

# Pathogenic and molecular characterisation of *Pythium* spp. inducing root rot symptoms in other crops intercropped with beans in Southwestern Uganda

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## ABSTRACT

**Objective:** In Southwestern Uganda, bean root rot epidemics associated with *Pythium* species are frequent despite the use of various management methods. This study set out to determine whether *Pythium* root rots affect other crops in bean cropping system of Southwestern Uganda and to characterise the *Pythium* species using Internal transcribed sequence (ITS) DNA primers.

**Methodology and Results:** Root rots were found to occur on maize, sorghum, peas and potato sampled from farmer's fields where they were found to be intercropped with beans affected by root rot. *Pythium* species were isolated using Corn meal agar (CMA). DNA was subsequently extracted and polymerase chain reaction (PCR) analysis carried out using ITS DNA region primers and then the PCR products were sequenced. Twenty-one *Pythium* species were isolated. Cross pathogenicity tests were done in a screen house using bean pathogenic *Pythium* species and *Pythium*, species derived from other crops intercropped with beans. The *Pythium* species were moderately to non-pathogenic in maize and millet while those in sorghum and peas were highly pathogenic.

**Conclusion and application of results:** This study found that other crops intercropped with beans in Southwestern Uganda were affected by *Pythium* root rots. Peas and sorghum were highly susceptible to the pathogenic *Pythium* species and could therefore be contributing to the continuous bean root rot epiphytotics in the region. On the other hand, maize and millet were non-pathogenic to the *Pythium* species. This knowledge could be used to advice farmers not to include sorghum and peas when intercropping with beans, as they seem to be contributing to the *Pythium* inoculum load. They could alternatively use maize and millet, as they are not susceptible to the pathogenic *Pythium* species.

**Key words:** *Pythium*, root rots, Southwestern Uganda, ITS DNA, pathogenic

## INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is the second most important source of human dietary protein and the third most important source of calories of all agricultural commodities produced in Eastern and Southern Africa (Sarikamis et al., 2009).

Eastern Africa has the highest bean production in sub-Saharan Africa at 75,369 metric tonnes per annum (FAO,2004).In Uganda, beans are grown throughout the country but especially in the cool highlands of Southwestern Uganda (Mukalazi,

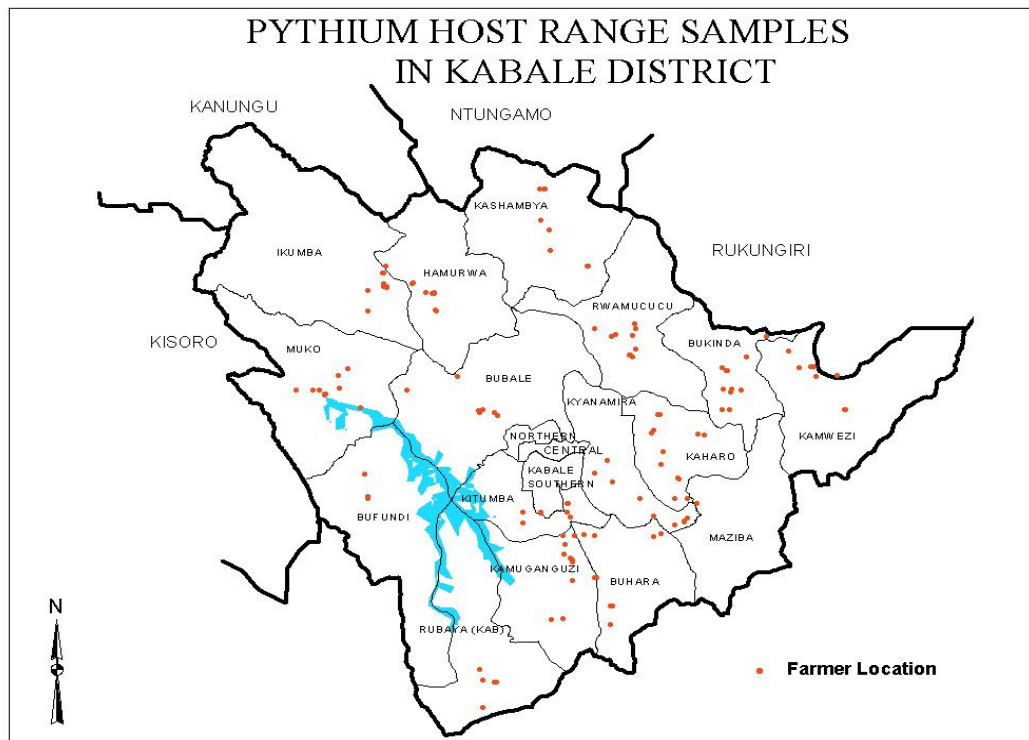
2004). These areas lie at an altitude of 800-2,300 metres above sea level. In most parts of Africa, common beans are grown as an intercrop with other food crops, especially maize. Such cropping methods with little or no added inputs such as fertilisers or pesticides, do not allow beans to realise their maximum yield potential (Nderitu *et al.* 1997). Consequently under such production systems, the biotic and abiotic constraints seriously reduce yield. One of the biotic constraints affecting bean production is root rots. The occurrence and severity of root rot disease is associated with high intensity of bean growing attributed to lack of crop rotation. Root rots are caused by one or more soil borne pathogens either alone or as a complex of two or more pathogens depending on environmental conditions (Rusuku *et al.*, 1997). Bean root rots are characterised by above ground symptoms such as poor seedling establishment, uneven growth, chlorosis and premature defoliation of severely infected plants (Abawi and Ludwig, 2005; CIAT 2005). A study done on farmers' fields in Southwestern Uganda, found differing root rot lesions on maize, sorghum, peas and beans (CIAT, 2005). In Southwestern Uganda, bean root rot epidemics associated with *Pythium* spp. are frequent (Rusuku *et al.*, 1997). Various management options such as use of resistant cultivars and crop rotation have been used to control root rots of beans (Abawi and Pastor-Corrales, 1990). In Africa, the integration of organic amendments, raised beds and resistant

