopy 7 days after germination of seed the oomycete hyphae was observed to attach on the outer surface of the root tissue and had began the process of penetration. Eight days after germination, there was presence of numerous oomycete hypha bearing appressorium like structure on the root surface tissue. Nine days after germination, the oomycete hypha was observed to form an intertwining network of hyphae within the epidermis (Plate 13).

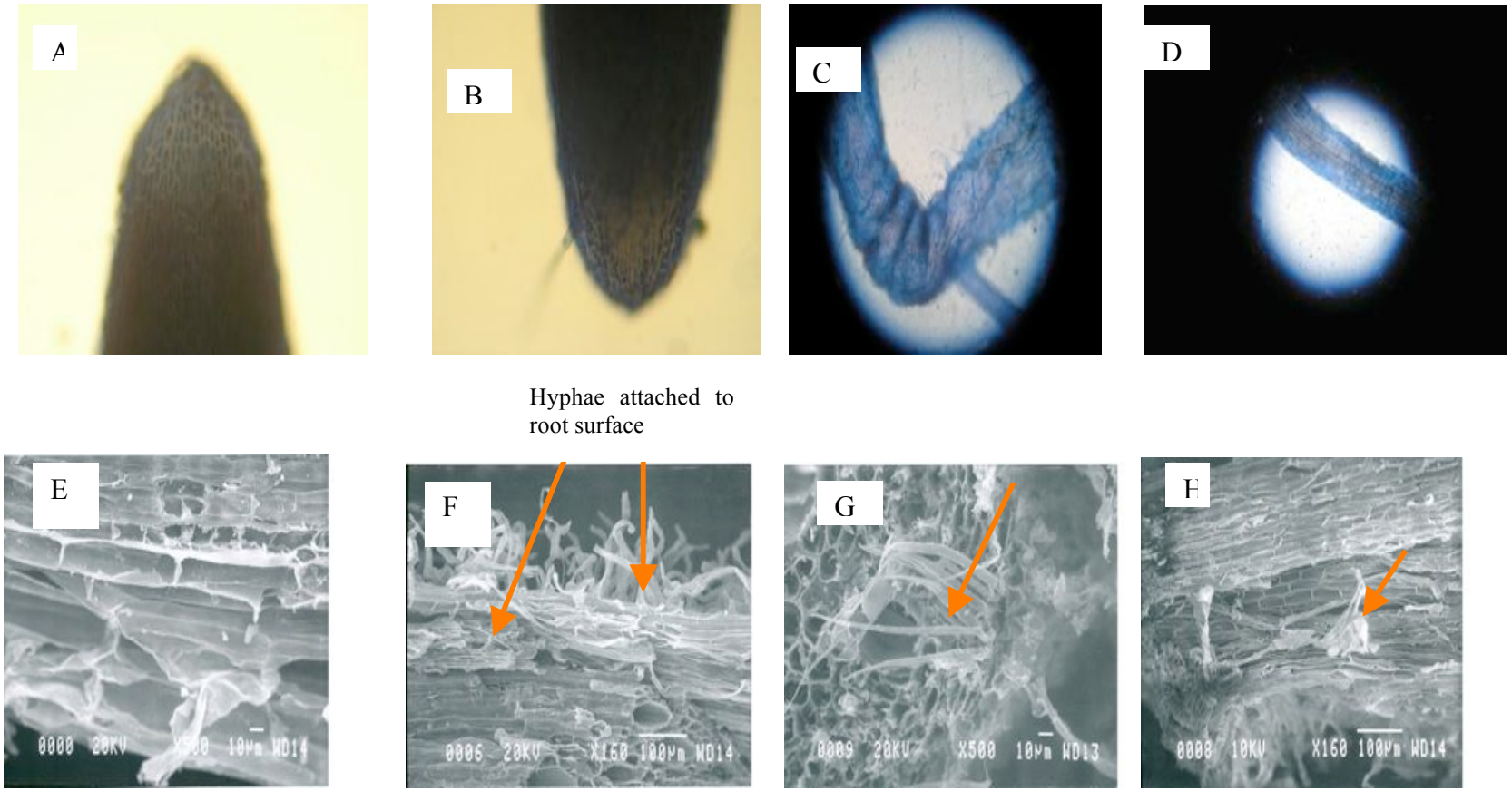

Plate 9: The panels marked A-D are pictures of light micrographs of susceptible bean variety (CAL 96) tissue inoculated with *Pythium irregulare*. A: non-inoculated sample. B: tissue collected 7 days after germination of seed; hyphae attached on root surface. C: tissue collected 8 days after germination of seed; only a small area of the endodermis did not stain blue indicating that it was not colonised by oomycete. D: tissue collected 9 days after germination of seed; epidermis and endodermis stained blue indicating colonisation by oomycete. The panels marked E-H are Scanning electron micrographs (SEM) of susceptible bean variety (CAL 96) tissue inoculated with *P.irregulare*. E: a non-inoculated control. F: infected root tissue taken 7 days after germination of seed showing hyphae attached on root surface. The arrows indicate the hyphae. G: infected root sample taken 8 days after germination of seed showing hyphae without appressorium in the endodermis. H: infeted tissue taken 9 days after germination of seed showing hyphae in epidermis and endodermis.

