

RENIFORM AND ROOT KNOT NEMATODES ON PASSIONFRUIT IN FIJI
[LOS NEMATODOS RENIFORME Y NODULADOR EN GRANADILLA EN FIJI]. M.F. Kirby, Agriculture Department, Koronivia Research Station, P.O. Box 77, Naisori, Fiji.

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ABSTRACT

A survey of Fiji's passionfruit crop, *Passiflora edulis* var. *flavicarpa*, showed *Rotylenchulus reniformis* to be present in 16 of 19 sites sampled, and in numbers exceeding 36,000 per 200 cm³ of soil. *Meloidogyne* sp. was recovered from only 1 sample. Reniform nematodes reproduced on passionfruit in a glasshouse experiment, and a significant reduction in vine weights was associated with the presence of this nematode. Leaves were chlorotic, and infected roots had a uniformly darker appearance when compared with controls. No plant growth reductions were associated with *M. arenaria*, *M. incognita*, and *M. javanica*, inoculated separately in a controlled experiment. Root galling occurred in response to all species. However, nematodes failed to develop beyond larval stages, and no evidence of reproduction was seen. It is concluded that reniform nematodes may be involved in a serious decline disease affecting passionfruit in Fiji. Evidence indicates that passionfruit is not a suitable host for the *Meloidogyne* species tested.

INTRODUCTION

The impact which phytoparasitic nematodes have on passionfruit, *Passiflora edulis* var. *flavicarpa*, in Fiji is unknown. In recent years a decline of a poorly defined etiology has affected the crop. Five years ago the economic production period of a planting was 4 years, and now it is only 18 months (2). Passionfruit decline has been primarily attributed to collar rot disease caused by *Phytophthora cinnamomi* and *nicotianae* var. *parasitica* (1), but there are no experimental data to support this hypothesis.

This is a report on survey and experimental work to determine the status of reniform nematodes, *Rotylenchulus reniformis*, and root knot nematodes, *Meloidogyne arenaria*, *M. incognita*, and *M. javanica*, as pests of passionfruit. The possible roles of these nematodes in passionfruit decline are discussed.

MATERIALS AND METHODS

As part of a country wide nematode survey on agricultural crops, soil samples from the root zones of passionfruit were collected and analyzed for the presence of phytoparasitic nematodes. From a single planting of passionfruit, 6 subsamples were collected and combined. Aliquots of 200 cc were processed within 5 days of collection by a modified Baermann technique. Soil was spread on Scotties tissue on raised wire supports set in 15 cm diam enamel pans and covered with water, and was left for 48 hrs at room temperature. Nematodes were collected on a 325 mesh (45 u openings) sieve. They were identified and numbers were estimated by counting 10% of the samples or a lesser diluted quantity when numbers were high.

To test pathogenicity and host susceptibility of passionfruit to the different nematodes, replicated pot trials in randomized complete block designs were conducted

under glasshouse conditions. Passionfruit seedlings, *P. edulis* var *flavicarpa*, were grown for 5 wks in steamed soil in seedling pots prior to the experiments. At 5 wks the plants were 15 cm high, and they were selected for uniformity within each experiment. The average daily maximum temperature in the glasshouse during the experimental periods was 32° C. To avoid cross contaminations, pots were raised off the bench and spaced 20 cm apart.

For the reniform nematode trial, naturally infested passionfruit soil was used. Soil was a Sigatoka clay loam from the flood plain of the Sigatoka River, Viti Levu I. Nematode counts were made from 100 cm³ aliquots from well mixed soil, and the different inoculum levels were subsequently made by thoroughly mixing measured quantities of steamed (nematode free) and unsteamed soil. Ten replicates of 3 treatments (0; 2,275; and 22,750 *R. reniformis*) were planted into 15.5 cm diam and 4000 cc capacity black plastic bags. Prior to and at the end of the experiment the soil was found to be free of detectable levels of other species of phytoparasitic nematodes. Other soil microfauna and flora were not controlled in the experiment.

The trial was harvested at 81 days, and measurements were taken of vine lengths and fresh vine and root weights. Nematodes from 2 g root samples were counted after a 48 hr incubation period (6). Nematodes per 100 cm³ of soil from each pot were counted after extraction as described above. Root, soil, and total nematodes per pot were estimated based on these counts.