varieties has been shown to be advantageous over single components in controlling the severity of root rots and yield (Buruchara and Scheidegger, 1993; CIAT, 1993). Studies on root rots have indicated that continuous cropping of beans, which is a common practice in East Africa, exacerbates the root rot problem (Rusuku *et al.*, 1997). In most cases due to population and land pressure, beans are often intercropped with other crops (Ocitti p'Obwoya, 1996). The objectives of this study are firstly to determine whether root rots occur in other crops in a bean based system in Southwestern Uganda. Secondly, to characterise using ITS DNA sequences those *Pythium* species responsible for *Pythium* root rots on those crops intercropped with beans. This is to ascertain if new *Pythium* species are implicated in bean root rot epidemics in the region. It is known that the bean-*Pythium* pathosystem has been subjected to selection pressure which could result in parasite population modification (CIAT, 1992). The aim of the study would also be to understand if other crop species in the system are influencing root rot epidemics. Since *Pythium* variability on beans is known, Koch's postulate will be used to investigate the cross pathogenicity properties of *Pythium* species on other crops in the bean-based system of Southwestern Uganda. The aim would be to generate information, which is critical for formulating integrated management options for bean root rot epidemics.

## MATERIALS AND METHODS

Sample collection and purification of *Pythium* spp.:  
Samples of crops intercropped with beans with or without

typical symptoms of root rot were collected from bean root rot fields in Southwestern Uganda (Fig 1).



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

Samples were picked from a pre-determined position using a grid distance of 5 to 10 km. In each of the sampled fields, crops were selected following a “W” pattern. Ten plants were sampled for tall crop species and twenty for short crop species. The sampling procedure was repeated in three different years/seasons. Root rot symptoms on crops were observed and noted during sampling. These symptoms included rotten stems in sorghum and reduced root system in pea and sweet potato (Table 2). Once the crop samples were collected, the isolation procedure described by White (1988) was used to isolate the *Pythium* species from root pieces of the crops. A selective medium was prepared by mixing corn meal agar, CMA (20g) and deionised water (1000ml) before autoclaving at 15 psi for 20 minutes. The antibiotic preparation (rifamycin (45 µg/ml) and pimarcin (100 µg/ml) were added on cooling the media to 40°C. Isolations from crop samples were done by first washing soil from plant tissues in a jet-stream of tap water, rinsing twice in sterile distilled water, blotting dry on new paper towel, and placing infected root pieces (approximately 0.5-2cm long) cut from expanding lesions on the prepared selective medium (CMA). These Petri dishes with CMA were incubated at room temperature (20-25°C) for 2-5 days. The *Pythium* mycelia developing from plant tissues were transferred onto potato dextrose agar (PDA) slants.