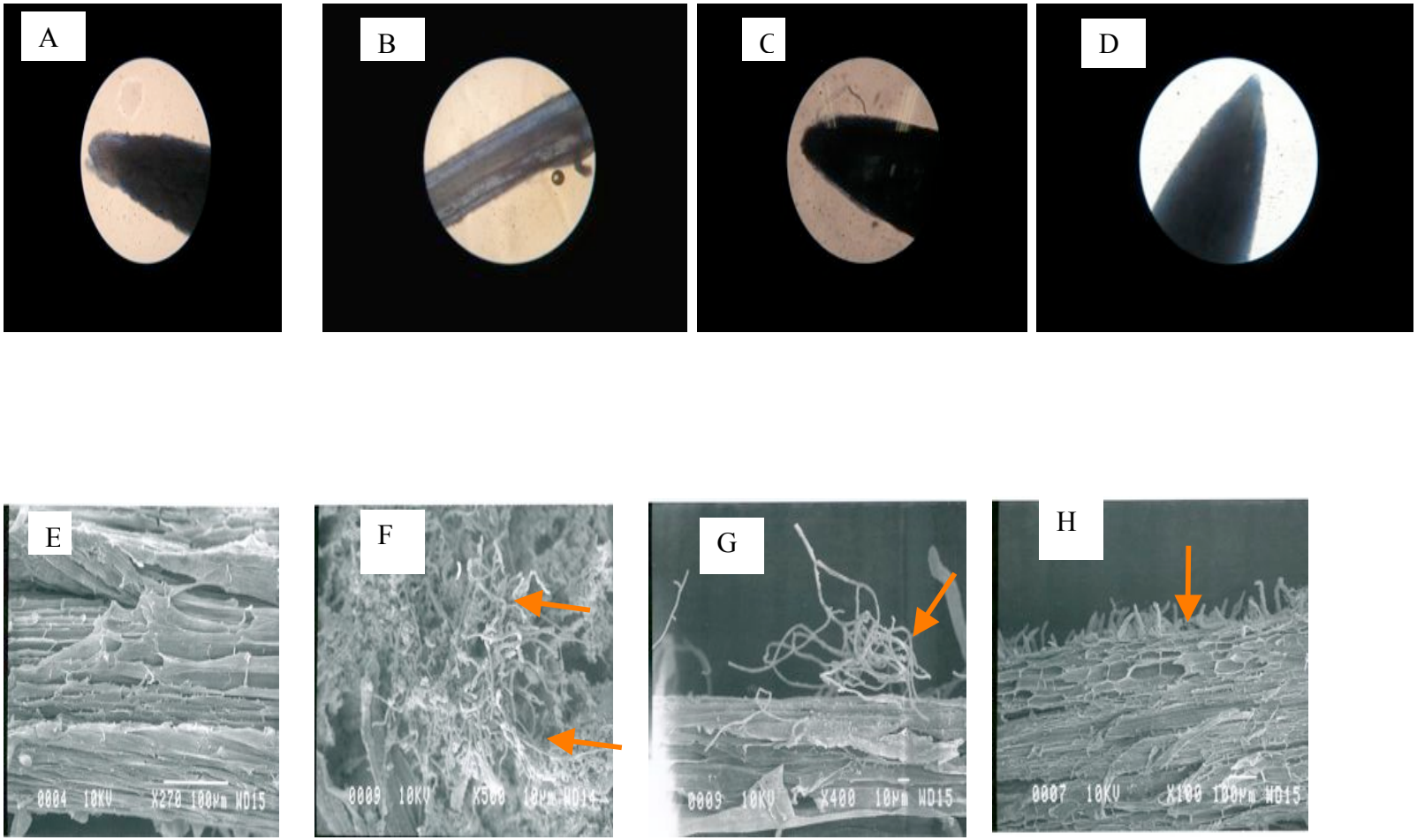

Plate 10: The panels marked A-D are pictures of light micrographs of susceptible bean variety (CAL 96) tissue inoculated with *Pythium ultimum*. A: non-inoculated sample. B: tissue collected 7 days after germination of seed, stained blue indicating colonisation by oomycete. C: tissue collected 8 days after germination of seed; hyphae attached on root surface. D: tissue collected 9 days after germination of seed, epidermal tissue was stained blue indicating colonisation by oomycete. The panels marked E-H are Scanning electron micrographs (SEM) of susceptible bean variety (CAL 96) tissue inoculated with *P.ultimum*. E: a non-inoculated control. F: infected root tissue taken 7 days after germination of seed showing extensive colonisation of epidermal tissue by thin oomycete hyphae. G: infected root sample taken 8 days after germination of seed showing thin hyphae penetrating the epidermis. H: infected tissue taken 9 days after germination of seed indicating short, thick hyphae attached on the root surface.

Light microscopy of *Pythium ultimum* infection on sorghum revealed 8 days after germination of seed, that the oomycete hypha was attached to the root tissue (Plate 14). Seven days after germination, the oomycete hyphae were observed to be intercellular; 8 days after germination, the hyphae were intracellular and finally 9 days after germination the hyphae was found distributed on the external surface of root tissue as well as in the endodermis and epidermis (Plate 14).

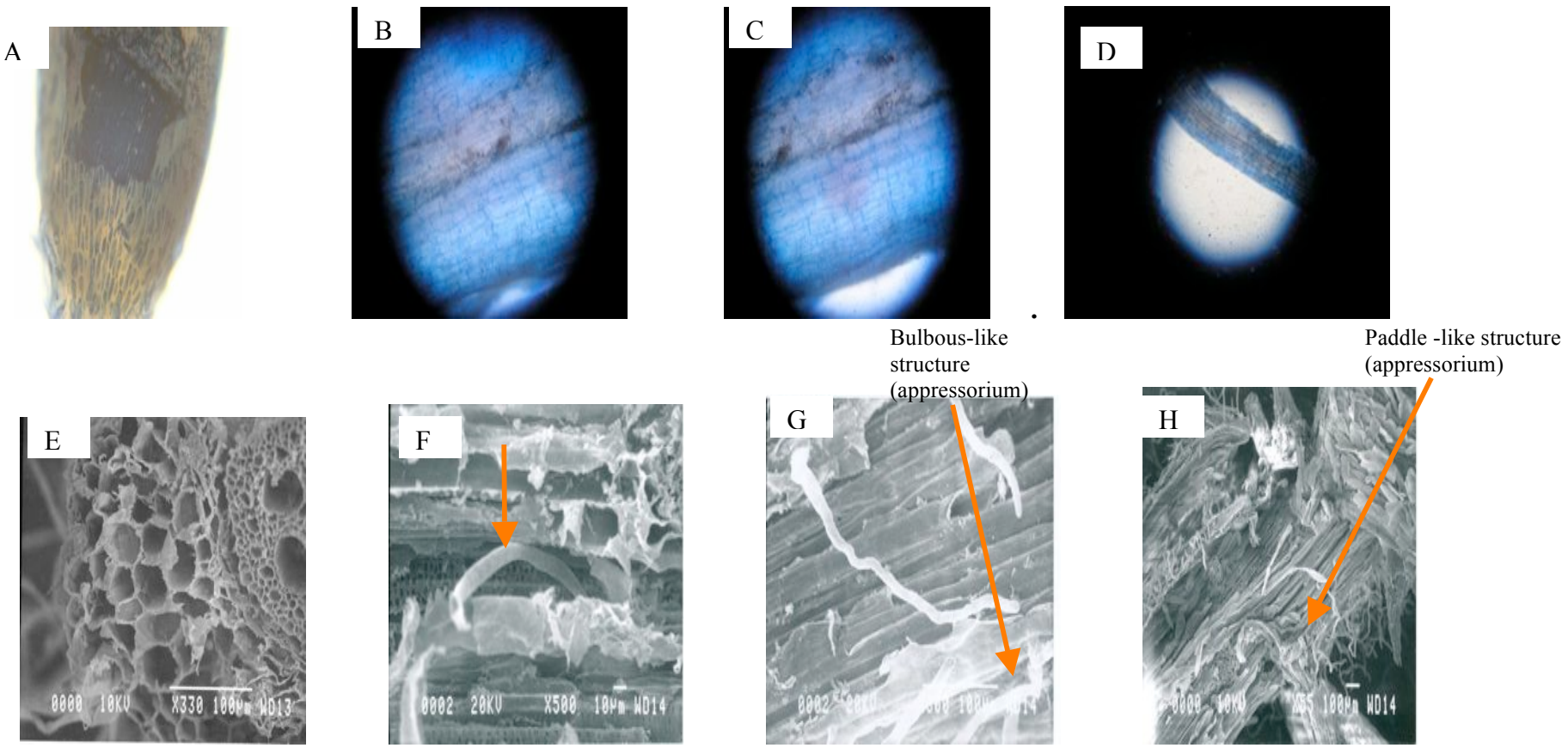

Plate 11: The panels marked A-D are pictures of light micrographs of maize tissue inoculated with *Pythium irregulare.*A: non-inoculated sample. B: tissue collected 7 days after germination of seed, epidermis stained blue indicating colonisation of epidermis by oomycete. C: tissue collected 8 days after germination of seed showed epidermal cells stained blue and enlargened. D: tissue collected 9 days after germination of seed showed epidermis stained blue indicating colonisation by oomycete.The panels marked E-H are Scanning electron micrographs (SEM) of maize inoculated with P.irregulare. E: a non-inoculated control. F: infected tissue taken 7 days after germination of seed showed hyphae growing intracellulary. The arrows in the diagram indicate hyphae. G: infected root sample taken 8 days after germination of seed showing hyphae which is long and bearing appressoria (bulbous-like structures). H: infected tissue taken 9 days after germination of seed showed shorter hyphae bearing paddle-like structures.

