Incidence and control of root rot disease of cashew seedlings, **Anacardium occidentale** in the nursery*

O. A. OLUNLOYO**

COMPENDIO

Durante la estación lluviosa de 1973 se observó en dos almácigos adyacentes, en la Estación Experimental de Gambari, Cocoa Research Institute de Nigeria, Ibadán, una severa pudrición radical de plántulas de marañón, Anacardium occidentale *L. Las plántulas estaban creciendo en bolsas de polietileno para ser trasplantadas a parcelas del campo. Fueron aisladas persistentemente, de las raíces infectadas de marañón,* Fusarium solani *(Mart.) Apel and Wr.,* Botryodiplodia theobromae *Pat.,* Gliocladiopsis *spp.* y Pythium ultimum *Trow.*

Las pruebas de patogenicidad mostraron que Pythium ultimum *fue la causa primaria de la enfermedad, mientras que* Botryodiplodia theobromae *and* Gliocladiopsis *fueron principalmente parásitos secundarios que no produjeron síntomas.* Fusarium solani *causó marchitamiento cuando se inoculó a las raíces con heridas pero fue incapaz de producir síntomas en raíces saludables. Esta es la primera vez que* Pythium ultimum *se registra como un organismo que causa pudrición de las raíces en plántulas de marañón en Nigeria. Se obtuvo un control químico completo con p-dimetilamino-bencenodiazo sulfonato de sodio (Dexon) aplicado a 113,6 kg/ha especialmente con el método de incorporación al suelo.* — El autor.

Introduction

DURING the rainy season (April to October) of 1973, a severe root rot disease of cashew seedlings, *Anacardium occidentale*, was observed in two adjacent nursery sheds at the Gambari Experimental Station, Cocoa Research Institute of Nigeria. The seedlings were being raised in polyethylene bags for transplanting into the field plots. Disease incidence of the seedlings was estimated at 10 to 15 per cent in the nursery. The primary symptoms are the yellowing of the lower leaves of 4 to 6 week-old cashew seedlings and conspicuous stunting of the plants. Examination of the diseased plants reveals the rotting of the root systems with infection starting from the root tips. Some stunted seedlings which are seemingly healthy usually have rotted root systems. Such plants can only survive temporarily on the reserved food material in the bulbous hypocotyl region of the seedlings and when the food reserve is exhausted, the seedlings die off.

There is no information on the occurrence of root rot of cashew seedlings. The objective of this study was to determine the causal agent and to control the disease.

Materials and methods

Isolation of the root rotting fungi

Infected cashew seedlings with distinctly recognisable symptoms of the root rot disease were collected from the plants grown in naturally infested soil contained in polyethylene bags arranged on the nursery beds. The roots were thoroughly washed with fine jets of tap water and blot dried on filter paper. These roots were separated into tap roots and the lateral roots. Each of these was

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** Plant Pathologist, Cocoa Research Institute of Nigeria, Gambari Experimental Station, P. M. B. 5244, Ibadan, Nigeria.

cut into 5-mm portions and then surface - disinfected in one per cent sodium hypochlorite solution for 30 seconds. The root pieces were washed thrice with sterile distilled water to remove the effect of sodium hypochlorite solution. The root pieces were plated on potato dextrose agar (PDA) contained in oven-dried petri-dishes and incubated for 3 days. Pure cultures of all the fungi that had grown were obtained for identification. The frequency of occurrence of the isolated fungi was determined from both the total number of the root pieces plated and the total number of fungi isolated.

Pathogenicity test

Seedlings raised from nuts of the tree selection Iwo 222 were used in the pathogenicity tests carried out in the glasshouse. The isolated fungi were tested by growing the fungi on potato dextrose agar contained in petri-dishes for seven days at 25°C. Inoculation of the seedlings at four stages of the plants' development were carried out as follows:

1 —One-day-old germinating nuts were inoculated by mixing two petri-dishes of the fungus with steam-sterilized garden soil in 20-cm clay pots approximately 5 cm below surface-disinfected cashew nuts. An additional 5 cm of steamed soil was used to cover the nuts.

2 —One-week-old seedling growing in stem-sterilized saw-dust in 10 cm diameter polyethylene bags with wide drainage holes were placed on sterilized garden soil in 20-cm clay pots inoculated by mixing two plates of the mycelial inoculum of each of the fungi with the top 5 cm of soil. When the roots of the young plants grew out through the drainage holes they eventually would touch the layer of the fungal inoculum in the pots.

