**CROP PROTECTION PROGRAMME**

# Bean Root Rot Disease Management in Uganda

**R8316 (ZA0586)**

## FINAL TECHNICAL REPORT

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## List of Abbreviations

AHI – African Highlands Initiative
ALS – Angular Leaf Spot
ASARECA – Association for Strengthening Agricultural Research in East and Central Africa
BAPPA – Beyond Agricultural Production to Poverty Alleviation
BCMNV – Bean Common Mosaic Necrotic Virus
BCMV - Bean Common Mosaic Virus
BRR –Bean Root Rot
CIAT – Centro Internacional de Agricultura Tropical
CIDA – Canadian International Development Agency
CPP – Crop Protection Programme
DFID – Department for International Development
ECABREN – East and Central Bean Research Network
FSP - *Fusarium solani* f. sp. *phaseoli*
FTR – Final Technical Report
HRI – Horticultural Research International
ICM – Integrated Crop Management
IDPM – Integrated Disease and Pest management
ITK – Indigenous Technical Knowledge
NAADS - National Agricultural Advisory Services
NARO – National Agricultural Research Organisation
NGO – Non-Government Organisation
PABRA – Pan African Bean Research Alliance
PCR – Polymerase Chain Reaction
PRAs – Participatory Rural Appraisals
RFLPs – Restriction Fragment Length Polymorphism
RIA – Rural Innovation Actvities
RKN – Root Knot Nematodes
SABRN – South Africa Bean Research Network
SADC – Southern African Development Community
SRO – Sub-Regional Organization
USAID – US Agency for International Development

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## Executive Summary

Root rots are the most serious constraint to bean production in Uganda. Demand for root rot management has been identified and uptake and promotion pathways are in place, but insufficient knowledge of the primary pathogens has continued to limit development. The overall objective of project R8316 was to underpin promotion of sustainable control of bean root rots through resistant varieties and soil amendments by addressing the key scientific constraints remaining in the project objectives. To this end, project outputs have been achieved as expected. Current and previous research on root rots has identified and determined the distribution of major pathogens responsible for root rots in great detail. This knowledge has been key in underpinning development of resistant varieties at CIAT. Valuable tools (cultural and molecular) for identification and quantification of *Fusarium solani* fsp *phaseoli* and *Pythium* spp have been have been developed and tested. The current project R8316 has developed multiplexing PCR detection method for key *Pythium* species pathogenic to beans, but also found to be affecting other important crops in the cropping system. As a result, a quantitative assay (combining classical and biotechnology techniques) can now be adapted for the most important species (e.g. *P*. *ultimum* var *ultimum*), which can then be used to evaluate crop and management options on inoculum levels of these pathogens in the soil. This will, in turn, generate information on more efficient and effective options, practices or components of integrated root rot management strategies. Assessing how these options affect or impact on the pathogen population levels and trend is key in assessing their potential effectiveness in managing bean root rots. The current project has generated information and techniques, which have prospects for application by researchers in the Pan African Bean Research Alliance (PABRA) region. Since it has also been shown that some of the *Pythium* species causing root rots in beans also affect crops such as sorghum and millets which are often grown in association/rotation with beans, information, methods, and techniques developed are therefore not only limited to researchers working on beans but have potentially for application by researchers working on other crops affected by *Pythium* pathogens. Development of protocols, research and training manuals on diagnostic and quantification methods should be valuable capacity development resources. Exposing these techniques to researchers will enhance their capacity to apply them and also facilitate a more effective assessment of management options. The proved effective promotional pathway provided by ECABREN and PABRA and the NGO partners in R8316 has been able to research some of the key fundamental constraints, knowledge of which will lead to continued improvements in the management of root rots.

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## Background

The common bean *Phaseolus vulgaris* is a vitally important food crop. It 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). Production is concentrated in the cool highlands of these areas and, as the most significant pulse crop in Uganda, beans are grown throughout the country but especially in the southwest. An important food to people of all income categories, beans are particularly valuable to the poor as a source of dietary protein because animal protein is often rare, or completely absent, from their diets. In East Africa, beans are primarily grown by the smallholder farmers, especially women, for home consumption, while any excess production is sold at market. Thus beans play an essential role in the sustainable livelihoods of smallholder farmers and their families, providing both food security and income generation.

Given the importance of *P. vulgaris* to the people of Africa, intensity of bean production is very high. In many areas there are several growing seasons per year, thus crops are grown with minimal rotation and limited or no fallow period. These practices have led to a decline in soil fertility, together with a concomitant rise in disease pressure, due to an increase in pathogen inoculum levels in the soil.

In South Western Uganda in 1994, bean production fell to 25% of its previous level, dropping further to 20% in 1995. Although this dramatic decrease in yield was attributed to the effects of a number of insect pests and diseases, root rot has now been identified as a major constraint to bean production (CIAT, 1986, 1992; Opio, 1999). Root rot is such a serious problem that in some seasons it is responsible for entire crop failures. Moreover, this problem is no longer restricted to SW Uganda; the fact that root rot is spreading means that its control is of high priority for the country. The increase in human population, land use and decline in soil fertility (which is positively linked with the occurrence of root rot), are expected to result in a sharp rise in the incidence and severity of root rots over time, unless efforts are made to develop sustainable management technologies.

Root rots are believed to be caused by one or more soil-borne pathogens that act either alone, or as a complex of two or more pathogens, depending on environmental conditions (Rusuku *et al*., 1997). Pathogens that have been isolated from plants displaying root rot symptoms include *Fusarium oxysporum*, *Rhizoctonia solani*, and *Pythium spp*. The increasing importance and widespread distribution of root rot illustrates the need to understand disease aetiology and to accurately diagnose the components of the pathogen complex responsible for root rots in different production areas.

*Fusarium oxysporum* is usually associated with bean wilt or “bean yellows”, a disease that caused serious crop losses of one of the most popular climbing bean

varieties in the Great Lakes Region (G 2333, also known as Umubano in Rwanda). Some five years after the variety had been introduced in SW Rwanda, farmers were forced to abandon growing it. Several races of this pathogen are known to exist worldwide and it is important that African isolates of *F. oxysporum* f. sp. *phaseoli* are compared to known races such that appropriate isolates can be used in future screening of bean genotypes. The Pan-Africa Bean Pathology Working Group sees the characterisation of pathogenic diversity of this pathogen as a priority. Fusarium wilt has not yet been reported in Uganda or Kenya, but the variety G 2333 has been released in both countries. Thus, the disease may be expected to become prevalent in the future, particularly if fungal inoculum is seed-borne (Buruchara *et al*., 1999).

In Rwanda, western Kenya and SW Uganda *Pythium* spp. are the fungal pathogens most frequently associated with severe root rot outbreaks. Consequently, identification of resistant germplasm has targeted resistance/tolerance to *Pythium* spp., and a few varieties are now available which grow well in areas where root rots are a serious problem. However, other pathogens known to cause root rots (such as *Fusarium* spp. and *R. solani*) may reduce the effectiveness of the available resistance. At least four *Pythium* spp. have been implicated in bean root rots, but their relative importance in the region and possible synergistic effects are unknown. Identification of *Pythium* spp. using morphological characters is both difficult and slow and, when extracted from soil, complicated by the presence of a wide range of other organisms. Identification to species level is a critical step towards identifying host resistance, and must also be carried out prior to the deployment of other disease management practices (e.g. rotation crops, which should be non-hosts to bean pathogens). One reason for the varied performance of certain bean varieties in different areas of East and Central Africa may be differences between the compositions of local pathogen populations. This further emphasises the need to identify the primary pathogen(s) in different areas, and to determine the level of variation within individual pathogen populations. Previous research (Project R7568) has sought to develop novel rapid and reliable molecular diagnostic tools for the identification and differentiation of *Fusarium* and *Pythium*. (See FTR, Spence, 2003). However, although progress in quantification was good for *Fusarium*, this proved to be more difficult for *Pythium*. The processes involved in evaluating large samples (culturing, RFLPs and sequencing) were slow and laborious, and because the selection of *Pythium* cultures for RFLPs was based on colony characteristics, which are often inaccurate, this led to the selection of both pathogenic and non-pathogenic forms. Limitations such as these mean that the need to develop rapid and accurate detection and quantification methods for *Pythium* spp. is still outstanding.

Beans crops are seldom grown in isolation, being routinely grown as components of complex crop systems. Surveys carried out in plot areas during R7568 showed that the major crops grown include beans (by 100% of the farmers), sorghum (99%), maize (94%), sweet potatoes (92%), Irish potatoes (47%), bananas (39%) and peas (39%). Other crops grown on a small scale by selected households included sugarcane, pineapples, passion fruit, yams, pumpkin, cabbages, cassava, tomatoes, carrots, onions, avocado and coffee. Climbing varieties are usually grown alone, while bush types are intercropped. Major crops intercropped with beans include maize (79%), sorghum (52%), peas (46%), Irish potatoes, sweet potatoes and yams. At least three crops exist in the same field at the same time. Crop rotation in the strict sense is rare, although farmers claim to practice it. Only dominance of crops in

the fields shifts according to seasons. Major rotations are beans-maize-sorghum (71%), beans-maize-beans (27%) and beans-Irish potato-sweet potato. Maize and sorghum are normally intercropped with beans and/or Irish potatoes such that the bean crop appears in the field season after season. All this implies that bean-based systems are almost always closely linked with other crops, either in association or in short (one or two season) period rotation. But of all these crops, bean appears to be most affected by *Pythium* root rots and *Fusarium solani* f. sp. *phaseoli* (FSP).

*Pythium* species have a wide host range and project R7568 identified a number of *Pythium* spp. known to affect beans in the areas examined. Also, a group of isolates which were highly virulent towards beans and with molecular characteristics very similar to isolates causing sudden death disease (SDD) in soybean were identified within *F. solani*. The fact that some of the other species known to be pathogenic on other crops were grouped together with non-pathogenic and fast growing isolates (using AFLP), implies that the latter could possibly be something other than FSP. We urgently need to understand the role(s) and significance of those crops used in rotation or intercropped with beans in the survival, host range, inoculum density and severity of root rot, and the implications of this for disease management. It is also important toe establish whether *P. vulgaris* is the only crop likely to cause an increase in the soil population of root rot pathogens, or whether it is simply a good indicator of the level of root rot pathogens present in soil which have been influenced by preceding or interplanting of different crops.

Project R7568 not only advanced knowledge of the biology of root rot, its causal agents and their epidemiology. It also made preliminary investigations into the interactions between root rot pathogens and other biotic factors which may influence levels of disease (e.g. root knot nematodes (RKN) and bean stem maggot). It is important that such studies of the relationship between root rot pathogens and other soil-borne disease-moderating organisms continue, such that disease management practices can be optimised.

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## Project Purpose

Root rots are the most serious constraint to bean production in Uganda. Demand for root rot management has been identified and uptake and promotion pathways are in place but knowledge of the primary pathogens is the developmental constraint. The overall objective of project R8316 was to underpin promotion of sustainable control of bean root rots through resistant varieties and soil amendments by addressing the key scientific constraints identified in previous project R7568. To this end, specific objectives were:

The development and implementation of rapid detection and quantification methods for *Pythium* spp., to support evaluation of resistant germplasm and disease management practices;

The elucidation of the role(s) and significance of other crops in bean-based cropping systems in the development of root rots, to promote/allow sustainable integrated disease management;

The follow-up characterisation of *Fusarium oxysporum* f. sp. *phaseoli* that causes wilts in some climbing beans in Rwanda, to support evaluation of resistant germplasm and risk assessment in other countries in the region;

Evaluation of interactions between root rot pathogens and other disease-moderating organisms in the soil, to improve disease management practices.

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### Beneficiaries

NARO is working to develop community-based seed production systems (11 groups have been established) and CIAT has developed manuals for initiating seed enterprises. Seed is also being multiplied in conjunction with seed companies and NGOs. NARO has conducted adoption studies (Most (74%) of the bean growing area in Eastern Uganda is planted with “improved beans”). The project will underpin such seed production and promotion initiatives.

The project will link with the National Agricultural Advisory Services (NAADS), a recent initiative in Uganda using new approaches to extension delivery so as to make it more efficient and effective. NAADS’s mission is to increase farmer access to information, knowledge and technology for profitable agriculture. NAADS have activities in southwest Uganda, where this project is also based.

Project R8316 also links with the RIA/BAPPA initiative, that is managed by CIAT and implemented through NGOs: AfricCare in Uganda, Concern Universal in Malawi, and a local NGO in Tanzania. Methodologies MoUs have been developed with these organisations. Under BAPPA the CIAT role is to develop and promote the process with the NGOs having the implementation role in addressing organisational capacity, farmer experimentation, social capital, environment and women’s empowerment.

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### Related Projects

This project is strongly linked to CPP project R7965 *Scaling up and scaling out bean IDPM promotion activities including pest tolerant and improved high yielding bean genotypes*. These CPP projects link with other bean research in Africa through the Pan African Bean Research Alliance (PABRA), East and Central African Bean Research Network (ECABRN), over-arching initiatives (through PABRA), CIAT and other donors (CIDA, SDC, USAID and Rockefeller Foundation). The project also links with the Rural Innovation Activities/ Beyond Agricultural Production to Poverty Alleviation (BAPPA) initiative that is managed by CIAT, and implemented through NGOs: AfriCare in Uganda, Concern Universal in Malawi, and a local NGO in Tanzania. In addition, the work links with the African Highlands Initiative (AHI) in Uganda, which has an office in the Kabale region.

PABRA are funded by a variety of donors, including CPP. Bean research has uptake pathways through the networks beyond 2005, as Rockefeller Foundation have verbally agreed that a further 3 years of funding will be forthcoming after 2003 to underpin breeding resistance to ALS and root rots as well as introducing biotechnology. Rockefeller has also funded training. USAID will also provide support for a further 3 years at a similar level beyond 2003. ASARECA has developed a conceptual framework for the networks, and USAID will use this as a guide to the network activities it will fund. CIDA has renewed its funding to PABRA as from June 2003 for 5 years for co-ordination, training, support to SROs and NGOs, capacity building and dissemination.

# Chapter 1: Development of quantification methods for *Pythium spp*.

## SUMMARY

The nature of any method to be used to quantify *Pythium* species is not only critical to epidemiological studies, but also to an understanding of the biological processes, technologies, practices and interactions that affect levels of bean root rot inoculum in the soil. This knowledge is critical in the deployment of appropriate resistant germplasm in other disease management practices. For these reasons, the two major components planned under this work included the development of a multiplex diagnostic assay for the identification of the major *Pythium* species associated with common bean disease, and also the development of a molecular-based quantitative assay for *Pythium* species.

## Part 1: The development of a multiplex PCR assay for positive identification of the main *Pythium* species associated with common bean

### 1.1.1 MATERIALS AND METHODS

**Primer design**:

The internal transcribed spacer region 1 (ITS 1) of the ribosomal DNA was targeted for the development of *Pythium* species-specific primers. DNA sequences from our own work (CIAT 2003), and from the data bank of Dr Andre Levesque (AAFC Canada) were compared for the 7 target *Pythium* species, and based on sequence differences in the ITS 1 region, species-specific primers were designed. We also took note of the primers that had been developed and reported by other groups, and where these matched what we had designed, the same primer sequences were synthesized. Where there were differences from our optimised sequences, the two primers, our own and the one reported were synthesized and tested for specificity by amplifying test strains, as well as isolates of the 6 species commonly found in association with beans.

**Primer optimization**:

The specificity of the primers to the target strain was tested by amplifying DNA of several *Pythium* species that we have in our collection. Once found to be specific, the primers were optimised, to select suitable primer combination that avoid formation of hierodule, and then these were optimised for cycling conditions including magnesium concentrations, DNA concentrations, enzyme concentrations, maximum annealing temperature possible and cycling conditions, etc.

