STUDIES OF CAUSAL AGENTS OF ROT IN *DIOSCOREA ROTUNDATA* POIR VAR. GBOKO (WHITE YAM)

L. O. Adimora, K. A. Oduro and H. B. Damptey

L. O. A. : Department of Biological Sciences, Rivers State University of Science & Technology, P.M.B. 5080, Port Harcourt, Nigeria; K. A. O.: Department of Crop Science, University of Ghana, Legon, Ghana; H. B. D. : Department of Botany, University of Cape Coast, Cape Coast, Ghana

Summary

Studies of the causal agents of rot in *Dioscorea rotundata* Poir var. Gboko in Port Harcourt, Nigeria, revealed that *Aspergillus niger*, *Aspergillus* sp., *Botrydiplodia theobromae*, *Fusarium* sp., *Penicillium* sp., and *Rhizopus stolonifera* caused the disease. Of these, *Fusarium* sp. exhibited the greatest potential for causing rot but recorded very low frequency of occurrence. *Rhizopus stolonifera*, on the other hand, recorded the highest mean frequency of occurrence but demonstrated the least ability to cause rot.

Introduction

Yam, *Dioscorea* sp. is one of the most important staple foodstuffs in West Africa. In Nigeria, Onwueme (1978) reported that yam is second only to cassava. Besides its use as food, many species are used as pharmaceutical bases (Adenuga, 1979). Thus the economic importance of yam cannot be overemphasized. Though there are usually large supplies of yams during the harvesting season which runs from October to January, yams are scarce and expensive from February. This is mainly due to inefficient storage techniques which influence diseases.

Coursey & Booth (1972) attribute diseases of stored yams to biotic and abiotic factors. The greatest cause of postharvest losses, however, has been attributed to attacks by micro-organisms (Coursey, 1967). Several fungi (Adeniji, 1970a) and a few bacteria (Mohamed, 1976) have repeatedly been isolated from rotting yams and have been shown to be the causal agents. These include *Botrydiplodia theobromae, Penicillium sclerotigenum, P. cyclopium, P. oxalicum, Fusarium moniliforme, Aspergillus niger, Rhizopus* sp. (Okafor, 1966; Ogundana, Naqvi & Ekundayo, 1970; Adeniji, 1970a; Adenuga, 1979).

Another important postharvest disease is the Internal Brown Spot which is caused by a virus (Mohamed, 1976). The extent of damage caused by the fungi just mentioned seems to differ from species to species (Adeniji, 1970b) and from locality to locality (Ricci, Coleno & Fevre, 1979). For instance while Coursey (1967) and Ogundana *et al.* (1970) rated *Botrydiplodia theobromae* as the most common and severe rot causing organism of stored yams, Adeniji (1970b) and Ricci *et al.* (1979) judged it as rather unimportant in the rotting of *Dioscorea trifida.*

Yam is consumed to a considerable extent in Port Harcourt in Rivers State at the southeastern part of Nigeria. The most preferred species is *Dioscorea rotundata* Poir var. Gboko (white yam). It has been reported that as much as 15 - 60 per cent of postharvest yam is lost through rotting in Nigeria (Anon., 1981). It was, therefore, decided to study the causal agents in order to devise control measures.

Experimental

Sources of yams

Three major yam markets in Port Harcourt, Creek Road, Mile 1 market, and Mile 3 market were the main source of yams used in this work.

Sampling

Partially rotted yams were purchased fortnightly from the three major markets. These were kept in the refrigerator for isolation studies. Laboratory temperatures throughout the experimental period ranged between 28 and 32 °C. A total of 177 rotting yam tubers from the three markets were sampled from August to March 1986 (Table 1).

TABLE 1

Number of partially rotten yam tubers sampled from the three major markets

Sampling months	*Number of yam tubers obtained*			
	Creek Road Market	*Mile One Market*	*Mile Three Market*	*Total*
August	20	10	8	38
September	18	9	6	33
October	18	10	7	35
November	10	4	5	19
January	10	8	7	25
February	10	4	4	18
March	5	2	2	9
Total	91	47	39	177

Media preparation

Isolation of organisms from decaying yam tubers were first attempted on water agar (WA) and then potato dextrose agar (PDA). The preparation of the latter was done by suspending 39 g of PDA (Merk) powder in 1 *l* distilled water in a 2-*l* flask. It was allowed to soak, shaken, boiled, and sterilized by autoclaving at 33 kg psi at 121°C for 15 min.

Water agar (WA) was prepared by suspending 20 g of Agar Agar (Oxoid) in 1 *l* of distilled water. This was allowed to soak, dissolved by boiling and then sterilized as above.

