AFRICA: BEAN PATHOLOGY

Activity 1. Characterization and distribution of Pythium spp associated with bean root rot in East Africa.

Rationale

Among approximately 100 known species of the genus *Pythium* include pathogenic, saprophytic and biological control groups. Our recent studies in Uganda have shown that over seven *Pythium* spp cause root rots on common beans, but their distribution and relative importance in other countries in East Africa are unknown. Characterization of *Pythium* species and their distribution is therefore considered a necessary pre-requisite in order to develop effective management strategies. However, identification of *Pythium* species using morphological or pathogenic characteristics is difficult given the large species numbers and their mixed occurrence in the soil. We have therefore continued with the characterization of Pythium spp, using molecular methods as a basis for developing simpler, accurate and rapid but reliable detection and characterization techniques. We therefore continued to characterize *Pythium* spp prevalent in Kenya and Rwanda

Methods: One hundred and thirty-four Pythium isolates obtained from root rot affected areas in Kenya and Rwanda were characterized by sequencing using the protocol of Levesque *et. al.* (1998). The DNA of isolates was amplified with universal eukaryotic primers targeting the internal transcribed spacer (ITS) regions and the 5.8S gene of nuclear ribosomal DNA. Purified template DNA was sequenced using an ABI prism automated sequencer. Sequences obtained were edited and compared to data of *Pythium* spp managed by Dr A. Levesque of the Agri-Food and Food and Agriculture Canada.

Results and Discussion: Out of 134 isolates characterized, 22 species were identified **(Table 1)**. Thirteen of these have been reported in our previous pathogen characterization studies in Uganda and Kenya but nine were new additions. All except three (*P. macrosporum, P. zingiberis, P. graminicola*) species were recovered from Rwanda with *P. ultimum* being the most frequent, followed by *P. torulosum* and *P. spinosum.* The three are pathogenic to beans. Fifteen of the 22 species were recovered from Kenya with *P. vexans* being the more frequent species, followed by *P. torulosum*, *P. irregular and P. ultimum.* Species distribution maps for Kenya and Rwanda are shown in **Figures 1 and 2** respectively. These results are consistent with past observations that overall *P. ultimum* is the most frequent species in the region. Pathogenicity of some of the new species is being determined to establish their role in the bean root rot problem in the region.

Table 1. Identification by sequencing of *Pythium* isolates obtained from bean growing areas associated with bean root rots in Kenya and Rwanda.

Species	Pythium Isolates		
	Kenya	Rwanda	Total
P. acanthicum	2	1	3
P. chamaehyphon	1	2	3
P. folliculosum	3	2	5
P. indigoferae	2	2	4
P. irregulare	9	1	10

Species	Pythium Isolates		
	Kenya	Rwanda	Total
P. lutarium	1	3	4
P. macrosporum	1	0	1
P. myriotylum	1	1	2
P. paroecandrum	3	3	6
P. torulosum	9	10	19
P. vexans	10	4	14
P. zingiberis	5	0	5
P. graminicola	4	0	4
P. spinosum	1	7	8
P. ultimum	5	23	28
P. arrhenomane	0	2	2
P. catenulatum	0	1	1
P. deliense	0	1	1
P. diclinum	0	2	2
P. dissotocum	0	3	3
P. rostratum	0	5	5
P. salpingophorum	0	4	4
Total	57	77	134