**Cycling conditions**:

The optimum conditions, taking into consideration the differences in annealing temperatures were: 0.2 mM dNTP, 0.5 µM each primer, 2 mM $Mg^{2+}$, 1U *Taq* DNA polymerase and 10-30 ng genomic DNA in a 12.5 µL PCR reaction volume.

**Electrophoresis**:

An optimum electrophoresis condition to be able to distinguish all *Pythium* species was in 1.5% agarose gel. The small fragment size might warrant higher concentrations of agarose, and these have to be optimised for local conditions before large-scale application.

**Suitability for detecting different *Pythium* species**:

Two approaches were followed to test the suitability of the multiplex PCR assay: (1) primers were mixed, added one at a time and used to amplify DNA from individual species, until all primers were part of the mix; (2) DNA from different species were mixed and amplified, first with individual primers, then with different primer combinations.

## 1.1.2 RESULTS AND DISCUSSION

**Multiplex PCR assay for *Pythium* species**:

A multiplex PCR assay was developed to simultaneously detect and identify 6 *Pythium* species that are pathogenic to beans. Multiplex PCR is a method for simultaneous amplification of several fragments in a single PCR and is one of the best molecular tools for species identification, as it enables the identification of several species by a single PCR followed by a single electrophoresis. The method uses 6 species specific primers that were developed based on differences in the internal transcribed spacer region 1 of the ribosomal DNA and one Oomycete specific primer that was previously developed by Dr. Andre Levesque, AAFC, Canada. The Oomycete specific primer is based on conserved sequences in the 5.8S ribosomal genes. The designed primers, their sequences and the target fragment size are shown in Table 1.1.

In all cases, the desired fragments were amplified from the desired species, but in some cases, multiple fragments were observed (Table 1.1; Plates 1.1 and 1.2). However, these fragments disappeared when the annealing temperature was increased. The annealing temperatures for the different primers ranged from 56 – 70, and as such, the multiplexing should take this into consideration, using primers with a similar annealing range together.

Currently different methods of DNA extractions from plant tissues are being tested to assess the effect of DNA purity. In addition, we are adopting different methods for extracting DNA from different soils with different clay content, so as to establish the suitability of the developed PCR assay for direct detection of these *Pythium* species directly in soil.

**Table 1.1**: Amplification results from the designed primers using genomic DNA from different *Pythium* species.

| ***Pythium* species** | **Primer name** | **Band Size (bp)** | **Primer sequence 5' to 3'** | **Source** |
|---|---|---|---|---|
| *P. spinosum* | Pspi (old) | 200 | TGT GTG TTG TGA TCG TGC CT | Wang *et al*., 2003 |
| *P. spinosum* | Psp1 (new) | 325 | TGT TGT GTG TCT GCG CCG TTG TTG G | This project |
| *P. ultimum* | PuK1 | 192 | ACG AAG GTT GGT CTG TTG | Kageyama *et al*., 1997 |
| *P. deliense* | Pdel1 | 182 | GCT GAA CGA AGG TGG GCT GCT | This project |
| *P. salpingophorum* | Psal1 | 217 | TTA TGT TCT GTG CCT TCT CTC G | This project |
| *P. oligandrum* | Po1 | 150 | TGC GTC TAT TTT GGA TGC GG | Wang *et al*., 2003 |
| *P. nodosum* | Pnod1 | 232 | ATC TGC TCT CTG TGC CTT TCG | This project |
| *P. torulosum* | Pto 1 | 177 | AGG TAG AGC TGC ATG TAA AAG T | Wang *et al*., 2003 |
| OOM-lo 5.8S47B | | | CGC ATT ACG TAT CGC AGT TCG CAG | Schurko *et al*., 2003 |

*All primers were paired with the oomycte specific primer OOM-Lo5.8S 47 B

**Plate 1.1**: Primers for different *Pythium* species were mixed, and DNA from individual *Pythium* species was added. In all cases, the desired fragment was amplified. Only with *P. oligandrum* were two fragments amplified, the desired 150 bp and a second larger fragment of approximately 400 bp. It appears that this primer anneals somewhere within the ITS regions.

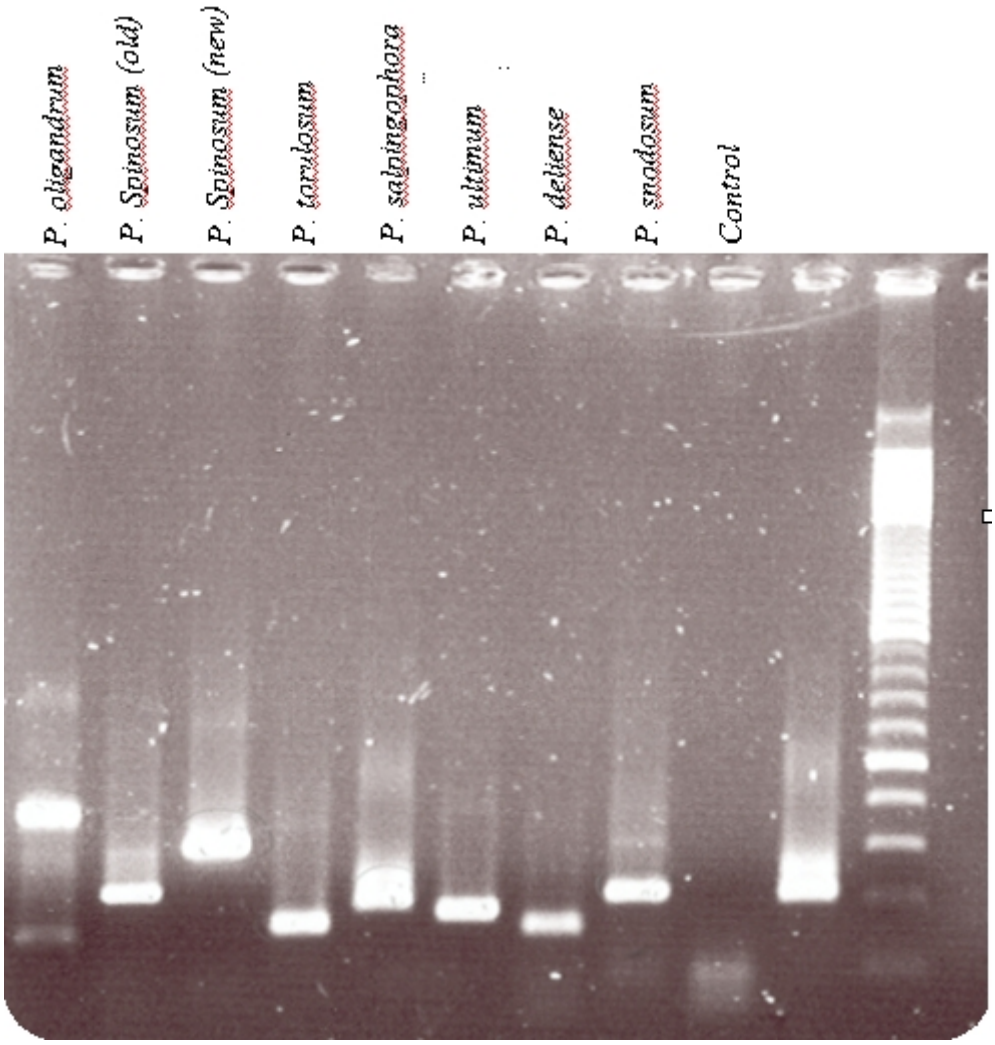
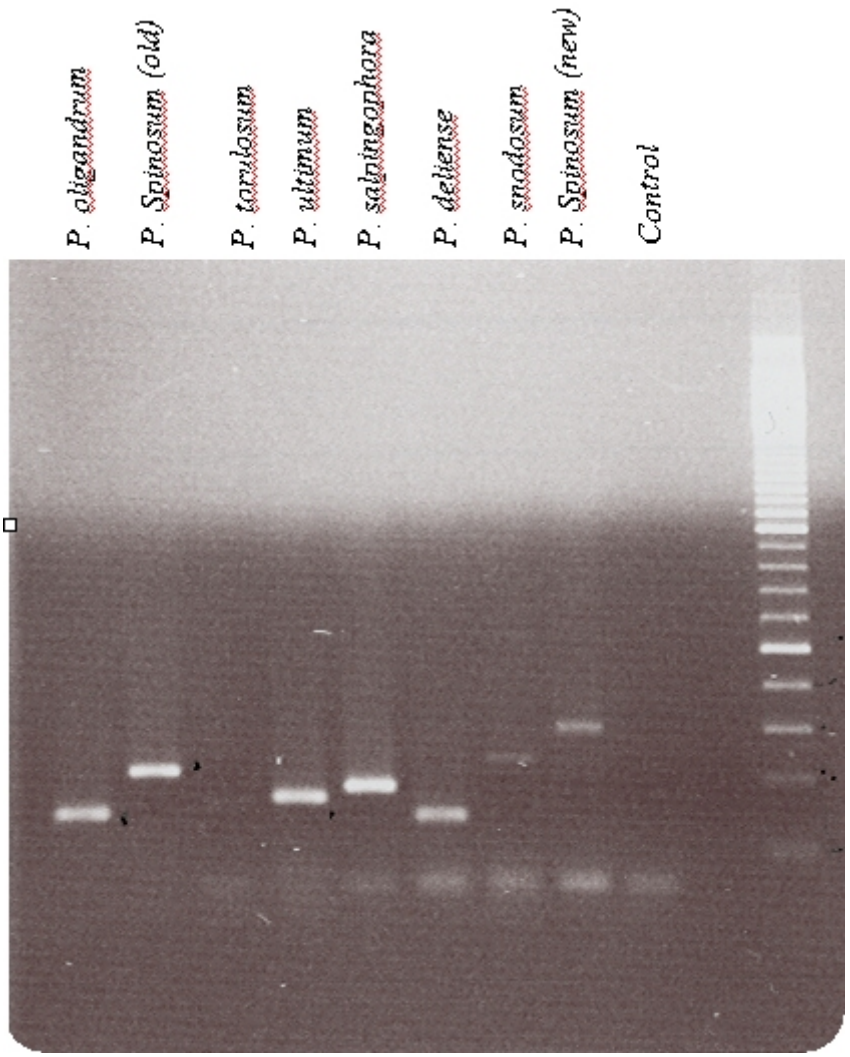


**Plate 1.2**: Simultaneous detection of 6 *Pythium* species using a multiplex PCR assay. DNA from different *Pythium* species was mixed and amplified using species-specific primers that target the ITS 1 region of ribosomal DNA.



# Part 2: Development of a molecular-based quantitative assay for *Pythium* species

## 1.2.1 MATERIALS AND METHODS

*Pythium ultimum* has been observed to be the most important species in Africa. We are currently developing a quantitative PCR assay for this species based on the competitive PCR technique. Amplification of the target *P. ultimum* DNA was done using the primers Puk1 and Puk3 previously developed by Kageyama *et al*. (1997), and using the following conditions: 0.5 µM of each primer, 0.2 mM dNTP mixture, ! x PCR buffer, 1.5 mM $Mg^{2+}$, 1U *Taq* DNA polymerase and 20 ng genomic DNA in a 12.5 µL PCR reaction volume. The cycling conditions were as follows: 94°C for 3 min, followed by 30 cycles of denaturation at 94°C for I min, annealing at 55°C for 1 min and extension at 72°C for 2 min, with a final extension at 72°C for 10 min.

### Creation of a heterologous internal standard

An internal standard was prepared by amplifying agenomic DNA extracted from the soil pathogen *Macrophomina phaseolina* with primers Puk1 and Puk3 primers (Kageyama et al, 1997) at low stringency. These two primers amplify a fragment of approximately 670 bp in *P. ultimum*. A band of approximately 600 bp was cut from the gel and amplified at high stringency (55°C) using the same primers. After confirming the size, the fragment was cleaned and cloned into pGEM T-easy vector and maintained in transformed *E. coli*. DNA for further studies was extracted and quantified for use as an internal control.

## 1.2.2 RESULTS AND DISCUSSION

### Generation of competitor DNA fragments

The amplification of *M. phaseolina* genomic DNA under low stringency annealing conditions resulted in the production of several PCR fragments, of which a 600 bp fragment that was cloned and used in competitive PCR assays. This fragment (~600 bp) was engineered to contain the priming sites of the two *P. ultimum*-specific primers, through low stringency amplification, followed by size selection before the fragment was cloned in *E. coli* (Plate 1.3). In competitive PCR, it is extremely important to have a competitor with a greater degree of similarity (both in length and sequence) to the target, so as to allow for more even amplification efficiencies. In addition, the two fragments be easily separated following electrophoresis. The usefulness of the designed heterologous probe was tested by amplifying different concentrations of target and competitor DNA (Plate 1.4). As the concentration of the target DNA increases, the intensity of the amplified competitor DNA decreases. By comparing the relative band intensities of the two fragments, a ratio is reached where the amount of target and competitor DNA are in a 1:1 ratio. This can then be extrapolated the amount of propagules of the target species that were used to obtain the DNA used in the 1:1 ratio.

**Competitive PCR assay**

We are in the process of testing the suitability of the designed competitive PCR assay for direct estimation of *P. ultimum* propagules directly in the soil. Several procedures for direct extraction of DNA from soils are currently being tested, by extracting known concentrations of DNA from soils differing in structure, clay and humus content. This is important, to optimize the conditions under which this assay could be applied.

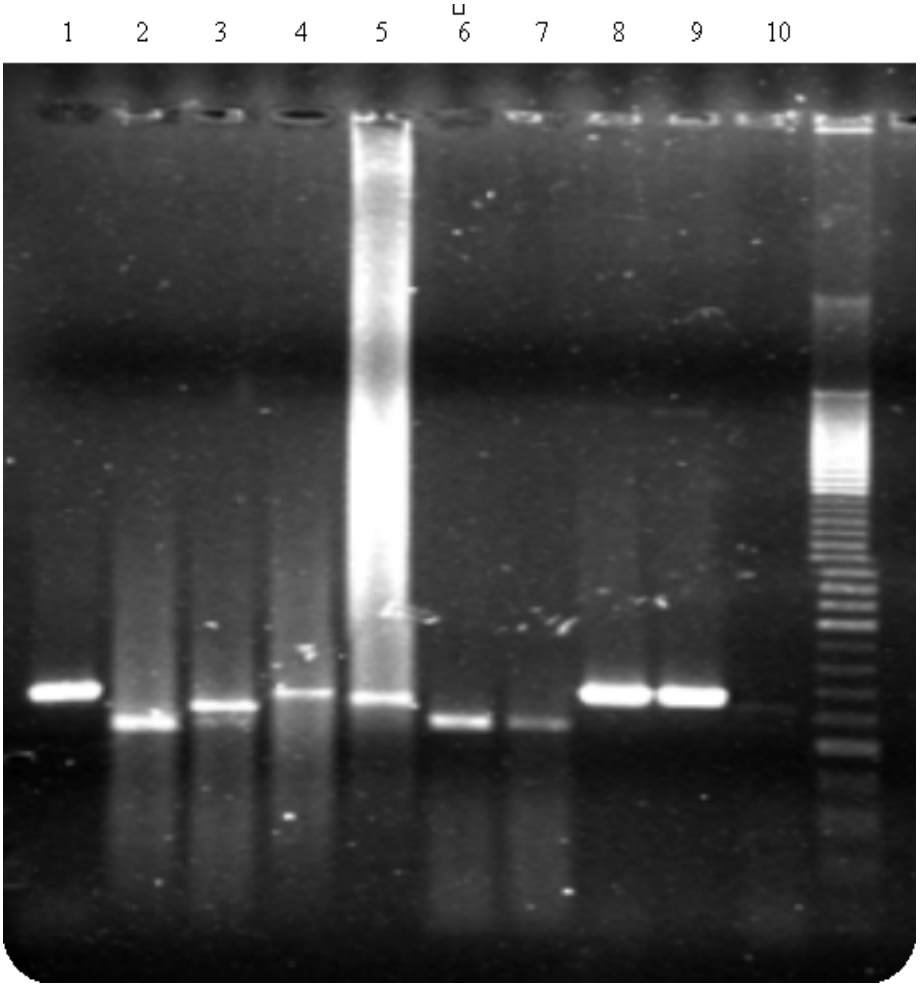



**Plate 1.3:** Ethidium bromide stained gel of products of candidate internal fragments for competitive PCR. Lanes 1, 8 and 9 represent *Pythium ultimum* DNA, while lanes 2 to 7 are different individual bands obtained from amplifying *Macrophomina phaseolina* DNA under low stringency conditions using *P. ultimum* specific primers Puk1 and Puk3. Fragments from lanes 2 and 3 were eventually cloned and subsequently tested for their suitability as internal controls in competitive PCR assays.