Isolation and identification of micro-organisms

Partially decaying yam tubers were cut into relatively big pieces with a kitchen knife. Sterile scalpel blades were then used to cut pieces of 4 cm x 1 cm from the outermost edges of the advancing rot margins. They were disinfected in 5 per cent sodium hypochlorite solution for 2 min, rinsed in sterilized distilled water and asceptically transferred into WA plates. The plates were tied up in clean cellophane bags and incubated under ambient condition in the laboratory.

The micro-organisms which developed were transferred onto slants of PDA if pure. If a mixed culture was encountered, however, the micro-organisms were repeatedly subcultured until individual pure cultures were obtained.

For identification of the micro-organisms pure cultures were mounted in lactophenol and morphological characters observed under microscope.

Pathogenicity test of isolates

All fungi isolated from the decaying yam tubers were tested for their pathogenicity. Healthy yam tubers were cleaned with tap water and surface disinfected by immersing the tubers in 5 per cent sodium hypochlorite solution for 3 min and allowed to dry at room temperature. Each tuber was then swabbed with 95 per cent ethanol. A sterile 5-mm cork borer was used to make bores on the yam tubers by inserting it 10 mm deep into the tubers to remove the tissues. The cork borer was kept sterile by dipping it in 95 per cent ethanol and flaming off after each use.

Inoculations were made by placing 5-mm discs from the margin of a 4-day old culture of isolates on PDA into the wound cavities. The tissue plugs were then replaced into their respective cavities after swabbing the surface of the cavities with 95 per cent ethanol. The wounds created during the process were sealed off with molten paraffin wax chips. Each tuber was inoculated at the same time with all the seven isolates, each in a different cavity. Controls were treated similarly except that sterile discs of PDA without inoculum were placed in the cavities.

All treatments had five replicates and all in-

oculated tubers were kept in a cupboard to prevent attacks from cocroaches and mice.

Twenty-eight days after inoculation the inoculated yam tubers were cut open from the head to the tail across the points of inoculation. Symptoms of any rot were recorded and compared with the symptoms present in the diseased tuber from which the isolates originated. Measurements of rot diameter from points of inoculum were done with a metre rule.

Koch's postulates were fulfilled by the re-isolation of each pathogen from the respective inoculated tubers. The micro-organisms were identified as described above.

Results

Micro-organisms associated with rotting of yam

From the isolation studies the fungi that could be identified were: *Aspergillus niger, Aspergillus* sp., *Botrydiplodia theobromae, Fusarium* sp., *Penicillium* sp. and *Rhizopus stolonifera.* Two other fungi which could not be identified, and a cocoal bacterium, were also isolated.

The frequency of occurrence of the micro-organisms from the yams are presented in Table 2. The percentage frequency is shown in Fig.1. Using Friedman's S test and Chi-square test there was a significant difference ($P = 0.05$) in the occurrence of the pathogens in the yam tubers. Further analysis using Scheffe's multiple comparism to determine the differences in frequency amongst the pathogens showed highly

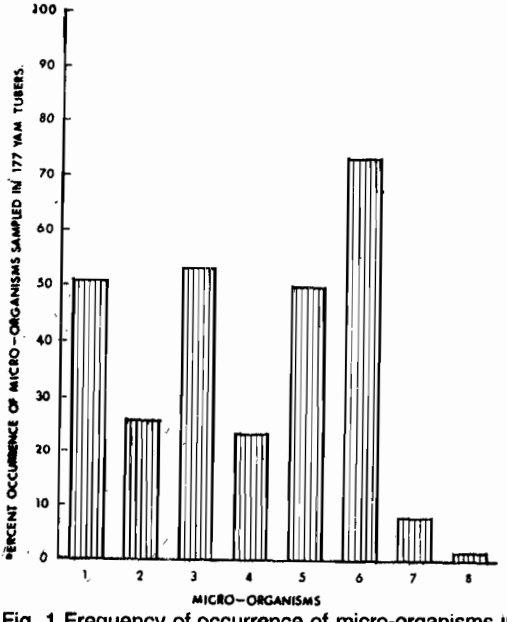

Fig. 1 Frequency of occurrence of micro-organisms in 177 yam tubers sampled from three markets in Port Harcourt. 1. *A. niger*, 2. *Aspergillus* sp., 3. *B. theobromae*, 4. *Fusarium* sp., 5. *Penicillum* sp., 6. *R. stolonifer*, 7. *Coccal bacterium*, 8. Two unidentified fungi.

significant differences ($P = 0.01$) in the frequency of occurrence of micro-organisms in the diseased yam tubers.

