CROP PROTECTION PROGRAMME

Bean Root Rot Disease Management in Uganda

DFID Project Ref. R8478, NR Int. Code ZA0689

FINAL TECHNICAL REPORT

1 April 2005 – 31 January 2006

Project Leader: Nicola Spence

Project team: Dr Robin Buruchara (CIAT), Mr John Carder (Warwick-HRI), Dr Gay Marris (CSL), Dr George Mahuku (CIAT), Dr Fina Opio (NAARI-NARO), Dr Sreenivasaprasad (Warwick-HRI),

International Development Team
Central Science Laboratory (DEFRA)

FTR completed March 2006

R8478 CROP PROTECTION PROGRAMME

Acknowledgements

The authors would like to acknowledge and thank the following persons who in one way or another contributed to the carrying out of this project:

- Dr A.C Levesque, collaborator
- The late Stephen Mayanja, a former research assistant,
- Virginia Gichuru, a PhD student,
- Walter Ocimati, an MSc student,
- Stephen Buah, a research assistant,
- Clare Mukankuzi, a research assistant.

R8478 CROP PROTECTION PROGRAMME

Table of Contents

R8478 CROP PROTECTION PROGRAMME

List of Tables, Figures and Plates

R8478 CROP PROTECTION PROGRAMME

List of Abbreviations

AHI	African Highlands Initiative
BAPPA	Beyond Agricultural Production to Poverty Alleviation
BRR	Bean Root Rot
CIAT	Centro Internacional de Agricultura Tropical
CPP	Crop Protection Programme
DFID	Department for International Development
ECABREN	East and Central Bean Research Network
EDTA	Ethylenediaminetetraacetic acid
FSP	*Fusarium solani* f. sp. *phaseoli*
FTR	Final Technical Report
FYM	Farm yard manure
GM	Green manure
HRI	Horticultural Research International
LSD	Least Significant Difference
MoUs	Memoranda of Understanding
NAADS	National Agricultural Advisory Services
NARO	National Agricultural Research Organisation
NARS	National Agricultural Research systems
NCBI	National Center for Biotechnology Information
NGO	Non-Government Organisation
PABRA	Pan African Bean Research Alliance
PDA	Potato Dextrose Agar
PCR	Polymerase Chain Reaction
RKN	Root Knot Nematodes
PRAs	Participatory Rural Appraisals
RFLPs	Restriction Fragment Length Polymorphism
SABRN	South Africa Bean Research Network
TBE	Tris Borate EDTA
TES	Tris, EDTA, and sodium chloride buffer
TSBF	Tropical Soil Biology and Fertility Institute

Executive Summary

Project R8478 sought to underpin promotion of sustainable control of bean root rots through resistant varieties and soil amendments by addressing key scientific constraints investigated in previous projects R7568 and R8316. In the first of three activities described below, methods for extracting DNA from various soil types are now being tested. By spiking samples with different concentrations of *P. ultimum* propagules, a standard curve for the estimation of *P. ultimum* oospores in soils is being established, using highly sensitive optimised molecular detection techniques. This will allow the relative effectiveness of available management options in reducing the inoculum levels of these pathogens in the soil to be critically assessed. Greenhouse trials to assess the effects of organic amendments (e.g. manure) have been initiated. These experiments are not yet complete, but the project team expect to see a correlation between incidence of root rots and the number of propagules estimated from the competitive PCR assay. This will then be used to determine the management option that has the greatest effect on *P. ultimum* levels. Final data will be available during March 2006, beyond the final date of the project.

Seventeen different isolates of species pathogenic to beans were screened for their potential use as biocontrol agents against the causative organisms of Bean root rot disease. Cultural assays designed to determine any antagonistic activities between them revealed that several isolates showed mutual inhibition on contact with the common soil fungus, *Mortierella* (isolate MS10). Screenhouse trials confirmed that MS10 was non-pathogenic to otherwise susceptible bean varieties, and a marked reduction in disease severity (up to 100%) was seen in treatments where MS10 was added as antagonist to pathogenic *Pythium* isolates. MS10 resulted in significant increases in root and shoot dry weights of infected plants, suggesting that *Mortierella* reduces the effects of disease by improving root development necessary for efficient nutrient uptake. Repeated application of MS10 could result in its build up in the soil, further suppressing root rot. It was also found that application of MS10 one week prior to planting reduced disease severity more than inoculation followed by immediate planting. Realizing the potential of MS10 and other candidate organisms will thus depend not only on developing a practical application method, but also upon inoculation time. The isolate that was antagonistic to *Pythium* species was identified as *Mortierella alpina*.

Manuals containing detailed protocols on identification and quantification of *Fusarium solani* fsp *phaseoli* and *Pythium* spp, to facilitate wider utilization of tools, methods and techniques that have been developed in previous CPP projects are being produced, particularly modifying protocols so that they will be useful under the sub-optimal conditions found in most NARS. Initial drafts of the diagnostic manual for *Pythium* and *Fusarium* spp have been developed. The information generated during project R8478 has been shared with Below Ground Biodiversity Project (hosted by the Tropical Soil Biology and Fertility Institute (TSBF) of CIAT). The project team have received requests for manuals from students and partners from Kenya, Uganda, Malawi and South Africa. Quantification protocols are being validated. The manuals containing detailed protocols on identification and quantification of *Fusarium* and *Pythium* species are already being shared with other researchers (e.g. TSBF) in other countries (Kenya and South Africa).

Background

The common bean *Phaseolus vulgaris* L. is the most important, widely grown (4 million ha annually) and consumed grain legume in Sub-Saharan Africa, mainly by resource poor farmers particularly women, hence the name “a woman’s 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 (Pacchio, 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, 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. Demand to address the root rot problem has been expressed by farmers, local political representatives, ministry of agriculture and NGO's (Africare, World Vision and Care); by bean networks (PABRA, ECABREN and SABRN) and other initiatives (AHI, BAPPA).

