SURVEY OF ROOT-INHABITING MICROORGANISMS ON DECLINING AND NONDECLINING BREADFRUIT (*ARTOCARPUS ALTILIS*) IN JAMAICA

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ABSTRACT

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A survey of phytoparasitic nematodes, fungi, and bacteria associated with roots of breadfruit (*Artocarpus altilis*) trees was conducted in Jamaica in 1991. Eighteen trees sampled did and 12 did not exhibit decline, characterized by premature fruit drop, leaf chlorosis and abscission, general unthriftiness, and branch dieback. Most trees of each category were parasitized by dense populations of *Pratylenchus coffeae*. *Helicotylenchus erythrinae*, *H. multicinctus*, and *Meloidogyne incognita* also occurred within roots in large populations. *Fusarium* species (*F. equiseti*, *F. oxysporum*, *F. pallido-roseum*, and *F. solani*) were detected frequently. *Pseudomonas* spp. occurred in most root samples, and all roots had vesicular-arbuscular mycorrhizae. Physical and chemical characteristics of the soil and nutrient concentrations within leaves were not relatable to tree health. Most trees exhibiting decline symptoms were more than 20 years old.

Key words: Artocarpus altilis, breadfruit, decline, nutrients, *Pratylenchus coffeae*, root-inhabiting microorganisms, tree age.

RESUMEN

Coates-Beckford, P. L. y M. J. Pereira. 1992. Muestreo de los microorganismos habitando las raíces de plantas del árbol del pan (*Artocarpus altilis*), mostrando o no el fenómeno de declinación lenta, en Jamaica. Nematrópica 22:55–63.

En 1991, se llevó a cabo en Jamaica un muestreo de nematodos fitoparásitos, hongos y bacterias asociados con las raíces de árbol del pan (*Artocarpus altilis*). Doce árboles muestreados no mostraron y 18 sí mostraron una declinación caracterizada por la caída prematura de los frutos, clorosis y abscición de las hojas, debilidad general, y muerte de las ramas. La mayoría de los árboles de ambas categorías estaban parasitados por altas poblaciones de *Pratylenchus coffeae*. *Helicotylenchus erythrinae*, *H. multicinctus* y *Meloidogyne incognita* también se encontraron en las raíces en poblaciones elevadas. Especies de *Fusarium* (*F. equiseti*, *F. oxysporum*, *F. pallido-roseum* y *F. solani*) fueron detectadas frecuentemente. Se encontraron *Pseudomonas* spp. en la mayoría de las muestras de raíces y todas las raíces muestreadas tenían micorrizas vesiculo-arbulsculares. Las características físicas y químicas del suelo y las concentraciones de nutrientes en las hojas no se correlacionaron con el estado sanitario de los árboles. La mayoría de los árboles mostrando síntomas de declinación tenían más de 20 años de edad.

Palabras clave: árbol del pan, *Artocarpus altilis*, declinación, edad del árbol, *Fusarium* spp., microorganismos de las raíces, nutrientes, *Pratylenchus coffeae*.

INTRODUCTION

Breadfruit [*Artocarpus altilis* (Parkinson) Fosberg] trees, collected in Tahiti and Timor and brought to Jamaica in 1793 by Captain Bligh (32), grew successfully and became distributed throughout the island. Most trees were seedless forms but a few were a seeded form called the breadnut.

Between 1958 and 1987, a serious decline disease killed all but 46 000 of the 2 000 000 breadfruit trees of Jamaica (22). Huricane Gilbert in 1988 killed or damaged many remaining trees. A simi-

lar lethal decline disease of breadfruit has occurred in the Pacific Basin (17,33).

Breadfruit trees can produce 700 fruits/year, each fruit weighing up to 4.5 kg (20). In Jamaica, the fruits are a staple food, eaten after being roasted or boiled, and also are fed to animals. In addition, the breadfruit provides timber, has potential for the manufacture of starch and alcohol, and reduces soil erosion (22).

