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Fungal pathogens of yams (*Dioscorea rotundata* Poir.) during storage in Nigeria

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Summary

Fungal pathogens responsible for yam losses during storage were investigated. One hundred and fifty yam tuber samples showing rot symptoms were collected from three agroecological zones representing the yam belt of Nigeria to identify the associated fungal pathogens. The 17 fungal isolates obtained from the infected tubers were *Nattrassia mangiferae, Fusarium oxysporum, Fusarium verticillium, Fusarium semitectum, Fusarium chlamydosporum, Fusarium solani, Fusarium dimenrum, Penicillium oxalicum, Rhizoctonia solani, Aspergillus niger, Aspergillus flavus, Lasidiodiplodia theobromae, Setosphaeria rostrata, Colletotrichum gloeosporioides, Trichoderma longibrachiatum Rhizopus stolonifer* and *Sclerotium rolfsii. Aspergillus niger* was the most predominant species in all the agroecological zones. On artificial inoculation, all the 17 fungal species were found to be pathogenic on healthy-looking yam tubers of the variety TDr 93-31 but none of the fungal isolates could initiate rot symptoms on unwounded healthy-looking yam tubers however, results showed that there were significant differences ($P < 0.05$) in virulence among pathogens from different agroecological zones when inoculated into healthy tubers of TDr 93-31. Rots caused by *Lasidiodiplodia theobromae* and *Aspergillus niger* were more severe on yams when inoculated and stored for 14 days. The isolation of more than one type of fungi from a particular tuber suggests the possibility of multiple infections and the cumulative effects of these fungi interactions may cause rapid and extensive rotting of tubers.

Key words: Yam, storage, fungi, pathogenicity, wounding

Introduction

Nigeria is the largest producer of yams (*Dioscorea species*) in the world (FAO, 2010). The tuber serves as a major source of food but, high percentages of tuber loss during storage pose serious threat to its availability (Olurinola *et al*., 1992). Sources of storage losses include microbial rots, pest infestation, physiological respiration and sprouting. Fungi are however, the most important of these factors and have been reported to be responsible for 80% of all storage rots (Degras, 1993).

Three fungal genera, *Botryodiplodia, Fusarium* and *Penicillium* have been reported to cause extensive rotting of tubers. These genera are highly pathogenic and are extremely widespread but the level of damage varies with the region, varieties and season (Degras, 1993).

Fusarium rots are often considered to be secondary to nematode attacks because they are superficial (Bridge, 1972). *Penicillium* species cause a hard but dry brown rot which turns wet and soft when invaded by bacteria (Adeniji, 1970). Lesions on infected tubers are usually covered with green sporulation (Degras, 1993). Fungal pathogens may cause infections either singly or in combination with several others. Other fungi that have been reported to cause storage rots of yams include *Rhizopus nodosus* (Ikotun, 1983), *Sclerotium rolfsii* (Ejechi & Ilondu, 1998) and *Rhizoctonia solani* (Green *et al*., 1995). The objective of the study was to identify the major fungal pathogens responsible for yam tuber rots during storage in Nigeria.

Material and Methods

Survey of yam growing areas of Nigeria for collection of rot samples

The yam belt of Nigeria was surveyed to obtain tubers showing rot symptoms during storage. The study area covered three ecological zones (the Guinea Savanna, the Forest/Savanna transition and the Forest zones). Five infected yam tubers showing rot symptoms were collected from each of the farmers' barns visited during the survey, kept in an individual sample bags and labeled. Fifty tubers were collected from each ecological zone giving a total of 150 tubers collected across the country for the study.

Isolation and identification of fungal pathogens

Tuber pieces (approximately 2 mm) were cut from tissues at the junction between healthy and infected portions, surface-sterilised in 0.05% sodium hypochlorite for 2 minutes and then rinsed twice (one minute each wash) in sterile distilled water . The tuber pieces were dried in layers of sterile paper towels in a laminar flow cabinet (Environmental Air Control, Inc. (EACI) Hagerstown, Maryland, USA) for 10 minutes. Tuber pieces were then plated on potato dextrose agar (PDA) incubated at 27°C and examined daily for fungal growth. Cultures were identified, samples that could not be identified were sent to CABI Bio-science (Diagnostic and Advisory Laboratory) Reading, UK for identification. Frequency of occurrence of each fungal isolate was recorded and expressed as a percentage of the total isolations made from each geographic zone.