3 —Two-week-old plants growing in steam-sterilized garden soil in 20-cm clay pots were inoculated by placing inoculum against the tap-root about 3 cm beneath the soil.

4 —Four-week-old plants in 20-cm clay pots containing sterile soil were also inoculated by inserting a small portion of the 7-day-old agar culture into a longitudinal slit made with the aid of a sterile scalpel at the crown of the root of each plant.

In the control series similar procedures were followed using sterile agar. All inoculation experiments were carried out in the glasshouse where temperature varied between 26°C and 30°C. Abundant soil moisture content was maintained with tap water.

Before planting, the nuts were soaked for 24 hours and only those that sank were used for planting while the floating ones were discarted. This procedure of soaking the nuts hastened germination. Twenty-five pots with two plants per pot were inoculated with each fungus while the same number of pots was alloted to the control. Plants were observed for signs of root rot for 8 weeks after inoculation. Re-isolations were made to confirm whether the inoculated fungus was the one producing the observed symptoms in the infected plants.

Chemical control experiment

Control of the root disease was carried out under the glasshouse conditions by using 5 per cent wettable powder P-dimethylaminobenzenediazo sodium sulphonate (Dexon). In the glasshouse experiments, naturally infested soils were collected from several locations in the vicinity of the nurseries at the Gambari Experimental Station. The soils were mixed and the resulting composite was used. Three kilogram of the infested soil was weighed into each of the 15 cm diameter and 30 cm deep black polyethylene bags. The presence or absence of the root rotting agent in the soil was tested by planting cashew nuts of the tree selection Iwo 222 in the polyethylene bags.

The chemical was applied at the rate of 12.5, 25 and 50 milligram per kilogram of the infested soil. These calculated amounts of the chemical were equivalent to 28.4, 56.8 and 113.6 kg/hectare respectively. The fungicide was applied to the soil by methods similar to those used by Olunloyo (17) as follows:

(a) incorporated with the soil before planting;

(b) applied at the seed (nut) level at the time of planting;

(c) added to the soil surface immediately after the nuts were sown.

Incorporation of Dexon with the soil was accomplished by using a hand trowel. All treatments were replicated ten times with two nuts sown per polyethylene bag. Untreated but infested soil and steam-sterilized soil in polyethylene bags similarly replicated served as two sets of controls for each treatment. Each nut was covered with two centimeters of soil after sowing. Water was applied to all the pots in the morning every day. The temperature in the grasshouse varied between 25°C and 30°C during the period of the experiments. In order to test the residual effects of Dexon in the same soil the second planting was carried out. Before the second planting in the glasshouse tests, however, the soils from all treatments receiving similar chemical dosage were combined, sieved and thoroughly mixed. The identity of the soil was maintained. Root rot disease rating was determined 16 weeks after germination. Root rot indices were assessed on the basis of disease ratings of 0 to 4 as follows:

0 — no infection;

1 — infection restricted to the root tips;

2 — infection restricted to the root branches and secondary roots;

3 — some of the roots including the main root totally diseased, the remainder only partly infected;

4 — entire root system infected.

In the root rot ratings, a disease index for each treatment replicate was attained by multiplying the number of plants in each class by the respective rating number; the totals were added and the sum was divided by the total number of plants in the replicate

Confirmation of the root infection was obtained by dipping the infected roots into one per cent (1.0 per cent) solution of 2, 3, 5-triphenyl-tetrazolium chloride (T.T.C.) used as an indicator to determine the extent of root infection by pythiaceaus fungi (9).

Results

Fusarium solani, Botryodiplodia theobromae, Gliocladiopsis species and *Pythium ultimum* were consistently isolated from the rotted root systems of cashew seedlings. *Pythium ultimum* was isolated more often from the lateral roots than the other fungi (Table 1).