**DNA extraction:** After two to three days, a plug of pure culture from Potato Dextrose Agar (PDA) was cut from the growing margin of cultures and placed in sterilised 20% V8 juice broth containing 2.5 g of CaCO<sub>3</sub>. After four days of incubation in darkness at room temperature, mycelia was harvested using sterilised forceps, filtered through a layer of 85-mm filter paper and blot dried of excess V8 juice with paper towels. DNA was extracted from the harvested mycelia according to the procedure described by Mahuku (2004). Mycelia were ground to a fine paste in a mortar containing TES extraction buffer (0.2 M Tris-HCl (pH8), 10mM EDTA (pH 8), 0.5M NaCl, 1 % SDS) and sterilized acid-washed sea sand. Additional TES buffer containing proteinase K was added and the mixture incubated at 65°C for 30 minutes. DNA was precipitated using ice-cold isopropanol and the pellet was washed twice with 70% ethanol, dried and dissolved in TE buffer (10mM Tris-HCl (pH8), 1mM EDTA). **Polymerase Chain Reaction:** PCR analysis was done using oomycete ITS (Internal Transcribed Sequence) region primers to differentiate *Pythium* from other closely related fungi (White *et al.* 1990). The PCR reaction was performed in 50 µl final reaction volume containing 5µl of 1.25 mM dNTP, 0.2µl of each primer (20µM) (18S (5'-TCC GTA GGT GGT GAA CCT GCG G-3') and 28S (5'-TCC TCC GCT TAT TGA TAT GC-3') 20 ng of DNA and

0.2 µl Taq DNA polymerase (5 U/µl) (CIAT Virology Laboratory, Cali, Colombia). Amplification was performed in a Primus 96 plus Thermal Cycler (MWG-Biotech, Germany), programmed for 35 cycles of denaturation at 94°C for 1 min, annealing at 68°C for 2 min, and extension at 72°C for 1 min. At the end of the amplification reaction, a final extension step was achieved at 72°C for 7 minutes. The products were run on 2% agarose gels containing 5mg/ml of ethidium bromide in a TBE (1 time concentrated) as the running solution. The electrophoretic migration was carried out during 2 h under a 100 V (voltage). The amplified products were visualised and photographed under UV light. To estimate the size of the PCR products, a 100bp (base pair) molecular ladder was used. The negative controls were PCR amplifications containing no DNA template. Sequencing of amplified DNA and *Pythium* identification: All the PCR products having a size of 800 bp were submitted to sequencing procedure. Residual primers and dNTPs in the PCR products were removed using QIAquick™ PCR purification spin columns, following the manufacturer's protocol (QIAGEN, Crawley, UK). Direct sequencing of the PCR amplified products was carried out using ITS 2 primer (White *et al.* 1990). Sequencing reaction of the double stranded DNA templates were carried out using the ABI Prism™ Dye terminator cycle sequencing ready reaction kit (Applied Biosystems, CA-USA). The products were purified by ethanol precipitation following the manufacturer's protocol and nucleotide sequences were determined by the ABI 377 automated sequencing technology (Applied Biosystems, CA-USA). Sequences from ITS 1 region of the ribosomal gene were edited using the BioEdit programme (DNASTAR Inc., Madison, Wis, USA). The ITS 1 sequences were aligned to sequences deposited at the National Centre for Biotechnology Information (NCBI Gene Ban) using blastN (<http://www.ncbi.nih.gov/BLAST>.) Multiple alignments of the sequenced ITS product was performed for comparison. *Pythium* sequences obtained were aligned with Clustal X (Thompson *et al.*, 1994). Consequently, sequences were saved in Phylip format and used for phylogenetic analysis. A neighbour-joining tree was drawn using Clustal X and the boot strapping done to generate trees using 1000 replications. The Tree View software was used to view trees. Pathogenicity analyses of the *Pythium* spp.: Cross pathogenicity tests of some crop species with bean pathogenic *Pythium* species as well as *Pythium* species pathogenic to other crops found in a bean-based system were done. These experiments