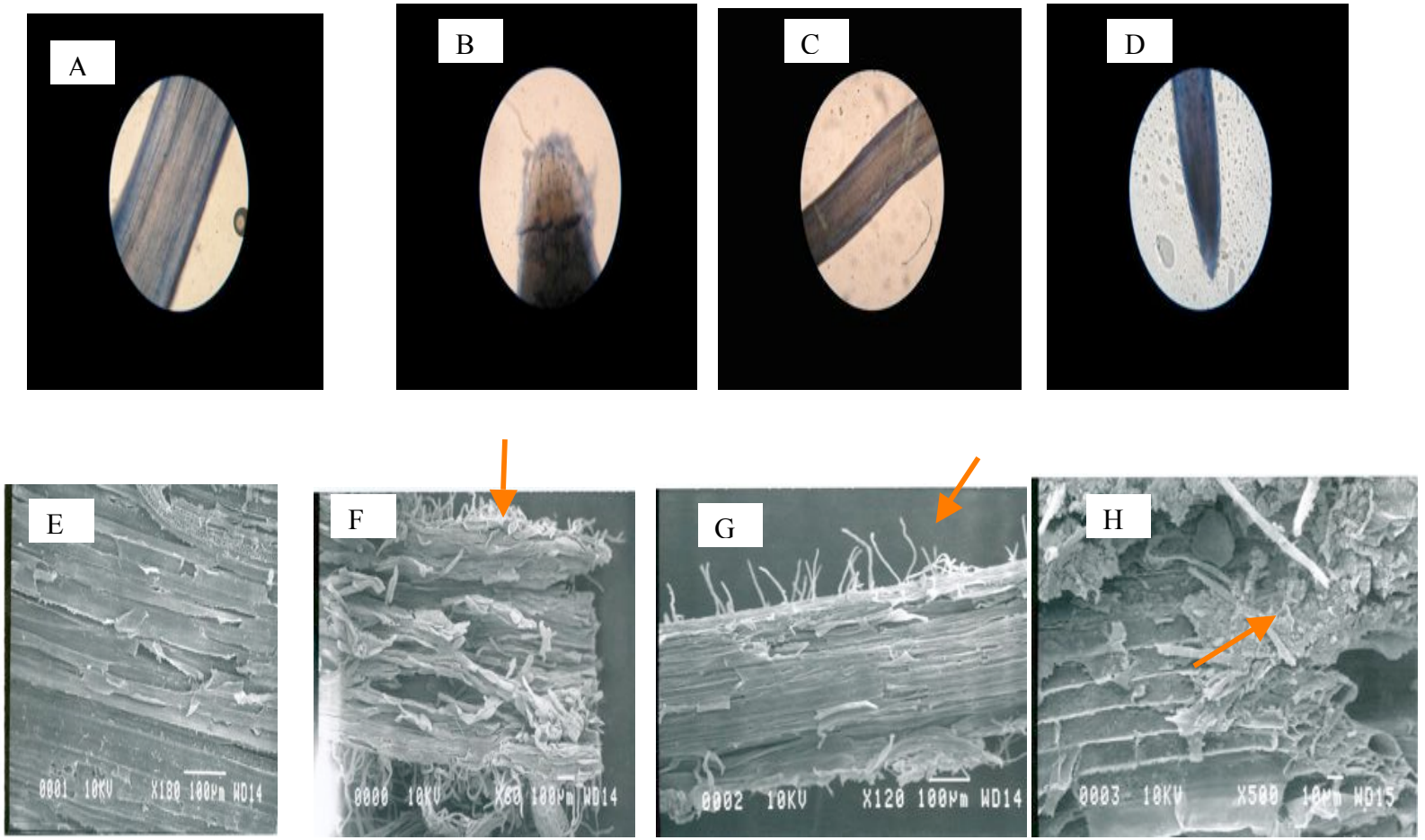

Plate 12: The panels marked A-D are pictures of light micrographs of maize tissue inoculated with *Pythium ultimum.* A: non- inoculated sample. B: tissue collected 7 days after germination of seed showed hyphae attached to the root surface. C: tissue collected 8 days after germination of seed showed epidermal cells stained blue indicating presence of oomycete. D: tissue collected 9 days after germination of seed showed epidermal cells stained blue. The panels marked E-H are Scanning electron micrographs (SEM) of maize tissue inoculated with *P.ultimum*. E: a non-inoculated control. F: infected tissue taken 7 days after germination of seed showed hyphae on root surface. The arrows indicate the hyphae. G: infected root sample taken 8 days after germination of seed showing few hyphae on root surface. H: infected tissue taken 9 days after germination of seed showed hyphae undergoing necrosis.

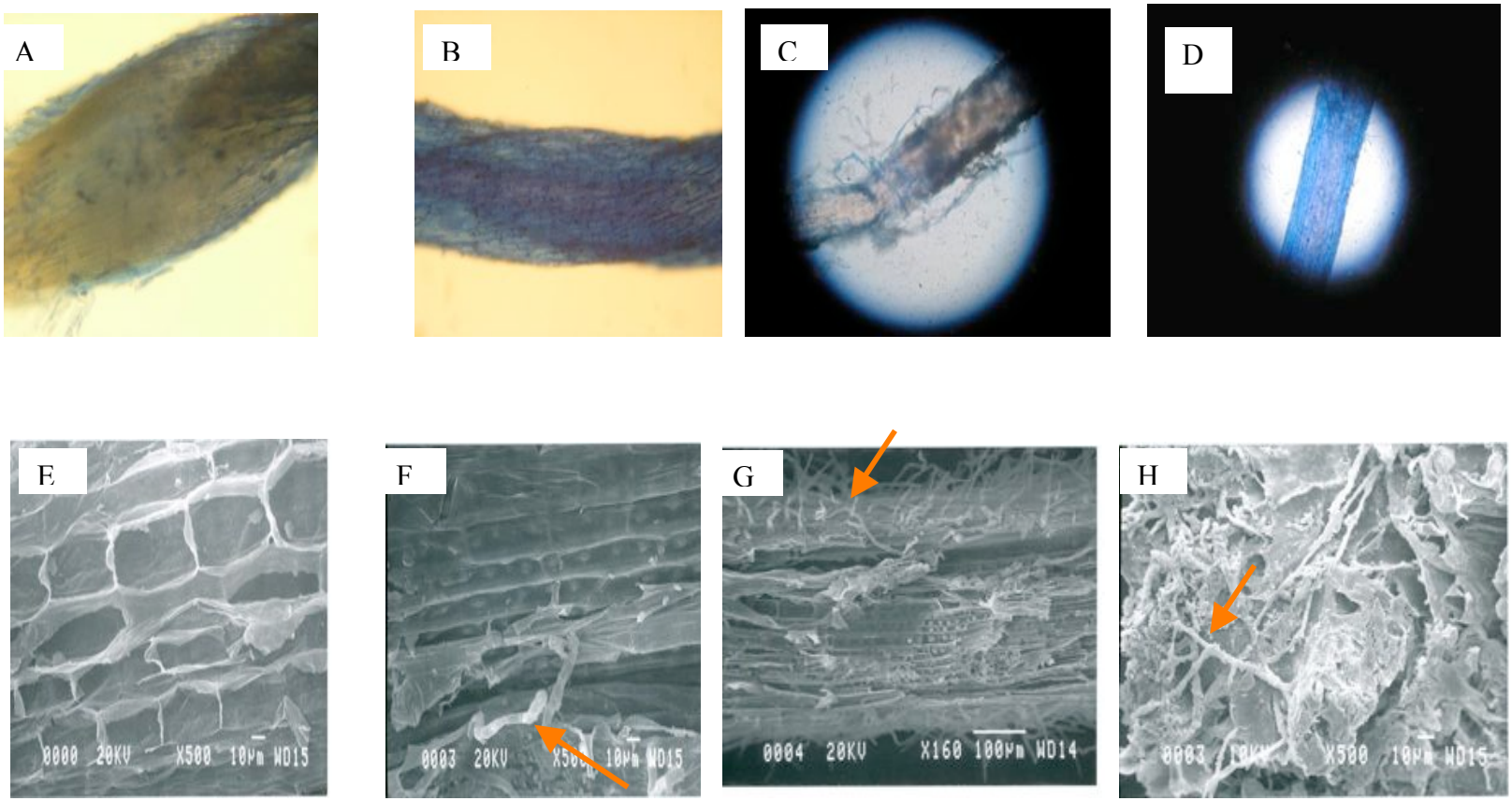

Plate 13: The panels marked A-D are pictures of light micrographs of sorghum tissue inoculated with *Pythium irregulare*. A: non-inoculated sample. B: tissue collected 7 days after germination of seed showed epidermis stained blue indicating colonisation by hyphae. C: tissue collected 8 days after germination of seed showed hyphae attached to root surface and penetrating epidermis. D: tissue collected 9 days after germination of seed showed epidermis and endodermis stained blue indicating colonisation by hyphae.The panels marked E-H are Scanning electron micrographs (SEM) of sorghum tissue inoculated with *P.irregulare*.E: a non-inoculated control. F: infected tissue taken 7 days after germination of seed showed hyphae bearing appressoria and the hyphae were penetrating the epidermis. The arrows indicate the hyphae. G: infected root sample taken 8 days after germination of seed showing numerous hyphae on root surface. H: infected root tissue taken 9 days after germination of seed showed hyphae forming an extensive network in the epidermis.