At least four *Pythium* spp. were previously 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* (another key causal agent of root rots) and *Pythium*. However, although progress in quantification was good for *Fusarium*, this proved to be more difficult for *Pythium*. The processes that involved 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.

Although the development of resistant host varieties remains a key area for research activity, other avenues for disease management need to be explored. Previous 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, such as the potentially antagonistic species *P. olgandrum*. 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.

Project Purpose

Root rots are the most serious threat 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 has been a persistent developmental constraint. The overall objective of project R8478 was to continue to underpin promotion of sustainable control of bean root rots through resistant varieties and soil amendments by addressing the key scientific constraints identified and investigated in previous projects R7568 and R8316. To this end, the specific aims of the current project, R8478, were as follows:

- Firstly, to adapt a quantitative assay (that combines classical and biotechnology techniques) for the most important root rot pathogens, e.g. *P*. *ultimum* var *ultimum*, and then use this assay to evaluate crop and management options effective in reducing/increasing the inoculum levels of these pathogens in the soil.
- Secondly, to assess the effects of alternative management options on the relative inoculum population of pathogenic species and that of potential antagonistic or bio-control species (e.g. *Pythium oligandrum*).
- Valuable tools (cultural and molecular) for identification and quantification of *Fusarium solani* fsp *phaseoli* and *Pythium* spp have been developed in previous CPP projects R7568 and R8316), and it has been shown that some of the *Pythium* species causing root rots in beans also affect other crops (e.g. sorghum, millets and peas often grown in association or in rotation with beans). This means that people/organisations who are likely to be interested in information, methods, and techniques developed for *Pythium* are therefore not only limited to researchers working on beans, but may include researchers working on other crops affected by *Pythium* pathogens in the PABRA region. The third aim was to develop manuals containing detailed protocols on identification and quantification of *F. solani* fsp *phaseoli* and *Pythium* spp based on our experiences during the past and current projects (R7568, R8316), to facilitate wider utilization of tools, methods and techniques that have been developed.

These research activities will benefit researchers of national bean programme in NARO (Uganda), bean networks (ECABREN and SABRN), and PABRA; researchers working on other crops affected by Pythium species, other service providers and NGOs.

Research Activities and Outputs

1. Develop and validate a quantitative assay for key *Pythium spp*

1.1 Develop and validate a quantitative assay combining classical and biotechnology techniques for key species of *Pythium* pathogenic to beans and bio-control species *P. oligandrum*.

The amplification of *M. phaseolina* genomic DNA under low stringency annealing conditions resulted in the production of several PCR fragments. Two fragments of 250 and 190 bp in size were excised from the gels, purified and cloned into pGEM T-easy vector. These fragments were 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 pBluescript. In competitive PCR, it is extremely important to have a competitor with a greater degree of similarity (size fragment) to the target, so as to allow for more even amplification efficiencies. In addition, the two fragments should be easily distinguished upon electrophoresis. The usefulness of the designed heterologous probe was tested by amplifying different concentrations of competitor DNA in the presence of a fixed amount of target DNA (Figure 1). As the concentration of competitor DNA decreases, the intensity of the amplified target DNA increases. 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. The sensitivity of detection was measured by amplifying known concentrations of the control DNA. The assay could detect down to 100 fg of target DNA (Figure 2).

To apply the developed competitive PCR assay for the estimation of *P. ultimum var ultimum* propagules in soil samples and hence, use it to evaluate crop and management options effective in reducing/increasing the inoculum levels of these pathogens in the soil, it is necessary to develop standard curves re. the efficacy of this method to detect oospores of *P. ultimum* in different soil types and conditions. Several methods for producing *P. ultimum* oospores were evaluated, and it was found that Corn meal agar amended with 100 ml of compost infusion, or a solution of cane molasses (30g/ml of molasses), were equally effective in producing *P. ultimum* oospores, each consistently giving 10^5 oospores/ml of medium.

A standard curve for the estimation of *P. ultimum* oospores in soils was developed based on the ratio between target DNA (obtained from known numbers of oospores) and control (competitor) DNA. Sterile field soil spiked with different concentrations of *P. ultimum* oospores was used for the development of the standard curve. The project team are still 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 *P. ultimum* DNA from soils are currently being tested, by extracting DNA from known concentrations of *P. ultimum*

oospores spiked into soils differing in clay, humus and organic matter content, so as to optimize the conditions under which this assay could be applied for the the estimation of *P. ultimum* propagules directly in soil.