were performed at the National Agricultural Research Laboratories, Kawanda. This site is located at 0°25' 05"N and 32° 31' 54" E at 1190 m above sea level (masl), average rainfall is 1224 mm per annum and average daily temperatures are 15.3°C (minimum) and 27.3°C (maximum). Four *Pythium* species previously found to be pathogenic on beans were used (Mukalazi, 2004). These isolates were MS 61 (*P.ultimum* var. *ultimum*), KAK 5B (*P.macrosporum*), VIHA 2A (*P.chamaehyphon*) and JM 29A (*P.pachycaule*). In addition, seven *Pythium* species obtained from crops intercropped with beans during sample collection in this study were used. These include *P.macrosporum*, *P.oligandrum*, *P.spinatum* (isolated from sorghum), *P.glomeratum* (isolated from potato), *P.arhenomanes* (isolated from maize), *P.heterothallicum* (isolated from sweet potato) and *P.ultimum*. Inoculum of the various *Pythium* species above was multiplied by plating mycelia on autoclaved millet grains (100g) mixed with 200 ml of water in 500 ml bottles. After two weeks of incubation under darkness at 25°C, presterilised soil was mixed with the infested millet at a ratio of 1:10 v/v in wooden trays of 42 x 72 cm. Each tray contained 10 plants of maize (*Zea mays*), sorghum (*Sorghum bicolor*), peas (*Pisum sativum*), CAL 96 (susceptible bean variety) and RWR 719 (resistant bean variety). The trays were set up in a Randomised Complete Block Design (RCBD) with three replications for each *Pythium* species. After germination, the seedlings were watered every day to provide a favourable environment for the pathogen establishment and development. Severity of root rots on seedlings was then estimated based on CIAT scale of 1-9 (Abawi and Pastor Corrales, 1990) where 1= no root symptoms, 3= a maximum of 10% of the hypocotyls and root tissue have lesions; 5=approximately 25% of the hypocotyls and root tissues have lesions and the root system suffers a considerable decay; 9=75% or more of the hypocotyls and root tissues have lesions and the root system suffers advanced stages of decay and considerable reduction. *Pythium* isolates which give a mean disease score of 1-2 were considered non-pathogenic; those, which gave a score of 3-5, were considered mildly pathogenic and those that gave a score of 6-9 were highly pathogenic and virulent. Since the crops' roots varied in size, for disease scoring, five plants of maize, peas and beans (CAL 96 and RWR 719) were used, ten plants were used for sorghum and millet. This was to minimise between host variations and enhance estimation of the error level.

**RESULTS**

The sampling of crops for *Pythium* root rot was done in Kabale district (Fig 1). In this study, the main crops found to be intercropped with beans are shown in Table 1. In most cases, beans were found to be the major crop grown followed by potato, maize, sorghum and sweet potato. Various intercropping combinations were found. For instance, sweet potato was intercropped with

potatoes. Potatoes, maize and sorghum were grown in association with two other crops. Every farm had 1 to 6 different crop species intercropped with beans. During sample collection in farmers' fields, various crops were found to have symptoms characteristic of *Pythium* root rot (Table 2)

**Table 1:** Frequencies of the major crops grown in Kabale district in 2004, 2005 and 2006 cropping seasons.

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

**Table 2:** Disease symptoms found on other crops intercropped with beans

	Symptoms
Beans	Root rot, Yellowing of leaves
Maize	Yellow and red spots on leaves, Stem rotting
Sorghum	Rotten stem, Red lesions on stem, Leaves with black spots Rotting roots, Stunting, Black purple leaves, Black roots, Drying of leaves
Peas	Reduced root system, Root rot, Wilted leaves, Drying of roots, Yellowing stem
Potato	Wilted leaves, Rotten stems, Rotten roots, Black spots on leaves, Tubers rotting, Yellowing of leaves
Sweet potato	Reduced root system

Using ribosomal DNA sequences of the ITS region, 21 *Pythium* species were found to be associated with root rot in other crops commonly intercropped with beans. Five of these *Pythium* species i.e. *P.spinosum*, *P.oligandrum*, *P.parvum*, *P.arrhenomanes* and *P.conidiophorum* had been found associated with bean root rot (Mukalazi,

2004). Fifteen of the species sequences in this study were new and were identified for the first time in Southwestern Uganda during this study. In addition, *P.ultimum* was the most abundant *Pythium* species found in this study (39%) (Table 3).