The most obvious symptom of decline is the dieback of branches, resulting in death of the trees after several years. Other symptoms are the presence of smaller and fewer leaves than in nondeclining trees, leaf chlorosis, reduced fruit production, and general unthriftiness coupled with excessive, premature fruit fall. Diseased trees usually occur in groups.

Trujillo (29) observed breadfruit diseases in several islands in the Pacific Basin and suggested that the dieback disease there, "Pingalap Disease", was not caused by microorganisms (28). He proposed that saline soils, salty sea spray, or typhoon damage, accentuated by drought and old age, were responsible for tree decline. However, in some islands, *Rhizoctonia solani* Kühn was consistently associated with feeder root dieback, while *Pythium* sp. and *Fomes* sp. were associated with brown rot of lateral roots (28). Infection by *Fomes* sp. sometimes caused a soft heart rot disease, and another basidiomycete, possibly *Corticium* sp., severely rotted the crowns and roots (28).

Because breadfruit is an important staple in Jamaica, efforts are being made to identify factors limiting production. Our objective was to determine if specific root parasites or nutrient deficiencies were associated with decline symptoms in trees throughout the island.

MATERIALS AND METHODS

From April to September, 1991, 30 breadfruit trees were selected for sampling. Six were from the northern parish of St. Ann, five and six from the southern parishes of St. Catherine and St. Andrew, respectively, seven from Portland in the east, and three from each of the western parishes of St. James and Trelawny. Eighteen trees exhibited and 12 did not exhibit dieback. One or more trees of each category were selected at each site. Some trees not exhibiting dieback had grown from root suckers of declining trees. A single breadnut tree growing in Portland was also examined.

The trees were categorized as young, mid-aged, and old if they were less than 20, 20–40, and more than 40 years old, respectively. For each tree, a cluster of leaves at the tip of a branch 2–4 m above soil level was collected for nutrient determination by the Rural Physical Planning Unit of the Ministry of Agriculture in Kingston, Jamaica. Also, a 1-kg sample of soil and associated roots, each consisting of two to nine combined, randomly selected subsamples, was taken to a depth of 30 cm in an area within the dripline of the tree. Samples were stored immediately at 4 C. Nematodes were extracted within 2 days and fungi and bacteria were isolated within 7 days of sample collection.

Roots were gently washed free from soil and cut into 1-cm lengths. Duplicate subsamples of up to 10 g were placed on a mist extraction apparatus for 2 days to extract motile nematodes (11) and the resulting suspension was collected. Some of the remaining feeder roots were stained with acid fuchin and examined microscopically to detect sedentary nematodes (12); others were stained with trypan blue to detect mycorrhizae (18).

Pieces of the feeder root system, 1.5–2.5 cm long, were dipped for 20–25 s in 0.5% sodium hypochlorite and for 10 s in each of five changes of sterile distilled water. Root pieces then were placed on Petri plates of carrot potato dextrose agar (CPDA) to isolate fungi (25) or on nutrient agar (NA) to isolate bacteria (4). The CPDA plates were incubated at 28 C under a 12 h/12 h light/dark cycle and the NA plates were incubated at 30 C in the dark. As soon as fungi or bacteria grew out from the roots, they were isolated in pure culture on the same medium.

The fungi were identified by morphological characters (2,8,9,10,14,26,30). Bacterial isolates with characteristics of phytopathogens (24) were identified by their behaviour on diagnostic media (24) and also by the Commonwealth Agricultural Bureau International Mycological Institute (CABIMI).

For loamy soils, nematodes were extracted for 2 days from duplicate 200-cm^3 subsamples by the Baermann tray tech-

Table 1. Number of nondeclining and declining trees of *Artocarpus altilis* that were sampled according to location, age, soil texture, soil pH, and soil electrical conductivity.

