MUARIK BULLETIN, Vol. 2. pp. 41- 48, 1999

Seed-borne fungi of cowpea: transmission and effect of *Macrophomina phaseolina*

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Abstract

Seed health testing by the blotter method was done to establish seed-borne fungi of cowpea seed samples collected from eastern Uganda. Twenty different fungal species were encountered on the seed samples of which *Fusarium semitectum, Phoma* sp., *Alternaria tenius, Aspergillus* sp., *Rhizopus* sp. *and Chaetomium* sp. were most common. was encountered more frequently in the first season than the second one, although its range of infection was low, 0.3-4.3%. Germination tests were done on 13 samples selected on the basis of their *M.phaseolina* infection levels and grouped as low (1.0-1.5%), moderate (1.8-2.5%) and high (2.8-4.3%) infection categories. There was no significant difference ($P \leq 0.05$) in germination percentage between chlorine treated and non-treated seed samples except in the high *M.phaseolina* infection category. In transmission studies, the fungus caused seed, stem and root rots. Affected seedlings wilted and later died and the transmission rate of the fungus was1:1.

Key words: *Fusarium semitectum, Macrophomina phaseolina,* seed, Uganda, *Vigna unguiculata*

Introduction

Cowpea (*Vigna unguiculata* (L.) Walp) is attacked by a number of seed-borne diseases leading to poor plant establishment and consequently heavy yield losses (Diekman, 1994). Most of the important diseases are fungal with some classes or genera being more frequent than others. One of the effects of seed-borne fungal infection is the reduction or elimination of germination capacity (Neergaard, 1979). This has been attributed to invasion of seed tissues and their consequent disruption (Sinha and Khare, 1977; Neergaard, 1979) resulting in the reduction of seed quality (Buruchara, 1990).

Among the seed-borne fungi of cowpea, *Macrophomina phaseolina* (Tassi) Goid, is often important, as it causes seed and seedling rot (Sinha and Khare, 1977). The fungus is seed-transmitted (Sinha and Khare, 1977), or soil-borne (Sinclair, 1982) and is commonly encountered on cowpea seeds in Uganda (Nakawuka *et al.*, 1997) but no studies have been conducted to determine its transmission rate from naturally infected seeds to seedlings. Therefore, the present work was undertaken to identify the kind and amount of fungi associated with cowpea seeds from eastern Uganda, and to determine the significance and seed transmission rate of *M. phaseolina.*

Materials and Methods

Seeds of two local cultivars, *Ebelat* and *Ecirikukwai,* and the introduced, "Kenyan/Black" were collected from eastern Uganda, a (major cowpea growing region of the country). Samples (240) were obtained from farmers' harvests of first (May) and second (November) seasons of 1996. The samples were collected from 3 major cowpea growing districts, from individual households situated 5-10 km apart and transcending the three districts. From each farmer's seed lot, primary samples were drawn

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by the hand method and used to make a composite sample of 1kg was drawn (ISTA, 1993). For subsequent studies, working samples were drawn using a mechanical seed divider (ISTA, 1993).

Seed health testing

The health status of the seeds was established using the blotter method (ISTA, 1993). Four hundred seeds from each sample were then plated on three layers of moist blotters in 9 cm diameter petri-dishes with 10 seeds/dish and incubated for 7 days under alternating cycles of 12 hours of darkness and 12 hours under near ultraviolet (NUV) light at 20-22 °C. Each incubated seed was then examined using a stereo microscope (x 50 - 60 magnification) for fungal growth. Fungi were identified according to their "habit characterers" (Mathur *et al.*, 1992). Infection level of each fungus was then computed as percentage of infected seeds in every 400 seeds per sample. The number of samples showing occurrence of a particular fungi was then computed as a percentage out of all samples tested (frequency of occurrence).

Germination test

Thirteen seed samples with natural *M.phaseolina* infection were selected and categorised into low (1-1.5%), moderate (1.8-2.5%) and high (2.8-4.3%) infection category. The categories were developed based on an earlier study of various field samples of cowpea using the blotter method which showed that seed-borne infestation levels ranged from about 1to over 4%. Four hundred seeds from each sample were divided into two parts of 200 seeds each. One part was surface disinfected with 1% sodium hypochlorite solution for 2 minutes before testing for germination using the "on-top of paper "method (ISTA, 1993). The other part was not treated but was also tested for germination.The relationship between germination capacity and levels of infection by *M. phaseolina* was then tested using correlation analysis.