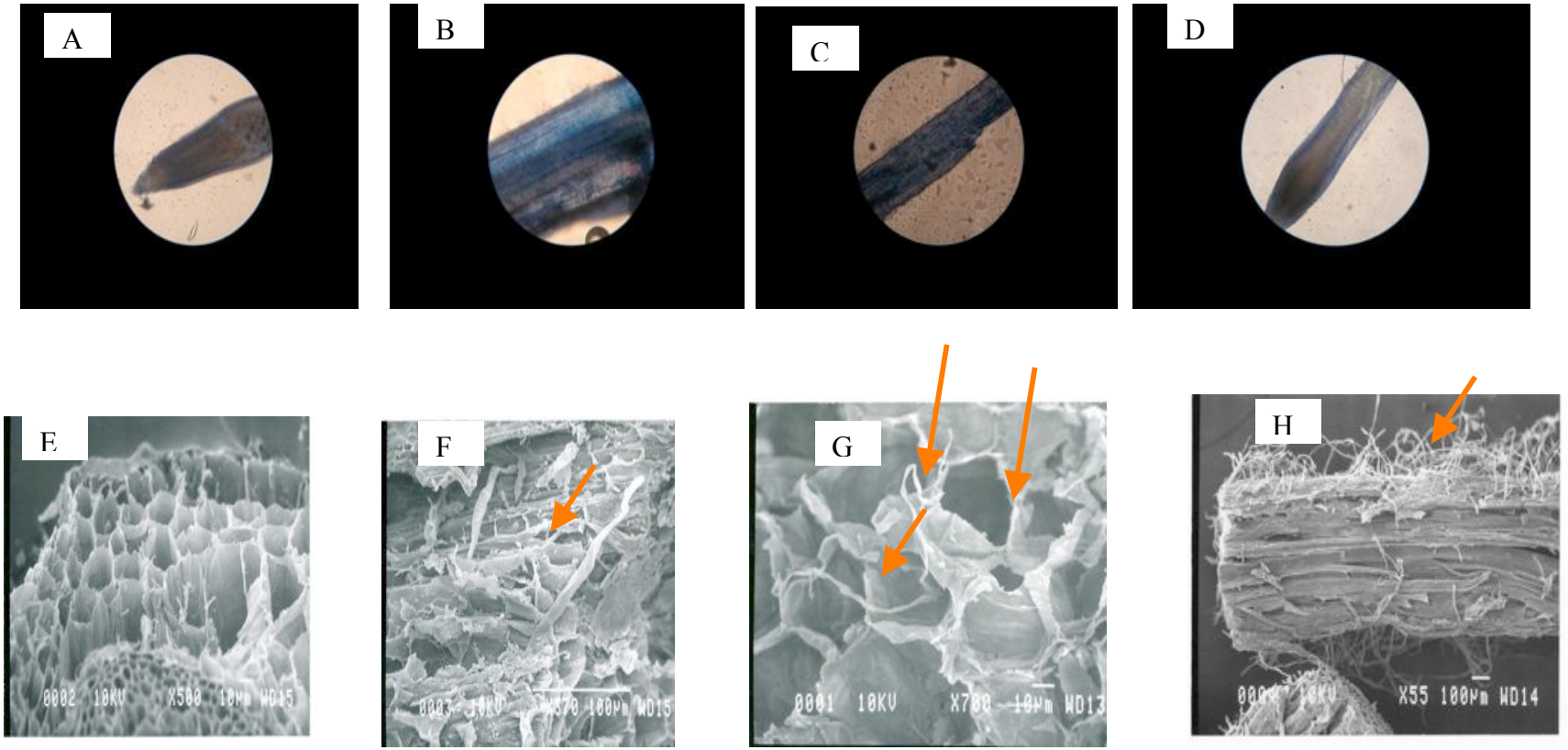

Plate 14: The panels marked A-D are pictures of light micrographs of sorghum tissue inoculated with *Pythium ultimum*. A: non-inoculated sample. B: tissue collected 7 days after germination of seed, epidermis stained blue indicating colonisation by hyphae.C: tissue collected 8 dyas after germination of seed, hyphae was attached to root surface. D: tissue collected 9 days after germination of seed, epidermis stained blue indicating colonization by hyphae. The panels marked E-H are Scanning electron micrographs (SEM) of sorghum tissue inoculated with *P.ultimum*. E: a non-inoculated control. F: infected tissue taken 7 days after germination of seed showed hyphae growing intercellularly. The arrows are pointing to hypahe. G: infected root sample taken 8 days after germination of seed showed that hyphae were intracellular. H; infected tissue taken 9 days after germination of seed had numerous hyphae on root surface.

4.5 Discussion

The symptoms of *Pythium* root rot in sorghum and maize have been described in previous sections of this thesis, but the histological and ultra structural aspects of the infection in these crops by *Pythium* species have not been documented. The ultra structural investigation of maize and sorghum with *Pythium irregulare* showed the attachment on root surface of oomycete hyphae. The hyphae had bulbous like structures which grew intracellularly. However with *Pythium ultimum* on maize, the oomycete hyphae underwent necrosis nine days after germination of seed. In contrast on sorghum, there was extensive colonisation of the epidermis and endodermis with oomycete hyphae. There is also evidence that sorghum and maize are both infected by *Pythium* species. However the difference is that in sorghum the pathogen grows while in maize the pathogen dies.

Pathogen adhesion to the host surface is an essential pre-penetration process that determines the success of infection and disease development (Isaac, 1992). Some pathogens specifically fungi produce mucilage (polysaccharides, glycoproteins, hexosamine polymers and xylans) to ensure close contact with the host (Isaac, 1992). The role of fungal exudates appears to be multiple. These roles include: attachment to the plant surface, sealing up of the penetration site, protection of appressoria against dessication and other limiting environmental factors and a reservoir for "penetrating enzymes" (Frezzi, 1968). Sometimes fungal pathogens are able to penetrate the tissue via the cuticle by mechanical force (Isaac, 1992) or through the effects of degrading enzymes such as cutinase in the case of *Fusarium solani. f. sp.pisi* (Woloshuck and

Kolattukudy, 1984). Apparently in *Rhizoctonia solani* the infection peg breaks the cuticle by mechanical pressure in bean hypocotyls (Kenning and Hanchey, 1980).

Results presented in plate 3 and 7 showed few oomycete hyphae in the epidermis with *Pythium irregulare*. Using electron microscopy, the early colonisation of bean tissue by *Pythium myriotylum, Pythium aphanidermatum* and *Pythium ultimum* have been characterised and found to involve the development of infection hyphae in the epidermal cell layer and the underlying cortex (Dow and Lumsden, 1975). In this study *Pythium ultimum* underwent necrosis in both resistant bean variety (AND 1062) and maize. In the case of susceptible bean variety (CAL 96) and sorghum, there was formation of appressoria, an appendage needed for pathogen entry into the plant. The susceptible bean variety (CAL 96) displayed infection of vascular system by *Pythium irregulare* while sorghum formed sub cuticular hyphae. The presence of sub cuticular hyphae has been reported for a number of plant pathogens for example *Venturia inaequalis* (Preece, 1962), *Phomophis leptostromiformis* (Williamson and Sivasithamparam, 1991).

In both susceptible bean variety (CAL 96) and sorghum the hyphae of these pathogens grew through the tissue primarily in an intracellular manner. In later stages of pathogenesis, the hypha is in the cortex and later grows intracellulary and intercellularly. There was also presence of primary and secondary hyphae in resistant bean variety (AND 1062) inoculated with *Pythium ultimum*. The fact that *Pythium ultimum* displayed presence of primary and secondary hypahe suggests that this *Pythium* species was more virulent than *Pythium irregulare*. Other cytological studies elsewhere have shown that *Colletotrichum sublineolum* has a two-stage

hemibiotrophic infection process on sorghum similar to that of *Colletotrichum lindemuthianum* on bean (O'Connell *et al*., 1985). The initial biotrophic phase is associated with primary hyphae which colonise many host cells giving rise to necrotrophic secondary hyphae (Wharton *et al*., 2001). Secondary hyphae have much thinner walls (50-60 nm) thick than mature primary hyphae in infection of sorghum by *Colletotrichum sublineolum*. *Pythium ultimum* infection was found to be highly virulent on spruce seedlings (*Picea abies (L.) Karst*) and *Pythium irregulare* was less virulent.

In this study *Pythium irregulare* formed appressoria when infecting maize and sorghum. During infection *Pythium* species are known to produce appressoria that penetrate the cuticle and epidermal cell walls mechanically (Adegbola and Hagedorn, 1969). Appresorium formation has been observed in *Phomopsis helianthi* (Mutanola, 1989) and *Asochyta pisi* (Heath and Wood, 1969). Different species of *Phoma* can infect plants with or without appressorium formation. In this case the behaviour of the fungus could be different on other parts of the plant, such as leaf petiole or base of the stem, where pathogen penetration might be accompanied by appressoria formation. The formation of appressorium is known to depend on different factors such as epicuticular waxes, rigidity and surface hardness (Höhl et *al*., 1990).

This study confirms that pathogenesis of *Pythium* species is influenced by host genotype and physical attributes. In this study sorghum was extensively infected by *Pythium* species in a similar manner to susceptible bean variety (CAL 96). Pathogenesis in maize and the resistant bean variety (AND 1062) were similar with evidence of attachment followed by necrosis of hyphae which prevents further

infection. This study confirms that sorghum is an alternative host of *Pythium* species. In addition in this study it was possible to confirm the presence of hemibiotropic infection process because of the presence of two types of hyphae with *Pythium ultimum*. This also indicates that this *Pythium* species was more virulent than *Pythium irregulare* because infection was mediated by the two types of oomycete hyphae which means that the two types of hyphae are necessary to cause infection in crops

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CHAPTER FIVE

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5 General Discussion

The first study characterised root rots found in bean, multi-crop systems of south western Uganda. The second study investigated pathogenicity of *Pythium* species isolated from beans and other crops species and tested on selected crops. The third study investigated *Pythium* infection process in selected crop host species.