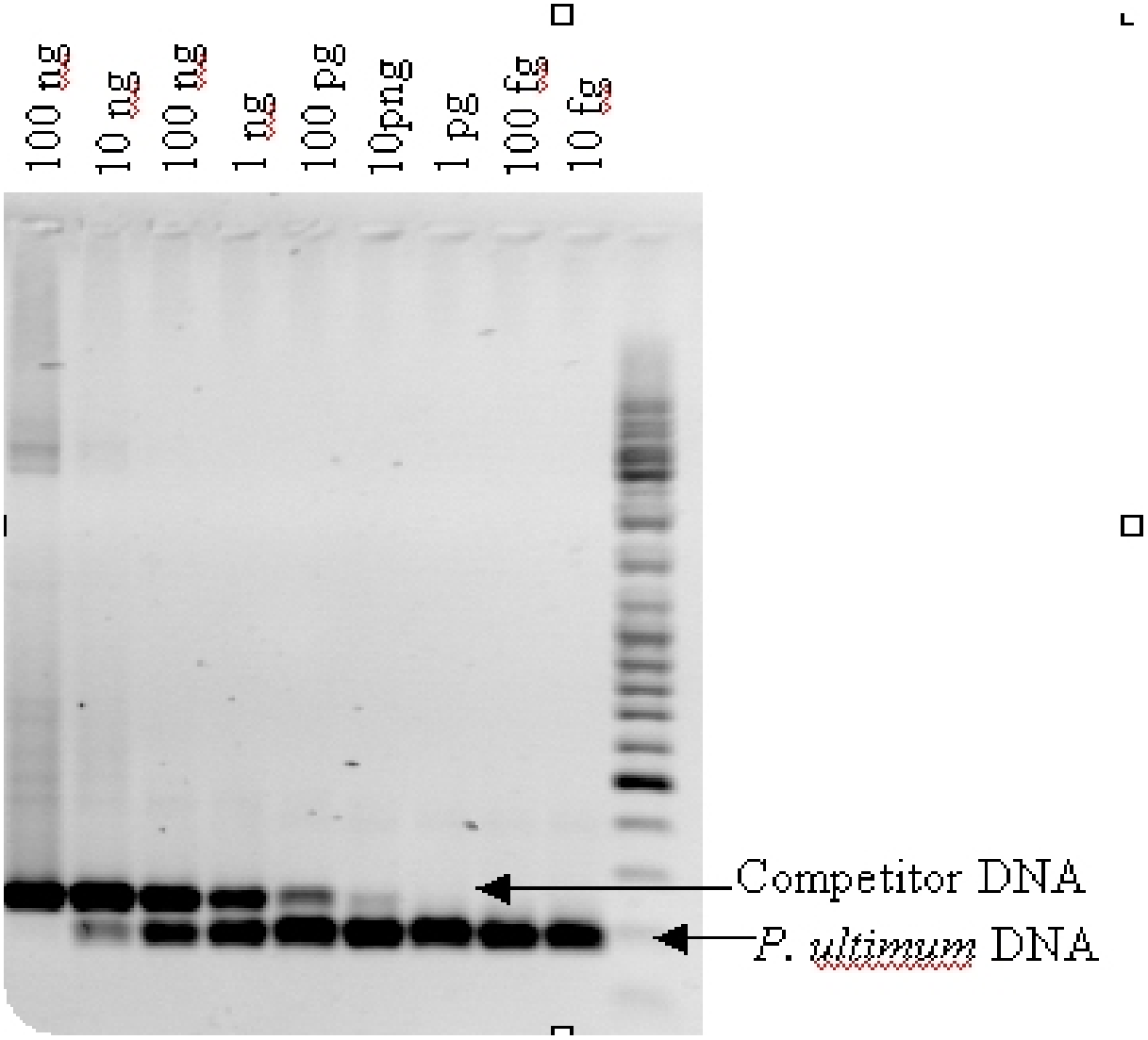


Figure 1. Competitive PCR products of *P. ultimum*.
A constant unknown concentration of *P. ultimum* DNA was co amplified in the presence of competitor DNA ranging from 100 ng to 10 fg. At 1 ng concentration of competitor DNA shows an almost 1:1 ratio of control: *P. ultimum* DNA concentration.

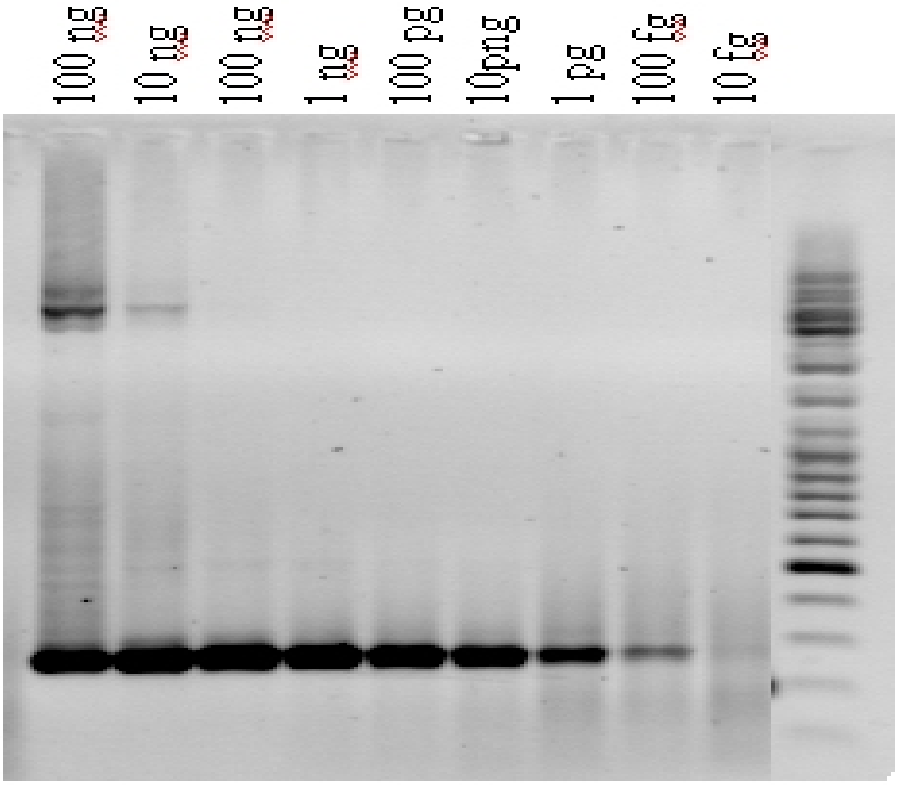


Figure 2. Sensitivity of the competitive PCR assay to detect target DNA.