Transmission test

Two hundred seeds were taken from each of the 13 samples and divided into 2 equal parts. One part was surface sterilised with 1% Sodium hypochlorite for 2 minutes before sowing in 0.5 kg sterile plastic bags containing sterilised soil/sand mixture. For each part of the seed samples, 5 replicates each of 20 seeds each were sown in bags supported on a sterile wire tray and thereafter seedlings were grown in a screenhouse set at 17 and 37°C minimum and maximum temperatures, respectively, and relative humidity of 70-76%. Prior to sowing, the sterilised soil/sand was tested for the presence of fungi, in particular *M. phaseolina*, using the soil agar plate method to ensure that there was no soil-borne inoculum. Soil agar media was prepared by dissolving 4.25 g of corn meal agar in 250 ml of water, sterilised for 15 minutes at 121°C and a pressure of 1.06 bars. The agar was left to cool in a lamina flow cabinet and poured into sterile glass petri-dishes. Sterile soil/sand was then sprinkled on the agar, covered tightly and incubated at 23°C. After 24, 36 and 48 hours, the petri-dishes were examined for presence of *M. phaseolina.*

Control tests were set up along side test samples using *M. phaseolina*-free seed samples. The set up was done following randomised complete block design with 5 replications. Seedlings were harvested 4 times at 10, 17, 24 and 31 days after sowing (DAS). For each harvest, 5 bags were randomly selected from each replicate and the seedlings/ungerminated seeds carefully removed from the soil. In the laboratory, the seedlings and ungerminated seeds were washed in sterile water to remove the soil. Under a lamina flow cabinet, they were surface sterilised with 1% Sodium hypochlorite for 1 minute, rinsed for 5 minutes in 3 changes of sterile water (Kabeere, 1995) and put on sterile wire trays to dry for 1 hour. The seedlings were cut into roots, stems and leaves before plating on 3 layers of moist blotter paper (Nakawuka, 1996) in 9 cm diameter sterile glass petri-dishes. The different plant parts were

plated in different dishes. The dishes were then incubated under alternating cycles of 12 h NUV light and 12 h darkness for 7 days, after which they were examined for the presence of fungi. The experiment was done twice and the data subjected to two-way analysis of variation (ANOVA) (Steel *et al.*, 1997). Where significant differences were detected, means were compared using Fisher's protected least significant differerence test (LSD) using the MSTATC computer program.

Results

Twenty different fungi were detected on the seeds: *Fusarium semitectum, F. solani, F. monoliforme, F. oxysporum, F. equiseti, Phoma* sp., *Alternaria tenius, A. zinniae A.sesamicola, Aspergillus* sp., *Rhizopus* sp., *Chaetomium* sp., *Macrophomina phaseolina, Phomopsis* sp.., *Colletotrichum dematium, C. lindemuthianum, Bipolaris* sp., *Nigrospora* sp., *Botryodiplodia theobromae,* and *Curvularia* sp. Generally infection levels were higher for the first than second season (Table 1). Infestation levels differed with cultivar, being generally higher in the Kenyan originated cultivar.

Effect of Macrophomina phaseolina on germination of cowpeas

Table 1. Occurrence of seed-borne fungi and their mean infection levels on three common cultivars of cowpeas grown in Eastern Uganda in 1996.

Fungi	*Ebelat*		*Ecirikukwai*		*"Kenya"*	
	Season 1	Season 2	Season 1	Season 2	Season 1	Season 2
Fusarium semitectum	43.0	20.0	40.7	39.5	69.7	10.0
F. solani	7.2	0.3	5.0	1.2	31.0	-
F. monoliforme	0.8	0.3	2.8	2.1	0.8	-
F. oxysporum	2.0	0.8	1.2	-	12.0	-
F. equiseti	0.5	5.9	0.3	10.7	-	1.8
Alternaria tenius	2.0	0.4	2.6	0.3	1.4	-
A. zinniae	0.7	1.7	2.0	1.9	0.5	0.3
A. sesamicola	0.6	0.6	-	2.2	-	0.5
Macrophomina phaseolina	0.9	0.9	1.3	-	1.8	-
Phoma spp.	2.8	0.9	6.1	0.7	1.3	0.3
Phomopsis spp.	1.3	0.4	0.7	0.3	0.3	-
Botryodiplodia theobromae	0.8	0.3	0.5	0.3	-	-
Bipolaris spp.	0.4	0.7	0.3	0.3	0.5	0.3
Colletotrichum dematium	0.5	27.1	0.3	6.9	-	31.5
C. lindemuthianum	0.5	6.5	-	4.5	-	6.1
Curvularia spp.	0.3	4.3	0.4	0.6	0.3	1.1
Acremonium spp.	0.8	0.4	-	0.3	-	2.0
Nigropora spp.	0.3	0.4	-	-	-	0.5
Aspergillus spp.	0.8	0.3	14.7	1.3	0.3	0.4
Rhizopus spp.	0.4	-	0.5	-	0.5	-
Chaetomium spp.	1.3	-	0.8	-	0.6	-
LSD (0.05)	0.7	0.6	0.6	0.7	0.5	0.6

For first season, total number of samples: *Ebelat*, n = 42, *Ecirikukwai*, n = 13 and "*Kenya*", n = 5. For second season total number of samples: *Ebelat*, n = 37, *Ecirikukwai*, n = 20 and "*Kenya*", n = 2.