**Table 3:** *Pythium* spp. isolated from other crops in a bean based cropping system of south-western Uganda identified by ITS sequencing

<i>Pythium</i> spp.	<i>Crops sampled</i>													
	Potato	Sorghum	Maize	Bean	Sweet potato	Cabbage	Peas	Tomatoes	Millet	Wheat	Bananas	Weed	Yam	Total
<i>P. ultimum</i>	15	15	11	2	1	5	2	-	-	2	1	-	1	55
<i>P. acanthicum</i>	5	3	-	1	-	-	-	-	-	-	-	1	-	10
<i>P. spinosum</i>	3	4	1	-	-	-	1	1	-	-	-	-	-	10
<i>P. torulosum</i>	2	-	2	1	-	-	1	-	-	-	-	-	-	6
<i>P. folliculosum</i>	1	5	3	5	-	-	1	-	-	-	-	-	-	15
<i>P. oligandrum</i>	1	4	2	-	-	-	-	-	-	-	-	-	-	7
<i>P. parvum</i>	-	2	-	-	-	2	-	-	2	-	-	-	-	6
<i>P. irregulare</i>	2	-	-	-	-	-	-	1	-	-	-	-	-	3
<i>P. glomeratum</i>	2	-	-	-	-	-	-	-	-	-	-	-	-	2
<i>P. heterothallicum</i>	1	1	-	1	2	-	-	-	-	-	-	-	-	5
<i>P. rostratum</i>	1	-	-	-	1	-	-	-	-	-	-	-	-	2
<i>P. arrhenomanes</i>	-	1	-	-	-	-	-	-	-	-	-	-	-	1
<i>P. macrosporum</i>	1	1	-	-	-	-	-	-	-	-	-	-	-	2
<i>P. mamillatum</i>	1	-	-	-	-	-	-	-	-	-	-	-	-	1
<i>P. orthogonon</i>	-	-	-	1	-	-	-	-	-	-	-	-	-	1
<i>P. arrhenomanes</i>	-	-	4	-	-	-	-	-	2	-	-	-	-	6
<i>P. conodiophorum</i>	-	-	-	5	-	-	-	-	-	-	-	-	-	5
<i>P. erinaceum</i>	-	1	-	-	-	-	-	-	-	-	-	-	-	1
<i>P. periplocum</i>	-	-	-	-	1	-	-	-	-	-	-	-	-	1
<i>P. vexans</i>	-	1	-	-	-	-	-	-	-	-	-	-	-	1
<i>P. pachycaule</i>	-	2	-	-	-	-	-	-	-	-	-	-	-	2
Total	29	23	12	7	5	5	4	2	4	2	1	1	1	142

Pathogenicity: The disease scores of root rot disease due to inoculation of various crops and bean plants in the screen house with bean pathogenic *Pythium* species is as indicated in Table 4. It was observed that maize and millet were moderately susceptible to bean pathogenic *Pythium* species (disease score of 3-5). However, sorghum and peas were highly susceptible to bean pathogenic *Pythium* species (disease score of 6-9). Root rot symptoms were observed in the trays inoculated with *Pythium* species and these symptoms were absent from the control trays without *Pythium* inoculum. Susceptible

bean variety (CAL 96) displayed typical root rot symptoms, which included waterish stem and reduced root system (Plate 1). Sorghum had red-black lesions on the roots. Millet displayed the formation of prop roots while peas had brownish watery systems and root rots. Furthermore, there were above ground symptoms commonly associated with *Pythium* infection. Stunting was visible in sorghum and millet. Maize plants showed leaf chlorosis while the millet leaves dried up at the shoot tips. Sorghum leaves exhibited anthocyanescence in the inoculated trays.