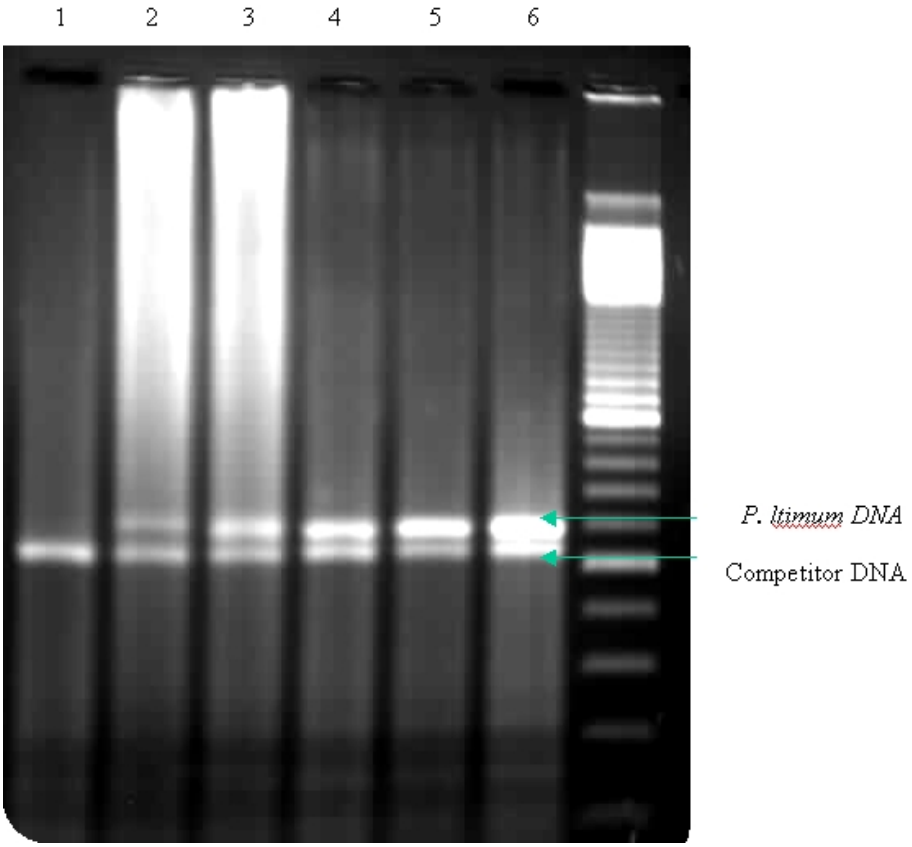



**Plate 1.4:** Ethidium bromide stained gel of products from competitive PCR of P. ultimum. A constant concentration of internal DNA was mixed with an unknown concentration of P. ultimum DNA. Lane 4 shows an almost 1:1 ratio of the internal DNA: *P. ultimum* DNA concentration.

# Chapter 2: Role and significance of other crops in a bean-based cropping system in the development of root rots understood

## SUMMARY

The major crops intercropped with beans in South-western Uganda include maize, sorghum, peas, Irish potatoes, sweet potatoes and yams. Crop rotation in the strict sense is rare, although farmers claim to practice it (Ampaire, 2003). Of all these crops used in rotation or inter-cropping, bean appears to be the most affected by *Pythium* root rot (CIAT, 2003). There is need to understand the role and significance of the other crops used in rotation with beans in affecting survival, host range, inoculum density and severity of root rots in beans and thus come up with effective disease management strategies.

The aims of the procedures described in the following Chapter were to understand the role and significance of crops used in rotation or intercropped with beans in the survival, host range, soil inoculum levels and importance of root rots in beans, and thus the implications of these factors in their management. In multi-cropping systems, this information is critical for ICM. To this end, the project sought to: Determine incidence and severity of root rots on crops associated with bean as intercrops or in rotation (Chapter 2, Part 1); characterise root rot pathogens (*Pythium* and *Fusarium spp* etc.) associated with these crops (Chapter 2, Part 2); determine pathogenicity of bean-pathogenic *Pythium spp*. and *Fusarium spp*. on major crops associated with beans (Chapter 2, Part 3); determine (if any) possible role of other crops in the increase in incidence and severity of bean root rots in a bean-based cropping system (Chapter 2, Part 4); determine the impact of bean root rot management practices on the productivity of other crops associated with beans (Chapter 2, Part 5); develop recommendation domains (Chapter 2, Part 6).

## Part 1: Incidence and severity of root rots on crops associated with bean as intercrops or in rotation

### 2.1.1 MATERIALS AND METHODS

Initial assessment of the incidence and severity of root rots was done during the surveys and sample collections in Kabale and Kisoro districts in South-western Uganda. However, since many of the samples crops were symptom-less, adjustments were made on the method used to assess incidence and severity. Assessment was done in a study based on three major crops (maize, sorghum and peas) grown in rotation and / or intercropped with beans. The three crops were grown in Rubaya in Kabale in a trial that also sought to assess different management options that are known to reduce root rots in beans. CAL 96, a susceptible bean cultivar was grown as a check. A split plot design in which the crops occupied the

sub-plots was used. Root rot incidence and severity in these crops was measured in the field.

### 2.1.2 RESULTS AND DISCUSSION

Lesions were observed on all crops examined in the trials which were similar to root rots in beans. Plant deaths were observed on all the four crops at 38 days after planting as shown by the changes in plant stands, with more deaths registered in sorghum and the check crop (beans) during the season (Figure 2.1). Higher lesion incidence and scores were recorded in sorghum (> 8) and beans (check) (> 7) than in maize (> 4) and peas (> 3) (Figure 2.2) indicating that sorghum and CAL 96 were most affected and are more susceptible to root rots than maize and peas. Sorghum and beans are major crops grown (in large acreage) in rotation in south west Uganda and their susceptibility indicates that they may be contributing to the soil inoculum build-up. The susceptibility of sorghum was unexpected but these results imply that effects of root rots on sorghum may be underestimated.

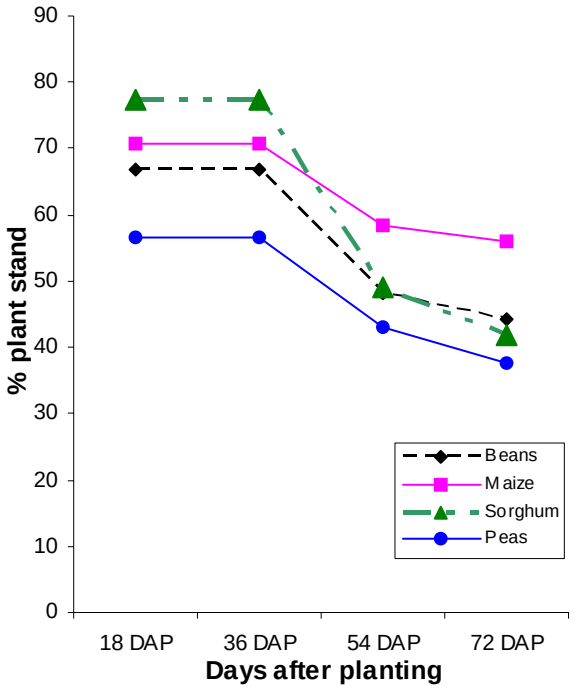



**Figure 2.1** Plants stand in root rot trials in Rubaya County, Kabale District, during season 2004 b

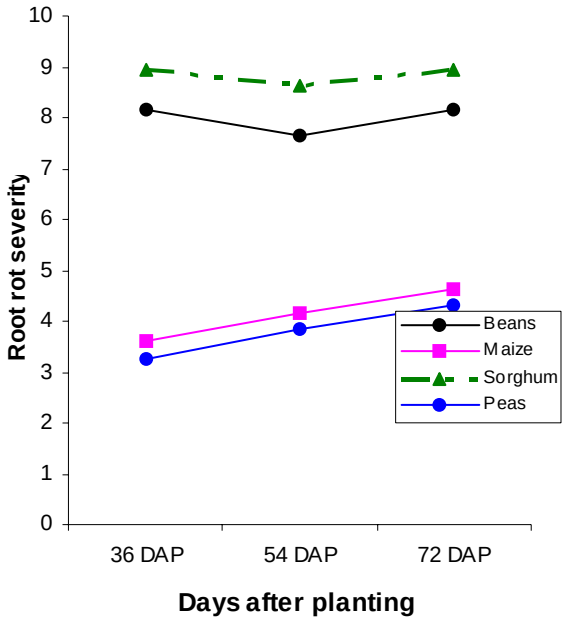



**Figure 2.2** Root rot severities in root rot trials in Rubaya County, Kabale District, during season 2004 b.

In beans, damping-off at seedling stage, yellowing of leaves, stunted growth and, in severe cases, death of plants were observed. These symptoms are a result of the destruction of the root tissues that is responsible for the uptake of water and mineral salts resulting in deficiency and death in severe cases. The roots had a mixture of brown, water soaked lesions with red lesions. Surviving plants produced no or few pods. Sorghum exhibited purpling and yellowing of leaves, death of leaf portions, stunting, extensive tillering and prop root development and plant death in severe cases (Plates 2.1 & 2.2). Purpling and yellowing could be due to nutrient deficiency as result of reduced root surface for absorption. Tillering and prop root development are mechanisms developed by the plant for more water and nutrient uptake, thus its survival. The infected roots were darkened and blackened. Red pigmentation was also noted in some roots (See Plates 2.1 and 2.2).

**Plate 2.1** Severely affected sorghum roots in control plots

**Plate 2.2** Sorghum roots with prop root development in plots amended with NPK

Purpling, stunting, leaf death, prop root development and plant death were also observed in maize. The roots showed greyish-brown lesions (Plate 2.3). Yellowing, stunting and death of plants in severe cases were observed in pea plants. Infected roots had brown lesions.

**Plate 2.3** Maize roots showing lesions

The high lesion scores can be attributed to the continuous cropping on the same piece of land that has led to the build up of root rot pathogens. High infection in beans may also be due to sorghum, peas and possibly other crops acting as alternative hosts for species pathogenic to beans (Gichuru, *et al*., 2004).

## Part 2: Characterisation of root rot pathogens (*Pythium* and *Fusarium spp* etc.) found on crops associated with beans

### 2.2.1 MATERIALS AND METHODS

Surveys were carried out during the short rain season of 2003 (November) and the long rain season of 2004 (April) and April 2005. Plant samples were collected from 16 sub-counties which included Ikumba, Hamurwa, Muko, Bufundi, Kashambya, Bubale, Rwamucucu, Rubaya, Kitumba, Kamuganguzi, Buhara, Kyanamira, Bukinda, Kaharo, Kamwezi and Kabale Central) in Kabale and Kisoro districts in Southwestern Uganda, which are most affected by bean root rots in Uganda. Twenty-one different crop hosts were sampled across various cropping systems. We successfully isolated *Pythium* spp. from 117 samples in the 2004 survey and 114 samples in the 2005 survey (Table 2.1).

**Table 2.1**: Crops sampled during the survey in Kabale district in April 2004 and 2005

| Crops sampled | No. of samples (2004) | No. of samples (2005) |
|---|---|---|
| 1. Beans | 40 | 3 |
| 2. Irish Potatoes | 22 | 41 |
| 3. Maize | 15 | 15 |
| 4. Sorghum | 12 | 20 |
| 5. Field Peas | 11 | 5 |
| 6. Sweetpotato | 6 | 6 |
| 7. Yams | 2 | 5 |
| 8. Weed | 2 | - |
| 9. Green pepper | 2 | 1 |
| 10. Carrot | 1 | 1 |
| 11. Amaranthus | 1 | - |
| 12. Cabbage | 1 | 5 |
| 13. Carrots | 1 | - |
| 14. Wheat | 1 | 2 |
| 15. Onions | 1 | - |
| 16. Millet | - | 3 |
| 17. Tomatoes | - | 3 |
| 18. Spring onions | - | 1 |
| 19. Pumpkin | - | 1 |
| 20. Tobacco | - | 1 |
| 21. Cauliflower | - | 1 |
| **Total** | **117** | **114** |

### Sample collection

Fields were sampled at a distance of 2 km apart and, in most cases, the targeted fields were where beans were already succumbing to bean root rot. In each field, the

farmer would be asked for permission to collect about 5 plants of each crop in his/her field that had some external symptoms of root rot e.g. rooting roots and stem, stunting etc. Although sampled bean fields were selected on the basis of bean root rot symptoms occurring on them, above ground symptoms on most crops were not apparent. It is only deficiency symptoms such as yellowing of leaves in cabbage, reduced root systems, lesions on the stems and roots, yellowing and stunting in the case of sorghum were used as the identifying factors for disease occurrence in these crops. Symptoms similar to those of beans were only observed on crops such as peas and Irish potatoes. Pictures of the various cropping systems were also taken. In each field, crops either having certain deficiency symptoms or symptoms of root rot as described above, were randomly sampled. Plants were sampled in a "W" zig-zag pattern over the whole field. These plants were uprooted with the soil, and all the roots shaken gently to remove the soil and packed in a labelled paper bag for isolation in the lab at Kawanda. A geographical positioning system (GPS) was used to record the spot location of the sampling fields for subsequent mapping.

**Isolation of the *Pythium* pathogen**

The samples were washed and left to dry. Pieces of apparently infected root were cut using a flamed sterilised scalpel blade, blot dried using a sterile paper towel, and placed on a selective Corn Meal Agar plate (20 gm corn meal agar, 0.1125g pimaricin, 0.03 g rifamycin, 1000ml distilled water). Positive samples, which showed growth of mycelia after 24-48 hours, were then sub-cultured onto Corn Meal Agar plate without antibiotics. After 24 hours the cultures were then transferred onto potato dextrose agar slants (PDA) and incubated at 25$^{o}$C for 48 hours

DNA was extracted from the plant tissue using the SDS extraction method (Doyle and Doyle, 1990). PCR analysis was done using the ITS primers i.e. ITS 1 and ITS 4. This amplification also provides a means to eliminate *Mortirella* spp. which is not distinguishable in culture from *Pythium* species. RFLP was run on clean *Pythium* samples in order to group possible similar species groups before selecting representative samples for sequencing.

**RFLP analysis**

Three enzymes were used to carry out the restriction of the PCR fragments: mainly MboI, HinfI and CfoI. MboI did not give as many bands as the other two enzymes and only data generated using Cfo I and Hinf I to assess variability amongst the various isolates was used. To act as checks we used *Pythium* isolates that had been previously sequenced and species determined were used in order to group unknown isolates.