The frequency of occurrence of the bacterium and the unidentified fungi was significantly lower ($P = 0.05$) than all the identified fungi. There was, however, no significant difference amongst *A. niger, B. theobromae, Penicillium* sp., and *R. stolonifera.* Also there was no significant differ-

TABLE 2

Occurrence of different micro-organisms in 177 diseased yam tubers sampled from three markets in Port Harcourt

	A. niger	*Aspergi-gillus sp.*	*B. theo-bromae*	*Fusa-rium sp.*	*Penici-llium sp.*	*R. stoloni-fera*	*Bacte-ria*	*Uniden-tified fungi*	*Total*
Creek Road Market	45	25	46	19	41	68	2	1	247
Mile One Market	27	14	29	13	31	32	4	1	151
Mile Three Market	18	7	19	10	16	30	8	1	109
Total	90	46	94	42	88	130	14	3	507

ence amongst *Aspergillus* sp., *B. theobromae, Fusarium* sp. and *Penicillium* sp. However, the frequency of occurrence of *R. stolonifera* was significantly higher ($P = 0.05$) than those of *Aspergillus* sp.

The various characteristics of the identified fungi were as follows:

Aspergillus niger. This fungus produced fawn rots with very dark charcoal-like margins on the yam tubers infected. On PDA the colonies spread rapidly with the white mycelium which turned dark brownish with age. Sometimes concentric zonations were shown where growth was taking place. The colourless or brownish conidiophores which were mostly smooth measured about 200-400 m long. The conidial heads and vesicles were globose and appeared brown to black and often very dark when seen en masse. The conidia were about 5μ in diameter.

Aspergillus *sp.* This second species of *Aspergillus* had ochre-coloured colonies on PDA. The mycelium which looked submerged was tinted to various shades of yellow and purple. The spore heads were larger and more conspicuous than *A. niger*. The heads, vesicles and conidia were globose. The conidia were between 3 and 5 μ in diameter, some produced masses of sclerotia with short, rough stalks.

Botrydiplodia theobromae. This fungus caused a firm decaying area at the centre of the yam tuber and this was usually surrounded by a dark brown margin between the diseased and healthy tissue. It produced greyish to black fluffy colonies in potato agar with abundant aereal mycelium. While sporulation readily occurred on the yam tuber, the mycelium was sterile on PDA. The pycnidium was simple and was 5μ in width. The conidiophore was haline, unbranched and septate. The conidia were hyaline and oblong initially while mature ones were septate and were about 20 μ along its longitudinal axis.

Fusarium *sp.* This fungus produced creamish dry rots surrounded by light brown margins with occasional wine red stripes in the decaying yam tuber. It grew with moderately greyish white mycelium with brown discoloration when growing on PDA. It produced both macro and micro conidia where the latter developed from elongated lateral philiades in the aerial mycelium. These microconidia were hyaline, cylindrical and wedge-shaped and measured 9 μ x 2 μ on the average. Macroconidia developed in about 4-7 days from branched and well developed conidiophore which themselves had well developed foot cells and were about 50 μ x 5 μ in size. In older cultures chlamydophores were present appearing smooth and oval.

Penicillium *sp.* It produced light tan to dark brown rots with dark brown margins on decaying yam tuber. The texture of the rotted area varied from soft to wet and dry to firm. In PDA its colonies were blue-green to bright green in colour with broad white margin during the first 4-6 days of growth. With age the colours turned purplish brown. The penicillin were smooth and fairly complex. Conidiophores were about 3 μ in diameter. Conidia were subglobose and less than 2μ.

Rhizopus stolonifera. It produced grey to brown caky rot surrounded by dark margin on decaying yam tubers. Colonies on PDA were white cottony at first, becoming heavily speckled by the presence of sporangia which turned brownish black with age. This fungus spread rapidly by means of stolons fixed at various points to the media base by rhizoids. It had aseptate smooth walled sporangiosphores which were up to 34 μ in diameter and about in length. They were simple and light brown and in groups of three arising from stolons adjoining the rhizoids. The stolons and globose sporangia were brownish and measured about 150 μ in diameter. The columelae are subglobose, dorsiventrally flattened, light brownish grey and when dehisced by pressure were umbrella-shaped.

Results of pathogenicity test

Pathogenicity test of isolates revealed that the six micro-organisms were able to rot the healthy

yam tubers into which they were inoculated. The rot colour symptoms described above, were similar to the varying colours that were associated with the infected yam tubers from which the rots were first observed. Morphological characteristics of re-isolated micro-organisms as seen under the microscope described above were similar to those used as inocula. The two unidentified fungi and the bacterium could not cause rotting of healthy yam tubers.