1.2. Evaluate the effect of management practices on soil inoculum of target species using the developed (quantitative) assay.

This activity is ongoing and started late because it is dependent on results from Activity 1. The activities include: (i) collection of soil samples from ongoing testing of different management options for *Pythium* root rots, (ii) evaluation of the incidence of *Pythium* root rots under screenhouse conditions using a highly susceptible bean genotype (to make sure that conditions are conducive for diseases development); (iii) testing of infected plants using PCR assay with species specific primers (to identify the species responsible for inciting disease); (iv) DNA extraction directly from a portion of the same soil and estimation of *P. ultimum* propagule levels using the competitive PCR assay. Disease incidence results for each soil (including controls that have not received any treatment) are compared with the estimated propagule levels to establish correlations between pathogen levels and disease levels and evaluate the effect that the management option has on *P. ultimum* propagule levels.

1.2.1. Materials and Methods

Soil samples have been collected from ongoing experiments to test the effect of different management options particularly organic amendments, such as farm yard manure and green manures etc. A susceptible genotype has been grown, and assessment carried out under constant controlled greenhouse conditions, selected to promote optimal disease development. Root rot incidence was measured according to the severity of disease symptoms/levels of mortality in infected plants. PCR with species-specific primers was used to distinguish between species, using DNA extracted from infected plants. DNA was extracted from portions of the soil used for growing on tests, and the number of *P. ultimum* propagules estimated using the competitive PCR assay developed in Activity 1.1. Competitive PCR assay results were correlated to disease incidence, and used to assess the effect of management options on the levels of *P. ultimum* in soils, when compared to control experiments (receiving no management option).

1.2.2. Results

These experiments are not yet complete, but the project team expect to see a correlation between incidence of root rots and the number of propagules estimated from the competitive PCR assay. This will then be used to determine the management option that has the greatest effect on *P. ultimum* propagules. The use of soil collected from plots receiving different treatments is used as an indirect measure of the effect of that treatment on the population levels of *P. ultimum* in the soil. These results would provide a further validation of the molecular quantitative assay developed under Activity 1.1 and its utility or application in evaluating the effect of different management options on *P. ultimum* propagule levels in the soil. It is anticipated that these results will be available in March 2006, regrettably after the end date of the project.

2. Assess the effects of management options on the relative inoculum population of pathogenic species, and that of potentially antagonistic or bio-control species.

2.1 Assess the effect of potential antagonistic species on species pathogenic to beans under screenhouse and field conditions by using both classical and multiplex PCR and quantitative assay to detect their levels.

2.1.1. Materials and Methods

Preserved isolates (MS46, MS49, MS11, MS47, MS34, DFD47, KIS4, MS15, MS27, MS6, MS66, KLE3A, MS61 (*P. ultimum*), VIH4, KB4, MS10 (*Mortierella*) and KB14) were screened for their potential use as biocontrol agents. The antagonistic activity of the isolates were determined in a dual culture assay in which opposite ends of the Potato dextrose agar (PDA) plate is inoculated with an isolate and incubated at 24^{o}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.

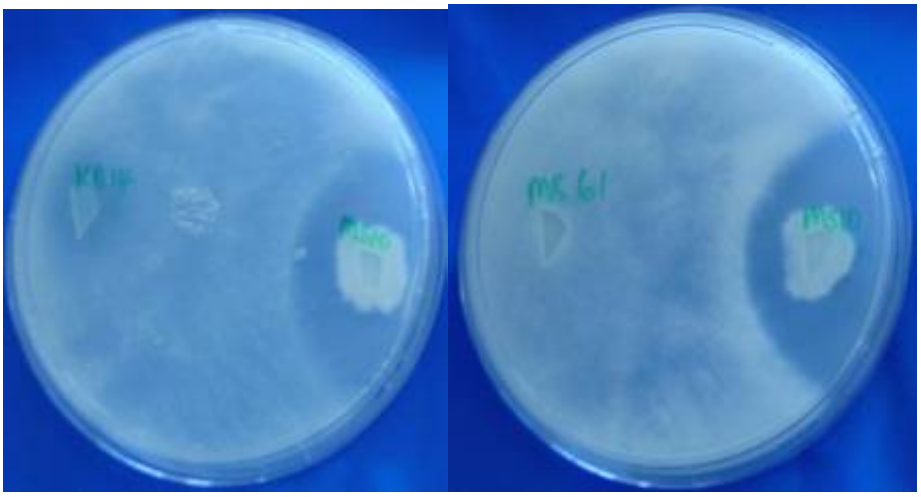


Plate 1: Level 3 of KB14 and MS61 vs MS10 on culture

In culture, four isolates (KB 14, KB 4, VIH 4 and MS 61) showed mutual inhibition on contact (Level 3) with MS10 whereby the space between the two organisms was small but clearly marked (Plate 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.

2.1.2. 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 with soil in a ratio of 1:8 v/v inoculum to soil, in a wooden flat tray of 42 cm x 72 cm, and left to stabilize for one week before antagonist MS10 was added in the same ratio and mixed with the soil. Planting was done after one week. MS10 was not added to control trays of each of the isolates which served to confirm their pathogenicity. 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 after which MS10 was added and planting done immediately. This was aimed at assessing the appropriate planting time after inoculation. Twenty seeds of susceptible bean varieties CAL96 and K20 as well as resistant bean variety (RWR 719) were planted in two rows, each row consisting 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 (Plates 2 and 3), rinsing the roots and scoring them according to the CIAT 1-9 scale with the following meanings: 1- no visible symptoms, 3- little symptoms, 5- moderate 7- severe 9- complete discolouration of the tap root.

Plate 2: KB4 alone

Plate 3: KB4 vs MS10

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).