**DNA sequencing**

DNA sequencing was carried out on 49 unknown *Pythium* isolates from various host crops collected from Kabale District in Uganda. Direct sequencing using the ITS1 primer (White, 1990) was carried out to identify the species. The isolates that have been sequenced are shown in the table below (Table 2.2):

**Table 2.2**: Samples on which DNA sequencing was carried out

| Identification number | Crop sampled |
|---|---|
| 1. KBL1 A | Irish potato |
| 2. KSB3Z | Irish potato |
| 3. KBL22 A | Irish potato |
| 4. MR8 | Irish potato |
| 5. KBL 21A | Irish potato |
| 6. KBL21 A | Irish potato |
| 7. BB13 | Irish potato |
| 8. KM8 | Irish potato |
| 9. KM1 | Irish potato |
| 10. BH10 | Irish potato |
| 11. KIS4 | Irish potato |
| 12. KB4 | Irish potato |
| 13. KSB3 | Maize |
| 14. KG5 | Maize |
| 15. KG4 | Maize |
| 16. RW10 | Maize |
| 17. KS9 | Maize |
| 18. KIS5 | Maize |
| 19. MR16a | Maize |
| 20. KMG 2b | Sorghum |
| 21. MR 16 | Sorghum |
| 22. Sec KAK5BII | Sorghum |
| 23. MZ 1 | Sorghum |
| 24. KBL12 A | Sorghum |
| 25. KBL 12 B | Sorghum |
| 26. MR-1 | Sorghum |
| 27. KS9 | Sorghum |
| 28. RB 11 | Sorghum |
| 29. KBL30A | Sorghum |
| 30. MZ1 | Sorghum |
| 31. IK10 | Sorghum |
| 32. RB4 | Sorghum |
| 33. KBL 11B | Millet |
| 34. KY7 | Pea |
| 35. BH9 | Peas |
| 36. MR16 | Peas |
| 37. RBY1(a) | Sweet potato |
| 38. KG2 | Sweet potato |
| 39. KBL18C | Wheat |
| 40. BH1 | Weed |
| 41. RB45 | Beans |
| 42. KSB2 | Beans |
| 43. MZ1 | Beans |
| 44. KMZ 2a | Beans |
| 45. RW 5(2) | Beans |
| 46. KG2 | Beans |

KBL = Kabale, MRa= Kisoro(district), KSB/KS = Kashambya, MZ = Maziba, KG/KMZ = Kamuganguzi, BH = Buhara, RB = Rubaya, RW = Rwamucucu, KIS = Kisoro, KY = Kyanamira, IK = Ikumba, HM = Hamurwa, BB = Bubale

## 2.2.2 RESULTS AND DISCUSSION

### Isolate collection

Isolates were collected from 16 sub-counties on crops shown in Tables 2.3 and 2.4. A map of Kabale showing locations where sample were collected from is shown in Figure 2.3.

**Table 2.3** Sites where possible *Pythium* samples were collected from during a survey in Uganda, 2004

| Sub-county | Parishes | Village | No. of samples collected |
|---|---|---|---|
| Rubaya | 2 | 2 | 14 |
| Maziba | 4 | 5 | 15 |
| Kyanamira | 3 | 8 | 28 |
| Kamuganguzi | 7 | 8 | 20 |
| Kashambya | 3 | 5 | 31 |
| Hamurwa | 1 | 1 | 24 |
| Kaharo | 5 | 7 | 29 |
| Bubale | 2 | 4 | 19 |
| Bukinda | 5 | 7 | 31 |
| Kitumba | 3 | 5 | 22 |
| Bufundi | 2 | 3 | 15 |
| Buhara | 4 | 4 | 24 |
| Muko | 3 | 4 | 23 |
| Kamwezi | 3 | 6 | 25 |
| Ikumba | 2 | 2 | 19 |
| Rwamucucu | 3 | 7 | 29 |

**Table 2.4** Sites where possible *Pythium* samples were collected from during a survey in Uganda, 2005

| Sub-county | Parishes | No. of samples collected |
|---|---|---|
| Rubaya | 4 | 25 |
| Maziba | 1 | 20 |
| Kyanamira | 3 | 20 |
| Kamuganguzi | 3 | 26 |
| Kashambya | 1 | 20 |
| Hamurwa | 4 | 18 |
| Kaharo | 5 | 17 |
| Bubale | 5 | 15 |
| Bukinda | 3 | 20 |
| Kitumba | 3 | 20 |
| Bufundi | 2 | 6 |
| Buhara | 2 | 16 |
| Muko | 5 | 13 |
| Kamwezi | 3 | 21 |
| Ikumba | 22 | 13 |
| Rwamucucu | 3 | 22 |

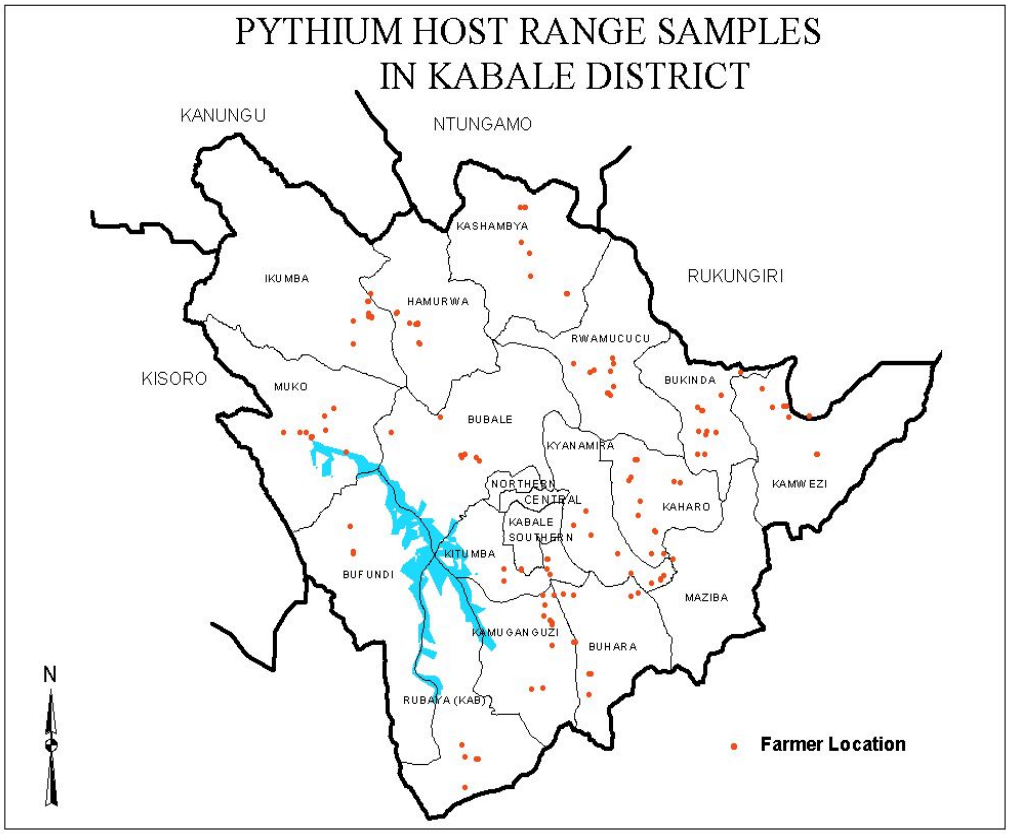



**Figure 2.3** A map indicating sites in Kabale district where samples were collected from different plants.

## RLFP results

*Pythium* specific primers were used to differentiate between *Pythium* and non-*Pythium* species. Restriction analysis of PCR products of apparently 48 *Pythium* isolates using restriction enzymes *Cfo* I, Hinf I, *Mbo* I grouped the isolates into 3 RFLP groups (Figure 2.4). Some *Pythium* isolates identified grouped themselves tighter, implying that RFLPs could not distinguish them. Isolates representing different RFLP groups have been selected for sequence analysis.

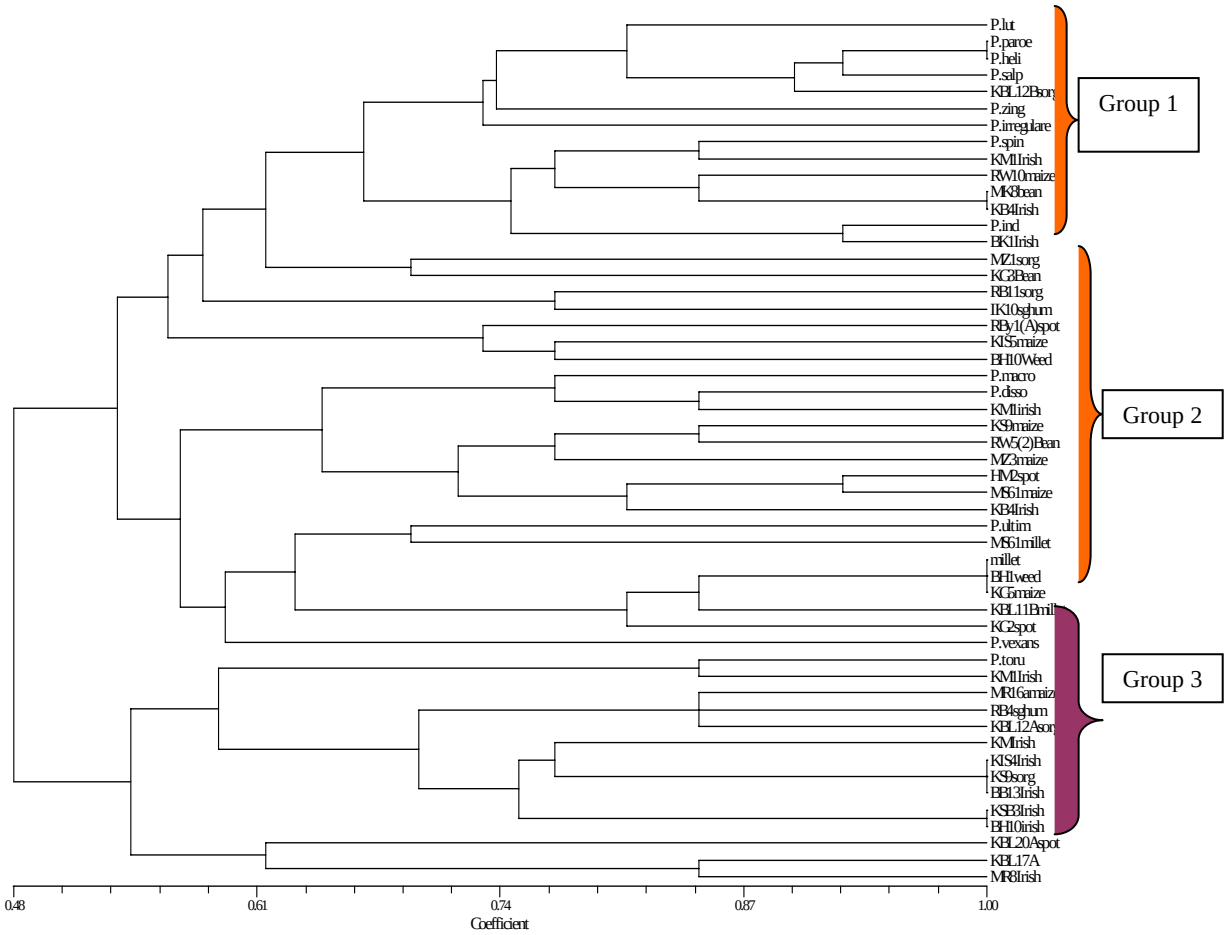



**Figure 2.4**: A dendogram showing similarities among *Cfo* I endonucleases for 48 *Pythium* isolates (36 from Uganda and 12 are identified *Pythium* checks; the number of major branches indicates the clusters/RFLP groupings.

**DNA Sequencing Results**

Of the 49 samples sequenced, 8 samples were contaminated or had short DNA fragments while five were not *Pythium* spp, i.e., three were *Verticilium coccosporum*, one was *Nectria haematococca* and another was *Fusarium oxysporum*.

Twelve *Pythium* spp. from the 34 *Pythium* samples sequenced were recorded on the different crops (Table 2.5). These included, *P. folliculosum, P. oligandrum, P. torulosum, P. acanthicum, P. ultimum, P. spinosum, P. rostratum, P. erinaceum , P. parvum, P. vexans, P. periplocum and P. conidiosporum*. Of these *P. torulosum, P. acanthicum, P. ultimum* were most abundant. Of the 12 *Pythium* spp, *P. erinaceum, P. parvum, P. periplocum and P. conidiosporum* have not been found on beans. *P. erinaceum* and *P. vexans* were found only on sorghum, *P. periplocum* was found only on sweet potato and *P. conidiosporum* on only beans. Irish potatoes and sorghum recorded the highest number of different *Pythium* spps but again the higher diversity on these crops could be due to the fact that more samples were collected from these crops than from other crops. *P. torulosum, P. ultimum, and P. spinosum* recorded on maize, Irish potatoes, peas and wheat, are pathogenic to beans. Whether these species infect beans has yet to be confirmed.

In peas, root rot may be caused by any one or a combination of several common soil fungi including *Pythium ultimum* (www.ipm.uiuc.edu/diseases/series 900). From our study, in addition to *P. ultimum*, another *Pythium* spp, *P. torulosum* was found in association with peas. *P. periplocum* which was found on sweet potato in our study has been reported on tomato (Bailey *et al.* 2002), indicating the diverse host range of this *Pythium* spp. *Pythium* has also been reported in wheat, i.e., *Pythium diclinum* which caused a damping-off disease in a wheat field in Kidwan village, El-Minia city, Egypt, during December, 2000. In our study *P. ultimum* was found associated with wheat but its pathogenicity is yet to be determined. *Pythium oligandrum* Drechs. was reported on maize, sorghum and Irish potato in this study. This *Pythium* spp is parasitic on many fungi from the same or other orders and is regarded as an antagonistic fungus. It can be utilised for biological control on a wide spectrum of crop plants (Brozova, 2002). *P rostratum* has been found to infect Irish potato *(Solanum tuberosum)* and sweet potato *(Ipomoea batatas)* (www.extento.hawaii.edu) and have also been recorded on the same crops in this study indicating that *P. rostratum* may be infectious to Irish potato and sweetpotato. *P. vexans* has been recorded on onion (*Allium cepa*), castor bean *(Ricinus communis)* and spinach *(Spinacia oleracea)* (www.extento.hawaii.edu). Several different, normally harmless, fungi in the soil can attack grain sorghum seed and seedlings. The organisms most commonly involved are *Fusarium, Pythium, Rhizoctonia, Aspergillus* and *Phoma*. From our study eight *Pythium* species are associated with sorghum. *P. acanthicum* was isolated from Irish potato (*Solanum tuberosum*) in Sweden (Levesque, 2004). We were also able to isolate this *Pythium* spp. from the same host in our study.

These results indicate that *Pythium* spp. do have a wide host range and seem to infect other crops which are intercrops of beans in the bean-based cropping system. Work is still going on to carry out complete characterisation of the isolates from the field (2004 and 2005 samples) as well as to determine cross pathogencity properties.

This work will be completed during the final phase of the project in R8478 (Bean root rot disease management in Uganda follow-on).

Pathogenicity tests will confirm whether the different *Pythium* spp associated with the different crops are pathogenic to those crops or just associated with those crops. The species confirmed through sequencing will be used to identify RFLP groups that would be generated from further collections.

No recovery of other *Fusarium solani* f sp *phaseoli* (FSP) has been made from other crops using selective media, however it belongs to the *Nectria haematococca Fusarium solani* species complex section *Martiella* of *Fusarium* that were identified in the sequencing. FSP was only isolated from beans.

