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Pathogenicity of *Pythium* species on hosts associated with bean-based cropping system in south western Uganda

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Abstract (212 words)

A pathosystem is a subsystem of an ecosystem and is characterised by the phenomenon of parasitism. The bean-*Pythium* pathosystem consists of the host (bean), the pathogen (*Pythium*) and their host-pathogen relation. Of interest is how the pathogen causes pathogenicity on other crops and beans. To investigate this, screen house experiments were set up to test the pathogenicity of *Pythium* species derived from bean and other crops grown in association with beans. Pathogenicity was tested on maize (*Zea mays*), millet (*Eleusine corcana*), sorghum (*Sorghum bicolor*), peas (*Pisum satium*), susceptible bean variety (CAL 96) and resistant bean variety (RWR 719). The results indicated that distinct symptoms were observed in the roots and shoots of test crop species which are characteristic of *Pythium* infection. For instance peas had brownish watery stems and roots Also bean-derived pathogenic *Pythium spp*. were found to be more virulent than *Pythium spp*. derived from other crop species.

Sorghum and peas had the highest disease scores upon infection by *Pythium spp.* We can conclude that there is cross pathogenicity among *Pythium spp*. especially affecting sorghum and peas. This phenomenon may account for the current root rot epiphytotics in south western Uganda and other similar agroecologies. An integrated disease management strategy that will deploy multi-non hosts to *Pythium* root rot is recommended.

Introduction

The cultivation of common beans (*Phaseolus vulgaris* L) in association with other crop species is a primary characteristic of bean production in East Africa and Africa as a whole (Muigai, 1990; Allen *et al*., 1989). Each farm household formulates its own cropping system based on resource endowment, experiences and priorities (Bashaasha *et al*., 1995). The crop most commonly associated in mixed cropping bean system includes maize, although the bean-banana association is common in Rwanda, Burundi, Uganda and the Kagera region of Tanzania (Allen *et al.*, 1989). Also important is growing beans in rotation with other crops. The major rotations in south western Uganda are: maize (*Zea mays*), sorghum (*Sorghum bicolor*), potatoes (*Solanum tuberosum*), sweet potato (*Ipomoea batatas*) and yam (*Dioscorea spp*.) (Ampaire, 2003). Bean root rot disease is considered one of the most important production constraints in south western Uganda (Mukalazi, 2004). This disease is caused by a complex of pathogens which include *Pythium, Fusarium spp* and *Rhizoctonia solanii* (Rusuku *et al.*, 1997).

As part of this study, *Pythium* species were isolated from other crops grown with beans during a survey in south western Uganda. These crops were selected from farms where beans were also growing or had been previously grown. In previous studies, eleven *Pythium spp.* were found to be associated with bean root rot in south western Uganda (Mukalazi, 2004). It has been reported in studies elsewhere that root rots occur also on maize (*Zea mays*), wheat (*Triticum aestivum),* garden peas (*Pisum satium*), potato (*Solanum tuberosum*) and cowpea (*Vigna unguiculata* L.) (Adandonon, 2004). Thus *Pythium spp.* are able to attack a wide range of crop species. For example sorghum seed may be attacked by *Pythium spp.* prior to emergence and germination (Vincelli and Hershmann, 2002). *Pythium* root rot is characterised by a reduction in seedling emergence, smaller and distorted first leaves, plant stunting, reduced tillering, loss of fine feeder roots and lower yields (Paulitz and Adams, 2003). The symptoms associated with *Pythium* infection in peas include the roots being destroyed and pale yellow leaves (Malvic and Babadoost, 2002). Monocrops also experience *Pythium* attack. The seedlings undergo pre-emergent or post emergent damping off (Kucharek, 2000). Beans suffer *Pythium* root rot attack at all growth stages but especially while the crop is young (2 to 3 weeks after planting) (Rusuku *et al.,* 1997). The symptoms in beans include seedling damping off, yellowing of leaves, stunted growth and rotting of roots (Otsyula *et al.,* 1998; Mukalazi, 2004). Given the multi-host pathogenicity of *Pythium spp*., the role of various crop species in pathogenesis cannot be precluded. The objective of this study was to investigate the pathogenicity of *Pythium spp.* (derived from other crops and beans) on maize, millet, sorghum and peas which are usually grown in association with beans in south western Uganda.