		Seedless trees (breadfruit)		Seeded tree (breadnut)
		Nondeclining	Declining	Declining
Location				
North	St. Ann	2	4	0
South	St. Andrew	4	2	0
	St. Catherine	2	3	0
East	Portland	2	5	1
West	St. James	1	2	0
	Trelawny	1	2	0
Tree age				
Young	< 20 years	8	2	0
Mid-aged	20–40 years	2	8	0
Old	> 40 years	2	8	1
Soil texture				
Clay		2	3	0
Clay loam		4	8	0
Stony loam		2	4	0
Sandy loam		4	3	1
Soil pH				
4.6–5.5		2	3	0
5.7–6.5		1	2	1
6.6–7.0		0	2	0
7.1–7.5		2	3	0
7.6–8.5		7	8	0
Electrical conductivity				
Low	(1-2 mmho/cm)	1	2	0
Very low	(< 1 mmho/cm)	11	16	1

nique (1). For clay soils, nematodes were extracted from duplicate subsamples by centrifugal flotation (1). Phytoparasitic nematodes were identified (16) and counted.

Duplicate determinations were made of the pH (23) and nitrate nitrogen (21), phosphorus (21), and potassium (3) concentrations in each soil sample. Also, the texture, and the electrical conductivity (EC), which was a measure of salinity, were determined by the Rural Physical Planning Unit.

All the above-mentioned determinations were carried out for a single declining breadnut tree growing in Portland.

RESULTS

In areas where breadfruit decline occurred, dieback was exhibited mainly by trees more than 20 years old. Declining and nondeclining trees occurred on a variety of soils, ranging from sandy loam to clay, and with pH values ranging from 4.6 to 8.3. (Table 1). However, most trees were from alkaline soils (pH > 7.1) and all were from nonsaline soils (EC < 4 mmho/cm) (27).

The fertility of the rhizosphere soil, based on analyses for nitrates, phosphates, and potash, were similar for nondeclining and declining plants (Table 2).

Table 2. Levels of nutrients in the rhizosphere soil and in leaves of nondeclining and declining trees of *Artocarpus altilis*.

	Seedless trees (breadfruit)				Seeded tree (breadnut)
	Nondeclining (n = 10)[y]		Declining (n = 14)		Declining (n = 1)
Nutrient	Mean	SE[z]	Mean	SE	
		Soil			
N (ppm)	18.70	2.87	18.60	2.60	12
P (ppm)	24.50	2.84	27.80	2.38	14
K (ppm)	45.70	4.99	57.10	4.10	47
		Leaves			
N (%)	2.50	0.13	2.53	0.10	2.80
P (%)	0.58	0.03	0.60	0.03	0.60
K (%)	1.81	0.17	2.06	0.14	1.87
Ca (%)	2.57	0.30	2.30	0.20	1.46
Mg (%)	0.55	0.05	0.57	0.05	0.45
Cu (ppm)	4.95	0.48	5.42	0.39	4.80
Fe (ppm)	97.22	6.78	104.98	6.46	195.50
Mn (ppm)	26.90	4.25	24.90	3.43	25.30
Zn (ppm)	13.19	1.28	13.68	1.06	18.10

[y]Number of trees sampled.
[z]Standard error of mean.

Also, the mean concentrations and ranges of five essential macroelements and four trace elements in the leaves of the two categories of trees were similar. In the single breadnut tree sampled, however, the concentrations of calcium and iron were lower and higher, respectively, than the minimum and maximum values noted for breadfruit (Table 2).

Feeder roots of half the trees of each category were much more difficult to locate than in the other trees. Vesicular-arbuscular mycorrhizal associations were present in all trees sampled (Table 3) and were detected easily. Roots of most nondeclining and of all the declining trees yielded two forms of *Pseudomonas*. Most isolates belonged to fluorescent group Va and one to group III (Table 3), groups consisting mainly of saprophytic and opportunistic phytoparasitic bacteria (CABIMI, personal communication). Roots of one declining plant yielded *Erwinia herbicola* (Geilinger) Dye which may

Table 3. Number of nondeclining and declining trees of *Artocarpus altilis* in which mycorrhizal associations and root-inhabiting bacteria and fungi were detected.