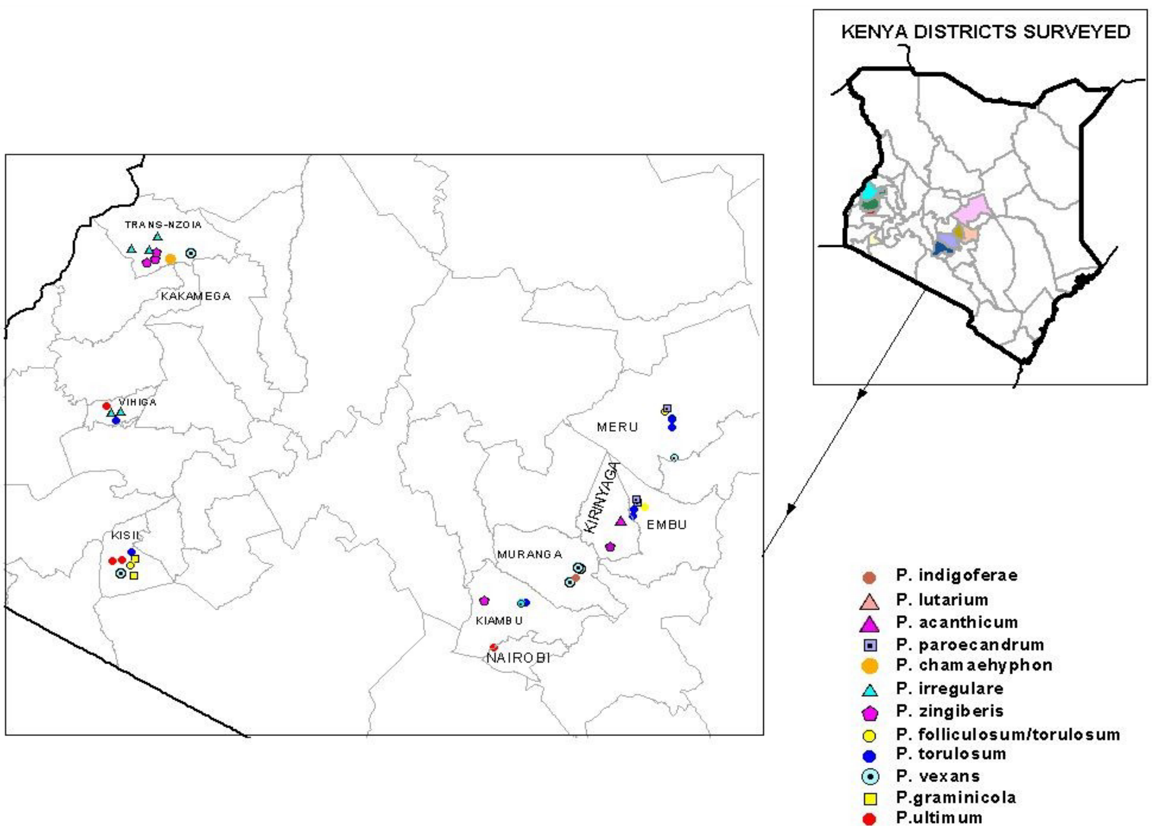

Figure 1. Distribution of Pythium species in some districts of Kenya where bean root rots is prevalent. Characterization was based on sequencing of Pythium isolates.

