RESEARCH NOTE/NOTA INVESTIGATIVA

PLANT-PARASITIC NEMATODES ASSOCIATED WITH BREADFRUIT, *ARTOCARPUS ALTILIS*, IN HAWAI'I

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ABSTRACT

Lau, J.-W., S. P. Marahatta, D. Ragone, K.-H. Wang, and B. S. Sipes. 2018. Plant-parasitic nematodes associated with breadfruit, *Artocarpus altilis*, in Hawai'i. Nematropica 48:172-178.

Twenty-five breadfruit plantings on the islands of Kaua'i, Maui, and O'ahu were surveyed for plant-parasitic nematodes. Soil and breadfruit root samples were collected from plantings in residences, parks, schools, botanical gardens, and breadfruit orchards. Soil samples were processed by elutriation and centrifugation for nematode extraction. Roots were subjected to mist chamber extraction for 7 days. Seven different genera of plant-parasitic nematodes (*Helicotylenchus, Meloidogyne, Mesocriconema, Paratylenchus, Pratylenchus, Rotylenchulus,* and *Tylenchorhynchus*) and one unidentified taxon in Heteroderidae were found. *Helicotylenchus dihystera, Rotylenchulus reniformis, Paratylenchus* sp., *Pratylenchus coffeae, Meloidogyne* spp., and a Heteroderid were found on all islands surveyed. *Helicotylenchus dihystera* was the most frequent species encountered, occurring at 68% of sites surveyed and was the most widespread plant-parasitic nematode of breadfruit on Kaua'i. On Maui, *H. dihystera*, *R. reniformis,* and *P. coffeae* were found in 40% of the samples collected. *Paratylenchus* was the most common plant-parasitic nematode to the island of O'ahu and was detected in 86% of the samples. *Meloidogyne* spp. were found in 43% of breadfruit sites on Oah'u, and 23% and 20% of that on Kaua'i and Maui, respectively, but in low abundance on all islands. Soil samples yielded *Mesocriconema* at sites on Kaua'i and O'ahu, and *Tylenchorhynchus* on O'ahu. Plant-parasitic nematodes were absent from 12% of the locations surveyed. An unidentified nematode in the Heteroderidae was detected in samples from each island, all from botanical gardens.

Key words: *Helicotylenchus dihystera,* Kaua'i, Maui, *Meloidogyne, Mesocriconema,* O'ahu, *Paratylenchus, Pratylenchus coffeae, Rotylenchulus reniformis, Tylenchorhynchus*

RESUMEN

Lau, J.-W., S. P. Marahatta, D. Ragone, K.-H. Wang, and B. S. Sipes. 2018. Nematodos parásitos de plantas asociados con la fruta del pan, *Artocarpus altilis*, en Hawai'i. Nematropica 48:172-178.

Veinticinco plantaciones de fruta de pan en las islas de Kaua'i, Maui y Oahu fueron examinadas en busca de nematodos parásitos de plantas. Las muestras de suelo y de la raíz de la fruta del pan se recolectaron de arboles en residencias, parques, escuelas, jardines botánicos y huertos de la fruta del pan. Las muestras de suelo se procesaron por elutriación y centrifugación para la extracción de nematodos. Las raíces se sometieron a extracción en cámara de niebla durante 7 días. Se encontraron siete géneros diferentes de nematodos parásitos de plantas (*Helicotylenchus*, *Meloidogyne*, *Mesocriconema*, *Paratylenchus*,

Pratylenchus, *Rotylenchulus* y *Tylenchorhynchus*) y un taxón no identificado en Heteroderidae. *Helicotylenchus dihystera*, *Rotylenchulus reniformis*, *Paratylenchus* sp., *Pratylenchus coffeae*, *Meloidogyne* spp., y un Heteroderid se encontraron en todas las islas estudiadas. *Helicotylenchus dihystera* fue la especie más frecuente encontrada, se presentó en el 68% de los sitios estudiados y fue el nematodo parásito-planta más extendido de la fruta de pan en Kauai. En Maui, se encontraron *H. dihystera*, *R. reniformis* y *P. coffeae* en el 40% de las muestras recolectadas. *Paratylenchus* fue el nematodo más común en la isla de Oahu y se detectó en el 86% de las muestras. *Meloidogyne* spp. se encontraron en el 43% de los sitios de la fruta del pan en Oah'u, y el 23% y el 20% de los de Kaua‘i y Maui, respectivamente, pero en baja abundancia en todas las islas. Las muestras de suelo produjeron *Mesocriconema* en sitios en Kaua'i y O'ahu, y *Tylenchorhynchus* en O‘ahu. Los nematodos parásitos de plantas estuvieron ausentes en el 12% de los lugares encuestados. Se detectó un nematodo no identificado en las Heteroderidae en muestras de cada isla, todas de jardines botánicos.