Discussion

Eight micro-organisms have been found to be associated with rotting of *Dioscorea rotundata* Poir var. Gboko in Port Harcourt. Six of these caused rotting of *D. rotundata*. These were *Aspergillus niger, Aspergillus* sp., *Botrydiplodia theobromae, Fusarium* sp., *Penicillium* sp. and *Rhizopus stolonifera.*

A.. niger caused more severe rotting than *Aspergillus* sp. This is in agreement with Adeniji (1970a) who also reported that *A. niger* was a major causal agent for yam decay. Similarly, it has been reported that *B. theobromae*, which also caused severe rotting in the present work, is a major cause of decay of yams in West Africa (Noon & Calhoun, 1981; Ogundana *et al.*, 1970; Coursey, 1967). In contrast, Adeniji (1970a) and Ricci *et al.* (1979) found this fungus to be a weak pathogen of yams when yams were kept at 25°C. Ricci *et al.* (1978) found that yam decay by *B. theobromae* was severest at 35°C. Since the temperature used in the present work was between 28°C and 32°C, it is likely the temperature influenced the severity of rotting by *B. theobromae.*

An interesting observation in this work was that although *Fusarium* sp. caused the most severe rotting, it recorded a very low frequency of occurrence in the diseased yam tubers sampled. Though Noon & Calhoun (1979, 1981) reported similar potential for causing rot of yams by *F. solani, F. oxysporum* and other *Fusarium* sp., they recorded a high mean percentage frequency of occurrence. In the present work *R. stolonifera* recorded the highest mean frequency of occurrence but showed the least ability to cause rot. Other workers have considered *R. stolonifera* as a saprophyte in the postharvest decay of yam. However, Noon & Calhoun (1979, 1981) noted that its potential to cause rot increased in combination with other fungi such as *F. solan;*, and *F. oxysporum.*

Since the work of Okafor (1966) other workers have found *Penicillium* sp. to be serious pathogen of yam (Adeniji, 1970b; Ricci *et al.*, 1979; Noon & Calhoun, 1979; 1981). In the present work, *Penicillium* sp. ranked fifth in terms of potential to cause rot. These inconsistencies may be attributed to differences in the species causing the rot in each experiment.

From this work it was observed that all the various pathogens entered the yam tubers through bruises. These might have occurred during harvesting, packaging and transportation. If, therefore, these operations could be done with minimum bruises, the incidence of yam decay could be substantially reduced.

References

ADENIJI, M. O. (1970a) Fungi associated with storage decay of yam in Nigeria. *Phytopathology* **60**, 590-599.

ADENIJI, M. O. (1970b) Influence of moisture and temperature on yam decay organisms. *Phytopathology* **60**, 1968-1969.

ADENUGA, A. O. (1979) Processing and storage of yam in Nigeria, pp. 110-118. In *Small scale processing and storage of tropical root crops* (ed. D. L. Plucketts). West View Agriculture Series, Boulder Colorado: West View Press.

ANON (1981) *Postharvest food losses in developing countries*, p. 206. Washington D.C.: National Academy of Sciences.

COURSEY, D.G. (1967) Yam storage - L. A. Review of yam storage practice and information on storage losses. *J. Stored Prod. Res.* **2**, 229-232.

COURSEY, D.G. & BOOT, R. H. (1972) The post harvest photopathology of perishable tropical produce. *Rev. Pl. Path.* **51**, 751-765.

MOHAMED, N. (1976) Virus-like particles and cytoplasmic inclusions associated with disease of *Dioscorea* sp. in the eastern Carribean. *Trop. Agric. Trin.* **53**, 341-351.

NOON, R. A. & CALHOUN (1979) Market and storage of yams imported to United Kingdom. *Phytopath. Z.* **94**, 289-302.

NOON, R. A. & CALHOUN (1981) Observations of the nature of chilling injury in yams. *Phytopath. Z.* **100**, 10-18.

OGUNDANA, S. K., NAQVI, S. H. Z. & EKUNDAYO, J. A. (1970) Fungi associated with soft rot of yams (*Dioscorea* sp.) in storage in Nigeria. Trans. Br. mycol. Soc. **54**, 445-451.

OKAFOR, N. (1966) Microbial rotting of soft yams (Dioscorea sp.) in Nigeria. *Exp. Agric.* **2**, 179-182.

ONWUEME, I. C. (1978) *The tropical tuber crops: yams, cassava, sweet potato and cocoyams.* Chechester: John Wiley and Sons. 234 pp.

RICCI, P., COLENO, A. & FEVRE (1978) Storage problems of cush-cush yams. II. Control of *Penicillium oxalicum* rots. *Ann. Phytopath.* **10**, 433-440.

RICCI, P., TOREGROSSA, J. P. & ARNOLIN, R. (1979) Storage problems of cush-cush yams. I. Postharvest decay. *Trop. Agric. Trin.* **56**, 41-48.

SMITH, G. (1969) *An introduction to industrial mycology,* 6th ed. London: Edward Arnold. 390 pp.

Received 15 Nov 89; revised 3 Jul 90.