2.1.3. Results and Discussion

Single isolate treatments of KB14 (*P. ultimum*), KB4 (*P. vexans*) and MS61 (*P. ultimum var. ultimum*) 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 1). VIH4 (*P. ultimum*) and MS10 (*Mortirella*) isolates were revealed as non-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 1. Effect of different isolate combinations on root rot severity on 3 bean varieties

Treatments	***Bean Varieties***			
	CAL 96	**K 20**	**RWR 719**	**Mean**
KB14[x]	9.00[a]	9.00[a]	1.00[j]	6.33
KB 14 vs MS10[x]	5.80[b]	3.90[e]	1.10[j]	3.60
KB 14 vs MS10[y]	4.50[d]	5.85[b]	1.00[j]	3.78
VIH4[x]	2.06[gh]	2.80[f]	1.00[j]	1.95
VIH4 vs MS10[x]	1.00[j]	1.00[j]	1.00[j]	1.00
VIH4 vs MS10[y]	1.00[j]	1.00[j]	1.00[j]	1.00
KB4[x]	9.00[a]	9.00[a]	1.00[j]	6.33
KB 4 vs MS10[x]	1.47[e]	1.00[j]	1.00[j]	1.16
KB 4 vs MS10[y]	2.59[fg]	1.75[hi]	1.00[j]	1.78
MS61[x]	9.00[a]	9.00[a]	1.00[j]	6.30
MS61 vs MS10[x]	1.11[j]	1.00[j]	1.00[j]	1.03
MS61 vs MS10[y]	4.06[de]	5.11[c]	1.00[j]	3.39
MS10[x]	1.00[j]	1.00[j]	1.00[j]	1.00
Mean	3.97	3.95	1.00	2.97

a) Means within column followed by similar letters are not significantly different at P< 0.05.
b) [x] = Planted one week after inoculation
c) [y] = Both isolates added same day and planted immediately

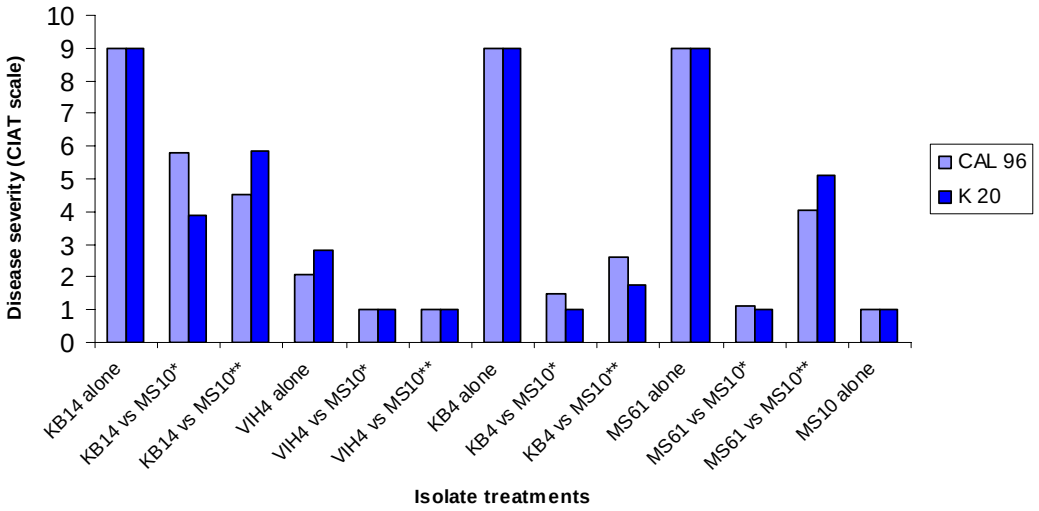
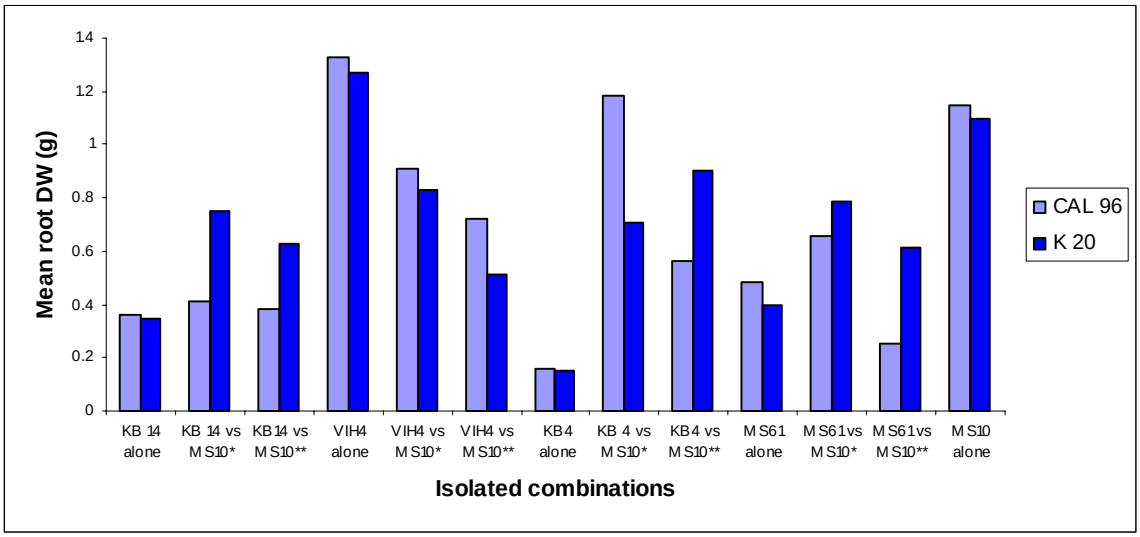