The objective of the first study was to investigate the role of mixed cropping systems on bean root rot epidemics in south western Uganda. Root rots were characterised in crops such as potato (*Solanum tuberosum*), peas (*Pisum satium*) and sorghum (*Sorghum bicolor*). Mixed cropping is a common phenomenon in east Africa and elsewhere it has been found to influence diversity in host populations and ultimately disease epidemics (Burdon *et al*., 1989). Root rots in these crops were found to be induced by a complex of pathogens which included *Pythium spp, Fusarium spp*. and *Verticillium coccosporum*. Using molecular techniques, a diversity of *Pythium* species were found on crops intercropped with beans. In eastern Africa, bean root rots are caused by a complex of two or more pathogens depending on suitable environmental conditions (CIAT 1992; Rusuku *et al*., 1997). This study implies that growing beans with other crops may be playing a crucial role in pathogen build up in the bean-*Pythium* pathosystem .For example potato and sorghum were found to have typical root rot symptoms and *Pythium* species were also isolated from them. Further

research carried out in this study found sorghum to be a susceptible crop. The information generated in the first part of the study will contribute towards the design of integrated management for bean root rot disease based on crop arrays that are best used by farmers in that area.

The objective of the second study was to investigate the contribution of various crop species to the bean root rot epiphytotics in south western Uganda. Sorghum and peas were found to be susceptible to *Pythium* root rot. Maize and millet were resistant to *Pythium* root rot. Majority of the *Pythium* species were playing a crucial role in post emergence damping off than on pre emergence damping off. Therefore the crop species that could form prop roots had a capacity to overcome disease therefore a higher root mass was obtained with cereals than with legumes. Other symptoms of *Pythium* infection included wilting and chlorosis of leaves. Bean derived *Pythium* species were found to be more virulent than Pythium species derived from other crops. This means that there is variation in the aggressiveness of the *Pythium* species on crops (Zhang and Yang, 2000). Root exudates appear to play a role also in influencing the level of disease. These exudates may support the growth of pathogens in the rhizosphere. The implication of this study is that in order to control bean root rot there is need to think of crop species that are unfavourable to the *Pythium* pathogen. In this study for instance maize and millet were unfavourable crops to *Pythium* species.

The objective of the third study was to confirm the actual pathogenicity of *Pythium* species using electron microscopy. Bean pathogenic *Pythium* species were pathogenic on sorghum. The infection pattern in sorghum was similar to a susceptibility reaction

in beans therefore this confirms that sorghum is an alternative host. In maize, the infection pattern was similar to a resistant response similar to that of resistant bean variety therefore this confirms that maize is a non host to *Pythium spp*. The presence of primary and secondary hyphae in *Pythium ultimum* implies a hemibiotrophic infection process (O' Connel *et al*., 1985). The implication of this study is that it would be advisable not to intercrop sorghum with beans since it is an alternative host of *Pythium* species.

5.1 Conclusions

Basing on the results and observations made during this study, the following conclusions can be drawn:

1. Cultivation of beans in mixed cropping systems with other crop species may in part be contributing to bean root rot epidemics. Some of the commonly cultivated crops such as sorghum and peas were found to be alternative hosts of pathogenic *Pythium* species implying that they contribute to pathogen inoculum load in the soil hence increased disease outbreaks. Hence farmers should be advised not to include sorghum and peas in rotation or intercropped with beans since they would be contributing to continuous bean root rot epiphytotics.
2. Some of the crops included in the mixed cropping system are poor hosts of pathogenic *Pythium* species. Maize and millet for example were not affected by *Pythium* species. Attempts to infect them resulted in necrosis of fungal hyphae. This suggests that such crops could be included in bean rotations in south western Uganda so as to reduce *Pythium* soil inoculum load.

3. Within the pathogenic *Pythium* species differences exist in pathogenicity even on the same hosts. This phenomenon suggests the possibility for directional selection leading to increase in species or even pathotype abundances among *Pythium* pathogenic species. This could lead to selection of virulent genes in the pathogens and hence increase the inoculum load of these pathogens in the soil thus continuing to affect the bean root rot epidemics.
4. Several *Pythium* species were isolated from bean and other crop hosts. Whereas some were pathogenic others were not. Given the multi-pathogenicity capacity of this genus, evolution of novel *Pythium* strains or pathogens on both beans and non-bean hosts cannot be precluded.
5. The similarity in disease reaction of resistant bean variety (RWR 719 and AND 1062) and maize to pathogenic *Pythium* infection suggests similarity in resistance reaction. Such a finding broadens the base for understanding pathogens of this important species by opening frontiers for studies in other well characterised crop species such as maize. Moreover the on going genome programs imply that such a study would benefit the maize genome sequencing project.

5.2 Future Perspectives

Basing on the results of this study the areas recommended to further understand bean *Pythium* pathosystems and bean root rot epiphytotics are:

1. The epidemiology of *Pythium* root rot is affected by environmental, host and cultural factors. This study has examined some environmental and host factors

however it would be of importance to investigate additional factors which may influence bean root rot epiphytotics such as soil moisture, soil temperature, soil compaction, organic matter, levels of herbicide use, plant density, poor seed quality and irrigation run off. These factors have been implicated in other pathosystems and may play a role in influencing the spatio-temporal aspects of bean root rot epiphytotics in south western Uganda.

2. Given the wide array of *Pythium* species found in the study and cross pathogenicity now confirmed it is of interest to further study pathogenicity-evolution in this multi-host-multi-pathogen pathosystem. The availability of new molecular tools could support testing of various hypotheses including that of divergent selection playing a role in pathogen evolution in this pathosystem.
3. What is clear from this study is that crop species react variously to *Pythium* attack. Some of the reactions are positive (susceptible) and others negative (resistant). It is therefore important to study how different crop combinations can be arrayed to manage the bean root rot disease in south western Uganda. This will contribute greatly to understanding which crops are suitable intercropped with beans as part of disease management of bean root rot epiphytotics in south western Uganda.

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APPENDICES

Appendix 1: Disease incidence of root rot in crops intercropped with beans

Crop	2005(%)	2006(%)
Beans	32.40[a]	19.80[a]
Cabbage	20.0a	110a
Cauliflower	20.0a	48.60[a]
Green pepper	20.0a	40.0a
Potato	20.0b	37.2[a]
Maize	20.0a	40.20[a]
Peas	20.20[a]	48.40[a]
Sorghum	20.0a	31.40a

Appendix 2: *Pythium* species recovered from various crop species in a bean based cropping system

Grouping on tree	Pathogen	Crop isolated from	Subcounty
A	*Fusarium spp.*	Millet	Bubale (BB)
A	*Fusarium oxysporum*	Cabbage	Kaharo (KH)
A	*Fusarium spp.*	Irish	Kisoro (KS)
A	*Fusarium equiseti*	Bean	Hamurwa (HM)
A	*Fusarium inc*	Bean	Buhara (BH)
B	*Pythium irregulare*	Irish	Bukinda (BK)
B	*Pythium irregulare*	Irish	Bubale (BB)
B	*Pythium irregulare*	Tomato	Bukinda (BB)
C	*Pythium spinosum*	Sorghum	Kyanamira(KY)
C	*Pythium spinosum*	Sorghum	Rubaya (RB)
C	*Pythium spinosum*	Sorghum	Muko (MK)
C	*Pythium spinosum*	Irish	Maziba (MZ)
D	*Pythium heterothallicum*	Sweet potato	Kamuganguzi (KG)
D	*Verticillium coccosporum*	Sorghum	Rubaya (RB)
E	*Pythium ultimum*	Maize	Kaharo(KH)
E	*Pythium ultimum*	Potato	Hamurwa(HM)
E	*Pythium ultimum*	Sorghum	Rwamucucu(RW)
E	*Pythium ultimum*	Sorghum	Kaharo(KH)
E	*Pythium ultimum*	Cabbage	Bubale(BB)
E	*Pythium ultimum*	Sweet potato	Kaharo(KH)
E	*Pythium ultimum*	Cabbage	Unknown
E	*Pythium ultimum*	Maize	Kabale(KB)
E	*Pythium ultimum*	Maize	Maziba(MZ)
E	*Pythium ultimum*	Banana	Bukinda(BK)
E	*Pythium ultimum*	Potato	Maziba(MZ)
E	*Pythium ultimum*	Potato	Ikumba(IK)
E	*Pythium ultimum*	Maize	Bubale(BB)
E	*Pythium ultimum*	Potato	Muko(MK)
E	*Pythium ultimum*	Potato	Kashambya(KS)
E	*Pythium ultimum*	Potato	Bufundi(BF)
E	*Pythium ultimum*	Sorghum	Kashambya(KS)
E	*Pythium ultimum*	Maize	Bufundi(BF)
F	*Pythium orthogonon*	Bean	Lira(L)
F	*Pythium parvum*	Cabbage	Bubale(BB)
F	*Pythium parvum*	Cabbage	Rubaya(RB)
F	*Pythium arrhenomanes*	Sorghum	Bukinda (BK)
F	*Pythium folliculosum*	Bean	Rwamucucu (RW)
F	*Pythium folliculosum*	Peas	Rwamucucu (RW)
F	*Pythium folliculosum*	Beans	Kaharo (KH)
F	*Pythium acanthicum*	Potato	Muko (MK)
F	*Pythium oligandrum*	Sorghum	Kyanamira (KY)
F	*Pythium oligandrum*	Maize	Rwamucucu (RW)