Palabras clave: *Helicotylenchus dihystera*, Kaua‘i, Maui, *Meloidogyne*, *Mesocriconema*, O‘ahu, *Paratylenchus*, *Pratylenchus coffeae*, *Rotylenchulus reniformis*, *Tylenchorhynchus*

For early Hawaiians, breadfruit or ‘ulu (*Artocarpus altilis* Parkinson (Fosberg)), was central to culture and sustenance. The breadfruit tree is a high-yielding, low-input starch crop for tropical and subtropical areas with hundreds of named varieties (Ragone, 1997). Transported in voyaging canoes when the first inhabitants arrived in the Hawaiian Islands, only one variety of breadfruit was known in the Hawaiian archipelago up until the early 20th Century (Rock, 1974; Meilleur *et al.,* 2004). Traditionally, seedless breadfruit was cultivated from root cuttings. However, *in vitro* propagation methods established by Shi *et al.* (2007) and Murch *et al*. (2008) allowed for mass cloning, making commercial distribution of breadfruit propagules much more feasible than in the past. Global concerns over food security with a focus on traditionally underutilized crops have increased cultivation of cloned breadfruit trees as an orchard crop. Breadfruit plantings have steadily increased over the past 8 yr in Hawai‘i, featured in private residences, parks, community centers, botanical gardens, and most recently in orchards. Between 2010-2016, more than 12,000 Ma‘afala cultivar trees were planted in Hawai‘i, including 10,000 trees distributed through the Plant a Tree of Life-Grow ‘Ulu project (Elevitch *et al.,* 2014, Ragone *et. al*, 2016).

Breadfruit trees have enjoyed the status of being a win-win plant: productive, low-input, and mostly free of pests and disease (Ragone, 1997; Taylor and Tuia, 2007). While it may be true that breadfruit is often little affected by pests or pathogens in cases of single specimen trees, intensive cultivation of breadfruit crops may exacerbate pathogen problems and amplify pathogen damage. For greater efficiency of harvesting, marketing, and maintenance, typically only a single variety is planted in a given orchard making a grove that is not only a monocrop, but genetically identical throughout. The lack of genetic diversity of traditional crops of Oceania has allowed for a build-up of pathogen populations responsible for deterioration of crop agronomic performance (Lebot, 1992). As stands of breadfruit orchards become more common in Hawai‘i, incorporating mixed plantings of breadfruit cultivars and diverse species may help mitigate disease problems.

While information is available on insect pests and plant pathogens that affect the breadfruit crown and fruit, below-ground pathogens are not as well understood. Information on plant-parasitic nematodes associated with breadfruit is limited. Surveys of plant-parasitic nematodes associated with breadfruit have been conducted in Jamaica (Hutton, 1976; Coates-Beckford and Perriera, 1992), Brazil (Sharma, 1976), and selected islands in the Pacific (Kirby *et al.,* 1980; Grandison, 1990, 1996; Grandison *et al.,* 2009), with an investigation on *Meloidogyne* spp. damaging breadfruit plants in Malaysia (Razak, 1978). *Aphelenchoides* sp., *Helicotylenchus dihystera, H. multicinctus, Hemicriconemoides cocophilus, Meloidogyne* sp., *Paratylenchus* sp., and *Xiphinema brevicolle* were present on the roots and rhizosphere of breadfruit

trees found in the Fiji, Kiribati, Niue, Western Samoa, Tonga, and Cook Islands (Orton Williams, 1980; Grandison, 1990). Kirby *et al.* (1980), Grandison (1996), and Grandison *et al.* (2009) reported *Achlysiella williamsi, Aphelenchoides bicaudatus, Criconemella denoudeni, C. onoensis, Ditylenchus* sp., *Gracilacus* sp., *Helicotylenchus dihystera, H. erythrinae, H. indicus, H. microcephalus, H. pseudorobustus, Helicotylenchus* spp., *Hemicriconemoides mangiferae, Heterodera* sp., *Meloidogyne incognita, Pratylenchus coffeae, P. loosi, Pratylenchus* sp., *Sphaeronema* sp., and *Xiphinema* sp. on breadfruit from islands of the Pacific including New Caledonia.