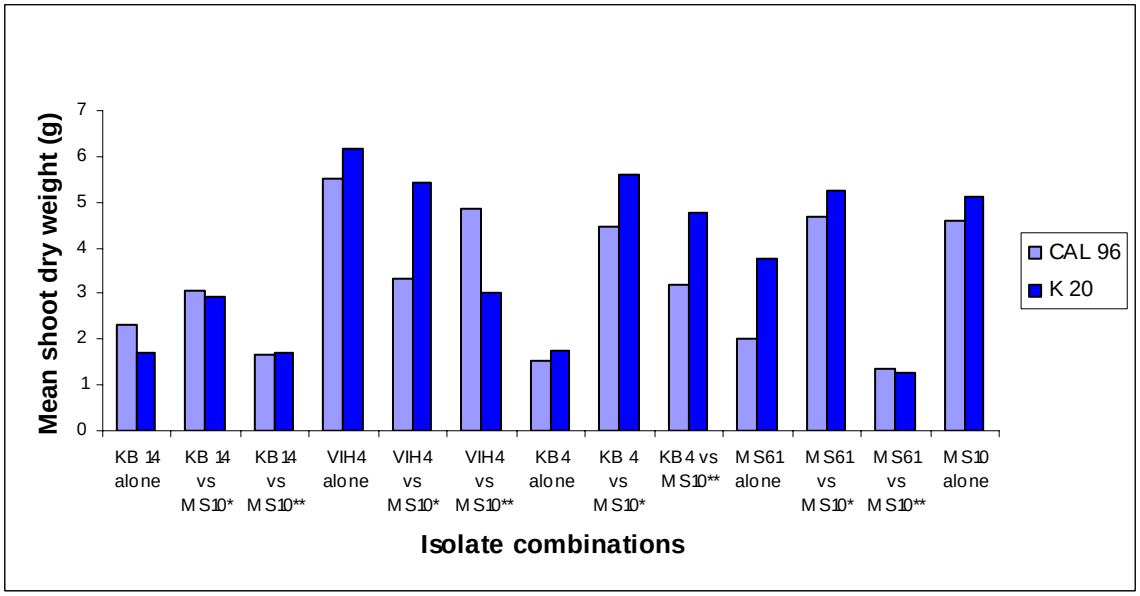
Figure 3. The effect of MS10 on pathogenic *Pythium* isolates*
(* = Planting done one week after addition of antagonist MS10; ** = Planting done immediately after addition of MS10)

The results further indicate that application of MS10 one week prior to planting reduces disease severity better than inoculation followed by immediate planting. The one week interval before planting probably allows the MS10 to colonize the soil and hamper further activity of the pathogens. Realizing the potential of MS10 and other candidate organisms will depend on developing a practical application method and inoculation time. Microbial parasites need time to find their host and infect. In further experiments, we will screen more isolates for antagonism and also determine the appropriate incubation time before planting.

Addition of MS10 into pathogenic isolates resulted in significant increase in root and shoot dry weights of susceptible varieties CAL 96 and K20 (Fig. 2 and 3). Comparatively high disease severity resulted in reduced root and shoot dry weight. For example KB4 has the highest disease severity and the lowest root dry weight. This indicates that isolate MS10 may have promise for reducing *Pythium* root rot because it improves root development necessary for efficient nutrient uptake by the plant as evidenced by increase in shoot dry weights.

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 that further suppress the root rot and increase root dry weight. However, inoculum carrier used in this experiment is millet grain, used at a ratio of 1:8 which may not be an economic level for farmers to apply. The minimum amount of inoculum needed to have the same efficacy needs to be determined in our further studies. These results obtained from the screen house experiments were with limited number of plants need to be validated in field situations.





* = Planting done one week after addition of antagonist MS10
** = Planting done immediately after addition of MS10

Figure 4. The effect of MS10 on mean root (top) and shoot (bottom) dry weight of susceptible bean varieties CAL 96 and K20

2.2. Assess the effect of management practices (organic amendments) on the levels and survival rate of these biocontrol agents under controlled and field conditions, using the multiplex and quantitative assays.

In previous project R8316 a multiplex PCR assay was developed to simultaneously detect and identify 6 *Pythium* species that are pathogenic to beans and *P. oligandrum*, a common bio-control agent. However, both field and greenhouse evaluations of other potential bio-control agents against pathogenic *Pythium* species showed that a common soil fungus, *Mortierella* sp., was more effective (see above). Moreover, isolates of *P. oligandrum* that were isolated from the region were found to have little or no effect on major species *Pythium* pathogenic to beans. Consequently, efforts were made to first characterize isolates of *Mortierella* that showed good bio-control properties.