**Table 2.5** *Pythium* species identified from isolates obtained from different crop samples in southwest Uganda

| Host | SS | *P. folliculosum* | *P. oligandrum* | *P. torulosum* | *P. acanthicum* | *P. ultimum* | *P. spinosum* | *P. rostratum* | *P. erinaceum* | *P. parvum* | *P. vexans* | *P. periplocum* | p. conidiosporum | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Maize | 4 | + | + | + | | | | | | | | | | 3 |
| Irish | 12 | + | + | + | + | + | + | + | | | | | | 7 |
| Sorghum | 10 | + | + | | + | + | + | | + | + | + | | | 8 |
| Sweet potato | 2 | | | | | | | + | | | | + | | 3 |
| Peas | 2 | | | + | | + | | | | | | | | 2 |
| Beans | 3 | | | + | + | | | | | | | | + | 3 |
| Weed | 1 | | | | + | | | | | | | | | 1 |
| Wheat | 1 | | | | | + | | | | | | | | 1 |
| Millet | 1 | | | | | | | | | + | | | | 1 |
| Total | 34 | 3 | 3 | 4 | 4 | 4 | 2 | 2 | 1 | 2 | 1 | 1 | 1 | |

SS = Samples sequenced

## Part 3: Pathogenicity of *Pythium spp*. and *Fusarium spp*. pathogenic to beans on major crops associated with beans.

### 2.3.1 MATERIALS AND METHODS

The objective of the study was to evaluate the pathogenicity of previously identified bean pathogenic *Pythium* species namely MS 61 (*P. ultimum var. ultimum*), VIH 2A (*P. chamaehyphon*.), KAK 5 B and JM 29 A (*P. pachycaule)*. Millet was used to culture the *Pythium* spp and then used to inoculate pre-sterilised soil using a ratio 1:10 v/v in a wooden flat of 42 cm x 72 cm replicated three times. A tray with non-infested millet was used as a control. This study was done in a screen house at CIAT-Kawanda Research Institute. The bean variety CAL 96 (highly susceptible) to *Pythium* root rot was used to evaluate the pathogenicity of the different isolates. The bean variety RWR 719 was used as a resistant check. Maize, sorghum and millet were planted in two rows of twelve plants each, replicated in three wooden flats. Cumulative emergence and stand was recorded one week after germination. Three weeks after planting, surviving plants were assessed for disease severity. To estimate disease severity the CIAT scale of 1-9 (Abawi and Pastor Corrales, 1990) was used with slight modifications for other crops apart from beans.

Other parameters that were measured after harvesting were root and shoot height and root and shoot dry matter content. Three trials were set up for this experiment over a period of three months. The data were subjected to analysis of variance. Means were separated by least significant difference (LSD) test using the Statistical Analysis System (SAS).

### 2.3.2 RESULTS/DISCUSSION

One of the significant findings during the survey carried out in Kabale district, was that root rot symptoms were observed on Irish potatoes and peas in addition to beans.

The different *Pythium* species tested invoked typical root rot symptoms on susceptible bean cultivar CAL 96 in screen house studies. As expected, cultivar RWR 719 was resistant. Sorghum exhibited severe stunting and purple color on leaves. These features were more pronounced with isolate KAK 5 B (*P. pachycaule*). Similarly, millet exhibited stunting as well as yellowing and drying of the leaf tips, unlike plants in un-inoculated control trays. Maize showed less pronounced effects characterized by reduced plant vigor and size. Peas were also severely affected by *Pythium* isolates showing stunting effects when compared to the control as well as rotting of roots. This effect was most severe with MS61 (*P.ultimum*). Similar results were obtained in a second trial. However, from external symptoms seen 1 week after planting, peas seemed to be succumbing severely to *Pythium* to the same degree or even more than CAL 96.

Symptoms on roots of sorghum were comprised of red-black lesions and discolorations, reduced root mass and length. Millets displayed some lesions and reduced root mass. Maize exhibited little if any lesions on roots 3 weeks after emergence although root mass was relatively lower than in the control trays. *Pythium* was re-isolated from roots of all crops grown in infected soil.

These screen house results showed that *Pythium* species used had an effect on the different crops tested. The most pathogenic *Pythium* isolates were *Pythium ultimum, P. pachycaule* and KAK 5B in descending order. The most affected crop were beans and sorghum, maize and millet in that decreasing order (Table 2.6). Maize exhibited an interesting reaction in that there was some reduction in both shoot and root mass but little necrosis on the latter. Stunting in crops is attributed to reduced capacity of roots (either due to damage or reduced amount) to support adequate water and food uptake. We can conclude from these observations that *Pythium* species pathogenic to beans cause damage to sorghum, millets and maize to varying degrees. This agrees with the notion that root rots caused by *Pythium* spp could be potentially important disease of graminaceous crops such as wheat, corn and sorghum.

**Table 2.6**: Disease reaction (severity) on major crops subjected to four *Pythium* species during the first trial

| Isolate | *Pythium* species | Disease severity | | | | | |
|---|---|---|---|---|---|---|---|
| | | CAL 96 | Maize | Sorghum | Millet | Peas | RWR 719 |
| MS61 | *P.ultimum* | 6.56[a] | 2.78[b] | 5.67[b] | 1.67[e] | 7.5[a] | 1.14[a] |
| Control | No *Pythium* spp | 1.00[e] | 1.30[d] | 1.00[d] | 1.00[d] | 2.0[e] | 1.00[a] |
| VIH 2A | *P. chamaehyphon* | 4.56[c] | 4.30[a] | 4.11[c] | 5.11[a] | 7.19[b] | 1.00[a] |
| KAK 5B | | 3.97[d] | 1.89[c] | 6.44[a] | 1.89[c] | 4.47[d] | 1.06[a] |
| JM 29A | *P. pachycaule* | 5.92[b] | 1.78[c] | 1.89[d] | 3.11[b] | 6.03[c] | 1.14+ |

a) Means within column followed by similar letters are not significantly different at P< 0.05. b) Disease scale (1-9) 1 = no root symptoms; 3 = 10% of the hypocotyle and root tissues have lesions; 5= 25% of the hypocotyle and root tissues lesions 7= 50% the hypocotyle and root tissues have lesions and the root system suffers a considerable decay; 9= 75% or more of the hypocotyle and root tissues have lesions and the root system suffers advanced stages of decay and considerable reduction (Abawi and Pastor Corrales, 1990).

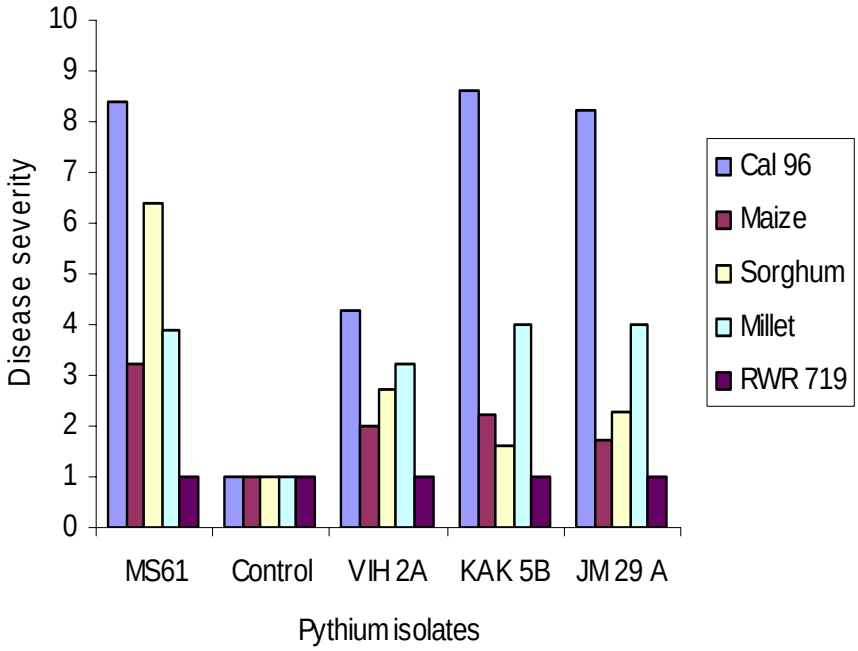



**Figure 2.5** Effect of various *Pythium* isolates on disease severity (based on CIAT of 1-9 or its adaptation) in the screen house at Kawanda during a second trial

With respect to the effect of root rots on dry matter of maize, sorghum, beans and peas, it was found that the root dry matter of the various crops was significantly different. In addition there was a significant difference in root dry matter, which varied depending on the *Pythium* species. The results correspond with root observations that showed maize to be least and sorghum most affected.

# Part 4: The impact of bean root rot management practices on the productivity of other crops associated with beans

## 2.4.1 MATERIALS AND METHODS

This study was carried out on farmer's fields in Rubaya sub-county, Kabale District in southwestern Uganda, an area considered as a hot spot for bean root rots. Fields with a history of high bean root rot incidence and severity were selected on the basis of discussions, interviews and experiences of farmers.

Crop selection was based upon its importance as a rotational crop or inter-crop to beans in the cropping system in the area (Plate 2.4). Of the crops grown as inter-crops or rotational crops in southwestern Uganda, the major ones included beans (100%), sorghum (99%), maize (94%), sweet potatoes (92%), solanum potatoes (47%) and peas (39%) (Ampaire *et al.*, 2003). The effect of root rot management practices were evaluated on sorghum (S), maize (M), peas (P) and beans was used as (B) as a susceptible control.

Four management options (farm yard manure, green manure, NPK fertilizer and the fungicide Ridomil) previously known to have useful effects against bean root rots were used. Seeds for local varieties of sorghum, maize and peas were obtained locally from farmers. A root rot susceptible bean variety (CAL 96) was used as a check. The organic manure (FYM) and green manure (Crotalaria) were applied on a dry weight basis at a rate of 5t/ha. NPK fertilizer was applied at a rate of 50KgN/ha. Fungicide (Ridomil) application was done as seed treatment as slurry at a rate of 2.5Kg/ha.

A split-plot design was used with soil amendments as main plots and crops as the sub-plots. Strips of 1m - 1.5m were left between the main-plots to minimize drift of soil amendments. Guard rows of 1m were left in between cereals and the short crops while guard rows of 0.6m were left between the short crops to minimize shading.

**Plate 2.4** A field showing bean, maize and peas intercropped

### 2.4.2 RESULTS AND DISCUSSION

As in screen house studies, infected sorghum plants exhibited stunted growth, purple leaves, shoot death and dark-red to black root lesions (see Plates 2.1 & 2.2). Significantly raised incidence and severities were observed in control plots, particularly at 54 and 72 days after planting. Amendments reduced these effects, and plant recovery was evident in plots amended with GM, FYM and NPK. Symptoms on maize were expressed as grey lesions on roots (see Plate 2.3), stunting and poor establishment. However, incidence and severity were low indicating that maize was less affected. As with sorghum, amendments and particularly FYM reduced severity and improved plant vigour and growth.

There was expression of root rot symptoms in all the crops with higher incidence being observed on sorghum after beans. Maize had the least incidence and severity. However, the extent of pathogenicity on maize has yet to be fully determined, despite isolations from all the crops of *Pythium*.

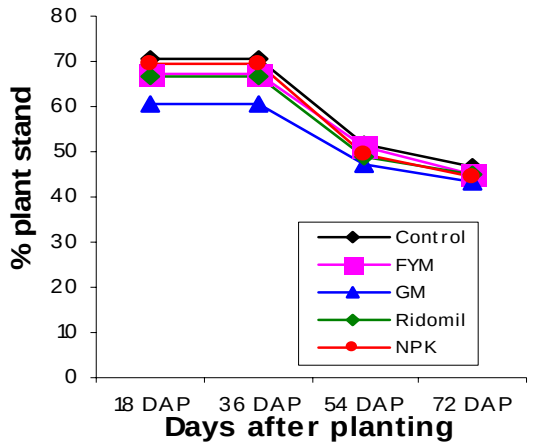
The management options evaluated affected the tested crops in different ways, but generally they reduced root rot incidence and severity considerably, relative to the control. FYM and Ridomil significantly reduced initial root rot infection on beans. In addition, FYM, GM and NPK, enhanced root (mass) growth.

Effect of the soil amendments on plant stand generally varied for all crops as shown in Figure 2.6a. FYM seemed more effective resulting in lower incidence and severity in all seasons compared to other treatments. Effectiveness of the management options varied with time in the season with Ridomil and FYM being more effective early in the season i.e. up to 36 days after planting, while GM from mid to late in the season. From 54 days after planting no significant difference in incidence and severity was observed in all the treatments. Generally, FYM was more effective in improving crop tolerance for all crops as shown in Figure 2.6b&c. This demonstrates the importance of adequate soil nutrient supply in enhancement of crop tolerance to root rot. In addition these amendments improve soil physical properties, which enhances plant tolerance, and create conditions unsuitable for the root rot pathogens. Manipulation of the variation in effectiveness of the amendments can therefore be exploited for developing a management strategy combining two or more compatible control methods. Figures 2.7, 2.8, 2.9 and 2.10 show the effects of the different amendments on the specific crops, i.e., beans, sorghum, maize and peas, respectively.

**Figure 2.6** Mean effects of bean root rot management practices on plant stand, root rot incidence and severity on all crops sampled grown in Rubaya County, Kabale District (2004 b)

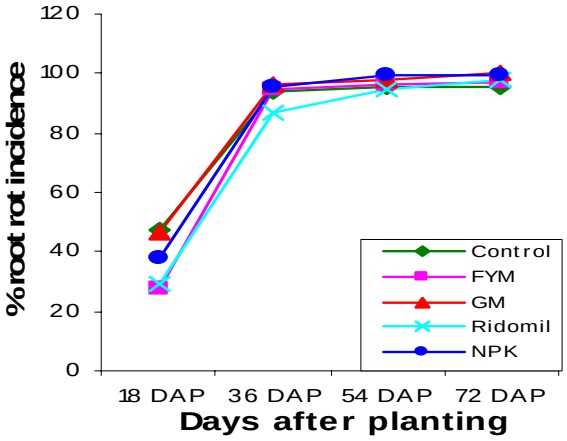
**a. Plant stand**

Death of plants occurred 36 days after planting. More deaths were observed in the control and less GM, FYM and Ridomil as shown by the gradient of decline in fig 2.5a. This shows that GM, FYM, Ridomil and NPK improve survival of the crops. GM and FYM achieve this through improving soil structure, nutrient availability and creating unfavourable conditions for the pathogens. Ridomil played a protective role against the root rots.




**b. Root rot incidence (%)**

Ridomil and FYM reduced root rots significantly between 18 and 36 days after planting when compared to NPK, GM and control. Control and GM (Clotararia) had the highest incidence between 18 and 36 days after planting. Thus ridomil and FYM were more protective against root rots. However, no difference was observed between the amendments and the control late in the season.




**c. Root rot severity (1-9 scale)**

The control and green manure treatments had a high root rot severity 36 days after planting with a drop in the rate of increase under green manure observed from 54 to 72 days after planting. Thus the effectiveness of green manure increased in the course of the season. This could be due to the incomplete decomposition early in the season. Just as with the incidence, there were lower severities with NPK, FYM, and ridomil in decreasing order. Ridomil was more protective to root rots. In general amendments reduced root rots in the crops.