In Brazil, *H. dihystera* and *Tylenchus leptosoma* were associated with the roots of the breadfruit tree (Sharma, 1976). In Malaysia, galls with *Meloidogyne* spp. have been reported to inhibit root development of feeder roots and root elongation causing "retarded growth, reduced trunk diameter, sparse branching, and general yellowing of the leaves and, in extreme cases, plant mortality" (Razak, 1978). *Meloidogyne* spp. were found associated with breadfruit in a survey conducted in Jamaica in the late 1950s (Hutton, 1976). In later surveys in Jamaica, Coates-Beckford and Perriera (1992) sampled declining and non-declining breadfruit trees and confirmed previous findings of plant-parasitic nematodes from other countries associated with breadfruit trees including: *P. coffeae, Pratylenchus* spp., *Helicotylencus erthrinae, H. multicinctus,* and *Meloidogyne* sp. The causal agent of slow decline disease in Jamaica, which causes decline and reduces production of mature breadfruit trees, is *P. coffeae* (Coates-Beckford and Perriera, 1992; Roberts-Nkrumah, 1994). To date, research on plant-parasitic nematodes found associated with breadfruit trees in Hawai'i is unavailable. The objective of this survey was to determine the incidence and abundance of plant-parasitic nematode fauna associated with the roots and soils of breadfruit trees on the islands of Kaua'i, Maui, and O'ahu.

Surveys of plant-parasitic nematodes associated with breadfruit were conducted from May 2013 to July 2015. A total of 25 soil and root samples were collected using a stratified random method from the islands of Kaua'i, Maui, and O'ahu. Three types of breadfruit production systems targeted for sampling included: commercial plantings, breadfruit growing near homesteads, and specimen trees in botanical gardens. Beneath the breadfruit canopy, soil and root samples were collected at four locations at a depth of 15 cm from the drip line.

With the exception of a few commercial plantings, most survey locations had single tree plantings. In commercial plantings, multiple trees were composited into a single sample to represent the entire orchard. Roots and soils were retrieved in a zig-zag pattern from 10 trees encompassing no more than 1 ha in area. Collected soil and root samples were gently mixed before 400 cm^3 of soil and 50 g of roots were subsampled and placed into plastic bags in a temperature-controlled vessel for transport to the laboratory at the University of Hawai'i at Mānoa. Breadfruit roots, identified by their red color, were separated from soil, cut into 2-cm long pieces, and 20 g were used for nematode extraction in a mist chamber (Seinhorst, 1956). Nematodes were collected from the mist chamber after 7 days. Soils were screened through a 4-mm mesh screen. Nematodes were then extracted from a 200 cm^3 subsample using a semi-automatic elutriator (Byrd *et al.,* 1976). All plant-parasitic nematodes were identified to the genus level using a Leica DMIRB inverted microscope. Nematode counts were adjusted to 200 cm^3 soil and 20 g root dry weight for comparisons. Mean, range, and "% frequency occurrence" of plant-parasitic nematodes by species were calculated (Table 1). To calculate "% frequency occurrence," positive sites were divided by the total number of sites surveyed.

Plant-parasitic nematodes found in soils associated with breadfruit on the islands of Kaua'i, Maui, and O'ahu included *Mesocriconema*, *Helicotylenchus dihystera*, *Meloidogyne, Paratylenchus*, *Pratylenchus coffeae, Rotylenchulus reniformis, Tylenchorhynchus,* and an unidentified taxon of Heteroderidae. Occurring at 68% of sites, *Helicotylenchus* was the most frequently occurring plant-parasitic nematode genus. *Paratylenchus* was the most frequently observed plant-parasitic nematode on breadfruit on O'ahu, occurring at 86% of sites surveyed. On the other hand, *H. dihystera*, *R. reniformis,* and *P. coffeae* were the most prevalent nematode genera found on Maui, present in 40% of locations surveyed. *Meloidogyne* was detected less frequently on breadfruit compared to the above mentioned plant-parasitic nematodes among the three islands surveyed, occurring on O'ahu at 43%,

Table 1. Mean number and range of plant-parasitic nematode species found on breadfruit from three Hawaiian Islands (20 g root samples and 250 cm^3 soil samples) and frequency of occurrence across surveyed sites.