2.2.1. Materials and Methods

A plug of pure culture from PDA was cut from the growing margin of cultures and placed in sterilized 20% V8 juice broth containing 2.5 g of $CaCO_3$ and incubated in darkness at room temperature for four days. Mycelia was harvested from V8 using sterilized forceps, blotted of excess juice, placed in sterile Eppendorf tubes and kept at -20ºC. DNA was extracted from harvested mycelia according to the procedure described by Mahuku (2004). Briefly, mycelia were ground to a fine paste in a mortar containing TES extraction buffer (0.2 M Tris-HCl [pH 8], 10 mM EDTA [pH 8], 0.5 M NaCl, 1% SDS) and sterilized acid-washed sea sand. Additional TES buffer containing Proteinase K was added and the mixture incubated at 65ºC for 30 min. DNA was precipitated using ice-cold isopropanol and the pellet was washed twice with 70% ethanol, dried and dissolved in TE buffer (10 mM Tris-HCl [pH 8], 1 mM EDTA). PCR analysis was performed using Oomycete ITS region primers to differentiate *Pythium* from other closely related fungi. The PCR reaction was performed in 50µl-reaction volumes containing 1X PCR buffer, 2mM $MgCl_2$, 0.1µM of each dNTP, 0.5 µM of each primer 18S and 28S, 20 ng of DNA, and 1.25 U Taq DNA polymerase. Amplification was performed in a Thermal Cycler programmed for 30 cycles of denaturation at 94ºC for 1 min, annealing at 68ºC for 2 min, and extension at 72ºC for 1 min, followed by a final extension for 8 min at 72ºC. The products were run on 2% agarose gels containing 5mg/ml of ethidium bromide. 1XTBE (Tris Borate EDTA buffer) was the running buffer at a voltage of 100V. At the end of electrophoresis, agarose was placed on a UV light table and a photo captured using a Polaroid camera. The amplified products were purified using QIAquick PCR purification Kit (QIAGEN, 1997) and sent to Guelp Molecular Supercenter at the University of Guelph, Ontario-Canada for automated sequencing. The resultant sequences were edited for accurate peak scorings and later subjected to a nucleotide-nucleotide blast search at National Center for Biotechnology Information (NCBI) website. The sequences were compared to 10 most relevant matches in the Gene Bank and the best match was taken as the species of the isolate.

2.2.2. Results and Discussion

The isolate that was antagonistic to *Pythium* species was identified as *Mortierella alpina*. PCR primers specific to this species will now be designed based on sequences differences in the internal transcribed spacer region 1 of the ribosomal DNA. When these have been developed, they will allow the relative incidence and survival rates of *Mortierella* to be monitored in the presence of various different soild amendments. This data will, in turn, provide insight into the effectiveness of this potential biocontrol agent, and which management options increase or decrease levels or effectiveness. This will lead to formulation of guidelines that will help in the management of pathogenic species while increasing the levels of beneficial ones in the soil.

2.3. Evaluate effect of different organic amendments (management strategies) on the interaction between potential biocontrol agents and pathogenic species (*P. ultimum*) on the population dynamics of the two, both under controlled conditions and under natural conditions.

Previous project 8316 undertook a study into the impact of bean root rot management practices on the productivity of other crops associated with beans This 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. Four management options (farm yard manure (FYM), green manure (GM), 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 FYM and GM 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.

As in screen house studies, infected control plants exhibited clear symptoms of root rot disease, including stunted growth, purple leaves, shoot death and dark-red to black root lesions (Plate 4). The management options evaluated affected the tested crops in different ways, but generally it was found that they reduced root rot incidence and severity considerably, relative to the control (Plate 5). Plant recovery was evident in plots amended with GM, FYM and NPK. FYM and Ridomil significantly reduced initial root rot infection on beans. In addition, FYM, GM and NPK, enhanced root (mass) growth Plate. FYM seemed the most effective, resulting in lower incidence and severity in all seasons compared to other treatments.

Together these findings demonstrate 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 could therefore be exploited for developing a management strategy combining two or more compatible control methods. The results reported under Activities 1.2 and 2.2 of the present study will allow levels of *P. ulltimum* and

*Mortierella to be*quantified. Hence, such techniques can be readily applied to assess the relative effects of different soil amendments on both pathogenic species, and also on their potential bio-control agents, respectively.

Plate 4. Severely affected roots in control plants

Plate 5. Root development in plant grown in amended plot

3. Develop manuals containing detailed protocols on identification and quantification of *Fusarium solani* fsp *phaseoli* and *Pythium* spp, disseminate them, and validate protocols that they contain.

3.1. The development of manuals

Valuable tools (cultural and molecular) for identification and quantification of *Fusarium solani* fsp *phaseoli* and *Pythium* spp have been developed in previous CPP projects (R7568, R8316), and it has been shown that some of the *Pythium* species causing root rots in beans also affect other crops (e.g. sorghum, millets and peas often grown in association or in rotation with beans). This means that people/organisations who are likely to be interested in information, methods, and techniques developed for *Pythium* are therefore not only limited to researchers working on beans, but may include researchers working on other crops affected by *Pythium* pathogens in the PABRA region. The dissemination of these techniques (through manuals and workshops) could greatly enhance researchers' capacity to apply them, and also facilitate a more effective assessment of management options. Development of protocols, research and training manuals on diagnostic and quantification methods should thus be valuable capacity development resources. This project sought to develop manuals containing detailed protocols on identification and quantification of *Fusarium solani* fsp *phaseoli* and *Pythium* spp, to facilitate wider utilization of tools, methods and techniques that have been developed. Special effort has been made to adapt and modify these protocols such that they will be useful under the sub-optimal conditions found in most NARS. Initial drafts of the diagnostic manual for *Pythium* and *Fusarium* spp have been developed .Sections dealing with quantification are not yet complete, pending final results (due March 2006).

3.2. Validate protocols with collaborators to ensure that they function and are reproducible under different environmental conditions and settings.

The information has been shared with Below Ground Biodiversity Project (hosted by the Tropical Soil Biology and Fertility Institute (TSBF) of CIAT). The project team have received requests for manuals from students and partners from Kenya, Uganda, Malawi and South Africa. Quantification protocols are being validated. The manuals containing detailed protocols on identification and quantification of *Fusarium* and *Pythium* species are already being shared with other researchers (e.g. TSBF) in other countries (Kenya and South Africa).

3.3. Publish and distribute the manuals to both bean and non-bean researchers.

The manuals will be published (and distributed) when all components are ready.

Contribution of Outputs to Developmental Impact

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 has underpinned such seed production and promotion initiatives. The project has also linked 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 was also based. Project R8478 also linked 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. The development of manuals for cultural and molecular identification and quantification of key root rot pathogens will be a valuable learning resource for researchers working in other regions and other production systems.