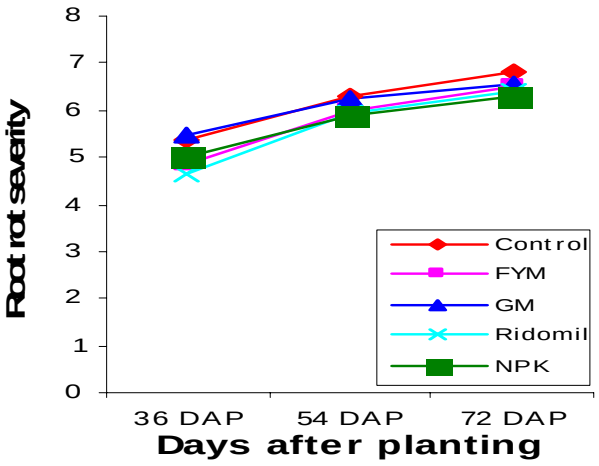
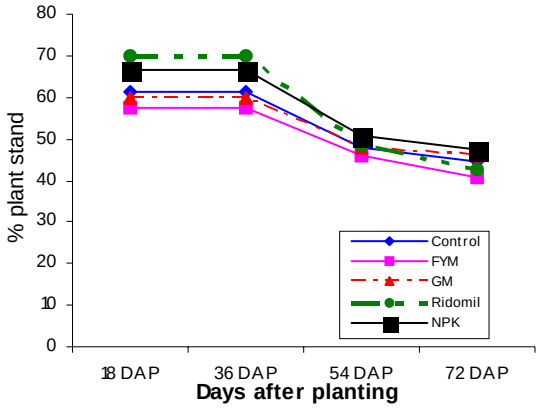


**Figure 2.7** Effect of bean root rot management practices on plant stand, root rot incidence and severity in beans (check) in Rubaya sub-county (2004 b)

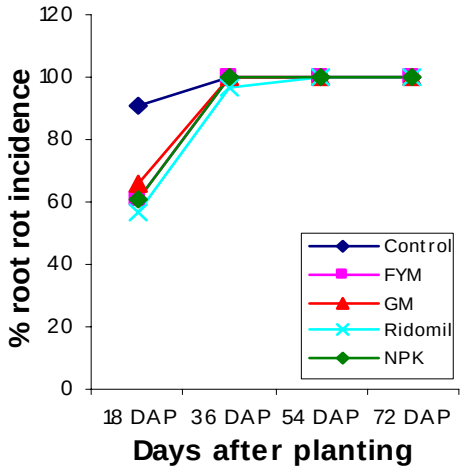
**a. Plant stand**

In the check crop (beans), the management practices did not have significantly different effects on root rots except for GM as shown by the gradient of decline in plant stand. More severe decline was observed under ridomil.




**b. Root rot incidence (%)**

All the treatments significantly reduced root rots early in the season relative to the control with an incidence of 8. Ridomil was more protective at 36 and 54 days after planting. However, after 36 days no significant differences were observed between the treatments. The amendments thus improved crop tolerance especially early in the season when the crops were at a susceptible stage of growth.




**c. Root rot severity (1-9 scale)**

Higher severities were observed in the control crop throughout the season, an indication that the amendments (particularly FYM and GM) were protective to beans though the effect of the amendments was not significantly different from the control.

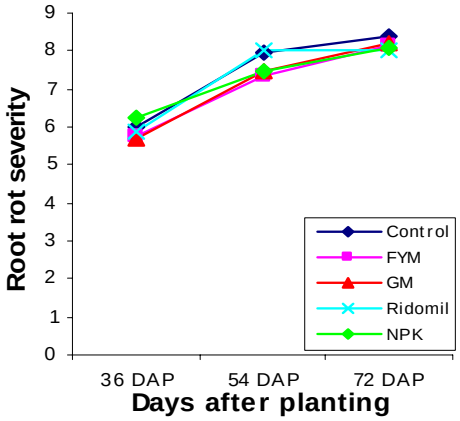
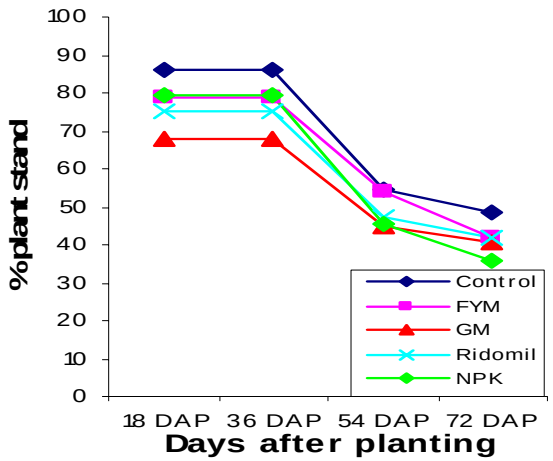


**Figure 2.8** Effect of bean root rot management practices on plant stand, root rot incidence and severity on sorghum in Rubaya County, Kabale District (2004b)

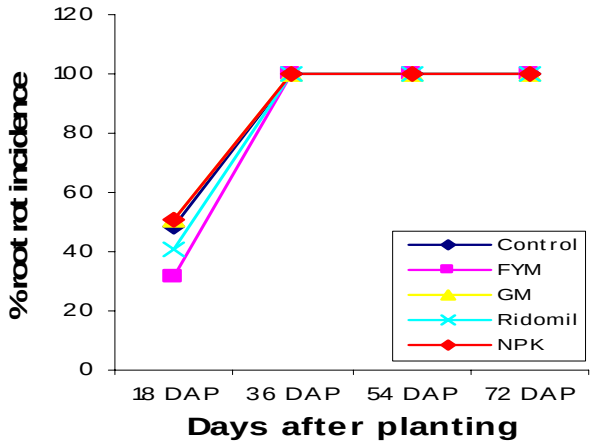
**a. Plant stand**

Survival of sorghum was more on FYM, GM and ridomil but less in NPK. This can be attributed to the protective effects of FYM, GM and ridomil. FYM and GM also improve soil conditions, enhance antagonists, and improve nutrient availability. However, effects of amendments did not vary significantly.




**b. Root rot incidence (%)**

FYM and ridomil had lower rot incidence compared to GM, control and NPK at 36 days after planting. No difference was observed between NPK, GM and the control at 18 days after planting. At 36 days all treatments had 100% incidence.




**b. Root rot severity (1-9 scale)**

Generally sorghum had high root rot severities of above 6 with the highest at 36 days after planting in control plots. Lower severity scores were observed under NPK, FYM and ridomil treatments. However, at 54 days, all had high disease severities (9).

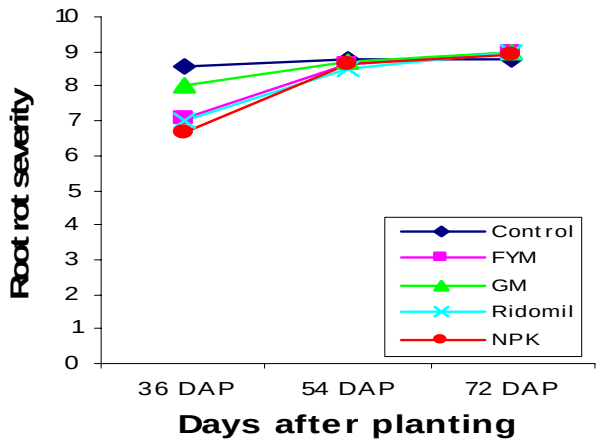
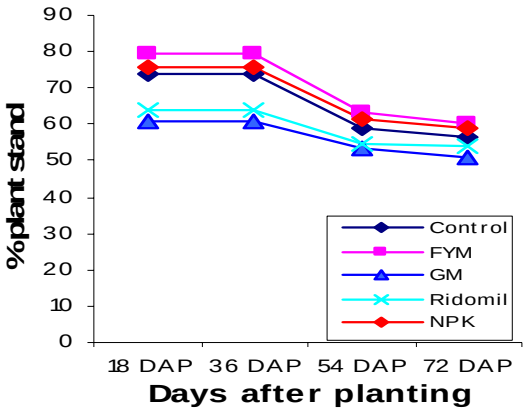


**Figure 2.9** Effect of bean root rot management practices on plant stand, root rot incidence and severity on maize in Rubaya County, Kabale District (2004 b)

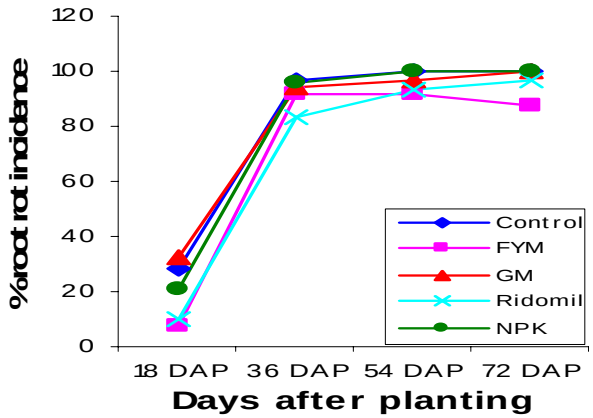
**a. Plant stand**

Lower numbers of maize deaths were observed in GM and ridomil with the highest in control plots as shown by the slope of the graphs after 36 days. It seemed that maize tolerance was improved by use of the soil amendments, with greater improvement under green manure and ridomil.




**b. Root rot incidence (%)**

Low incidences were observed after 18 days with the lowest under ridomil and FYM plots. The highest incidences were observed in GM followed by control and NPK plots. No significant difference was observed between them. No significant difference was observed between the treatments after 36 days.




**c. Root rot severity (1-9 scale)**

Maize under Ridomil, FYM, NPK and GM respectively improved crop tolerance throughout the season as shown by the graph

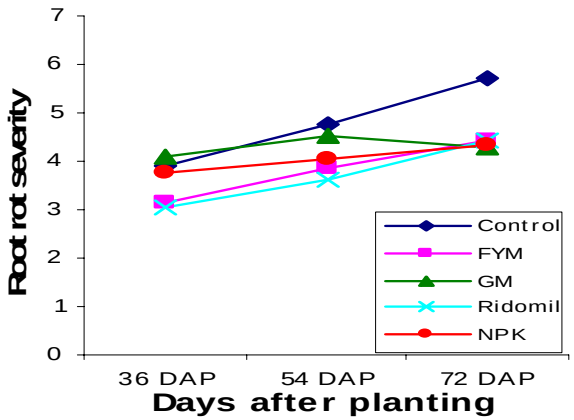
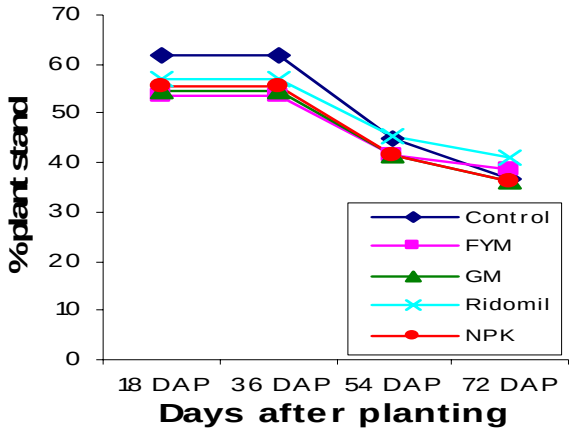


**Figure 2.10** Effect of bean root rot management practices on plant stand, root rot incidence and severity on peas in Rubaya County, Kabale District (2004 b)

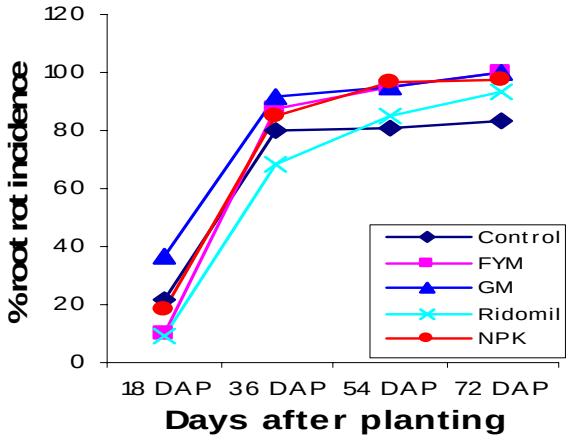
**a. Plant stand**

Plant death was observed more in the control than in other treatments. Better plant survival was recorded under Ridomil followed by FYM an indication that they offered more protection to peas.



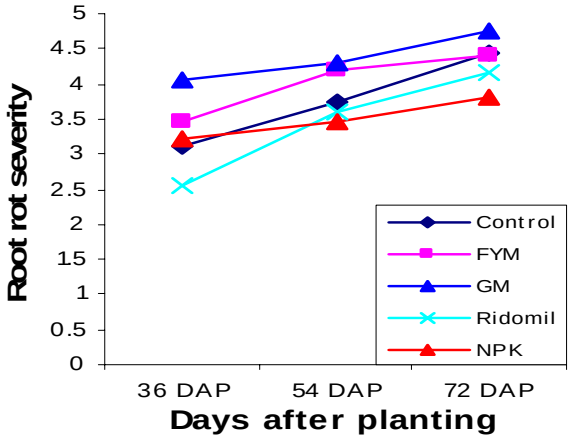

**b. Root rot incidence (%)**

Higher root rot incidence in peas was observed under GM throughout the season. Thus green manure reduced pea tolerance to root rots. FYM and Ridomil had the lowest incidences after 36 days. However, higher incidences were observed in the amendments late in the season compared to the control.




**c. Root rot severity (1-9 scale)**

Low root rot severity was only observed in ridomil and NPK plots while GM and ridomil had higher severities than the control, a result similar to that of incidence in fig 2.9b above. This could be due a negative interaction between the crop and these amendments or a positive interaction between them and the root rot pathogens..



**The effect of bean root rot management practices on dry matter production for maize and sorghum**

Management practices improved dry matter yield in crops (Table 2.7). This is probably because protective action (Ridomil, FYM and GM), enhancement of nutrient availability (NPK, GM and FYM), and improvement of soil physical and biological environment (GM and FYM). Improved DMP in sorghum due to Ridomil is probably due to its protective effect against *Pythium* species.

**Table 2.7** Effects of bean root rot management practices on mean dry matter production (72 days after planting) for beans, maize, sorghum and peas from Rubaya, Kabale District, 2004a and 2004b season.

| Treatment | Dry matter (g) | | | | | |
|---|---|---|---|---|---|---|
| | 2004A | | | 2004B | | |
| | **Maize** | **Sorghum** | Beans | Maize | Sorghum | Peas |
| Control | 106.9 | 18.5 | 9.71 a | 12.71 a | 2.81 a | 15.04 a |
| Farm Yard Manure | 128.3 | 42.7 | 12.81 | 28.65 b | 6.24 b | 25.30 b |
| Green Manure | 138.4 | 38.9 | 14.85 b | 20.19 c | 3.44 | 14.66 a |
| Ridomil | 112.5 | 46.1 | 12.20 | 14.65 a | 2.70 a | 16.15 a |
| NPK | 163.2 | 48.8 | 13.21 b | 23.08 c | 3.29 | 19.76 c |
| | L.S.D (P < 0.05) = (32.20) | | | L.S.D (P<0.05) = 3.385 | | |

[1]Means of the same letter(s) and no letters within columns do not differ significantly at P<0.05

Overall the different management options evaluated influenced severity of root damage and other growth parameters on crops grown in association with beans. This implies that using these options does not only contribute to the management of bean root rots, but is also beneficial to other crops. Ridomil exhibited more effect in root rot control especially early in the season though it did not significantly improve dry matter production except for sorghum during 2004 A season. However, the effects of these amendments were much less where severity was high as in sorghum, and beans. Moreover, FYM and green manure resulted in increased root rot incidence and severity in peas.

# Part 5: Determine (if any) possible role of other crops in the increase in incidence and severity of bean root rots in a bean-based cropping system

## 2.5.1 MATERIALS AND METHODS

Initial activities meant to signal if other crops grown in association with beans play some role in the observed increase or problem of bean root rots have been described in Sections 2.1.1, 2.2.1, 2.3.1 and 2.4.1.