F	*Pythium arrhenomanes*	Millet	Rubaya (RB)
F	*Pythium pachycaule*	Sorghum	Muko (MK)
F	*Pythium conidiophorum*	Bean	Kabale (KB)
F	*Pythium arrhenomanes*	Maize	Kaharo (KH)
F	*Pythium vexans*	Sweet potato	Kabale (KB)
F	*Pythium spinosum*	Millet	Rubaya (RB)
F	*Pythium folliculosum*	Bean	Muko (MK)
F	*Pythium folliculosum*	Sorghum	Muko (MK)
F	*Pythium folliculosum*	Sorghum	Buhara (BH)
F	*Pythium folliculosum*	Maize	Bufundi (BF)
F	*Pythium parvum*	Millet	Rubaya (RB)
F	*Pythium irregulare*	Bean	Muko (MK)
F	*Pythium sylvaticum*	Sorghum	Bufundi (BF)
F	*Pythium spinosum*	Sorghum	Bukinda (BK)
F	*Pythium spinosum*	Bean	Bufundi (BF)
F	*Pythium ultimum*	Cabbage	Kaharo (KH)
F	*Pythium spinosum*	Bean	Bufundi (BF)
F	*Pythium spinosum*	Sorghum	Rubaya (RB)
G	*Pythium ultimum*	Maize	Bufundi(BF)
G	*Pythium ultimum*	Potato	Bukinda (BK)
G	*Pythium ultimum*	Bean	Bufundi (BF)
G	*Pythium ultimum*	Sorghum	Bukinda (BK)
G	*Pythium ultimum*	Cabbage	Kaharo (KH)
G	*Pythium ultimum*	Yam	Bufundi (BF)
G	*Pythium ultimum*	Potato	Rubaya (RB)
G	*Pythium ultimum*	Maize	Bukinda (BK)
G	*Pythium ultimum*	Maize	Muko (MK)
G	*Pythium ultimum*	Wheat	Rubaya (RB)
G	*Pythium ultimum*	Sorghum	Kaharo (KH)
G	*Pythium ultimum*	Wheat	Buhara (BH)
G	*Pythium ultimum*	Maize	Bukinda (BK)
G	*Pythium ultimum*	Sorghum	Bukinda (BK)
G	*Pythium ultimum*	Sorghum	Kaharo (KH)
G	*Pythium ultimum*	Potato	Bufundi (BF)
G	*Pythium ultimum*	Potato	Kaharo (KH)
G	*Pythium conidiophorum*	Bean	
G	*Pythium ulitmum*	Maize	Bufundi (BF)
G	*Pythium ultimum*	Maize	Bukinda (BK)

Appendix 3: Mean emergence scores of crop species inoculated with bean derived pathogenic *Pythium* species

	Crop Species															
	Trial One								Trial Two							
Pythium species	Resistant bean variety [b]	Susceptible bean variety[a]	Maize	Sorghum	Millet	Peas	LSD (P≤ 0.05) [d]	CV (%) [e]	Resistant bean variety [b]	Susceptible bean variety [a]	Maize	Sorghum	Millet	Peas	LSD (P≤ 0.05) [d]	CV (%) [e]
No *Pythium* species [c]	16.00	16.00	16.00	16.00	16.00	16.00	0	0	16.00	16.00	16.00	16.00	16.00	15.00	0	0
P.ultimum var. *ultimum*	15.67	14.00	15.33	16.00	16.00	14.33	1.56	5.6	15.33	11.00	15.00	15.33	16.00	8.30	1.94	7.3
P.chamaehyphon	15.00	14.33	15.67	16.00	16.00	14.33	1.94	6.9	14.00	9.67	15.33	12.67	16.00	8.70	1.41	5.7
P.macrosporum	15.67	12.33	16.00	16.00	16.00	15.00	1.05	3.8	15.00	10.00	15.33	13.67	16.00	9.30	2.90	11.4
P.pachycaule	14.33	11.33	15.33	16.00	16.00	15.00	2.15	8.1	12.67	7.33	15.00	14.67	16.00	2.70	3.04	12.7
LSD (P≤ 0.05) [d]	2.00	2.70	0.81	0.00	0.00				2.10	3.87	0.66	1.88	0.0	10.40		
CV (%) [e]	7.1	10.9	2.9	0.0	0.0				7.9	19.7	2.4	7.1	0.0	64.9		

a= RWR 719 (resistant bean variety) (Mukalazi, 2004)
b= CAL 96 (susceptible bean variety) (Mukalazi, 2004)
c= Non inoculated control
d= Least Significance Difference (Steel *et al.,* 1997)
e= % Coefficient of Variation

Appendix 3 continued: Mean emergence scores of crop species inoculated with bean derived pathogenic *Pythium* species

	Crop Species							
	Trial three							
***Pythium* species**	**Resistant bean variety [b]**	**Susceptible bean variety[a]**	**Maize**	**Sorghum**	**Millet**	**Peas**	**LSD (P≤ 0.05) [d]**	**CV (%) [e]**
No *Pythium* species [c]	16.00	16.00	16.00	16.00	16.00	15.00	0	0
P.ultimum var. *ultimum*	13.67	8.67	13.33	12.00	15.33	8.30	2.49	10.8
P.chamaehyphon	13.33	8.00	14.67	11.33	15.33	8.70	1.88	8.2
P.macrosporum	11.00	6.00	14.00	12.00	15.33	9.30	4.10	19.3
P.pachycaule	11.00	5.00	14.33	9.00	15.67	2.70	2.53	12.6
LSD (P≤ 0.05) [d]	2.25	4.01	2.00	2.34	1.49	10.40		
CV (%) [e]	9.5	25.3	7.6	10.7	5.3	64.9		

a= RWR 719 (resistant bean variety) (Mukalazi, 2004)
b= CAL 96 (susceptible bean variety) (Mukalazi, 2004)
c= Non inoculated control
d= Least Significance Difference (Steel *et al.,* 1997)
e= % Coefficient of Variation

Appendix 4: Mean root dry matter scores of crop species inoculated with bean derived pathogenic *Pythium* species

	Crop species															
	Trial one								Trial two							
Pythium species	Resistant bean variety [b]	Susceptible bean variety [a]	Maize	Sorghum	Millet	Peas	LSD (P≤ 0.05) [d]	CV (%) [e]	Resistant bean variety[b]	Susceptible bean variety [a]	Maize	Sorghum	Millet	Peas	LSD (P≤ 0.05) [d]	CV (%) [e]
No *Pythium* species [c]	0.05	0.09	0.13	0.01	0.01	0.08	0.05	45.8	0.04a	0.04a	0.08a	0.01a	0.00a	0.05	0.02	35.0
P.ultimum var. *ultimum*	0.07	0.09	0.15	0.02	0.05	0.05	0.05	36.0	0.04a	0.05a	0.11a	0.01a	0.01a	0.04	0.03	30.7
P.chamaehyphon	0.07	0.06	0.17	0.03	0.03	0.05	0.05	34.3	0.04a	0.07a	0.13a	0.02a	0.02a	0.05	0.02	22.3
P.macrosporum	0.07	0.08	0.14	0.02	0.02	0.08	0.03	25.5	0.04a	0.07a	0.13a	0.02a	0.01a	0.06	0.02	20.5
P.pachycaule	0.08	0.11	0.17	0.03	0.03	0.07	0.02	14.2	0.01a	0.08a	0.12a	0.02a	0.01a	0.05	0.03	31.7
LSD (P≤ 0.05) [d]	0.05	0.06	0.04	0.02	0.02	0.02			0.02	0.03	0.04	0.00	0.01	0.04		
CV (%) [e]	37.0	39.1	14.4	42.3	44.3	13.4			30.0	24.1	20.3	0.0	41.9			

a= RWR 719 (resistant bean variety) (Mukalazi, 2004)
b= CAL 96 (susceptible bean variety) (Mukalazi, 2004)
c= Non inoculated control
d= Least Significance Difference (Steel *et al.,* 1997)
e= % Coefficient of Variation