	Seedless trees (breadfruit)		Seeded tree (breadnut)
Microorganism	Nondeclining (n = 12)[z]	Declining (n = 18)	Declining (n = 1)
Vesicular-arbuscular mycorrhizae			
Glomus tenuis	12	18	1
Bacteria			
Pseudomonas spp. (Groups III and VA)	9	18	1
Erwinia herbicola	1	0	0
Fungi			
Alternaria sp.	1	1	0
Aspergillus glaucus and *A. niger*	4	4	0
Botryodiplodia theobromae	1	7	0
Cephalosporium sp.	0	2	0
Curvularia verruculosa	0	1	0
Fusarium equiseti, *F. oxysporum*, *F. pallido-roseum*, and *F. solani*	5	10	1
Gloeosporium sp.	1	0	0
Monilia sp.	2	1	0
Mucoraceae	3	7	1
Myrothecium sp.	0	1	0
Nigrospora sp.	2	1	0
Penicillium sp.	1	1	0
Phoma sp.	1	1	0
Pythium sp.	0	1	0
Rhizoctonia sp.	1	0	0
Thielaviopsis paradoxa	1	0	0
Trichoderma viride	3	6	0
Unidentified	7	7	0

[z]Number of trees sampled.

also be an opportuistic phytopathogen (CABIMI, personal communication). In addition, fungi belonging to over 20 genera were isolated from all root samples (Table 3). Some genera contained well-known root pathogens (15,31). The most frequently isolated fungi, the Fusaria, including *Fusarium equiseti* (Corda) Sacc., *F. oxysporum* Schlecht, *F. pallido-roseum* (Cooke) Sacc., and *F. solani* (Mart.) Sacc., were detected in about half the trees in each category. There was a significantly higher percentage detection of *Botryodiplodia theobromae* Pat. in the roots of declining than in nondeclining trees.

The predominant phytoparasitic nematodes extracted from root samples were *Pratylenchus coffeae* (Zimmermann) Filipjev & Schuurmans Stekhoven, *Helicotylenchus erythrinae* (Zimmermann) Golden, *H. multicinctus* (Cobb) Golden, and *Aphelenchoides* sp. *Pratylenchus coffeae* had the largest mean population density in the roots of breadfruit trees both with and without dieback (Table 4). The breadnut sample also had a large population of *P. coffeae* as well as an equally large population of *Meloidogyne incognita* (Kofoid & White) Chitwood associated with the roots, which were heavily galled. *Meloidogyne incognita* was encountered only occasionally in breadfruit roots. Whenever *Pratylenchus* spp. were not detected in the roots, *Helicotylenchus* spp. were present. Both genera were detected in the same samples in some instances.

Several additional species of phytonematodes were detected in the rhizosphere soil but not in the roots (Tables 4, 5). Of these, *Rotylenchulus reniformis* Linford & Oliveira had the highest percentage occurrence (83%) and highest mean population in soil samples. The next most frequently encountered nematode in soil only was *Tylenchorhynchus* sp. These nematodes may have reproduced

Table 4. Mean density, range, and percentage occurrence of nematodes in the roots of nondeclining and declining trees of *Artocarpus altilis*.

	Seedless trees (breadfruit)						Seeded tree (breadnut)
	Nondeclining (n = 12)[y]			Declining (n = 18)			Declining (n = 1)
Nematode	Mean[z]	Range[z]	Occurrence (%)	Mean	Range	Occurrence (%)	
Aphelenchoides sp.	44	0–417	42	9	0–63	28	0
Helicotylenchus erythrinae and *H. multicinctus*	11	0–35	42	9	0–63	33	0
Meloidogyne incognita	4	0–43	8	0	0–0	0	113
Pratylenchus coffeae	304	0–1233	58	210	0–719	67	107
Tylenchus sp.	0	0–0	0	2	0–53	11	7

[y]Number of trees sampled.
[z]Nematodes/10 g fresh roots.

Table 5. Mean density, range, and percentage occurrence of nematodes in the rhizosphere soil of nondeclining and declining trees of *Artocarpus altilis*.