Subsequent activities will involve long term studies where comparisons will be made between sorghum and beans, key crops in the systems and which are affected by certain *Pythium* spp on the inoculum levels of the latter under different production practices. This will include establishing the effect of sorghum (under monocrop and in rotation with other crops) on the inoculum levels of *Pythium* species which are pathogenic to beans.

## 2.5.2 RESULTS AND DISCUSSION

The combined results obtained above (see Sections 2.1.2, 2.2.2, 2.3.2 and 2.4.2) point to the fact that *Pythium* species pathogenic to beans affect other crops particularly sorghum, which is major rotation crop to beans in south west Uganda. This implies that both crops may be contributing to the increase of the inoculum or survival of the bean pathogens in the soil. Practicing rotation particularly between sorghum and beans as is the case in southwest Uganda and believing that this is useful in reducing the effects on the bean root rots seems not to be the case. This is also based on the notion that cereals and legumes are less likely to share pathogens. It seems that some of the crops affected by *Pythium* species pathogenic to beans may be acting as alternate hosts of the same (*Pythium*) pathogens (in absence of the bean crop), and thus abet or contribute to the increase of the inoculum. A long-term study to empirically document population changes of bean *Pythium* pathogens under different crop conditions is under consideration. This work will be completed during the final phase of the project in R8478 (Bean root rot disease management in Uganda follow-on).

# Part 6: Recommendation domains

- Some crops (sorghum, peas etc) in the bean based systems are affected by pathogens that affect beans, but their effect may be less appreciated relative to beans. From sections, 2.1.2, 2.2.2, 2.3.2 and 2.4.2, it was shown that several pathogenic *Pythium* spp i.e., *P. spinosum*, *P. ultimum* and *P. torulosum* known to be pathogenic to beans are found in association with major crops usually grown in association with beans. This implies that they may act as alternate hosts to these pathogens and increase the inoculum levels in the soil hence leading to greater bean losses due to *Pythium* This was originally not considered in designing control measures for root rots.

- Rotation involving beans and some cereals such sorghum is not helpful in reducing bean root rots. Since major pathogenic *Pythium* spp to beans have been found on crops often grown in association with beans, their use as rotation crops in the management of *Pythium* root rot may not be effective and in most cases may actually increase the disease levels on beans. Instead of controlling the pathogens by acting as non-hosts they would instead sustain or even increase pathogen inoculum levels in the soil. There is hence a need to study the levels of inoculum build-up of *Pythium* spp. as a result of crop rotation and intercropping.

- Management practices geared towards root rots are equally useful to other crops in the system. From the study on the effect of management options for bean *Pythium* root rot, it was shown that the control options for *Pythium* root rot also control root rot in other crops. Therefore, for a crop system involving cereals and legumes, similar root rot management practices may be applied. Growing of root rot resistant varieties for root rot should be practiced for all crops grown in a bean cropping system.

- Therefore, in developing management strategies for *Pythium* root rot, it would be advantageous to consider a systems' approach rather than a commodity. Management approaches directed to only beans may not take into consideration other hosts (e.g. sorghum) of *Pythium* spp. often found in bean cropping systems, hence control would not be effective.

# Chapter 3: Follow-up characterization of *Fusarium oxysorum* fsp *phaseoli*

## SUMMARY

The pathogenicity of and molecular variation within a collection of isolates of *F. oxysporum* f. sp. *phaseoli* was determined in a previous project R7568 *Characterisation and epidemiology of root rot diseases caused by Fusarium and Pythium spp. In beans in Uganda* (see final Technical report). Some correlation between molecular groups and virulence towards bean varieties (particularly the African variety G2333) was identified. However, it was clear from this earlier work that an examination of more isolates (especially African examples) was needed before any firm conclusions can be drawn, and that the risk to the popular climber G2333 in other countries in the region must also be assessed. The following Chapter describes how isolates of *F. oxysporum* were collected from Ugandan and Rwandan bean plants which showed yellowing of foliage, and how the pathogenicity/virulence of these isolates were tested on bean varieties G2333 and A211 (a CIAT wilt-differential line). The molecular variation within and between groups of pathogenic and non-pathogenic isolates was subsequently examined, and pathogen-specific (ideally race-specific) PCR primers were developed.

### 3.1 MATERIALS AND METHODS

#### 3.1.1 Collection of isolates of *F. oxysporum* from Ugandan and Rwandan bean plants showing yellowing of foliage

Collection of *F. oxysporum* f. sp. *phaseoli* used for the study included fifty isolates from Uganda, eleven from Rwanda, four from the Democratic republic of Congo, two from Kenya and five from South Africa. In addition, for the purposes of comparison, fourteen further reference isolates were used, of which eight were examples from beans from other geographic locations including Spain, Columbia, Brazil and USA as well as culture collections CBS and ATCC, and six were examples from different hosts. For long-term storage the isolates were maintained as agar plugs stored in water and held at 5˚ C. These isolates were characterised using PCR based molecular methods, particularly Simple Sequence Repeat (SSR)-PCR. For molecular characterisation, genomic DNA was extracted from mycelial material grown in liquid culture. A range of SSR- primers representing different sequences and annealing temperatures were used for genetic characterisation and grouping of the isolates following standard PCR conditions.

## 3.2 RESULTS AND DISCUSSION

### 3.2.1 Molecular variation within and between groups of pathogenic and non-pathogenic isolates

The *F. oxysporum* f. sp. *phaseoli* isolates revealed both simple as well as complex molecular fingerprints with different SSR primers. Consensus analysis of the fingerprint profiles generated with different primers for each isolate enabled their genetic grouping (groups G1 – G4), and revealed interesting trends in relation to their geographic origin and pathogenicity as discussed below. For example, of the 50 *F. oxysporum* f. sp. *phaseoli* isolates from Uganda, 44 clustered into group G1, three represented G2 (TG015, TG020 and TG032), while isolates TG036, MbGT007B and Mbgt015 each displayed unique fingerprints suggesting that these were distinct genotypes within this collection. Among the eleven Rwandan isolates, four belonged to G1 (RW2F, RW17, RW18 and RW22), five to G3 (RW4, RW7, RW9, RW19 and RW23) while the other two (RW1 and RW25) were distinct genotypes. Of the four isolates from Congo, two each belonged to genetic groups G3 (DRC1a and DRC2c) and G4 (DRC1b and DRC2a); two isolates from Kenya were distinct from each other and clustered with groups G1 (UGKS-2F) and G3 (UGZ-16); four South African isolates belonged to group G1 (RSA57, I763, I767 and I770a) while the fifth isolate RSA15 was a distinct genotype. Thus clear genetic variation was observed among the isolates representing the pathogen *F. oxysporum* f. sp. *phaseoli* within each country, despite a limited number of examples in some cases. Overall, the majority (66) of the *F. oxysporum* f. sp. *phaseoli* isolates representing the pathogen populations in different African countries clustered into four major groups G1 to G4 referred to above with six other isolates displaying distinct genotypes. It is possible that these genotypes represent additional genetic groups within larger pathogen populations from these geographic locations. Interestingly, a number of *F. oxysporum* f. sp. *phaseoli* isolates from different countries belonged to the same genetic groups, while others belonged to distinct groups restricted to a country. For example, G1 isolates were present in Uganda, Rwanda, South Africa as well as Kenya. Similarly, G3 isolates were recorded from Rwanda, Congo and Kenya. On the other hand, isolates belonging to groups G2 and G4 were present only in Uganda and Congo, respectively. These observations suggest potential common origin and spread of some of the pathogen isolates, and the close association of others to particular agro-ecological conditions. Full details of the isolate origins are available within the project datasets.

### 3.2.2 Relatedness of molecular groups and pathogen virulence and Efforts to develop pathogen-specific PCR

In glasshouse tests**,** *F. oxysporum* f. sp. *phaseoli* isolates RW4, RW7, RW23 from Rwanda and DRC1b from Congo showed vascular browning in the lower stems of the plants reflecting varying levels of pathogenicity on the popular climber variety G2333. Interestingly, RW4, RW7, RW23 originated from similar plant samples within Rwanda and belonged to the same genetic group G3; isolate DRC1b, on the other hand, belonged to the genetic group G4. Isolates TG012 (G1) and TG032 (G2), both

from Uganda showed no vascular browning on G2333. However, a diverse collection of isolates from different countries have been identified as belonging to the genetic group G1 and examination of these characterised isolates is likely to reveal their virulence patterns. To develop pathogen-specific PCR, some of the unique bands from isolates RW7 and DRC1b were reamplified by stab PCR and gel purified. In specificity assays, however, the bands tested did not provide sufficient resolution and cross reacted with other isolates. As this approach has been shown to be effective with other systems to develop pathogen-specific PCR, it is likely that the bands tested did not contain sufficient level of sequence differences and analysis of other bands from the characterised isolates could lead to the development of diagnostic assays.

# Chapter 4: Identification of disease moderating interactions between organisms in the soil

## SUMMARY

A number of beneficial interactions were identified in R7568 (both negative and positive) in both laboratory and greenhouse studies. There is therefore a need to explore the potentially useful interactions at field levels, in order to exploit positive interactions and reduce the impact of negative ones. The activities described in the following Chapter sought to evaluate the effects of beneficial interactions under field conditions, and to assess the potential for use of candidate organisms.

## Part 1: An evaluation of the effects of beneficial interactions under field conditions

### 4.1.1 MATERIALS AND METHODS

#### 4.1.1.1 Laboratory screening

Preserved *P ultimum* isolates MS46, MS49, MS11, MS47, MS34, DFD47, KIS4, MS15, MS27, MS6, MS66, KLE3A, MS61 were evaluated against an organism previously shown to be a potential biocontrol agent (MS10). Potato dextrose agar (PDA) was used because of its ability to stimulate rapid fungal growth. The antagonistic activity of the isolates were determined in a dual culture assay in which opposite ends of the Petri dish was inoculated with an isolate and incubated at $24^0$C. Qualitative data of interactions were recorded after 48 h of incubation. Each level is explained thus: Level 1-Mutual intermingling of the two organisms; Level 2-Inhibition of one organism on contact and the other organism continues to grow unchanged or at a reduced rate through the colony of the inhibited organism; Level 3-Mutual inhibition on contact, the space between the two organisms is small but clearly marked; Level 4; Inhibition of one organism at a distance, the antagonist continues to grow at unchanged or at a reduced rate; Level 5-Mutual inhibition at a distance

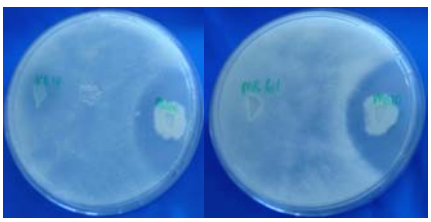


**Plate. 4.1** Level 3 of KB14 and MS61 vs MS10 on culture

In culture, four isolates showed mutual inhibition on contact (Level 3) with MS10 whereby the space between the two organisms was small but clearly marked (Plate 4.1 above). Inoculum of each isolate was raised independently on millet grains. 200g millet grains were mixed with 200ml tap water in screw capped bottles/polythene bags and autoclaved twice for 1 hour on 2 consecutive days. After cooling (overnight) the sterilized finger millet was inoculated with 5 mm discs of actively growing isolate to raise inoculum. The isolates were incubated at room temperature in a sterile environment for 12 days when millet grains were visibly colonized by the fungi.

**4.1.1.2 Screen house evaluation**.

The isolates were evaluated alone and in combination in the greenhouse.

In the first set of experiments, the isolates were each mixed soil in a ratio of 1:8 v/v inoculum to soil (Pyndji *et al*., 1996) in a wooden flat tray of 42 cm x 72 cm and left to stabilize for one week before antagonist MS10 was added and mixed with the soil. Planting was done after one week.

In the next set of experiments inocula of the five isolates (KB 14, KB 4, VIH 4, MS 61, and MS10) were each mixed with pre-sterilised soil in a ratio of 1:8 v/v inoculum to soil in wooden flat trays and left to stabilize for one week before planting. This was aimed at assessing the pathogenicity of the selected isolates on beans.

Twenty seeds of susceptible bean varieties CAL96 and K20 as well as resistant bean variety (RWR 719) were planted in two rows. Each row consisted of 10 plants. A germination count was taken after full emergence from the soil. Disease evaluation was done 21 days after planting by uprooting the plants, rinsing the roots (Plates 4.2 and 4.3) and scoring them according to the CIAT 1-9 with the following meanings: 1- no visible symptoms, 3- little symptoms, 5- moderate 7- severe 9 complete discolouration of the tap root.


**Plate 4.2** Unaffected roots


**Plate 4.3** Infected roots

The data was analyzed using GENSTAT for windows statistical package 7th Edition in accordance with the general analysis of variance design. The significance of the difference between means of isolate treatments was determined using the Least Significance Difference (LSD) values at 5% probability ($P$<0.05).

### 4.1.2 RESULTS AND DISCUSSION

Single isolate treatments of KB14, KB4 and MS61 showed severe root rot disease in CAL 96 confirming that these isolates are indeed pathogenic to beans with no significant difference ($P$<0.05) between the three treatments (Table 4.1). VIH4 and MS10 isolates were not pathogenic to both susceptible bean varieties. Marked reduction in disease severity was observed in treatments where MS10 was added as antagonist to the pathogenic *Pythium* isolates KB14, KB4 and MS61. The highest disease severity was recorded in susceptible variety CAL 96 followed by K20. RWR 719 which is a resistant variety had the least severity.

**Table 4.1** Effect of different isolate combinations on root rot severity on 3 bean varieties

| ***Treatments*** | ***Varieties*** | | | |
|---|---|---|---|---|
| | **CAL 96** | **K 20** | **RWR 719** | **Mean*** |
| KB14 + MS10 | 2.05 | 2.00 | 1.05 | 1.70[c] |
| KB 4 + MS10 | 2.15 | 2.15 | 1.00 | 1.77[c] |
| VIH4 + MS10 | 1.00 | 1.00 | 1.00 | 1.00[c] |
| MS61 + MS10 | 2.10 | 2.15 | 1.00 | 1.75[c] |
| KB14 | 7.25 | 4.15 | 4.40 | 5.27[ab] |
| KB4 | 7.90 | 7.55 | 2.75 | 6.07[a] |
| VIH4 | 2.00 | 2.00 | 1.00 | 1.67[c] |
| MS61 | 7.85 | 3.15 | 1.00 | 4.00[a] |
| MS10 | 1.00 | 1.00 | 1.00 | 1.00[c] |
| **Mean*** | 3.70[x] | 2.79 | 1.58 | |

*Means followed by the same letter are not significantly different from each other.

Addition of MS10 into pathogenic isolates resulted in significant increases in root dry weight of susceptible varieties CAL 96 and K20 (Figure 4.1). This indicates that isolate MS10 is a candidate for bio-control because it improves root development. Comparatively high disease severity resulted in reduced root dry weight. For example KB4 has the highest disease severity and the lowest root dry weights.

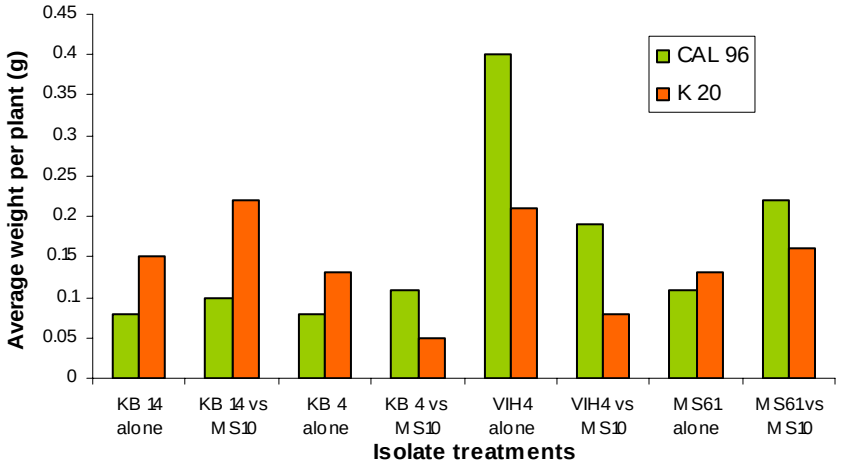



**Figure 4.1** Effect of isolate combinations on root dry weight per plant of susceptible bean varieties CAL 96 and K20

Results obtained in this study showed that significant reduction in root rot severity is possible with supplementation of MS10. Further application of MS10 could result in its build up in the soil to levels the further suppress the root rot and increase root dry weight. These results obtained from the greenhouse experiments are being validated in field situations.

