INTERACTIVE EFFECTS OF *ROTYLENCHULUS RENIFORMIS* AND *PHYTOPHTHORA PALMIVORA*[1] ON PAPAYA (*CARICA PAPAYA* L.) SURVIVAL AND GROWTH IN GREENHOUSE POTS

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While we recognize that *Phytophthora* species have been placed in the Stramenopiles, we will refer to *P. palmivora* as a fungus for clarity[1].

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ABSTRACT

Alston, D. G., B. S. Sipes, J. Uchida[3], D. P. Schmitt, and C. L. Chia. 2003. Interactive effects of *Rotylenchulus reniformis* and *Phytophthora palmivora* on papaya (*Carica papaya* L.) survival and growth in greenhouse pots. Nematropica 33:73-85.

Individual and interactive effects of the pathogen complex, *Rotylenchulus reniformis* and *Phytophthora palmivora*, were investigated on papaya (*Carica papaya*) grown in greenhouse pots (10-cm-diam) for 3-4 months. *P. palmivora* was a more virulent pathogen than *R. reniformis* and caused moderate to severe mortality (27-73%) at an inoculum density of 1,000 zoospores per pot. No lethal or sublethal effects of *P. palmivora* on papaya at lower inoculum densities were evident. *R. reniformis* stimulated papaya growth at inoculum densities below 500 juveniles per pot and only moderately decreased growth (1-2 g loss in plant dry weight for every $\log_{10}$ increase in initial population density) at higher densities (1,000-10,000 juveniles per pot). Synergistic interactions were observed between the two pathogens on papaya mortality, root ratings, and plant dry weight in some experiments. *R. reniformis* predisposed papaya to attack by *P. palmivora*, although *P. palmivora* had indirect negative effects on *R. reniformis* by reducing nematode reproduction through reduced quantity and quality of host roots. Four-wk-old plants were more susceptible to the pathogen complex, especially to *P. palmivora*, than 8-wk-old plants.

Key words: Carica papaya, papaya, growth suppression, pest interaction, *Rotylenchulus reniformis*, reniform nematode, *Phytophthora palmivora*, root-rotting pathogen.

RESUMEN

Alston, D. G., B. S. Sipes, J. Uchida[3], D. P. Schmitt, and C. L. Chia. 2003. Efectos interactivos de *Rotylenchulus reniformis* y *Phytophthora palmivora* en papaya (*Carica papaya* L.): sobrevivencia y crecimiento en macetas de invernadero. Nematropica 33:73-85.

Efectos individuales e interactivos del complejo de los patógenos *Rotylenchulus reniformis* y *Phytophthora palmivora*, fueron investigados en papaya (*Carica papaya*) cultivada en macetas de invernadero (diámetro de 10 cm) por 3-4 meses. *P. palmivora* era un patógeno mas virulente que *R. reniformis* y causó mortalidad moderada a severa (27-73%) usando una densidad de inóculo de 1,000 zoosporas por maceta. No se encontraron efectos letales o sub-letales evidentes de *P. palmivora* en papaya usando densidades de inóculo más bajas. *R. reniformis* estimuló el crecimiento de la papaya usando densidades de inóculo menores de 500 juveniles por maceta, y solamente disminuyó el crecimiento (1-2 g de pérdida de peso seco de planta por cada $\log_{10}$ incremento de población inicial) usando densidades más altas (1,000-10,000 juveniles por maceta). Interacciones sinergísticas entre los dos patógenos fueron observadas en el efecto sobre la mortalidad de la papaya, índices de las raices, y el peso seco de las plantas en algunos experimentos. *R. reniformis* causó la predisposición de la papaya al ataque por *P. palmivora*, aunque *P. palmivora* tenía efectos negativos sobre el crecimiento de *R. reniformis*, reduciendo su reproducción por medio de la reducción de la cantidad y calidad de las raices del huésped. Plantas de cuatro semanas eran más susceptibles al complejo de patógenos, y especialmente a *P. palmivora*, que plantas de 8 semanas.

Palablas claves: Carica papaya, papaya, supresión del crecimiento, interacción de plagas, *Rotylenchulus reniformis*, nemátodo reniforme, *Phytophthora palmivora*, patógeno de podredumbre de raices.

INTRODUCTION

Papaya (*Carica papaya* L.) production is affected by a number of soil pathogens that may increase loss of transplanted seedlings and reduce crop vigor, growth, longevity and yield. In Hawaii, poor papaya growth has been associated with two important groups of soil pathogens, root and crown rotting fungus (*Phytophthora* spp.) and plant-parasitic nematodes (*Rotylenchulus reniformis* (Linford and Oliveira), *Meloidogyne* spp., and perhaps *Helicotylenchus* and *Pratylenchus* spp.) (Schmitt and Ferreira, unpublished; Ogata, personal communication).

Phytophthora palmivora Butler as a soil pathogen primarily causes losses during the seedling stage when papaya roots are most susceptible to attack by the pathogen (Ko, 1982). A common inoculum source of *P. palmivora* is older trees with infected fruit and stems. This inoculum is a common cause of papaya replant problems as chlamydospores from fruits survive in the soil for long periods (Hunter and Buddenhagen, 1969; Ko, 1982).

Likewise for plant-parasitic nematodes, initial soil population density (P_i) at planting is predictive of reduced plant growth and yield loss (Seinhorst, 1965; Barker and Olthof, 1976). Surveys of papaya fields in 1998 and 1999 on Oahu (19 fields) and Maui (5 fields) found moderate to high densities of *R. reniformis* in soil during and immediately following papaya production (1,000-8,000 juveniles per 250 cm^3 soil), whereas generally lower densities were detected following sugarcane or fallow (0-2,000 juveniles per 250 cm^3 soil) (Alston *et al.*, unpublished). Replanting a papaya field with papaya soon after destruction of the previous crop would expose roots of small seedlings to high populations of *R. reniformis*.