Pathogenicity of fungal isolates in wounded whole tubers

A 7-day old culture of each of the 17 fungal isolates obtained from infected tubers were inoculated into healthy-looking tubers of *D. rotundata* variety TDr 93-31 to determine their pathogenicity on wounded tubers using a sterile 6 mm-diameter cork borer at three points (proximal, middle and distal regions). Each tuber was bored into (about 10 mm deep) with a 6 mm sterile cork borer to scoop-out tissue in three replicates of five tubers per replicate. The control set-up consisted of tubers inoculated with blank agar discs. Wounds were sealed with petroleum jelly. All inoculated tubers were enclosed in polyethylene bags, moistened with sterile distilled water to maintain high humidity. Inoculated tubers were maintained at 27 + 2^{o}C for 14 days. The tubers were assessed for rot development by cutting through the points of inoculation. Pathogens were re-isolated and their cultural characteristics were compared with those of the original isolates.

Pathogenicity of fungal isolates in unwounded whole tubers

Fungal pathogens were inoculated on to tubers and maintained and assessed as described above without wounding but firmly pressed to make adequate contact with the bark of the tuber to ascertain whether they could penetrate healthy-looking tubers in the absence of visible wounds. Blank agar discs were placed the same way on some tubers as control and at the end of incubation period, hyphae and spores at the point of inoculation were cultured on freshly prepared PDA and the different fungi obtained were compared with the original isolates.

Variability in virulence among fungal isolates of rotted yam tubers from different agroecologies

The 17 fungal pathogens obtained were assessed for variability in virulence by inoculating into healthy-looking tubers of TDr 93-31, control tubers were inoculated with blank agar discs with five tubers inoculated per replicate using randomised complete block design with three replicates, all tubers were maintained as described above and lesion diameter measured using a 30-cm rule.

Results

Rot symptoms and associated fungi

Fungi isolated from rotted samples collected across the yam belt of Nigeria were *Nattrassia mangiferae* (Syd. & P. Syd.) L. Sutton & Dyko; *Fusarium oxysporum* Schlecht. Emend. Snyd. & Hans; *Fusarium verticillium* Sheldon; *Fusarium semitectum* Berk. & Rav.; *Fusarium chlamydosporum* Wollenw. & Reinking; *Fusarium solani* (Mart.) Appel & Wollenw. Snyd. Hans.; *Fusarium dimenrum* Penzig; *Penicillium oxalicum* Currie and Thom; *Rhizoctonia solani* Kuhn; *Aspergillus niger* van Tiegh; *Aspergillus flavus* Link; *Lasidiodiplodia theobromae* Pat.; *Setosphaeria rostrata* K.J. Leonard (*Exserohilum anamorph*); *Colletotrichum gloeosporioides* Penz.; *Trichoderma longibrachiatum* Rifai; *Rhizopus stolonifer* Lind and *Sclerotium rolfsii* Sacc. Frequencies of isolation (Table 1) ranged from 2–50% in the Guinea Savanna, 0–50% in the Forest/Savanna transition zone to 0–72% in the rain forest. *A. niger* was the most predominant species in all the agroecological zones followed by *N. mangiferae*, *L. theobromae* and *P. oxalicum*. Other dominant species were *Fusarium oxysporum*, *F. verticillium* and *Sclerotium rolfsii* while *Setosphaeria rostrata* was only isolated from tubers collected from the Forest/Savanna transition zone.

Table 1. *Frequency of isolation (%) of fungal pathogens from rotted yam tubers from three ecological zones in Nigeria*

Fungus	Frequency (%) of isolation *		
	Guinea Savanna Zone	Forest/Savanna Transition Zone	Forest Zone
Aspergillus flavus	4	4	6
Aspergillus niger	50	50	72
Lasidiodiplodia theobromae	10	10	6
Colletotrichum gloeosporioides	Ni**	6	6
Fusarium chlamydosporum	2	2	Ni
Fusarium dimenrum	10	4	Ni
Fusarium verticillium	8	6	6
Fusarium oxysporum	16	4	11
Fusarium semitectum	2	2	Ni
Fusarium solani	4	2	Ni
Nattrassia mangiferae	42	28	6
Penicillium oxalicum	12	14	17
Rhizoctonia solani	10	10	Ni
Rhizopus stolonifer	6	24	28
Sclerotium rolfsii	Ni	2	11
Setosphaeria rostrata	Ni	2	Ni
Trichoderma longibrachiatum	4	8	6

* Fifty rotted tubers were used for isolation from each ecological zone.
**Ni=fungus not isolated.