Materials and Methods

Experiments were conducted under controlled conditions in a screen house at the National Agricultural Research Laboratories, Kawanda, Uganda (N. A. L. I). Five *Pythium spp.* isolated during surveys in south western Uganda were used. The *Pythium spp*. included *Pythium macrosporum, Pythium oligandrum, Pythium spinosum* isolated from sorghum*, Pythium arrhenomanes* isolated from maize and *Pythium heterothallicum* isolated from sweet potato. These *Pythium* species were grown on selective medium of corn meal agar (CMA) (Oxoid Ltd, Basingtoke and Hampshire, England) plates. After 2-3 days, plugs were cut from growing margins of the cultures and used to inoculate autoclaved millet (100 g) mixed with water (200 ml) so as to raise inoculum. After two weeks, pre sterilised soil was mixed with the infested millet in a ratio of 1:10 v/v in wooden trays of 42 cm x 72 cm. The trays were set up in a completely randomised design (CRD). Seed of maize, millet and sorghum, peas, susceptible bean variety (CAL 96) and resistant (RWR 719) respectively were grown on trays with inoculum. After germination, the seedlings were watered every day to provide a favourable environment for pathogen establishment and development. The data collected included emergence of seedlings, disease severity of roots and root dry matter. Emergence was determined by counting the number of seedlings which emerged one week after planting. Disease severity was determined three weeks after emergence of the seedlings. The surviving plants were harvested and washed with water to remove soil. Disease severity of root rots was then estimated based on the CIAT scale of 1-9 (Abawi and Pastor Corrales, 1990), where 1 = no root symptoms; 3 =a maximum of 10 % of the hypocotyls and root tissues have lesions; 5 = approximately 25 % of the hypocotyls and root tissues have lesions and the root system suffers a considerable decay; 9 =75% or more of the hypocotyls and root tissues have lesions and the root system suffers advanced stages of decay and considerable reduction. Isolates that had a mean disease score of 1-2, were considered non pathogenic; those with a score of 3-5 were considered mildly pathogenic and those that gave a score of 6-9 were highly pathogenic and virulent.

Data analysis: Emergence and disease severity scores data were subjected to analysis of variance (ANOVA) using Genstat software package (Lawes Agricultural Trust Rothamsted Experimental station, 1995). Means were compared using Fisher's protected least significant difference test at 5 % probability level (Steel *et al.*, 1997).

Results and discussion

Pathogenicity of *Pythium* species was assessed by three parameters mainly: symptomatology, pre-emergence damping off and post emergence damping off. The aim of this study was to investigate cross pathogenicity of *Pythium spp.* among diverse crop families commonly included in bean mixed cropping systems in south western Uganda.

Symptomatology

Various root and shoot symptoms were observed on the crop species in experiments where both bean derived *Pythium spp.* and *Pythium spp.* derived from other crops were inoculated. Peas and susceptible bean variety (CAL 96) had brownish watery stems and roots (Plate 1). Sorghum was observed to have red-black lesions on the roots (Plate 2). Millet had prop roots. There were also above ground symptoms associated with *Pythium* infection. Stunting was visible in sorghum and millet. Millet also had leaves drying at the tips. Maize had leaf chlorosis. Sorghum leaves exhibited anthocyanesence in the inoculated trays. The study found that bean-derived *Pythium* pathogens were more adapted for pathogenicity on beans than other crop species. A similar pattern was observed for *Pythium spp*. derived from other crops which were more virulent on other original hosts. This data suggests that host species appear to influence parasitic fitness of *Pythium spp.* In other studies, 14 isolates of *P. aphanidermatum* and *P. myriotylum* were tested for pathogenicity on twelve crops. Results indicated that the two species caused different levels of disease on any one crop (McCarter and Littrel, 1970). Moreover, genetic analysis of *Pythium spp*. indicates that their genotypes shift in response to different crops. This could mean that individual strains of *Pythium* species are better adapted at infecting some crops than others (Harvey, 2004). This study also found evidence of cross pathogenicity of *Pythium spp*. to crops found in bean based cropping system of south western Uganda. Sorghum and peas were found to be reservoirs of these pathogens. This implies that these diverse hosts may subsequently act as inoculum sources. This could allude to the structure of the kernel which could be providing an effective mechanical barrier to pathogen entry.