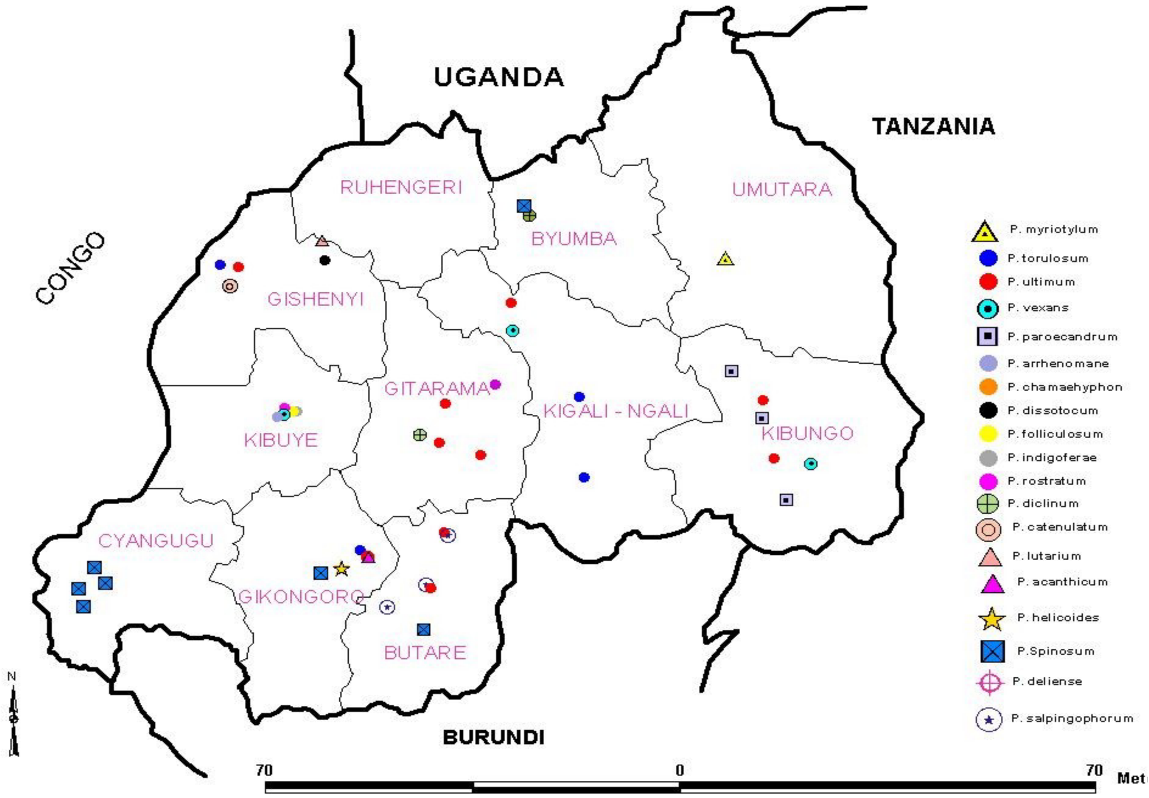

Figure 2. Distribution of Pythium species in Rwanda where bean root rots is prevalent Characterization was based on sequencing of *Pythium* isolates.

Progress towards achieving output milestones

- Pythium isolates (134) from root rot affected areas in Kenya and Rwanda were characterized by sequencing of ITS-1 region. *P. ultimum* was the most frequent occurring species followed by *P. torulosum*.
- Pythium distribution maps showing relative importance of characterized species in Kenya and Rwanda were developed.

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Activity 2. Developing integrated pest management components of root rots.

Pathogenicity of *Pythium* spp and effects of management options for root rots on crops grown in association with beans in southwest Uganda

Rationale

Beans is one of the crops grown under the intensive agricultural system in southwest Uganda. Others include sorghum, maize, sweet potatoes, Irish potatoes, bananas and peas. Crop rotation in the strict sense is rare. Dominance of crops in the field shifts according to season. Rotations commonly practiced include beans-maize-sorghum, beans-maize-beans and beans-Irish potato/maize-sweet potato (Edidah, 2003). Maize and sorghum are also intercropped with beans and/or Irish potatoes such that the bean crop appears in the field season after season. However, of all these crops, beans are most affected by root rots. In recent years this has resulted in the decline in bean production in the area. Given that some of the root rot causing pathogens (e.g. Pythium spp) are known to have a wide host range, some of the questions asked are: do crops grown in association or in rotation with beans play any role in the pathogen survival, inoculum density and severity of root rots in beans?; is bean the only crop in the system that is affected or is it simply a good indicator of the level of root rot pathogens?; to what extent are other crops in the system affected by bean pathogens?; what are the effects of management options for bean root rots on other crops? To address these questions, we initiated studies to characterize Pythium spp associated with major crops found in the bean based systems; to determine pathogenicity of some Pythium species on these crops; and to determine the effects of management options for bean root rot on crops grown in association with beans.

Materials and Methods

***Pathogenicity studies*:** Three *Pythium* species pathogenic to beans (*P. ultimum, P. chamaehyphon, P. pachycaule)* were artificially inoculated on three crops commonly associated with beans namely: sorghum, millets and maize. Autoclaved millet (100 g) was mixed with 200 ml of water in a 500-ml bottle and subsequently used to raise the fungi. After two weeks of incubation, the infested millet was mixed with pre-sterilised soil at a ratio 1:10 v/v in wooden trays. Maize, sorghum and millet were planted in two rows of twelve plants and replicated in three trays. Bean varieties CAL 96 and RWR 719 were used as susceptible and resistant checks respectively. Cumulative emergence and plant stand was recorded one week after germination. Three weeks after germination, plants were assessed for any root and shoot symptoms that may be associated with *Pythium* infection.

Effect of management practices: Four crops; beans (B), sorghum (S), maize (M), and peas (P) were subjected to four amendments i.e., farm yard manure (FYM), green manure (GM), inorganic fertilizer (NPK), fungicide (Ridomil) in farmers fields in Rubaya, Kabale district, southwest Uganda. Sorghum, maize and peas seed were obtained locally from farmers. A root rot susceptible bean variety (CAL 96) was used as a check. Farm yard manure and green manure (Crotalaria) were applied on a dry weight basis at a rate of 5t/ha and their nutrient level determined. NPK fertilizer was applied at a rate of 50 kg of N/ha. Ridomilwas applied as seed treatment (slurry) at a rate of 2.5 kg/ha. Qualitative data was obtained through field observations and photography. Quantitative data collected included: emergence, plant stand, disease incidence and severity at different times during the growing season, plant vigor and yield parameters (dry matter poduction). Disease severity was evaluated according to a CIAT nine-point scale where 1 is resistant and 9 susceptible (Abawi and Pastor Corralles, 1990)..