Future Prospects

Once the standard curve for the estimation of *P. ultimum* oospores has been established, it will be possible to critically assess relative effects of soil amendments in reducing inoculum levels of pathogenic species in the soil. This in turn, will allow the best soil management options to be recognised. Now that the potential bio-control agent screened in R8478 has been identified, specific PCR primers can be designed based on sequences differences in the internal transcribed spacer region 1 of the ribosomal DNA. Once developed, these will allow the relative incidence and survival rates of *Mortierella* to be monitored in the presence of various different soil amendments. This data will, in turn, provide insight into the effectiveness of this potential biocontrol agent, and which management options increase or decrease levels or effectiveness. This will lead to formulation of guidelines that will help in the management of pathogenic species while increasing the levels of beneficial ones in the soil. Valuable tools (cultural and molecular) for identification and quantification of *Fusarium solani* fsp *phaseoli* and *Pythium* spp have been developed in previous CPP projects (R7568 and R8316), and it has been shown that some of the *Pythium* species causing root rots in beans also affect other crops (e.g. sorghum, millets and peas often grown in association or in rotation with beans). This means that the manuals produced in project R8478 should usefully be distributed not only to researchers working on beans, but also to researchers working on other crops affected by *Pythium* pathogens in the PABRA region. There now exists the potential for the development of further diagnostic training resources to be used in other parts of Africa, to underpin epidemiology studies on plant pathogens.

References

Abawi, G.S. & Pastor-Corrales, M.A. (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.

Buruchara, R. A., Pastor-Corrales, M. A. & U. Scheidegger, U. (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

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

Hendrix, F. F. Jr. & Campbell, W. A. (1973) Pythiums as plant pathogens. *Annu. Rev. Phytopathol*. **11**:77-98.

Hendrix, F. F. Jr. & Campbell, W. A. (1983). Some Pythiaceous fungi. New roles for old organisms. Pages 123-160. *In*: Zoosporic Plant Pathogens. A Modern Perspective. S. T. Buczacki, ed. Academic Press, London.

Mahuku, G. S. (2004) A simple extraction method suitable for PCR-based analysis of plant, fungal, and bacterial DNA. *Plant Mol. Biol. Rep.,* .**22**: 1–11.

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.

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.

Dissemination Outputs

Published Papers/Conference Proceedings etc.

Buruchara, R.A. (2003) Integrated management strategies for bean root rots in Africa. Centro Internacional de Agricultura Tropical (CIAT). (Highlights: CIAT in Africa no. 2).

Buruchara, R. A. (2005) Application of biotechnology in bean disease management. CIAT in Africa Highlights 2p

Buruchara, R. A., Mahuku, G., Mukalazi, J. & A. Levesque, A. (2005) *Pythium* species associated with Pythium root rot of beans (*Phaseolus vulgaris* L) in Eastern Africa. 2005. Poster presented at the American Pythopathological Society, Annual Meeting, July 30 – August 3, Austin, TX, USA.

Gichuru, V., Ocimati, W., Buruchara, R., Okori, P., Tusiime, G., Opio, F. & Ugen, M. A. (2005) The role of other crops in developing integrated pest management (IPM) of bean root rots. Pan Africa Workshop on Integrated Pest and Nutrient Management, 31 October – 3rd November 2005.

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. Proceedings of the VIth African Crop Science Conference, 12-17 Oct 2003, Nairobi, Kenya.

Mukulazi, J. (2004) *Pathogen variation and quantification of* Pythium *species in bean fields in Uganda. Makerere University, Uganda, PhD thesis.*

Opio, F., Buruchara, R. A. & Ugen, M. (2005) Integrated disease management for the bean root rot disease in Uganda. 2005. Pan Africa Workshop on Integrated Pest and Nutrient Management, 31 October – 3rd November 2005, Kampala, Uganda.

Spence, N.J., Buruchara, R. A., Mahuku, G., Carder, J., S. Sreenivasaprasad, S., Opio, F. & Marris, G.C. (2006) Bean root rot disease management in Uganda. In: Perspectives on Pests. Achievement of research under the UK DFID Crop Protection Programme. pp. 34-35.

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. Proceedings of the VIth African Crop Science Conference, 12-17 Oct 2003, Nairobi, Kenya.

Tusiime, G. (2004) *Pathogen variation and quantification of* Fusarium *species in bean fields in Uganda*. Makerere University, Uganda, PhD thesis.

Manuscripts in submission

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

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

Ocimati, W., Tusiime, G., Buruchara, R. A., Opio, F. & Ugen, M. The effects of different management practices of bean root rots on root rots of major crops in the bean based cropping system of southwestern Uganda. *In submission to African Crop Science Journal.*

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 Phytopathololologische Zeitschrift*.

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

Annual Reports

Annual Report 2005 (CIAT print and on the web):

- Characterization and distribution of *Pythium spp*. associated with other crops in a bean based cropping system in southwestern Uganda
- Pathogenicity of *Pythium* spp on beans, Kawanda, screen house
- Pathogenicity of *Pythium spp* and effects of management options for root rots on crops grown in association with beans in southwest Uganda
- Assessment of the potential of candidate organism as a biocontrol agent against Pythium root rot
- Develop multiplex PCR assay for simultaneous detection of 6 *Pythium* species in common bean soils.
- Development of a molecular-based quantitative assay for *Pythium* species

Manuals

The manuals containing detailed protocols on identification and quantification of Fusarium and Pythium species are already being shared with other researchers (e.g. TSBF) in other countries (Kenya and South Africa)

Project Progress Reports

Crop Protection Programme PPR1 – April-September 2005