Pathogenicity of isolated fungi in wounded yam tubers

All 17 fungal species caused rot in the tubers. N. mangiferae caused soft, wet brown rot, *L. theobromae* caused soft, wet grey rot, *R. solani* caused soft, brown rot while *S. rolfsii* caused soft rot with dirty white colouration. *P. oxalicum*, *C. gloeosporioides, A. niger, A. flavus, T. longibrachiatum* and *S. rostrata* caused dry dark brown rot. *F. dimenrum, F. verticillium, F. oxysporum, F. semitectum, F. chlamydosporum, F. solani* caused soft rot with light to dark brown colouration and *R stolonifer* caused soft rot with greyish colouration.

Pathogenicity of fungi in unwounded tubers

None of the 17 fungal species could initiate rot symptoms on unwounded tubers. When tubers were cut at the point of inoculation, there were no macroscopic symptoms below the periderm layer. Tuber pieces from points of inoculation, when surface-sterilised and plated onto PDA, did not produce any fungus.

Table 2. *Virulence variation in rot - causing fungi isolated from rotted yam tubers in three agroecologies*

Pathogen	Guinea Savanna Rot virulence* diameter (mm)		Forest/Savanna Rot virulence diameter (mm)		Rain forest Rot virulence diameter (mm)	
Nattrassia mangiferae	18.3 b-e	M	24.7 a	H	21.7 d	H
Fusarium dimenrum	15.0 de	M	16.0 bcd	M	Ni**	-
Fusarium verticillium	17.3 b-e	M	16.7 bcd	M	17.3 be	M
Fusarium oxysporum	19.0 b-e	M	16.7 bcd	M	17.3 cd	M
Fusarium semitectum	19.7 bcd	M	13.3 cd	L	13.0 ef	L
Fusarium chlamydosporum	18.0 b-e	M	14.0 cd	L	Ni	-
Fusarium solani	17.3 b-e	M	14.0 cd	L	Ni	-
Lasidiodiplodia theobromae	20.7 bc	H	19.3 abc	M	22.0 d	H
Penicillium oxalicum	21.3 b	H	12.7 cd	L	12.0 f	L
Rhizoctonia solani	25.0 a	H	14.3 e	L	Ni	-
Setosphaeria rostrata	Ni	-	18.0 bcd	M	Ni	-
Aspergillus niger	18.3 bc	M	25.7 a	H	30.0 a	H
Aspergillus flavus	16.7 cde	M	25.0 a	H	16.7 cde	M
Trichoderma longibrachiatum	14.7 e	L	14.0 cd	L	12.3 f	L
Rhizopus stolonifer	16.0 cde	M	14.3 cd	L	11.7 f	L
Sclerotium rolfsii	Ni	-	21.3 ab	H	18.7 cd	M
Colletotrichum gloeosporioides	Ni	-	25.7 a	H	14.0 def	L

* Virulence: H = high, rot diameter > 20 mm; M = moderate, rot diameter >15 < 20 mm; L = low, rot diameter < 15 mm.

**Ni = Fungus not isolated from the agroecology

Means followed by the same letter within a column are not significantly different at $P < 0.05$ (using Duncan's Multiple Range Test).

Variability in virulence among fungal pathogens obtained from different agroecologies

There were significant differences ($P < 0.05$) in virulence among pathogens from different agroecological zones. In the Guinea Savanna zone, *L. theobromae, P. oxalicum* and *R. solanwere* highly virulent (20.7–25.0 mm) while *T. longibrachiatum* (14.7 mm) was slightly virulent (Table 2). In the Forest/Savanna transition zone, the highly virulent species were *N. mangiferae, A. flavus,*

A. niger, S. rolfsii and *C. gloeosporioides* while *F. dimenrum, F. verticillium, F. oxysporum, L. theobromae* and *S. rostata* were moderately virulent (16.0–19.3 mm). *P. oxalicum, F. semitectum, F. chlamydosporum, F. solani, T. longibrachiatum* and *R. stolonifer* were less virulent. In the rain Forest zone, *N. mangiferae, L. theobromea* and *A. niger* were highly virulent (21.7–30.0 mm) while *R. stolonifer, P. oxalicum, T. longibrachiatum, F. semitectum* and *C. gloeosporioides*) were low in virulence (11.7–14.0 mm).