Pre-emergence damping off

This parameter was assessed by the level of emergence of seedlings one week after planting. Emergence of crop species inoculated with bean pathogenic *Pythium spp.* resulted in high emergence with millet and low emergence with peas (Figure 1). There was variation in the emergence of various crop species with the different *Pythium spp.* (Figure 2).

Emergence of crops inoculated with *Pythium* species derived from other crops resulted in high emergence of seedlings of various crop species (Figure 3). Millet and sorghum had high emergence. *Pythium oligandrum* was the pathogen that caused the most damage since it resulted in the least emergence of crop seedlings (Figure 4). Pre emergence results from the study indicated that millet and sorghum had greater emergence of seedlings than maize and peas. This suggests that millet and sorghum seeds are able to effectively inhibit pathogen entry.

Post emergence damping off

This parameter was assessed by disease severity on the roots. From disease severity data it was evident that bean derived *Pythium* species were more virulent than *Pythium spp*. derived from other crop species. Bean pathogenic *Pythium* spp. inoculated on sorghum gave disease scores of 8.08 and 7.92 with *P. chamaehyphon* and *P. macrosporum* respectively (Table 1). *Pythium spp.* derived from other crops when isolated on sorghum gave scores of 6.69 and 7.06 on *P. arrhenomanes* and *P. heterothallicum* respectively (Table 2). With both bean derived *Pythium spp.* and *Pythium spp.* derived from other crops, sorghum and peas had the most root damage indicated by highest disease severity scores. Post emergence results indicated that sorghum and peas were more susceptible to *Pythium spp.* than maize and millet seedlings. Millet seedlings were able to counteract *Pythium* infection through formation of additional rots. This aids the plant in nutrition due to it being compromised by infection (CIAT, 2005). Maize seedling is also able to recover from *Pythium* infection because despite above ground symptoms indicating infection, the roots were free of the pathogen. Also maize and millet may produce biochemicals in their cells and tissues which are either toxic to the pathogen and inhibit the growth of the pathogen (Pearson and Parkinson, 1961).

Taken together it appears that *Pythium spp.* are more adapted to attack on young tissue than on seed because pre emergence damage to crops was not as severe as post emergence damage. *Pythium* infects germinating seeds and seedlings of all major grain crops and pastures. It also causes a reduction and discoloration of the root system and a complete rotting and decay of fibrous rootlets (Abawi *et al*., 1985). Other pathogens that cause seed and seedling rot include: - *Rhizoctonia solanii* and *Fusarium spp*. *Rhizoctonia solanii* may infect seeds before germination resulting in seed decay. Lesions on young seedling expand rapidly and result in damping off (Abawi *et al*., 1985; Adandonon, 2004). Typical symptoms are small, sunken lesions that are light to dark brown (Harman, 2001). Initial symptoms of *Fusarium* root rot appear as longitudinal, narrow, reddish lesions or streaks on the hypocotyls and primary root one to two weeks after seedling emergence. As infection progresses, lesions become numerous, coalesce and the entire underground stem and root system becomes covered with reddish brown superficial lesions (Lager, 2002).

This study can be used to recommend that farmers in south western Uganda should not intercrop beans with sorghum and peas since these crop species are infected by *Pythium spp*. Conversely, the farmers could intercrop beans with maize and millet since these crops are resistant to *Pythium* damping off.

In order to reduce pre emergence damping off due to *Pythium* infection farmers could make use of fungicides especially for seeds of diverse crop species. Other methods of control for *Pythium* root rot such as organic amendments can be applied on diverse crop species to improve seedling vigour and reduce disease incidence.