Germination percentage ranged from 74-81% and 72-87% for non-surface sterilised and surface sterilised samples, respectively, in the low infection (LI) category. For the medium infection (MI) category, it ranged from 73-88% and 77-89% for non treated and chlorine treatments, respectively. And, in the high infection (HI) category, germination ranged from 60-79% and 80-84% for non treated and chlorine treatments respectively (Table 2).

Percentage of abnormal seedlings ranged from 14-16% and 11-16% for the low infection category, 8-21% and 10-12% for the medium infestation category, and 15-33% and 12-15% for the high infection category for the non treated and chlorine treatments, respectively (Table 2). The abnormal seedlings showed defects such as of deformed tap roots, twisted epicotyl and hypocotyl, decaying seedlings, malformed primary leaves and stunted roots (Plate 1).

There was no significant correlation between germination and *F. semitectum* infection for the low and high infection categories (Table 3), but there was a negative but weak correlation between germination and *M.phaseolina* infection levels for the low infection and high infection categories (r = -0.652, P = 0.211; r = -0.410, P = 0.697, respectively) (Table 3).

Table 2. Germination percentage of cowpea seed samples with different levels of Macrophomina phaseolina infection.

	% Germination		% of other categories			
	normal seedlings		abnormal seedlings		dead seeds	
Variety	NT	CL	NT	CL	NT	CL
Low infection (1.0-1.5%)						
SC.102 (*Ecirikukwai*)	81.3	85.8	13.7	10.5	5.0	3.7
PB.103 *	73.8	86.7	18.8	10.7	7.4	2.7
SC.208 *	76.5	72.2	16.3	16.2	7.2	11.7
SC.219 (*Ebelat*)	77.3	77.5	15.5	12.2	7.2	10.3
KB.212	76.7	80.0	16.0	14.7	7.3	5.3
LSD(0.05)	6.8	NS	3.7			
Moderate infection (1.8-2.5%)						
SC.114 (*Ecirikukwai*)	87.2	84.3	9.3	11.8	3.5	3.8
PB.120 *	88.2	88.3	7.8	9.8	4.0	1.8
SC.220 (*Ebelat*)	81.0	80.7	14.2	11.5	4.8	7.8
PB.204 *	72.8	76.3	20.7	11.7	6.5	12.0
PB.208 *	79.2	82.0	12.8	9.5	8.0	8.5
LSD(0.05)	NS	9.1	NS			
High infection (2.8-4.3%)						
KK.107 (*Ebelat*)	59.3	83.8	32.8	13.0	7.8	3.2
KB.113 *	63.0	79.3	29.5	14.6	7.5	6.0
PB.211 (*Kenya*)	79.0	81.0	15.0	11.7	6.0	7.3
LSD(0.05)	21.7	14.5	NS			

Percentage germination based on 200 seeds per treatment per sample, ** pooled data for 3 experimental repeats, NT = Non-surface disinfected, CL = treated with 1% Sodium hypochlorite (surface disinfected).

Transmission rate of Macrophomina phaseolina

M. phaseolina was isolated from diseased seedlings and from ungerminated dead seeds. After incubation of the seedling parts, the fungus was detected mainly on stems but sometimes on leaves. After 17 DAS, roots and ungerminated seeds usually decomposed. There were no *M.phaseolina* symptoms on the control samples in all the 3 infection categories.The transmission rate of *M.*

Table 3. Correlation coefficient describing the relationships between germination capacity and infection levels of M.phaseolina.

Infection category	Infection level %	Germination percentage*	Correlation coefficient	P value
low infection	1.3	83.1	-0.652	0.211
moderate infection	2.0	80.8	0.460	0.421
high infection	4.3	71.6	-0.410	0.697

* grouped mean for the non-treated and chlorine treated samples.