Discussion

Seventeen fungi were found to be associated with rot in the three agroecologies. Of these, *B. theobromae, A. niger, S. rolfsii, R. solani* and *N. mangiferae* are highly pathogenic and present in all agroecologies. These fungi except *N. mangiferae* have been earlier implicated in rot development in yam tuber in storage (Adeniji, 1970; Ogundana *et al*., 1970; Ikotun 1983; Sangoyomi *et al*., 2002). Of all the 17 fungal pathogens, *S. rostrata* occurred only in Forest/ Savanna transition agroecology. This implies the possibility of the fungus being localised or adapted to the environmental conditions of the environment. None of the 17 fungi tested for pathogenicity was able to penetrate intact yam tubers. This implies that wounding of tubers by agents such as rodents, insect pests and nematodes and or mechanical damage are required in infections to take place. This agrees with the report of Bridge (1972) and Morse *et al*. (2000) that nematode infestation and insect pest attack predispose yam to infection by fungi. The magnitude of rots varied with responsible organisms. Rots caused by *L. theobromae* and *A. niger* were severe on yams in storage. This agrees with the reports of Adeniji (1970) and Ogundana *et al*. (1970), that *A. niger* and *L. theobromae* cause severe decay of tubers in Nigeria. Their studies however, did not report *S. rolfsii, R. solani* and *N. mangiferae* as important rot pathogens.

The isolation of more than one type of fungus from a particular tuber suggests the possibility of multiple infections. The cumulative effects of various fungi may cause rapid and extensive rottening of the tubers. Fungi are abundant in the environment but not all are pathogenic. However, it is important to do further work on the significance of *L. theobromae, A. niger, R. solani, S. rolfsii* and *N. mangiferae* that were found to be highly pathogenic on yam during storage.

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References

Adeniji M O. 1970. Fungi associated with storage decay of yam in Nigeria. *Phytopathology* **60**:590–592.

Bridge J. 1972. Nematode problems with yams (*Dioscorea* spp.) in Nigeria. *Pest Articles and News Summaries* **18**(1):89–91.

Degras L. 1993. *The yam* (A Tropical Root Crop). London: Macmillan Press. 408 pp.

Ejechi B O, Ilondu M E. 1998. Control of yam tuber (*Dioscorea rotundata*) rot agent *Sclerotium rolfsii* with camwood (*Baphia nitida* Lodd) sawdust extract. *African Journal of Root and Tuber Crops* **3**:13–15.

FAO Report. 2010. *Food and Agricultural Organization Production Yearbook* http://faostat.fao.org/site/339/default.aspx (Accessed 14th September 2012).

Green K R, Sangoyomi T E, Amusa N A. 1995. Importance of *Rhizoctonia solani* as a pathogen of Yam (*Dioscorea rotundata*) in Nigeria. In *Proceedings of the 6th Triennial Symposium of the International Society of Tropical root Crops-Africa Branch*, pp. 412–418.
Ikotun T. 1983. Post-harvest microbial rot of yams in Nigeria. *Fitopathologia Brasileira* **8**:1–7.
Morse S, Acholo M, McNamara N, Oliver R. 2000. Control of storage insects as a means of limiting yam tuber fungal rots. *Journal of Stored Products Research* **36**:37–45.
Ogundana S K, Naqvi S H Z, Ekundayo J A. 1970. Fungi associated with soft rot of yams in storage in Nigeria. *Transactions of the British Mycological Society* **54**:445–451.
Olurinola P, Ehinmidu J, Bonire J. 1992. Antifungal activity of normal-tributylin acetate against some common yam rot fungi. *Applied and Environmental Microbiology* **58**:758–760.
Sangoyomi T E, Ekpo E J A, Asiedu R. 2002. First Report of *Nattrassia mangiferae* as a post harvest fungal pathogen of white Yam (*Dioscorea rotundata*) in Nigeria. *Plant Disease* **86**(8):919.