	Soil			Roots			Frequency of occurrence (%)
	Kaua'i	O'ahu	Maui	Kaua'i	O'ahu	Maui	
	13[y]	7	5	8	7	5	
Helicotylenchus	98 (0-570)[z]	614 (20-1380)	72 (0-168)	41 (0-214)	140 (0-683)	39 (0-66)	68
Rotylenchulus	367 (0-1032)	83 (0-290)	47 (0-96)	28 (0-290)	5 (0-20)	16 (0-32)	52
Paratylenchus	24 (0-270)	54 (0-92)	58 (0-58)	32 (0-236)	31 (0-94)	45 (0-170)	44
Pratylenchus	-	63 (0-154)	36 (0-120)	6 (0-20)	423 (0-2942)	1385 (42-2518)	36
Meloidogyne	18 (0-156)	56 (0-177)	12 (0-62)	3 (0-10)	-	12 (0-82)	28
Heteroderidae	42 (0-168)	27 (0-60)	207 (0-610)	-	-	-	12
Mesocriconema	0 (0-6)	1 (0-10)	-	-	-	-	8
Tylenchorhynchus	-	1(0-8)	-	-	-	-	4

[y] Number of samples.

[z] outside ( ) = mean, inside ( ) = range, and - = specified nematode not found.

Kauaʻi at 23%, and Maui at 20% (Fig. 1). An unidentified species of nematode in the Heteroderidae family was detected at one site on each island, all of which were sampled from botanical gardens. *Mesocriconema* was less prevalent in samples, only observed at one location on each Kauaʻi, Maui, and Oʻahu. *Tylenchorhynchus* was found at one location on Oʻahu. Plant-parasitic nematodes were absent from 12% of locations surveyed, including one site on Kauaʻi and two on Maui. The most diverse occurrence of plant-parasitic nematode genera was collected at Waimea Arboretum, Oʻahu, where six different nematode genera were detected and *Pratylenchus* was the dominant species. Survey findings of plant-parasitic nematodes associated with breadfruit in Hawaiʻi are consistent with previous studies (Hutton, 1976; Sharma, 1976; Kirby *et al.*, 1980; Orton Williams, 1980; Grandison, 1990, 1996; Coates-Beckford and Perierra, 1992; Grandison *et al.,* 2009) where commonly occurring plant-parasitic nematodes, including *Meloidogyne, Pratylenchus, Rotylenchulus*, and *Helicotylenchus,* were associated with breadfruit. However, detection of *Meloidogyne* were considerably less frequent in our survey from Hawaiʻi, at just 28% of surveyed locations. Most of the breadfruit varieties surveyed in this project were either Maʻafala or Hawaiian ʻulu. Low occurrence and abundance of *Meloidogyne* may be the result of breadfruit varieties that were surveyed in Hawaiʻi being poor hosts to *Meloidogyne*. It would be important to conduct a pathogenicity test of specific breadfruit cultivars to *Meloidogyne* spp. and other species of plant-parasitic nematodes.

Pratylenchus were mainly found in the roots of breadfruit surveyed. Root extraction from the mist chamber yielded as much as 2,942 *Pratylenchus* nematodes per 20 g of roots on a site on Oʻahu, whereas elutriation of 200 cm^3 of soil yielded only 36 *Pratylenchus* nematodes from the same site. Results of *Pratylenchus* from root and soil extraction from Maui were similar. Since Coates-Beckford and Perriera (1992) cited *P. coffeae* as the cause of slow decline disease of breadfruit in Jamaica, the lesion nematodes found during our survey warranted a species identification. Measurements of 10 females and 10 males and a molecular analysis of 10 females, 4 males, and 2 juvenile lesion nematodes identified the species as *P. coffeae*. Whether *P. coffeae* is as devastating to breadfruit in Hawaiʻi as it is in Jamaica, is still not known. Future studies should also examine pathogenicity of *P. coffeae* on breadfruit cultivars commonly grown in Hawaiʻi and interactions of *P. coffeae* with other pathogenic