A second experiment was conducted with cultures of *Meloidogyne*, each started from a single egg mass. The original collection information for the cultures is as follows: a) *M. arenaria* (No. 00-1) collected from the roots of cherry, *Prunus* sp., at Bemana Locality, Sigatoka Valley, Viti Levu I. on 20 VII 1976; b) *M. arenaria* (No. PP-2) collected in soil around eggplant, *Solanum melongena*, at Tore Locality, Sigatoka Valley on 20 VII 1976; c) *M. incognita* (No. J-2) collected in soil around black pepper, *Piper nigrum*, at Naduruloulou Res. Stn., Viti Levu I. on 6 IV 1976; d) and *M. javanica* (No. HH-5) collected in soil around the roots of kava, *Piper methysticum*, at Wainigata Res. Stn., Vanua Levu I. on 8 VI 1976. Nematodes were identified based on female posterior cuticular pattern morphology as well as by differential host responses (5). Cultures were maintained on tomato plants, *Lycopersicon esculentum* 'Rutgers', in a glasshouse and with bimonthly transfers to fresh tomato seedlings. The potting mix was composed of river sand, sawdust, and dried cow dung (2:1:1 V/V). Soils were fumigated with methyl bromide at 0.675 kg per m³ for 48 hrs. *Meloidogyne* eggs to be used as inoculum were collected from 65 day old tomato plants by disrupting egg masses in dilute sodium hypochlorite and collecting eggs on nested 200/500 mesh sieves (3). Egg concentrations in the resulting suspensions were determined by dilution-counting methods and appropriate volumes of egg suspensions were pipetted into the root zones of transplanted seedlings to give inoculum levels of 40,000 and 400,000 eggs per pot. Controls were inoculated with a nematode free filtrate of the mixture. Four replicates of 9 treatments were planted into 17 cm top diam and 2300 cm³ capacity plastic pots.

The *Meloidogyne* trial was harvested at 60 days, and measurements were taken of vine lengths and fresh vine weights. Root galling was quantified by use of a visual indexing system where 0 - no infection, 1 - light infection, and 10 - severe infection (8). Root samples (3 g) were processed by a sodium hypochlorite-blender method (4) for extracting root knot nematode eggs. Nematodes per 100 cm³ soil samples from each pot were extracted and counted.

In order to compare the inoculum potential of the 4 *Meloidogyne* cultures, a trial was concurrently carried out using 'Rutgers' tomato seedlings and inoculum from each culture of 10,000 eggs per pot. The experiment was a randomized complete block with 4 replicates of 4 treatments. At 60 days, plants were harvested and root systems rated for the amount of root knot (8).

RESULTS AND DISCUSSION

Survey. Soil samples from passionfruit were taken from 19 farms, and 14 of these from the Sigatoka Valley where the industry is centered. Reniform nematodes were present in 16 samples including all those from the Sigatoka Valley, and counts from 11 samples exceeded 1,000 nematodes per 200 cm³ soil. A high of over 36,000 nematodes per 200 cm³ was recorded. *R. reniformis* was positively identified by microscopic examination from passionfruit roots from the field. Root knot nematodes were detected in only 1 of 19 samples.

Reniform nematode glasshouse experiment. Results of the reniform nematode experiment are summarized in Table 1. A 23% reduction in fresh weights of vines resulted in plants receiving the high level of inoculum (significant at $P = 0.01$). Reductions in averages of other growth parameters were not significant. Nematode counts from soil and root samples at harvest were used to estimate total nematodes for the combined soil-root system. These values (Table 1) represent over 700 and 1000% population increases from initial low and high inoculum levels, respectively, during the 81 day trial period.

Top and root symptoms were visually examined at harvest. Leaves of plants receiving high inoculum levels were uniformly chlorotic and distinguishable within each replicate from controls on this basis. Root systems were darker overall than controls, and low inoculum treatments were intermediate in this respect. Reniform nematode females fed simiendoparasitically on the roots.

The observed reductions in plant growth and associated increases in nematode populations suggest a pathogenic relation of reniform nematodes to passionfruit, although involvement of other soil microorganisms is not ruled out. This is believed to be the first report on pathogenicity of reniform nematodes to passionfruit.

Root knot nematode glasshouse experiment. Although our surveys gave no evidence of root knot nematode problems on passionfruit, the availability of cultures of *Meloidogyne* prompted further study. Experimental results are summarized in Table 2. None of the species reduced growth as measured by vine lengths and fresh weights. Root knot indices were assigned for galling without consideration of egg production. A light to moderate amount of galling occurred in response to all species, and the level of galling was proportional to inoculum level. However no eggs were extracted from 3 g root samples from each plant. Dissections of root from all the treatments did not reveal any eggs, and only larval stages were seen. From 100 cm³ soil samples processed for each plant, no larvae were found in the controls or in those inoculated with *M.*

Table 1. Growth responses of passionfruit, and nematode counts following inoculations with *Rotylenchulus reniformis*¹.