Appendix 4 continued: Mean root dry matter scores of crop species inoculated with bean derived pathogenic *Pythium* species

	Crop species							
	Trial three							
Pythium species	Resistant bean variety [b]	Susceptible bean variety [a]	Maize	Sorghum	Millet	Peas	LSD (P≤ 0.05) [d]	CV (%) [e]
No *Pythium* species [c]	0.03	0.02	0.04	0.01	0.00	0.05	0.03	78.0
P.ultimum var. *ultimum*	0.03	0.04	0.06	0.01	0.00	0.04	0.05	94.0
P.chamaehyphon	0.03	0.06	0.12	0.01	0.01	0.05	0.02	26.7
P.macrosporum	0.03	0.04	0.11	0.01	0.01	0.06	0.03	41.1
P.pachycaule	0.04	0.07	0.11	0.01	0.01	0.05	0.03	29.0
LSD (P≤ 0.05) [d]	0.02	0.03	0.06	0.01	0.01	0.04		
CV (%) [e]	41.2	41.0	35.5	0.01	41.9	48.1		

a= RWR 719 (resistant bean variety) (Mukalazi, 2004)
b= CAL 96 (susceptible bean variety) (Mukalazi, 2004)
c= Non inoculated control
d= Least Significance Difference (Steel *et al.,* 1997)
e= % Coefficient of Variation

Appendix 5: Mean emergence scores of crop species inoculated with three *Pythium* species isolated from crops intercropped with beans

	Crop species															
	Trial one								Trial two							
	Resistant bean variety[b]	Susceptible bean variety[a]	Maize	Sorghum	Millet	Peas	LSD(P≤ 0.05) [d]	CV (%) [e]	Resistant bean variety[b]	Susceptible bean variety[a]	Maize	Sorghum	Millet	Peas	LSD(P≤ 0.05) [d]	CV (%) [e]
Pythium species																
No *Pythium* species [c]	16.00	16.00	16.00	16.00	16.00	16.00	0	0	12.00	13.33	12.00	16.00	16.00	14.00	3.5	14.2
P.macrosporum	16.00	16.00	16.00	16.00	16.00	16.00	0	0	10.00	7.00	12.33	16.00	16.00	13.00	3.51	15.9
P.glomeratum	16.00	16.00	15.33	16.00	16.00	16.00	0.84	3.0	13.33	12.00	12.33	16.00	16.00	13.33	2.72	11.0
P.ultimum	16.00	16.00	14.33	16.00	16.00	16.00	1.11	4.0	8.30	12.00	13.33	16.00	16.00	14.67	2.52	10.6
LSD(P≤ 0.05) [d]	0.0	0.0	1.80	0.0	0.0	0.0			4.68	3.44	3.26	0.0	0.0	4.48		
CV (%) [e]	0.0	0.0	6.2	0.0	0.0	0.0			22.7	16.5	13.9	0.0	0.0	17.3		

a= RWR 719 (resistant bean variety) (Mukalazi, 2004)
b= CAL 96 (susceptible bean variety) (Mukalazi, 2004)
c= Non inoculated control
d= Least Significance Difference (Steel *et al.,* 1997)
e= % Coefficient of Variation

Appendix 5 continued: Mean emergence scores of crop species inoculated with three *Pythium* species isolated from crops intercropped with beans

	Crop species							
	Trial three							
	Resistant bean variety[b]	Susceptible bean variety[a]	Maize	Sorghum	Millet	Peas	LSD(P≤ 0.05) [d]	CV (%) [e]
Pythium species								
No *Pythium* species [c]	8.33	10.33	13.33	16.00	16.00	10.70	2.22	10.0
P.macrosporum	6.67	3.67	11.00	15.33	12.70	8.30	4.52	26.4
P.glomeratum	7.67	7.50	9.33	15.33	10.70	10.70	6.32	34.5
P.ultimum	5.33	5.00	10.67	16.00	9.70	7.70		
LSD(P≤ 0.05) [d]	3.56	6.01	2.49	1.22	6.66	6.88	4.84	30.0
CV (%) [e]	27.0	47.0	11.9	4.10	28.90	39.1		

a= RWR 719 (resistant bean variety) (Mukalazi, 2004)
b= CAL 96 (susceptible bean variety) (Mukalazi, 2004)
c= Non inoculated control
d= Least Significance Difference (Steel *et al.,* 1997)
e= % Coefficient of Variation

Appendix 6: Mean root dry matter scores of crop species inoculated with three *Pythium* species isolated from crops intercropped with beans

	Crop species															
	Trial one								Trial two							
	Resistant bean variety [a]	Susceptible bean variety [b]	Maize	Sorghum	Millet	Peas	LSD(P≤ 0.05) [d]	CV (%) [e]	Resistant bean variety[b]	Susceptible bean variety[a]	Maize	Sorghum	Millet	Peas	LSD(P≤ 0.05) [d]	CV (%) [e]
Pythium species																
No *Pythium* species [c]	0.07a	0.18a	0.29ab	0.03a	0.01a	0.05a	0.03	38.7	0.05a	0.11a	0.13a	0.02a	0.01a	0.04a	0.02	25.3
P.macrosporum	0.07a	0.11a	0.15a	0.03a	0.01a	0.04a	0.23	106.1	0.06a	0.10a	0.12a	0.01a	0.01a	0.03a	0.03	27.7
P.glomeratum	0.07a	0.13a	0.16a	0.02a	0.02a	0.05a	0.15	84.4	0.05a	0.12a	0.12a	0.01a	0.01a	0.04a	0.03	26.3
P.ultimum	0.08a	0.15a	0.18a	0.02a	0.02a	0.05a	0.04	27.0	0.06a	0.11a	0.12a	0.01a	0.01a	0.03a	0.01	12.4
LSD(P≤ 0.05) [d]	0.03	0.07	0.11	0.02	0.02	0.02			0.03	0.05	0.03	0.01	0.01	0.01		
CV (%) [e]	21.4	25.7	30.8	41.0	60.9	23.1			24.6	24.0	12.5	28.8	26.6	14.0		

a= RWR 719 (resistant bean variety) (Mukalazi, 2004)
b= CAL 96 (susceptible bean variety) (Mukalazi, 2004)
c= Non inoculated control
d= Least Significance Difference (Steel *et al.,* 1997)
e= % Coefficient of Variation

Appendix 6 continued: Mean root dry matter scores of crop species inoculated with three *Pythium* species from crops intercropped with beans

	Crop species							
	Trial three							
Pythium species	Resistant bean variety[b]	Susceptible bean variety[a]	Maize	Sorghum	Millet	Peas	LSD(P≤ 0.05) [d]	CV (%) [e]
No *Pythium* species [c]	0.03	0.08	0.11	0.01	0.01	0.03	0.02	27.3
P.macrosporum	0.03	0.04	0.18	0.01	0.01	0.03	0.03	44.5
P.glomeratum	0.03	0.09	0.09	0.01	0.01	0.03	0.04	53.6
P.ultimum	0.05	0.07	0.09	0.01	0.01	0.03	0.02	20.1
LSD(P≤ 0.05) [d]	0.01	0.03	0.03	0.01	0.0	0.02		
CV (%) [e]	13.9	25.8	13.9	26.6	0.0	26.5		

a= RWR 719 (resistant bean variety) (Mukalazi, 2004)
b= CAL 96 (susceptible bean variety) (Mukalazi, 2004)
c= Non inoculated control
d= Least Significance Difference (Steel *et al.,* 1997)
e= % Coefficient of Variation

Appendix 7: Mean emergence scores of crop species inoculated with five *Pythium* species isolated from crops intercropped with beans