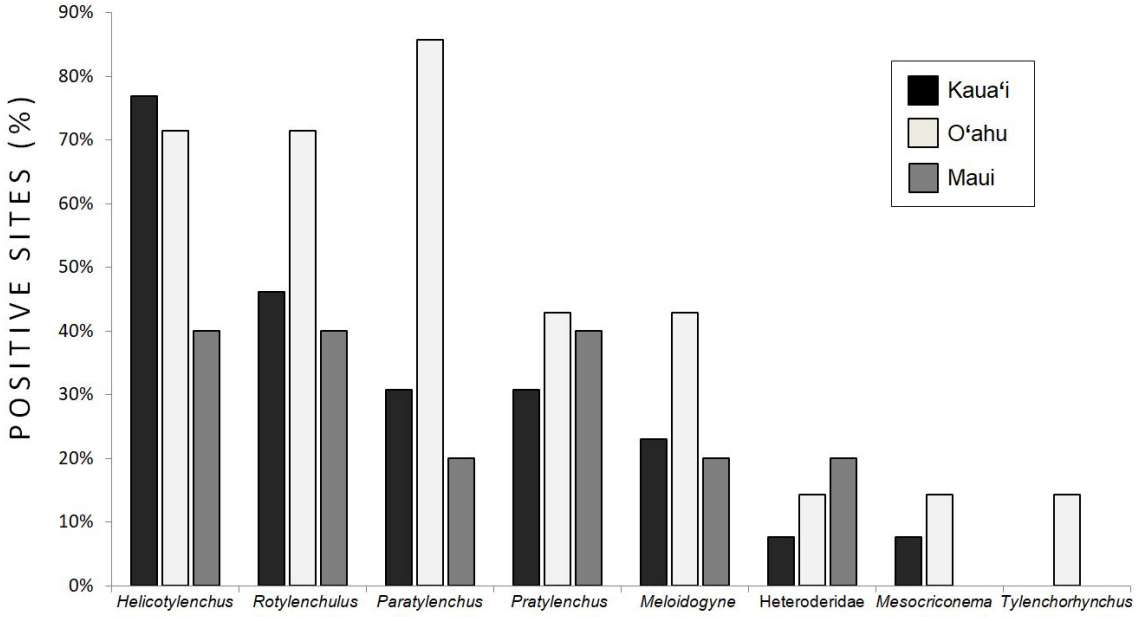

Fig. 1. Plant-parasitic nematode occurrence on breadfruit sites in Hawaii by island.

fungi and phytoparasitic bacteria mentioned to be correlated with declining breadfruit as stated by Coates-Beckford & Perriera (1992). Nonetheless, these findings suggest that breadfruit farmers should not accept breadfruit plants from root cuttings if nematode contamination is a risk.

Furthermore, reniform nematode was first described in Hawai‘i with a wide host range (Linford and Oliveira, 1940; Linford and Yap, 1940), thus it was no surprise that *Rotylenchulus* had a wide incidence in the soils associated with breadfruit on all islands. Also worth noting was the presence of the Heteroderid. Cyst nematodes are not common in Hawai‘i. Traditional propagation from root cuttings combined with *Heterodera* presence specific only to botanical gardens could be indicative of the diversity of propagules of various plant species collected from different locales planted in these gardens. Lastly, *Helicotylenchus* and *Paratylenchus* were found from many locations on all the islands. Since both *Paratylenchus* and *H. dihystera* were found primarily in soils and are not major plant pathogens of tree crops, it is not likely that these nematodes are problematic to breadfruit.

Plant-parasitic nematodes are prevalent in breadfruit roots and associated soil. Presence of specific plant-parasitic nematodes genera are an indicator of pathogen problems. However, pathogenicity tests are needed to identify the level of virulence and damage of the specific nematodes commonly detected in this survey. Sourcing new plants from root cuttings can spread endoparasitic nematodes to new planting areas, thus the use of *in vitro* breadfruit propagation material free of nematodes should be recommended to farmers. The occurrence and abundance of specific nematode genera associated with breadfruit will aid in developing specific nematode management approaches appropriate to successfully growing breadfruit in Hawai‘i.

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LITERATURE CITED

Byrd Jr., D. W., K. R. Barker, H. Ferris, C. J. Nusbaum, W. E. Griffin, R. H. Small, and C. A. Stone. 1976. Two semi-automatic elutriators for extracting nematodes and certain fungi from soil. Journal of Nematology 8:206.

Coates-Beckford, P. L., and M. J. Pereira. 1992. Survey of root-inhabiting microorganisms on declining and nondeclining breadfruit (*Artocarpus altilis*) in Jamaica. Nematropica 22:55-63.