Treatments	Vine lengths (cm)	Vine fresh weights (g)	Root fresh weights (g)	Nemas per 2 g roots	Nemas per 100 cm ³ soil	Nemas per pot
Control	129.8	30.3	4.9	0	0	0
2,275 Nemas	114.0	28.6	4.4	90	400	16,199
22,750 Nemas	113.8	23.4	3.5	1100	5810	236,228
LSD 0.05	ns	3.91	ns			
0.01	ns	5.29	ns			

¹Means of 10 replications

Table 2. Growth responses of passionfruit, and root knot indices following inoculation with *Meloidogyne* spp.¹

Treatments ²	Vine lengths (cm)	Vine fresh weights (g)	Root knot indices
Control	197.2	44.9	0.0
<i>M. arenaria</i> (00-1) Low	226.5	43.2	2.0
High	203.2	42.4	2.8
<i>M. arenaria</i> (PP-2) Low	267.8	50.1	1.2
High	218.8	40.8	3.8
<i>M. incognita</i> Low	264.5	44.6	3.0
High	251.8	42.8	4.5
<i>M. javanica</i> Low	240.0	43.2	3.0
High	260.8	43.1	3.0
LSD 0.05	ns	ns	

¹Means of 4 replications.

²Inoculum levels: low = 40,000 eggs; High = 400,000 eggs.

³Rating scale: 0 = no galling; 1 = light galling; 10 = heavy galling.

arenaria. In 5 samples of treatments with *M. incognita* and *M. javanica* from which larvae were recovered, the highest count was 45. This represents a total population in the pot of 1,035 nematodes which, in the absence of reproduction, is assumed to be the survivors from the original inoculum.

Root knot indices on tomato plants inoculated with 10,000 eggs each as a test of the uniformity of inoculum potential of the 4 cultures averaged 5.0 (*M. arenaria*, No. 00-1), 5.6 (*M. arenaria*, No. PP-2), 5.5 (*M. incognita*), 4.6 (*M. javanica*). Differences were not significant at $P = 0.05$. Prolific egg production was observed for all plants. It was concluded that inoculum potentials of the 4 cultures were uniform.

The foregoing evidence suggests that passionfruit is a nonhost for the *Meloidogyne* species tested. Contrasted with this is the report by De Villiers and Milne (7) which indicated that "*Meloidogyne* sp. was the nematode most often found on *Passiflora edulis* in a survey of passionfruit-growing areas in Transvaal, South Africa". They may have been working with a different species of *Meloidogyne* or a different passionfruit variety. Seemingly the species of nematodes tested in our study might successfully attack passionfruit under another set of environmental conditions, or they could cause harmful physiological disfunction without being able to reproduce.

CONCLUSIONS

The reniform nematode likely contributes to passionfruit decline in Fiji. Loss assessment and control trials in the field are needed, and a study should be made of the possi-

ble association of this nematode with collar rot pathogens in the overall disease syndrome. Root knot nematodes are not pests of passionfruit in Fiji, and it is further concluded that passionfruit, *P. edulis* var *flavicarpa*, is not a suitable host for *M. arenaria*, *M. incognita*, and *M. javanica*.

RESUMEN

Un reconocimiento del cultivo de la granadilla (*Passiflora edulis* var. *flavicarpa*) en Fiji demostró la presencia del *Rotylenchulus reniformis* en 16 de los 19 puntos examinados ocurriendo en densidades de más de 36000 por 200 cm³ de suelo. *Meloidogyne* sp se presentó en una sola muestra. En pruebas de invernadero los nematodos reniformes se reprodujeron en granadilla causando una disminución en el peso de la enredaderas. Las hojas mostraron clorosis y las raíces infectadas tenían una apariencia uniformemente más oscura que las de los testigos. En otros experimentos, no se demostraron reducciones de crecimiento en la presencia de *M. arenaria*, *M. incognita*, y *M. javanica* aunque se registró nodulación de las raíces; los nematodos no pasaron de los estados larvales y no hubo evidencia de reproducción. Se concluye que los nematodos reniformes están involucrados en una enfermedad de decaimiento de la granadilla en Fiji. Los resultados indican que la granadilla no es buena hospedera para las especies de *Meloidogyne* examinadas.

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