Results of the pathogenicity tests shown on Table 2 indicated that *Pythium* species was the primary organism associated with the root rot of cashew seedlings. *Fusarium solani, Gliocladiopsis* species and *Botryodiplodia theobromae* did not infect the healthy seedlings at any of the stages of the plant's development, but *Fusarium solani* induced wilting when inoculated by wounding 6-week-old plants. *Pythium* species was pathogenic at the four stages of the development of the plant. Observations of the seedlings inoculated in the glasshouse indicated that the radicle was infected shortly after emergence from the nut although varying degrees of radicle elongation occurred before attack. Some germinating seedlings were attacked in the neck region or at the growing root tip. Such seedlings died when neck region was invaded, separating the immature root system from the cotyledons. Some seedlings attacked in this manner emerged from the soil but eventually damped-off. Seedlings attacked at the root tip at the late stages of their development survived attack by forming secondary roots above the infection area and such plants appeared capable of developing into normal, healthy, matured plants. In more advanced infection of older seedlings, the entire root system was destroyed producing similar symptoms observed in the nursery (Figs. 1 and 2). Most of the germinating seedlings were killed within 3 weeks after inoculation and those inoculated by wounding 6-week-old seedlings developed roots necrosis within six weeks. Plants that tolerated infection beyond the seedlings stage had stunted growth.

Isolations from the inoculated roots yielded the original *Pythium ultimum*. All the plants in the control remained healthy.

Control of the root rot disease was achieved with p-dimethylaminobenzenediazo sodium sulphonate (Dexon) applied at 113.6 kg/ha especially when the chemi-

Table 1.—Fungi isolated from the infected root tissues of cashew seedlings in the nursery.

Fungus	Isolation from a 1/		
	Tap root	Lateral root	Hypocotyl
1. *Fusarium solani* (Mart.) Appel. & Wr.	71	66	28
2. *Gliocladiopsis* sp.	26	4	—
3. *Pythium ultimum* Trow	45	81	—
4. *Botryodiplodia theobromae* Pat.	31	—	6

1/ Figure represent number of times fungus was isolated from 100 pieces of infected root plated on potato dextrose agar (PDA).

Table 2.—Pathogenicity of *Fusarium solani, Gliocladiopsis* sp., *Pythium ultimum* and *Botryodiplodia theobromae* on cashew. Fifty plants inoculated in each trial. Figures represent per cent of root rot.

Stage of plant at inoculation	*Fusarium solani*	*Gliocladiopsis* spp.	*Pythium ultimum*	*B. theobromae*
One day old germinating nuts	0	0	100	0
One week old seedlings	0	0	92	0
2 week old	0	0	88	0
4 week old	66	0	100	0

Fig. 1.—*Cashew seedlings with rotted root systems (left) and healthy seedlings with normal root systems (right)*

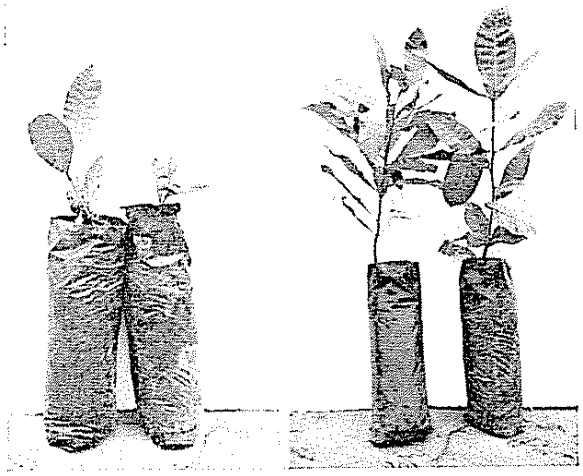

Fig 2 —*The characteristic stunting effect of* Pythium ultimum *on cashew seedlings (left) compared with normal seedlings (right)*

Table 3 —Root rot ratings of cashew seedlings grown in soil naturally infested by *Pythium ultimum* and treated with Dexon.

	Method of chemical treatment	Dosage applied (kg/ha)	Average root rot index 1/	
			First planting	Second planting
1	Incorporated with soil	28.4	1.5	0.8 a
	before planting	56.8	0.5	0.3 a
		113.6	0.0	0.0
2	Applied at seed level at	28.4	2.8	2.2 c
	the time of planting	56.8	1.6	1.0 a
		113.6	1.0	1.0 a
3	Added to the soil surface	28.4	2.0	1.5 b
	immediately after the	56.8	0.8	0.5 a
	nuts were sown	113.6	0.5	0.0
4	No chemical (control)	—	4.0	4.0 d
5	Steam-sterilized soil	—	0.0	0.0
	(control)			

1/ Indices followed by the same letter are not significantly different at the 5% as determined by Duncan's Multiple Range Test. Root rot index of: — 0 no infection; 1 — infection restricted to the root tips; 2 — infection restricted to the root branches and secondary roots; 3 — some of the roots including the main root totally infected, the remainder only partly infected; 4 — entire root system infected

cal was incorporated with the soil before planting (Table 3). No top injury of the plant was observed in any of the three methods of application of the chemical. Observations showed that only plants whose root systems showed root rot ratings of 3 or 4 were stunted and later killed by *Pythium* while those plants with root rot rating of 2 or less grew normally. Steam sterilization of the infested soil was also very effective in controlling the organism. All plants grown in untreated infested soil had root rot rating of 4. This is the first time *Pythium ultimum* is being reported as a root rot organism on cashew seedlings in Nigeria.