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Plates

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 & Pastor-Corrales, 1990).

Plate 2: Root damage on sorghum right hand panel, and roots from non inoculated control (sorghum). This root damage was used to assess severity of disease on a 1-9 scale (Abawi & Pastor-Corrales, 1990).

Figures

Figure 1: Emergence scores of crop species after inoculation with bean pathogenic *Pythium* species.

Figure 2: Damage by bean pathogenic *Pythium* species on various crop species.

Figure 3: Emergence scores of crop species after inoculation with *Pythium* species from non bean crops.

Figure 4: Damage by *Pythium* species isolated from non bean crops on various crop species.

Plate 1:

Plate 2:

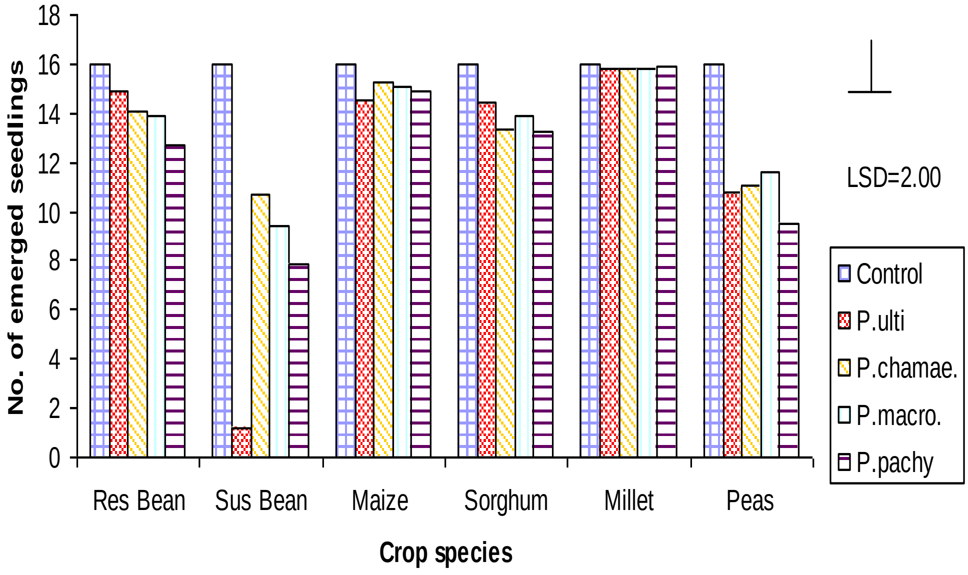


Figure 1:

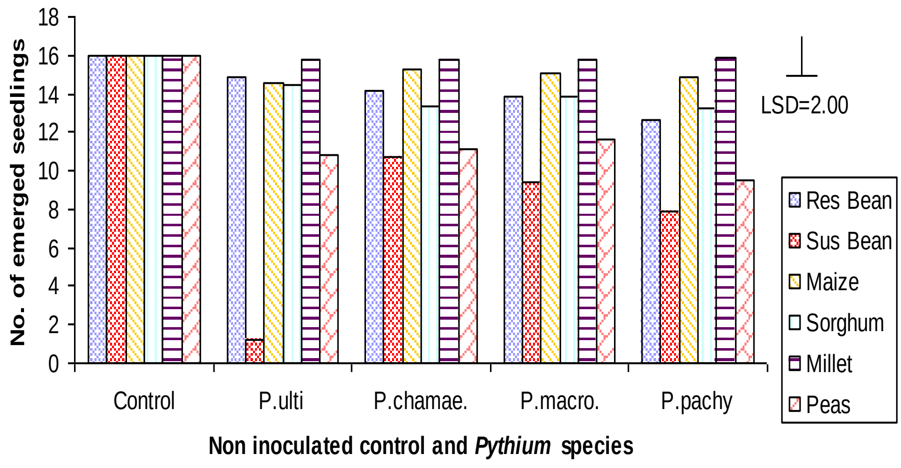


Figure 2:

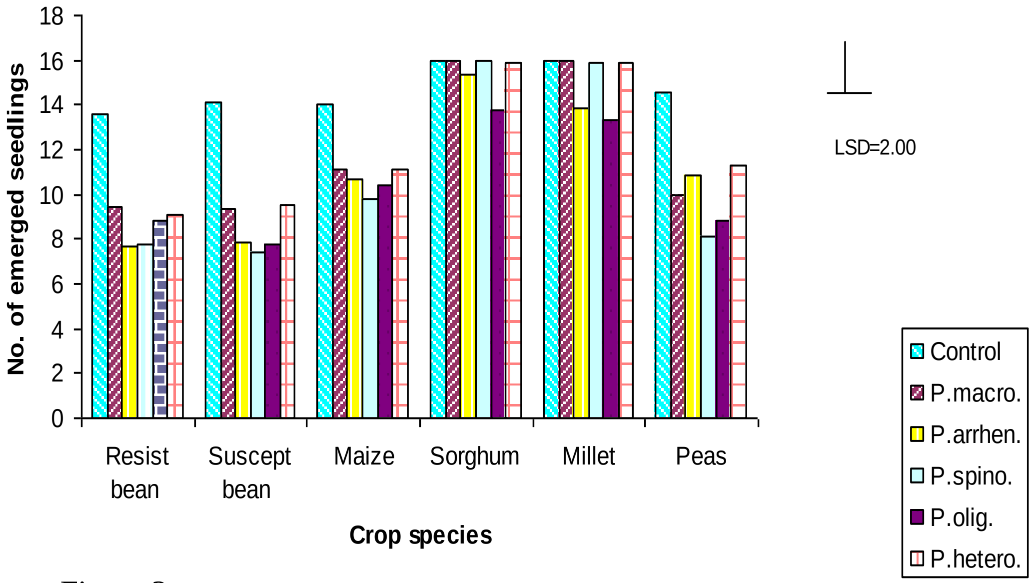
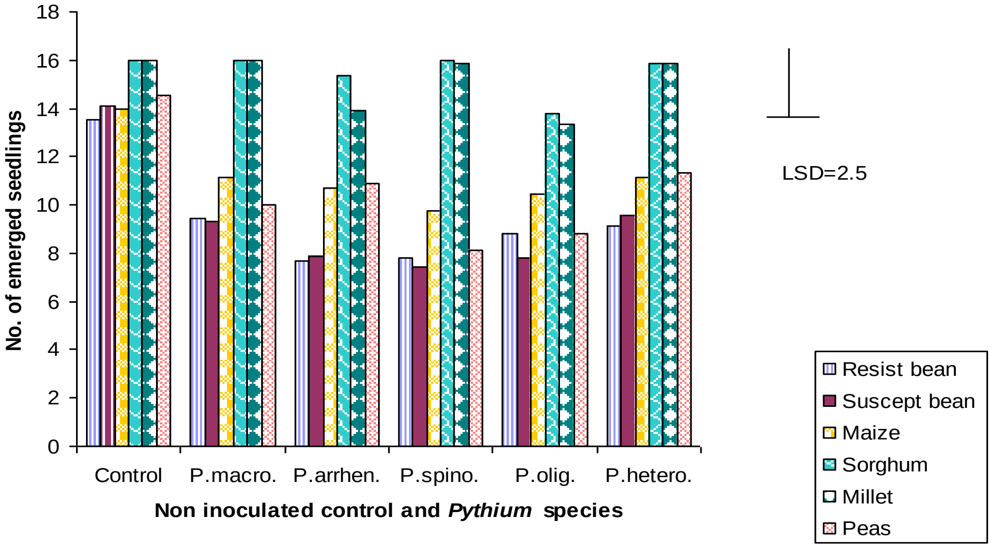


Figure 3:



. Figure 4:

Table 1: Mean disease scores of crop species after inoculation with bean pathogenic *Pythium* species

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

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

Table 2: Mean disease scores of crop species inoculated with *Pythium* species isolated from other crops

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

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