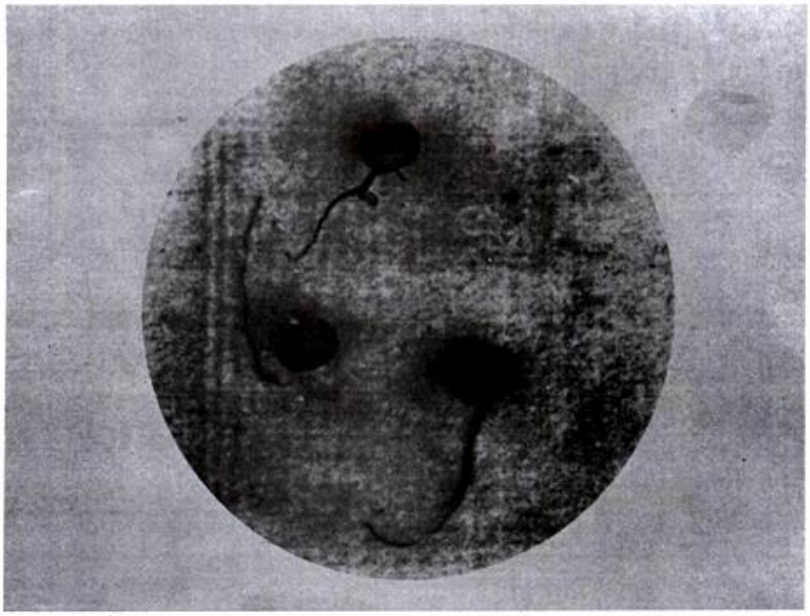

Plate 1. Abnormal seedlings with M.phaseolina infection.

Table 4. Transmission rate of Macrophomina phaseolina from infected seeds to seedlings.

Infection level	SC. 102	PB. 103	CS. 208	SC. 219	KB. 212	SC. 114	PB. 120	CS. 220	PB. 204	PB. 208	KK. 107	KB. 113	PB. 211
Initial infection level (%)	1.25	1.25	1.50	1.25	1.50	2.25	1.75	2.00	1.75	1.75	4.25	2.75	2.75
Transmission*	1.00	1.00	2.00	1.00	1.00	2.00	1.50	2.00	2.00	1.50	3.50	2.50	2.00
Transmission ratio[a]	1:1	1:1	1:1	1:1	2:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1

* = percent recovery *of M.phaseolina* based on 100 seeds per sample and is the grouped mean for the non treated (NT) and Sodium hypochlorine (Cl) treatments.

a = ratio of the initial infection level to percent recovery of *M. phaseolina.*

phaseolina, is given as a ratio of diseased seedlings to the initial level of infection, was approximately 1:1 for all the 3 levels of *M. phaseolina* infection (Table 4).

Discussion

Infection of cowpea seeds by seed-borne fungi was generally higher during the first than the second season crop. This could be due to the fact that the first season's rains were longer than the second season and therefore more humid. Humid conditions generally favour most fungal epidemics (Williams, 1975).

The high infection level of *Fusarium semitectum*, a weak pathogen (Nakawuka *et al.*,1997), is probably due to contamination of the seeds from infected plant debris or soil. *Aspergillus* sp. and *Rhizopus* sp. also had high infection levels. These are storage fungi (Neergaard,1979), whose infestation increase with the storage duration and are known to decrease seed germination by invading the embryos of stored seed. In addition, they produce mycotoxins (Singh *et al.*, 1991) which greatly reduce seed quality as regards consumption.

Macrophomina phaseolina, with pycinidia, was recorded on many samples from all the districts, implying its widespread occurrence. The presence of *M.phaseolina* on cowpea seeds is potentially important because it implies that the areas where the seed samples were collected from could be considered as reservoirs of the pathogen. The negative association between *M.phaseolina* and seed germination indicates that *M.phaseolina* adversely affects seed health. But, because the correlation coefficients were weak, higher levels (> 4.3%) are probably needed to cause significant reduction in seed germination. This is supported by the fact that germination significantly improved as a result of seed treatment with chlorine only for the high *M. phaseolina* infected (HI) samples. This was probably because these samples had high levels of fungal infection and the effect of pre-treatment was therefore apparent.

In the screenhouse, it was observed that all seedlings that showed *M.phaseolina* symptoms eventually died. This suggests that the seed-borne inoculum led to death of the infected seeds/seedlings and the pathogen had a high transmission rate of 1:1. This corroborates Songa *et al.* (1997) findings that most seedlings infected with *M.phaseolina* do not survive to maturity. The occurrence of such a wide range of seed-borne fungi on cowpea seeds suggest that cowpea is susceptible to many fungal diseases which are likely to affect its germination and subsequent growth and development. Therefore, seed health testing and sorting are necessary to ensure that appropriate control measures such as seed treatment are taken. This can help in averting crop failures due to seed-borne pathogens.

Acknowledgement

We thank the Agriculture Extension Programme of the Ministry of Agriculture for funding the research. We also thank The Uganda Seed Project and Ministry of Agriculture's Plant Protection and Quarantine section, both based at Kawanda Agricultural Research Institute, for use of their research facilities.

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