**Table 4:** Mean disease scores of crop species after inoculation with bean pathogenic *Pythium* species

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



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

In addition, disease scores of root rot disease due to inoculation of various crops and bean plants with *Pythium* species isolated from crops intercropped with beans is indicated in Table 5. Peas were moderately pathogenic to *P. glomeratum* and *P. ultimum* (disease score 3-5). However, peas were highly pathogenic to

*P. macrosporum*, *P. arrhenomanes* and *P. heterothallicum*. Maize was moderately pathogenic to all *Pythium* isolates used. Millet was non-pathogenic. The susceptible bean variety (CAL 96) was non pathogenic to moderately pathogenic

**Table 5:** Mean disease scores of crop species after inoculation with eight *Pythium* species from crops intercropped with beans

<i>Pythium</i> species	Crop Host Plants							LSD( P≤ 0.05) <sup>d</sup>	CV (%) <sup>e</sup>
	<sup>a</sup> Resistant bean variety	<sup>b</sup> Susceptible bean variety	Maize	Sorghum	Millet	Peas			
<i>Pythium</i> species									
Non inoculated control <sup>c</sup>	1.03	1.11	1.08	1.00	1.00	1.61	0.29	54.8	
<i>Pythium macrosporum</i>	3.28	3.19	4.31	6.36	1.06	6.17	1.00	52.4	
<i>Pythium arrhenomanes</i>	2.03	1.78	2.42	6.69	2.11	7.61	0.91	51.7	
<i>Pythium spinosum</i>	1.75	2.39	2.89	6.69	1.94	1.75	0.72	42.1	
<i>Pythium oligandrum</i>	3.47	0.94	3.31	6.72	1.44	3.94	1.01	69.4	
<i>Pythium heterothallicum</i>	2.17	4.53	3.44	7.06	1.25	7.69	0.93	45.9	
<i>P.glomeratum</i>	2.39	1.73	3.17	6.19	1.00	4.14	1.03	70.4	
<i>P.ultimum</i>	2.61	2.06	2.89	6.53	1.00	4.67	0.94	61.7	
LSD( P≤ 0.05) <sup>d</sup>	0.95	0.47	0.42	0.41	0.61	0.72			
CV (%) <sup>e</sup>	36.9	32.9	15.8	60.4	49.3	24.7			

## DISCUSSION

In this study, a number of food crops were found to be intercropped with beans. In Africa, beans are largely intercropped (Ocitti p'Obwoya, 1996). For example in Rwanda, beans are seasonally rotated with sorghum or sweet potato (Rusuku *et al.*, 1997). In Southwestern Uganda, beans are grown in an intensive agricultural system together with sorghum, maize, sweet potato, potatoes, bananas and garden peas (Ampaire, 2003). Intercropping sweet potato with beans is the most popular sweet potato based intercropping system in Uganda (Bashaasha *et al.*, 1995). Moreover, intercropping beans is an important strategy for food security, improves labour use efficiency, enhances soil nutrients because of the different demands made by the mixture of crops (Ocitti p'Obwoya, 1996). During this study, some of the crops intercropped with beans were found to also suffer from root rot disease. This correlates with other observations which indicate that most of the root rot pathogens of beans in fact attack many crop species (Agrios, 1997). There has been speculation that continuous intercropping of beans which is common in Southwestern Uganda and other areas in Africa has increased the root rot problem (Rusuku *et al.*, 1997). Hence those crops with root rot symptoms and even those without but that had been intercropped with beans were isolated for *Pythium* causing root rot. This