	Crop species															
	Trial one								Trial two							
Pythium species	Resistant bean variety[b]	Susceptible bean variety[a]	Maize	Sorghum	Millet	Peas	LSD(P≤ 0.05) [d]	CV (%) [e]	Resistant bean variety[b]	Susceptible bean variety[a]	Maize	Sorghum	Millet	Peas	LSD(P≤ 0.05) [d]	CV (%) e
No *Pythium* species	16.00	16.00	16.00	16.00	16.00	16.00	0	0	16.00	16.00	16.00	16.00	16.00	16.00	0	0
P.macrosporum	10.67	12.67	14.00	16.00	16.00	13.00	0.94	3.8	9.33	10.00	8.33	16.00	16.00	10.67	1.19	5.7
P.arrhenomanes	12.67	11.33	14.00	16.00	16.00	12.67	2.41	9.8	12.67	9.67	7.00	16.00	16.00	12.00	4.67	23.2
P.spinosum	10.67	10.33	11.67	16.00	16.00	9.67	1.51	6.9	10.67	9.67	7.00	16.00	16.00	8.33	2.44	12.1
P.oligandrum	11.67	11.00	12.33	16.00	16.00	13.00	2.01	8.5	11.67	10.00	8.67	16.00	16.00	7.67	2.75	13.7
P.heterothallicum	12.00	12.67	13.00	16.00	16.00	15.00	2.22	8.8	11.67	10.33	8.67	16.00	16.00	10.00	2.05	9.3
LSD(P≤ 0.05) [d]	1.97	2.44	2.48	0.0	0.0	1.39			1.88	0.73	2.30	0.0	0.0	2.91		
CV (%) [e]	9.0	11.1	10.3	0.0	0.0	5.9			8.8	3.7	13.9	0.0	0.0	15.2		

a= RWR 719 (resistant bean variety) (Mukalazi, 2004)
b= CAL 96 (susceptible bean variety) (Mukalazi, 2004)
c= Non inoculated control
d= Least Significance Difference (Steel *et al.,* 1997)
e= % Coefficient of Variation

Appendix 7 continued: Mean emergence scores of crop species inoculated with five *Pythium* species from crops intercropped with beans

	Crop species							
	Trial three							
	Resistant bean variety[b]	Susceptible bean variety[a]	Maize	Sorghum	Millet	Peas	LSD(P≤ 0.05) [d]	CV (%) [e]
Pythium species								
No *Pythium* species	11.67	10.3	10.0	16.00	16.00	8.67	1.26	5.8
P.macrosporum	6.33	5.33	10.67	16.00	16.00	8.67	2.55	13.7
P.arrhenomanes	8.67	4.00	7.00	14.00	12.00	2.67	6.32	44.1
P.spinosum	6.33	3.33	7.00	16.00	15.67	4.33	1.83	11.7
P.oligandrum	8.33	3.00	8.67	15.67	15.67	5.33	5.49	44.5
P.heterothallicum	8.33	5.67	8.67	15.67	15.67	5.33	3.56	20.2
LSD(P≤ 0.05) [d]	2.41	3.28	2.62	4.40	5.97	3.75		
CV (%) [e]	17.8	34.9	17.0	17.0	23.9	34.6		

a= RWR 719 (resistant bean variety) (Mukalazi, 2004)
b= CAL 96 (susceptible bean variety) (Mukalazi, 2004)
c= Non inoculated control
d= Least Significance Difference (Steel *et al.,* 1997)
e= % Coefficient of Variation

Appendix 8: Mean root dry matter scores of crop species inoculated with five *Pythium* species isolated from crops intercropped with beans

	Crop species															
	Trial one								Trial two							
Pythium species	RWR 719[b]	Cal 96[a]	Maize	Sorghum	Millet	Peas	LSD(P≤ 0.05) [d]	CV (%) [e]	Cal 96[a]	Maize	Sorghum	Millet	RWR 719[b]	Peas	LSD(P≤ 0.05) [d]	CV (%) [e]
No *Pythium* species	0.03	0.07	0.06	0.01	0.01	0.04	0.02	25.3	0.07a	0.06a	0.01a	0.03a	0.03ab	0.04a	0.03	38.3
P.macrosporum	0.06	0.09	0.12	0.01	0.00	0.04	0.03	34.6	0.14a	0.06a	0.13a	0.00a	0.07a	0.18a	0.23	132.5
P.arrhenomanes	0.05	0.16	0.13	0.02	0.02	0.05	0.03	24.9	0.15a	0.20a	0.02a	0.01a	0.05a	0.04a	0.18	129.3
P.spinosum	0.06	0.10	0.11	0.02	0.01	0.04	0.03	31.7	0.15a	0.06a	0.02a	0.01a	0.10a	0.04a	0.04	33.2
P.oligandrum	0.08	0.10	0.11	0.02	0.01	0.04	0.06	50.0	0.15a	0.06a	0.02a	0.01a	0.08a	0.05a	0.02	21.3
P.heterothallicum	0.08	0.14	0.11	0.02	0.01	0.04	0.03	28.7	0.12a	0.05a	0.02a	0.01a	0.07a	0.05a	0.03	28.8
LSD(P≤ 0.05) [d]	0.02	0.06	0.06	0.01	0.01	0.02			0.05	0.18	0.15	0.02	0.04	0.18		
CV (%) [e]	22.3	31.7	28.4	30.6	54.7	19.1			19.9	124.4	225.3	110.8	36.6	149.4		

a= RWR 719 (resistant bean variety) (Mukalazi, 2004)
b= CAL 96 (susceptible bean variety) (Mukalazi, 2004)
c= Non inoculated control
d= Least Significance Difference (Steel *et al.,* 1997)
e= % Coefficient of Variation

Appendix 8 continued: Mean root dry matter scores of cereals and legumes inoculated with five *Pythium* species isolated from crops intercropped with beans

	Crop species							
	Trial three							
	Resistant bean variety[b]	Susceptible bean variety[a]	Maize	Sorghum	Millet	Peas	LSD(P≤ 0.05) [d]	CV (%) [e]
Pythium species								
No *Pythium* species	0.03	0.07	0.06	0.01	0.0	0.04	0.02	28.5
P.macrosporum	0.08	0.04	0.16	0.01	0.01	0.04	0.05	50.8
P.arrhenomanes	0.07	0.09	0.14	0.01	0.01	0.05	0.04	39.0
P.spinosum	0.06	0.11	0.11	0.01	0.01	0.05	0.02	16.7
P.oligandrum	0.06	0.12	0.15	0.01	0.01	0.05	0.01	6.4
P.heterothallicum	0.07	0.07	0.11	0.01	0.01	0.05	0.03	30.3
LSD(P≤ 0.05) [d]	0.02	0.04	0.06	0.01	0.01	0.01		
CV (%) [e]	20.4	25.2	28.3	30.0	57.7	15.4		

a= RWR 719 (resistant bean variety) (Mukalazi, 2004)
b= CAL 96 (susceptible bean variety) (Mukalazi, 2004)
c= Non inoculated control
d= Least Significance Difference (Steel *et al.,* 1997)
e= % Coefficient of Variation

Appendix 9: Data input form for bean root rot and intercrop survey

Date_____District_____County_____

SubCounty_____Parish_____Village_____Farmer____

No. of fields_____No. of affected fields_____

Production system **(A)** Sparse/moderate/intense_____

(B) Main intercrop if any_____

Crop types**(C):** Bananas__Maize___Sorghum_____Millet_____Cassava___;

Others_____ _____ _____

Source of planting seeds for beans (own, neighbour, local market) _____

Is it certified seed? _____

Weeding **(D)** None/systematic/sporadic____

(E) Type of fertilisers used if any (Yes/No)_____

(F) Amount of manure used if any (Yes/No)_____

(G) Type of manure used if any (Yes/No)_____

Does the farmer have bean root rot disease on his/her farm? (Yes/No)_____

What bean variety does s/he grow?_____

Describe symptom on stem and root
(1) Root_____
(2) stem_____

Annual yield (kg/acre) prior to disease (season1)_____(season 2)_____

Yield (kg/acre) after onset of disease (season1)_____season (2)_____

Coordinates (N)_____(E)_____Altitude_____**(H)**Topography _____

Plot	Plot size	Crop (main crop)	Beans	Describe spatial arrangement	No. diseased Plant	No. healthy Plants	Other seen disease	Remarks

Appendix 10: The authour working with an electron microscopy (left picture) and Hummer Spattering System (right) at CIAT, Cali, Colombia