# Part 2: Assessment of the potential for use of candidate organisms

### 4.2.1 OBJECTIVE

It is desirable to develop safer alternative to the traditional synthetic pesticides which are both expensive and hazardous to man and to the environment. We therefore seek to identify naturally occurring bio-control agents against *Pythium* root rot.

### 4.2.1 MATERIALS AND METHODS

Given results that were obtained in Part 1 above, efforts are being made to assess possible options of using the fungus under field conditions. This includes the time of application in relation to planting of the beans, placement methods, effective dosages, and the carrier materials. Other evaluations under consideration are the efficacy of the biocontrol agent under different field conditions and seasons.

### 4.2.2 RESULTS AND DISCUSSION

Results obtained in Part 1 are encouraging. Antagonistic effects observed under the laboratory conditions have also been demonstrated under screenhouse conditions. Initial trials were hit by drought resulting in crop failure. This work will be completed during the final phase of the project in R8478 (Bean root rot disease management in Uganda follow-on).

## References

**Abawi, G.S. & M.A. Pastor-Corrales, 1990**. Root rots of beans in Latin America and Africa: Diagnosis, research methodologies, and management strategies. Centro Internacional de Agricultura Tropical, Cali, Colombia. 114 pp.

**Ampaire, E.L., 2003.** Farmer's indigenous technical knowledge of bean disease management and communication systems in South-western Uganda. MSc. Thesis. Makerere University, Kampala, Uganda.127pp.

**Ampai re, E. L, Semana A. R, Maggaret N. M, 2003.** Farmers' indigenous technical Knowledge of bean disease management and communication systems in Southwestern Uganda. 2003.

**Bailey, A.M., Mitchell, D.J., Manjunath, K.L., Nolasco, G and C.L. Niblett. (2002).** Identification to the species level of the plant pathogens Phytophthora and *Pythium* by using unique sequences of the ITS1 region of ribosomal DNA as capture probes for PCR ELISA .FEMS Microbiology Letters 207 (2002) 153-158.

**Brozova, J.,** Review: Exploitation of the Mycoparisitic Fungus *Pythium* oligandrum in Plant Protection .Plant Protection 2002.Vol 38,No.1:29-35.

**Buruchara, R. A., M. A. Pastor-Corrales, & U. Scheidegger, 1999.** Fusarium wilt disease caused by *Fusarium oxysporum* f. sp. *phaseoli* on a common bean cultivar, G 2333 in Rwanda and the Democratic Republic of Congo. Plant Disease 83: 397.

**CIAT (1986)** Bean Diagnostic surveys. Pages 260-276 In: Annual Report, Bean Program. Centro Internacional de Agricultura Tropical, Cali, Colombia.

**CIAT (1992)** Root rots. Pages 112-116 In: Annual Report, Bean Program. Centro Internacional de Agricultura Tropical, Cali, Colombia

**CIAT, 2003.** Africa: Bean Pathology. pp 201-224. In: Annual Report, Bean Program. Centro International de Agricultural Tropical, Colombia

**Doyle , J. J and Doyle J.L., 1990.** Isolation of plant DNA from fresh tissue. Focus 12: 13-15

**GenStat Release 7.1 (PC/Windows XP), 2003**, Lawes Agricultural Trust (Rothamsted Experimental Station)

**Kageyama, K., Ohyama, A., and Hyakumachi, M. 1997.** Detection of *Pythium ultimum* using polymerase chain reaction with species-specific primers. Plant Disease 81: 1155-1160.

**Levesque, A.C and DeCock, W.A.M. 2004.** Molecular phylogeny and taxonomy of the genus Pythium.Mycol.Res.108(12):1363-1383

**Opio,** F. (1999) Management of bean root rot in Uganda. Report of subproject funded by African Highlands Initiative.

**Pachico, D., 1993.** The demand for bean technology. In: G. Henry (Ed.) Trends in CIAT commodities 1993. CIAT, Cali, Colombia.

**Pyndji, M.M., G.S. Abawi, and R. A. Buruchara, 1996.** Root rot pathogen of beans in the highlands of southwestern Uganda and the evaluation of bean germplasm for resistance to root rot. PhD. Department of plant pathogy, New York State Agricultural experimental station Cornell University.

**Rusuku, G., R. A. Buruchara, M. Gatabazi & M. A. Pastor-Corrales, 1997.** Occurrence and distribution in Rwanda of soil-borne fungi pathogenic to the common bean. Plant Disease 81: 445-449.

**Spence, N. (2003)** Characterisation and epidemiology of root rot diseases caused by Fusarium and *Pythium* spp. in beans in Uganda R7568 Final Technical report. 145pp.

**Wang, P. H., Wang, Y.T., and White, J.G. 2003.** Species-specific PCR primers for *Pythium* developed from ribosomal ITS1 region. Letters in Applied Microbiology 37: 127-13

**White, T.J., Bruns, T., Lee, S.B. and Taylar, J. 1990.** Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (Eds.). PCR Protocols, A Guide to Materials and Application. San Diego: Academic Press, 315-322.

www.extento.hawaii.edu *Pythium* primer.Pathogens and hosts

www.ipm.uiuc.edu/diseases/series 900 University of Illinois Extension Report on plant disease

# R8316 CROP PROTECTION PROGRAMME

## APPENDIX I: KEY DATASETS GENERATED

CSL (2005) CDs of JPEG files: Library of images collected for purpose of project: Bean root rot disease management in Uganda, primarily supplied by N. Spence *et al*.

MUKALAZI, J. (2004) Dataset. Morphological grouping of Ugandan *Pythium* isolates obtained from April 2000 collection. Microsoft Excel Worksheet, Makerere University, Kampala, Uganda.

MUKALAZI, J. (2004) Dataset. ITS DNA sizes (approximate base pairs) and number of restriction sites for isolates in the RFLP groupings using endonucleases on *Pythium* isolates. Microsoft Excel Worksheet, Makerere University, Kampala, Uganda.

MUKALAZI, J. (2004) Dendrogram showing major branches/indicating clusters and RFLP groupings. Microsoft Excel Worksheet, Makerere University, Kampala, Uganda.

MUKALAZI, J. (2004) Dataset. *Pythium* species identified from DNA sequence analysis. Microsoft Excel Worksheet, Makerere University, Kampala, Uganda.

MUKALAZI, J. (2004) Dataset. Alignment of the ITS1 sequences of *Pythium* spp. identified from Ugandan samples. Microsoft Excel Worksheet, Makerere University, Kampala, Uganda.

MUKALAZI, J. (2004) Map. Geographical distribution of *Pythium* species, Kabale District. Makerere University, Kampala, Uganda.

MUKALAZI, J. (2004) Map. Geographical distribution of *Pythium* species, Kisoro District. Makerere University, Kampala, Uganda.

MUKALAZI, J. (2004) Map. Geographical distribution of *Pythium* species, Mbale and Sironko Districts. Makerere University, Kampala, Uganda.

MUKALAZI, J. (2004) Dataset. Pathogenicity of *Pythium* isolates in different RFLP groupings. Microsoft Excel Worksheet, Makerere University, Kampala, Uganda.

MUKALAZI, J. (2004) Dataset. Pathogenicity of *Pythium* isolates identified from sequence analysis of bean varieties. Microsoft Excel Worksheet, Makerere University, Kampala, Uganda.

MUKALAZI, J. (2004) Dataset. Comparative graphical representations of the effects of manure and *Calliandra* on *Pythium* root rot disease progress in susceptible and tolerant bean varieties. Microsoft Excel Worksheet, Makerere University, Kampala, Uganda.

TUSIIME, G. (2004) Dataset. Morphological grouping of Ugandan *Fusarium* isolates obtained from April 2000 collection. Microsoft Excel Worksheet, Makerere University, Kampala, Uganda.

TUSIIME, G. (2004) Dataset. ITS DNA sizes (approximate base pairs) and number of restriction sites for isolates in the RFLP groupings using endonucleases on *Fusarium* isolates. Microsoft Excel Worksheet, Makerere University, Kampala, Uganda.

TUSIIME, G. (2004) Dendrogram showing major branches/indicating clusters and RFLP groupings. Microsoft Excel Worksheet, Makerere University, Kampala, Uganda.

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TUSIIME, G. (2004) Map. Geographical distribution of *Fusarium* species, Kisoro District. Makerere University, Kampala, Uganda.

TUSIIME, G. (2004) Map. Geographical distribution of *Fusarium* species, Mbale and Sironko Districts. Makerere University, Kampala, Uganda.

TUSIIME, G. (2004) Dataset. Pathogenicity of *Fusarium* isolates in different RFLP groupings. Microsoft Excel Worksheet, Makerere University, Kampala, Uganda.

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TUSIIME, G. (2004) Dataset. Comparative graphical representations of the effects of manure and *Calliandra* on *Fusarium* root rot disease progress in susceptible and tolerant bean varieties. Microsoft Excel Worksheet, Makerere University, Kampala, Uganda.

# R8316 CROP PROTECTION PROGRAMME

## APPENDIX II: DISSEMINATIONS

### Publications

Mukulazi, J. (2004) Pathogen variation and quantification of *Pythium* species in bean fields in Uganda. Thesis submitted for the degree of Doctor Of Philosophy of Makerere University.

Tusiime, G. (2004) Pathogen variation and quantification of *Fusarium* species in bean fields in Uganda. Thesis submitted for the degree of Doctor Of Philosophy of Makerere University.

Tusiime, G., Buruchara, R. A., Carder, J., Spence, N. J., Opio, F. & Adipala, E. Amplified fragment length polymorphism (AFLP) derived primers detects *Fusarium solani* f. sp. *phaseoli* in common beans. *In submission to Phytopathol. Z.*

Tusiime, G., Buruchara, R. A., Adipala, E., Carder, J., Spence, N. & Opio, F. Genetic variation in *Fusarium solani* isolated from beans and soil associated with *Fusarium* root rot disease in southwestern Uganda. *In submission to Phytopathol. Z.*

Mukalazi, J., Adipala, E., Buruchara, R. A., Carder, J., Opio, F., Pettitt, T. & Spence, N. J. Variation, identification and detection of *Pythium* species associated with bean root rot disease in Uganda. *In submission to Phytopathol. Z.*

Mukalazi, J., Adipala, E., Buruchara, R. A., Carder, J., Opio, F., Pettitt, T. & Spence, N. J. Quantification of *Pythium* species pathogenic to bean and the impact organic amendments on soil pathogen populations in Uganda. *In submission to Phytopathol. Z.*

# Internal Reports

### *Project Progress Reports*

Project Progress Report 1. April 2003 – September 2003
Project Progress Report 2. October 2003 – December 2003
Project Progress Report 1. April 2004 – September 2004
Project Progress Report 2. October 2004 – December 2004

### *Internal Progress Reports*

Ocimati W., Buruchara, R.A. Geoffrey, T., Opio, F., Ugen, M., and Spence, N. 2004. Effects of different management practices against *Pythium* root rots on sorghum, peas and maize crops under bean bean-based cropping system in south-western Uganda. Progress Report. 60pp. Kampala, Uganda.

Gichuru, V., Buruchara, R.A., Geoffrey, T., Opio, F., Ugen, M., and Spence, N. 2004. Symptomatology and characterization of *Pythium* species, recovered from crops grown in association with beans in south-western Uganda. Progress Report. 17pp. Kampala, Uganda.

### *Annual Reports*

CPP Annual Report 2003

Annual Report 2003 (CIAT print and on the web):
- Characterization and distribution of *Pythium* spp causing root rots in Eastern Africa.
- Effect of organic amendments on bean *Fusarium* root rot disease and soil inoculum levels.
- Development and use of dilution plating method to quantify the effect of organic amendments on the inoculum levels of *Pythium* spp and severity of *Pythium* root rots.

Annual Report 2004 (CIAT print and on the web):
- Pathogenicty of *Pythium* spp and effects of management options fro root rots on crops grown in association with beans in south west Uganda.

# Other Dissemination of Results

**Student seminars**
**(Kawanda Agricultural Research Institute) (2004)**

Ocimati W. Effects of management options for *Pythium* root rots on selected crops grown in association with beans in southwest Uganda.

Gichuru, V. Characterization and pathogenicity of *Pythium* isolates on crops, which are intercrops of beans in South Western Uganda.

**Conferences**

Tusiime, G., Buruchara, R., Adipala, E., Carder, J., Spence, N. & Opio, F. (2003). Variation of *Fusarium solani* from beans with root rot symptoms inferred from AFLP analysis, pathogenicity and DNA sequences. Presented at the African Crop Science Conference, 12-17 Oct 2003, Nairobi, Kenya.

Mukalazi, J., Adipala, E., Buruchara, R., Carder, J., Opio, F. & Spence, N.J. (2003) Variation and identification of *Pythium* species associated with bean root rot disease in Uganda. Presented at the African Crop Science Conference, 12-17 Oct 2003, Nairobi, Kenya.

Buruchara, R. G. Mahuku, J. Mukalazi, R. Otsyula & A. Levesque. Characterization of *Pythium* species associated with *Pythium* root rot of beans *Phaseolus vulgaris* (L) in Eastern Africa and identification of resistant genotypes (2005). Presented at: Biotechnology, Breeding and Seed Systems for African Crops. The Rockefeller Foundation and the Kenya Agricultural Research Institute (KARI), Nairobi, Kenya 24-27 January 2005.

Kimani, P. M., Buruchara, R., Muthamia, J., Mbikayi, A., Namayanja, A., Otsyula, R. & Blair, M. (2005) Selection of marketable bean lines with improved resistance to angular leaf spot, root rot and yield potential for smallholder farmers in eastern and central Africa. Presented at: Biotechnology, Breeding and Seed Systems for African Crops. The Rockefeller Foundation and the Kenya Agricultural Research Institute (KARI), Nairobi, Kenya 24-27 January 2005.

Musoni, A., Kimani, P. M., Buruchara, R., Narla, R. D. & Wagara, I. N. (2005). Characterisation and breeding for resistance against angular leaf spot, root rot and anthracnose pathogens in climbing beans. Presented at: Biotechnology, Breeding and Seed Systems for African Crops. The Rockefeller Foundation and the Kenya Agricultural Research Institute (KARI), Nairobi, Kenya 24-27 January 2005.

Opio, F., Ugen, M., Namayanja, I., Mugagga, I. & Mawejje, D. (2005). Improving food security in south-western Uganda by transferring and promoting resistant varieties and integrated management packages for bean root rot. Presented at: Biotechnology, Breeding and Seed Systems for African Crops. The Rockefeller

Foundation and the Kenya Agricultural Research Institute (KARI), Nairobi, Kenya 24-27 January 2005.

Otsyula, Rubaihayo, P., Buruchara, R., Mahuku, G. & Kimani, P. (2005). Inheritance and genetic characterization of resistance for use in developmnt of *Pythium* root rot-resistant bean varieties. Presented at: Biotechnology, Breeding and Seed Systems for African Crops. The Rockefeller Foundation and the Kenya Agricultural Research Institute (KARI), Nairobi, Kenya 24-27 January 2005.