was done using ITS ribosomal DNA sequences. Sequence analysis of selected DNA fragments to determine species relationship using ITS DNA regions has been more accurate than any other technique (White *et al.*, 1990). Analysing sequences especially the ITS region is a main focus of oomycetes (Levesque *et al.*, 2004). Fifteen new *Pythium* species were identified from other crops during this study. *Pythium* pathogens cause root rot or seedling rot in a number of legumes and other crops (Harman, 2001; Harvey, 2004). In this study, *P.ultimum* was the most abundant *Pythium* species found. In other studies done in the same area, *P.ultimum* was also found to be the most abundant *Pythium* species where beans are grown (Mukalazi, 2004). Other *Pythium* species also found in this study on other crops were *P.torulolum*, *P.folliculosum*, *P.acanthicum*, *P.spinosum* and *P.oligandrum*. Previously, *P.torulolum* and *P.folliculosum* have been isolated from monocotyledons, bryophytes, green algae, soil and occasionally from dicotyledons and conifers (Levesque *et al.*, 2004). In previous studies, *P.acanthicum* and *P.oligandrum* were found to be pathogens of mainly dicotyledons while also being mycoparasites (Chellemi *et al.*, 2000). In studies done in Southwestern Uganda by Mukalazi (2004) found *P.spinosum* to cause severe root rot in beans. DNA sequence analysis showed diverse clades and subgroups



that did not correspond to host or origin of occurrence of *Pythium* isolates. These results are similar to Cilliers *et al.* (2000) who compared ribosomal (rDNA) of *Sclerotium rolfsii* isolates and reported that there was no apparent clustering according to host/geographic origin. This study for the first time provides evidence to suggest that *Pythium* species are found on diverse crop species, which are usually grown with beans. This may play a role in affecting inoculum level and hence playing a role in the increase of bean root rot epidemics in Southwestern Uganda. In order to characterise these epidemics further, pathogenicity tests in the screen house were done using bean pathogenic *Pythium* species and *Pythium* species derived from other crops. Based on the data from the pathogenicity tests using bean pathogenic *Pythium* species, maize and millet were found to be moderately pathogenic while sorghum and peas were highly pathogenic. Further, using *Pythium* species derived from other crops, the reaction was similar with maize and millet being moderately pathogenic to being non-pathogenic while sorghum and peas were highly pathogenic. Symptoms observed because of inoculation with *Pythium* species included red-black roots in sorghum, prop roots in millet, peas had brownish watery stems as well as root rot. When vascular tissues are invaded by *Pythium* mycelium, they become discoloured (Agrios, 1997). If *Pythium* root rot occurs early in the life of the crop, it may cause an infected root to produce multiple roots

(Kucharek, 2000) as was observed in millet during this study. *Pythium* infection also results in wilting and older leaves of infected plants become yellow. This symptom is used as a positive diagnosis of *Pythium* root rot (Chase, 1999). The symptoms associated with *Pythium* infection in peas include the roots being destroyed and pale yellow leaves (Malvic and Babadoost, 2002). The pathogenicity studies indicate that cross pathogenicity is taking place whereby *Pythium* species derived from beans affect beans and other crops. In addition, *Pythium* species derived from other crops are pathogenic to beans and the host crops as well. The pathogenicity studies also indicate that *Pythium* species do not considerably affect the cereals (millet and maize) while they do affect sorghum and peas. This is an indication that sorghum and peas, which are part of the bean pathosystem in Southwestern Uganda, are contributing to continuous root rot epidemics since they too are affected. Maize and millet however would be good intercrops, as they are not affected. This study therefore provided the following information:

- i) That other crops intercropped with beans in Southwestern Uganda are affected by root rots.
- ii) These root rots can be attributed to *Pythium* species.
- iii) Peas and sorghum are affected by *Pythium* species and could thus be contributing to continuous bean root rot epidemics in Southwestern Uganda.

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## REFERENCES

- Abawi GS, Ludwig JW, 2005. Effect of three crop rotations with and without deep ploughing on root rot severity and yield of beans. Annual Report Bean Improvement Cooperative 48:118-119.
- Abawi GS, Pastor -Corrales MA, 1990. Root rots of beans in Latin America and Africa: diagnosis, research, methodologies and management strategies. Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia. 114 pp.
- Agrios GN. 1997. Plant pathology. Fourth edition. Academic Press, San Diego, California.
- Ampaire E, 2003. Farmers' Indigenous technical knowledge of bean disease management and communication systems in south western Uganda. M.Sc. thesis. Makerere University, Kampala. 127 pp.
- Bashaasha B, Mwanga R, Ocitti p'Obwoya C, Ewell PT, 1995. Sweet potato in the farming and food systems of Uganda: A farm survey report, International Potato Centre (CIP), National Agricultural Research Organisation (NARO). 63 pp.
- Buruchara RA, Pastor-Corrales MA, Scheidegger U, 1999. *Fusarium* wilt disease caused by *Fusarium oxysporum* f. sp. *Phaseoli* on a common bean cultivar, G2333 in Rwanda and the Democratic Republic of Congo. Plant Disease 83:397.
- Chase AR, 1999. *Pythium* root rot on ornamentals. Western Connection Turf and Ornamentals 1:8
- Chellemi DO, Mitchell DJ, Kannwischer-Mitchell ME, 2000. *Pythium* spp. associated with bell pepper