	Seedless trees (breadfruit)						Seeded tree (breadnut)
	Nondeclining (n = 12)[y]			Declining (n = 18)			Declining (n = 1)
Nematode	Mean[z]	Range[z]	Occurrence (%)	Mean	Range	Occurrence (%)	
Aphelenchoides sp.	53	0–275	75	25	0–50	72	0
Aphelenchus sp.	2	0–25	8	0	0–0	0	0
Ditylenchus sp.	0	0–0	0	3	0–50	6	0
Helicotylenchus erythrinae and *H. multicinctus*	57	0–175	75	34	0–388	56	0
Hemicycliophora sp.	3	0–38	8	0	0–0	0	0
Hoplolaimus sp.	3	0–38	8	3	0–50	6	0
Longidorus sp.	0	0–0	0	1	0–13	6	0
Macroposthonia sp.	2	0–13	17	0	0–0	0	0
Meloidogyne incognita	41	0–300	17	8	0–38	28	0
Pratylenchus coffeae and *Pratylenchus* sp.	61	0–213	75	76	0–325	67	0
Rotylenchulus reniformis	108	0–775	83	115	0–550	83	350
Trichodorus sp.	0	0–0	0	3	0–50	6	0
Tylenchorhynchus sp.	102	0–700	67	79	0–325	78	300
Tylenchus sp.	37	0–275	50	18	0–75	44	25
Xiphinema americanum	2	0–25	8	0	0–0	0	0

[y]Number of trees sampled.
[z]Nematodes/100 cm^3 soil.

on associated weeds. There were no obvious significant differences in the soil population densities of plant parasitic nematodes between nondeclining and declining plants.

DISCUSSION

The similarities in the ranges of soil texture, pH, and electrical conductivity, and of soil and leaf nutrient concentrations for nondeclining and declining plants indicated that these characteristics were not playing a major role in breadfruit decline disease. The beneficial mycorrhizal associations, being easily detected, did not appear to be scant and thereby adversely affecting nutrient uptake. The age of the tree, however, seemed to be a significant factor, dieback being prevalent in old and mid-aged trees whereas the young root suckers of the declining trees appeared healthy. This observation was confirmed by several persons living in the areas with decline and was noted in the Pacific Basin by Trujillo (29).

The presence of several potentially pathogenic fungi, including four species of *Fusarium* and *B. theobromae* (15), the possibly opportunistic phytoparasitic bacteria, *Pseudomonas* spp. and *E. herbicola*, a

dense population of phytoparasitic nematodes, in particular *P. coffeae*, and probably the interaction between these microorganisms (19), could have increasingly detrimental effects on the quantity and function of the roots during tree maturation. *Rhizoctonia* sp. was detected in only one nondeclining tree in contrast to Trujillo's (28) observation of constant association of *R. solani* with breadfruit root dieback in the Pacific. Although plants categorized as nondeclining appeared healthy, the excessive fruit fall reported could be an early symptom of decline (6), to be followed by general unthriftiness, branch dieback, and death. Hence, root-inhabiting microorganisms could be adversely affecting those plants with fruit drop but not exhibiting dieback.

The results of our survey confirm previous reports (5,13), according to which *P. coffeae* and other species of *Pratylenchus, Helicotylenchus nannus* Steiner, *H. erythrinae, H. multicinctus, R. reniformis,* and *Meloidogyne* spp. were the most common species associated with breadfruit roots in Jamaica. *Pratylenchus* spp., especially after years of parasitizing breadfruit roots, could be playing a role in breadfruit decline similar to that played by the burrowing nematode [*Radopholus similis* (Cobb) Thorne] in spreading decline of citrus (7). It is also possible, however, that other disease agents such as shoot-inhabiting microorganisms, fastidious bacteria, protozoa, viruses, or mycoplasmas, which would not be detected by the methods used, could be involved. Trujillo (29) associated neither plant-pathogenic nematodes nor bacteria with breadfruit decline in the Pacific Basin. Further work is necessary to elucidate the roles of the various root-inhabiting microorganisms in breadfruit decline.

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