Results and Discussion

Pathogenicity studies: The different *Pythium* species 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.

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 affected crop was beans and then sorghum, millet and maize in that decreasing order. 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 tentatively conclude from these preliminary observations that *Pythium* species pathogenic to beans cause damage to sorghum, millets and maize to varying degrees. Further investigation to elucidate these interactions is going-on.

Effects of management options on incidence and severity of root rots: The management options evaluated affected the crops in different ways. FYM and ridomil significantly reduced initial root rot infection on beans. High incidence of root rots was observed with GM and attributed to interactions between the root rot pathogens and soil micro-organisms. But FYM, GM and NPK, enhanced root (mass) growth in beans, contrary to observations in control plots.

As in screen house studies, infected sorghum plants exhibited stunted growth, purple leaves, shoot death and dark-red to black root lesions **(Figure 1)**. Significantly high incidence and severities were observed in control plots particularly 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 **(Figure 2)**, stunting and poor establishment. However, incidence and severity were low indicating that maize was less affected **(Figure 3a)**. As with sorghum, amendments and particularly FYM reduced severity **(Figure 3b)** and improved plant vigor and growth.

Figure 1. **(Left) Severely affected sorghum roots (control plots) and (Right) sorghum root with prop root development in plots amended with NPK**

Figure 2. **Maize roots showing root lesions.**

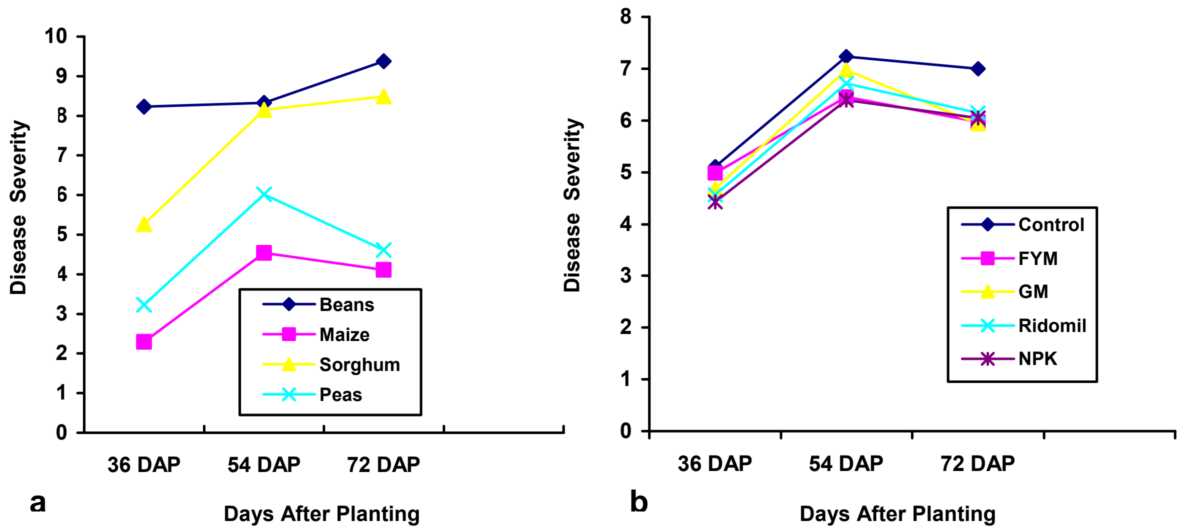

Figure 3. **Disease severity in crops over the season (left), and effects of different root rot management practices on root rots over the season (right).**

NPK, GM, FYM and ridomil (in sorghum) improved dry matter production (DMP) in both maize and sorghum **(Table 1).** Improved DMP in sorghum due to ridomil is probably due to its protective effect against *Pythium* species.

Table 1. **The effect of different soil amendments on mean dry matter production (72 days after planting) for maize and sorghum. Rubaya, Kabale, 2004 season A.**

Crop/Treatment	Dry Matter (g)	
	Maize	Sorghum
Control	106.9	18.5
Farm yard Manure	128.3	42.7
Green Manure	138.4	38.9
Ridomil	112.5	46.1
NPK	163.2	48.8
	L.s.d at $p < 0.05$ (32.20)	

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 the use these options do not only contribute in the management of bean root rots, but are also beneficial to other crops. Studies are underway to further define this contribution**.**

Progress towards achieving output milestones

- Pythium species pathogenic to beans were shown to cause damage on cereal crops grown in association with beans implying that they may be hosts of the pathogens.

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