- production in Florida. *Plant Disease* 84:1271-1274.
- CIAT, 1992. *Pathology in Africa*. CIAT Annual Report, 1992. Bean Programme, Cali, Colombia. 385 pp.
- CIAT, 1993. Trends in CIAT commodities, 1993. Working Document No.128. Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia.
- CIAT, 2005. Annual Report, 2005. Bean Improvement for the Tropics. Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia.
- Cilliers AJ, Herselman L, Pretorius ZA, 2000. Genetic variability within and among mycelial compatibility groups of *Sclerotium rolfsii* in South Africa. *Phytopathology* 90: 1026-1031.
- FAO, 2004. FAOSTAT database. <http://faostat.fao.org>.
- Harman GE, 2001. *Pythium* spp. Compendium of pea diseases. Kraft JM, Pflieger FL, Ed). St. Paul Mn, USA: American Phytopathological Society.
- Harvey P, 2004. Crop rotation would reduce *Pythium* root rot. Cropping Disease Management. [http://www.clw.csiro.au/publications/farming\\_ahead/2004/154.pdf](http://www.clw.csiro.au/publications/farming_ahead/2004/154.pdf)
- Kucharek T, 2000. Diseases of Agronomic and vegetable crops caused by *Pythium*. *Plant Pathology Fact Sheet*.
- Levesque CA, De Cock AWAM. 2004. Molecular phylogeny and taxonomy of the genus *Pythium*. *Mycologia Research* 108:1363-1383.
- Mahuku G, 2004. A simple extraction method suitable for PCR-based analysis of plant, fungal and bacterial DNA. *Plant Molecular Biology* 22:71-81.
- Malvic, D, Babadoost M, 2002. Root rot of peas. Report on Plant Diseases. [http://ipm.edu/diseases/series\\_900/rpd911/index.html](http://ipm.edu/diseases/series_900/rpd911/index.html).
- Mukalazi, J. 2004. Pathogen variation and quantification of *Pythium* spp. in bean fields in Uganda. PhD thesis. Makerere University, Kampala, Uganda. 146 pp.
- Nderitu JH, Buruchara RA, Ampofo JKO, 1997. Relationships between bean stem maggot, bean root rots and soil fertility. Literature review with emphasis on research in eastern and central Africa. African Highlands Initiative (AHI), Nairobi, KE. (Technical report series no.4). 16 pp.
- Ocitti p'Obwoya CN, 1996. Agronomic studies of sweet potato intercropped with bean. PhD. Thesis. Makerere University.
- Rusuku G, Buruchara RA, Gatabazi M, Pastor-Corrales MA, 1997. Effect of crop rotation on *Pythium ultimum* and other *Pythium* species in the soil. *Phytopathology* 52:27.
- Sarikamis, G F, Yasar M, Bakir K, Kazan A. Ergul, 2009. Genetic characterization of green bean (*Phaseolus vulgaris*) genotypes from eastern Turkey. *Genetics and Molecular Research* 8(3): 880-887.
- Thompson JD, Higgins DG, Gibson TJ, 1994. Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22: 4673-4680.
- White JG, 1988. Studies on the biology and control of cavity spot of carrots. *Annals of Applied Biology* 113: 259-268.
- White TJ, Bruns T, Lee S, Taylor J, 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protocols, A Guide to Methods and Application*. Innis, M. A., Gelfand, D. H., Sninsky, J. J., and White, T. J. (Eds Academic Press, San Diego). pp. 315-322.