Discussion

This investigation showed that *Pythium ultimum* Trow was the primary cause of the root rot disease of cashew seedlings in the nurseries. The pathogen, when operating under favourable condition of abundant soil moisture, usually caused severe root damage and consequent death of the infected seedlings. This observation supported the findings of some workers who reported that abundant soil water favoured diseases of seedlings and ornamentals incited by *Pythium* species (3, 4, 8, 10, 13, 20). The importance of *P. ultimum* as pathogen of pea roots (1, 5, 10, 19), cotton seedlings (2), corn seedlings (7), muskmelon (14), poinsettia (21), wheat (22), beans (11) and the foliage of highland bentgrass (16) was well documented.

Fusarium solani, Gliocladiopsis species and *Botryodiplodia theobromae* were only secondary parasites which only came in after the root systems of the seedlings had been infected by *P. ultimum*. However, *Fusarium solani* induced wilting of the seedlings only when injured roots were inoculated with the organism causing 66 per cent of the 4 - week old cashew seedlings inoculated by wounding to die off (Table 2). This suggested that special care should be taken to prevent root damage during the process of transplanting the seedlings into the experimental plots as any root injury would serve as an entry point to soil parasite such as *Fusarium solani* which could cause the plant to wilt.

Root rot disease of cashew seedlings could be a limiting factor in the establishment of the plants in old plantations which might have been naturally infested by *Pythium* species. It is, therefore, suggested that before any transplanting is made estimation of the inoculum potential of *Pythium* in such plantations should be carried out using a procedure applied by Olunloyo (17) to determine the inoculum level of *Phytophthora parasitica* Dast. var. *nicotianae* (Breda de Hann) Tucker, the causal organism of root and stem rot disease of roselle fibre crop in three plantations in Nigeria. Such estimation of the inoculum level could serve as a warning to cashew farmers who might not be aware that a field had already been naturally infested by *Pythium* and that planting of cashew directly in such areas could lead to a greater loss. As far as the author is aware, this is the first time *P. ultimum* is being reported as a root rot organism of cashew seedlings in Nigeria.

Complete chemical control of the disease was achieved when p-dimethylaminobenzenediazo sodium sulphonate (Dexon) was applied at the rate of 113.6 kg/hectare especially by soil incorporation method in the glasshouse tests. Dexon had been reported to be highly effective against some phycomycetous pathogens of seedlings such as *Aphanomyces enteiches* Drechsler (6, 12, 18), and roots such as *Pythium ultimum, Pythium aphanidermatum* (Edson) Fitzp. and *Aphanomyces cochlioides* Drechsler (12, 15), *Phytophthora cinnamomi* Rands (23) and *Phytophthora parasitica* var *nicotianae* (17).

Summary

A severe root rot disease of cashew seedlings, *Anacardium occidentale* L., was observed in two adjacent nursery sheds during the rainy season of 1973 at the Gambari Experimental Station, Cocoa Research Institute of Nigeria, Ibadan. The seedlings were being raised in polyethylene bags for transplanting into the field plots. *Fusarium solani* (Mart.) Apel and Wr., *Botryodiplodia theobromae* Pat., *Gliocladiopsia* spp. and *Pythium ultimum* Trow were consistently isolated from the infected roots of cashew seedlings.

Pathogenicity tests showed that *Pythium ultimum* was the primary cause of the disease while *Botryodiplodia theobromae,* and *Gliocladiopsis* were mainly secondary parasites which did not produce any symptoms. *Fusarium solani* caused wilting when inoculated into wounded roots but was unable to produce any symptoms on healthy roots. This is the first time *Pythium ultimum* is being reported as a root rot organism of cashew seedlings in Nigeria. Complete chemical control of the disease was achieved with p-dimethylaminobenzenediazo sodium sulphonate (Dexon) applied at 113.6 kg/hectare especially by soil incorporation method.

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