Elevitch, C. R., D. Ragone, and I. Cole. 2014. Breadfruit production guide: Recommended practices for growing, harvesting, and handling. Kalaheo, Kaua‘i, Hawai‘i, USA: Breadfruit Institute, National Tropical Botanical Garden.

Grandison, G. S. 1990. Report on a survey of plant parasitic nematodes in the Cook Islands (Southern Group). South Pacific Commission, Noumea, New Caledonia.

Grandison, G. S. 1996. Plant-parasitic nematodes of American Samoa. South Pacific Commission Technical Paper No. 205. Noumea: South Pacific Commission.

Grandison, G. S., S. Lebegin, and Z. L. Desprez. 2009. Plant-parasitic nematodes on economic crops of New Caledonia. Australasian Plant Pathology 38:408-410.

Hutton, D. G. April 1976. Country report on the status of root-knot nematode problems and research. Pp. 67-79 *in* Proceedings Regional Planning Conference of the International *Meloidogyne* Project, Region I.

Kirby, M. F., M. E. Kirby, M. R. Siddiqi, and P. A. Loof. 1980. Fiji nematode survey report: Plant-parasitic nematode distributions and host associations. Bulletin No. 68. Ministry of Agriculture and Fisheries, Fiji.

Lebot, V. 1992. Genetic vulnerability of Oceania’s traditional crops. Experimental Agriculture 28:309-323.

Linford, M. B., and J. M. Oliveira. 1940. *Rotylenchulus reniformis*, nov. gen., n. sp., a

nematode parasite of roots. Proceedings of the Helminthological Society of Washington 7:35-42.

Linford, M. B., and F. Yap. 1940. Some host plants of the reniform nematode in Hawaii. Proceedings of the Helminthological Society of Washington 7:42-44.

Meilleur, B. A., R. R. Jones, C. A. Titchenal, and A. S. Huang. 2004. Hawaiian breadfruit: Ethnobotany, nutrition, and human ecology. College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa, Honolulu, HI. 61 p.

Murch, S. J., D. Ragone, W. L. Shi, A. R. Alan, and P. K. Saxena. 2008. *In vitro* conservation and sustained production of breadfruit (*Artocarpus altilis,* Moraceae): modern technologies for a traditional tropical crop. Naturwissenschaften 95:99-107.

Orton Williams, K. J. 1980. Plant-parasitic nematodes of the Pacific. Technical Report Vol. 8, UNDP/FAO-SPEC Survey of Agricultural Pests and Diseases in the South Pacific. Commonwealth Institute of Helminthology, England.

Razak, A. R. 1978. Variation in plant response, gall size and form induced by *Meloidogyne* on some Malaysian crops. Kasetsart Journal (Thailand) 12:43-45.

Ragone, D. 1997. Breadfruit (*Artocarpus altilis* (Parkinson) Fosberg). Promoting the conservation and use of undertutilized and neglected crops. International Plant Genetic Resources Institute, Rome.

Ragone, D., C. Elevitch, and A. Dean. 2016. Revitalizing breadfruit in Hawaii - A model for encouraging the cultivation and use of breadfruit in the tropics. Tropical Agriculture 93:213-224.

Roberts-Nkrumah, L. B. 1994. Breadfruit and breadnut production [in the Caribbean]. Regional Workshop on Tropical Fruits, Grenada, May 1994.

Rock, J. 1974. The indigenous trees of the Hawaiian Islands. Lawai, Hawai‘i: Pacific Tropical Botanical Garden. 548 p.

Seinhorst, J. W. 1956. The quantitative extraction of nematodes from soil. Nematologica l:249-267.

Sharma, R. D. 1976. Nematodes of the cocoa region of Bahia, Brazil, VI. Nematodes associated with tropical fruit trees. Reunião de Nematologia 2:108-123.

Shi, W. L., P. K. Saxena, S. J. Murch, and, D. Ragone. 2007. Mass-propagation and bioreactor-based technologies for germplasm conservation, evaluation and international distribution of breadfruit. Acta Horticulturae 757:169-176.

Taylor, M. B., and V. S. Tuia. 2007. Breadfruit in the Pacific Region. International Symposium on Breadfruit Research and Development. Acta Horticulturae 757:43-50. DOI: 10.17660/ActaHortic.2007.757.4757:43-5

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