Very little research has addressed papaya yield loss associated with a complex of soil pathogens (Younge and Plucknett, 1981). A few researchers have determined yield loss from each pathogen group alone, for *Phytophthora* (Hunter and Buddenhagen, 1969) and nematodes (Ahmad, 1989; McSorley, 1992). No economic thresholds have been established for soil pathogens in papaya. Current management practices include planting into virgin soil (Ko, 1971; Ko, 1982), fallow, crop rotation, and soil fumigation (Younge and Plucknett, 1981). All of these approaches have challenges to their efficacy and some can be prohibitive in cost. Fumigants are generally ineffective in rocky and porous lava soils of Hawaii (Ko, 1982).

This study was undertaken to determine the effects of a common soil pathogen complex on papaya survival and growth. *Rotylenchulus reniformis* and *Phytophthora palmivora* were selected because both commonly occur in Hawaii papaya fields, interactive effects may occur as they attack papaya roots, and little is known about the relationship of their population densities to papaya growth. The effect of plant age at infection is also of interest in understanding tolerance of papaya to pathogen attack and in establishing economic thresholds for the field. We hypothesized: 1) that increasing initial population density (P_i) of both pathogens would decrease papaya growth, 2) *P. palmivora* would cause plant mortality at and above a certain P_i and cause damage below that level, 3) *R. reniformis* would predispose papaya roots to the fungus and cause additive or synergistic stresses, 4) presence of the fungus would decrease nematode reproduction on papaya roots, and 5) younger plants would be more susceptible to both pathogens than older plants.

MATERIALS AND METHODS

Pathogen inoculum and experimental design:

All experiments were conducted in the University of Hawaii Magoon Greenhouse from October 1998 to July 1999. 'UH Rainbow' papaya seeds (transgenic hybrid with resistance to papaya ringspot virus) were germinated in vermiculite and transplanted as single plants into 10-cm-diam terracotta pots in 1 part soil:1 part sand mix, 3 to 4 weeks after seeding. Seedlings were fertilized (20/20/20) immediately after transplant. *R. reniformis* inoculum was collected from pineapple soil at Whitmore, Oahu and cultured on 'California Blackeye' cowpea in the greenhouse. Nematode eggs and vermiform stages were collected from cowpea roots with a bleach shaking method (Hussey and Barker, 1973). *P. palmivora* (UH isolate H1226) was collected from diseased papaya roots and maintained on V-8 juice agar under fluorescent light (Aragaki and Hine, 1963). To collect inoculum, water was added to the cultures, the agar surface was rubbed with a spatula, water from several plates were combined in a beaker, chilled at 16°C for 20 min to prompt zoospore release from sporangia, and a subsample of the liquid vortexed to stimulate zoospore encystment. Inoculum was calibrated by counting zoospores with a haemocytometer and adjusted to the desired inoculum density. Once calibrated, nematode and pathogen inoculum was kept in uniform suspension and pipetted into small dimples pressed into the soil within 1 cm of the base of papaya plants.

All experiments used a completely randomized design with four or five replications. Experiments with both pathogens used a factorial design to separate main and interaction effects. Plant growth data were compared among treatments with analysis of variance (Proc Glm, SAS Institute Inc., 1990) and means for significant main effects were separated with the Waller-Duncan *k*-ratio *t*-test. Frequency of plant mortality and root ratings were compared among treatments with Fisher's Exact Test. All data were square root transformed [sqrt(x+1)] before analysis, but actual means are presented.

Experiment 1: Nematode P_i*:*

The first experiment was conducted to determine a range of *R. reniformis* initial population density (P_i) that would cause growth reductions in greenhouse grown papaya within a 3 to 4-month period. Papaya plants were inoculated with *R. reniformis* at 5 weeks after seeding and P_i treatments were 0, 500, 1,000, and 3,000 egg and vermiform stages per pot. Inoculum contained 5% juvenile stages and egg hatch after 7 days at 25°C was 35.6%. Fresh and dry weights of papaya shoots and roots were measured at 15 wk after seeding. Final *R. reniformis* population density (P_f) was determined by extracting nematode egg and vermiform stages from roots (Hussey and Barker, 1973) and from the soil with a combination of elutriation (Byrd *et al.*, 1976) and sugar flotation (Jenkins, 1964).

Experiment 2: Nematode and Fungus Interaction:

The second experiment evaluated the interactive effects of *R. reniformis* and *P. palmivora* on papaya growth and survival. Papaya plants were inoculated with *R. reniformis* at 4 wk and with *P. palmivora* at 11 wk after seeding. *R. reniformis* P_i treatments were 0, 1,000, 5,000 and 20,000 egg and juvenile stages (4% of total) per pot. Egg viability as measured by hatch after 7 days at 25°C was 49.3%. One thousand zoospores of *P. palmivora* per pot caused a moderate frequency of plant mortality in a preliminary test; consequently P_i levels were set at 0 and 1,000 zoospores per pot. Plants were harvested at 14 wk after seeding. Percentage plant mortality and fresh and dry shoot and root dry weights, and nematode P_f values were determined as previously described.

Experiment 3: Interaction of Nematode, Fungus and Plant Age:

This experiment evaluated the influence of papaya age of 4 versus 8 wk after seeding on susceptibility to the pathogen complex. In addition, a comparison of simultaneous and sequential inoculation of the pathogens was conducted to evaluate predisposition of papaya to the fungus by previous nematode infection. Nematode P_i treatments were 0, 5,000, and 20,000 egg and juvenile stages (6% of total) per pot. Egg hatch after 7 days at 25°C was 43%. Fungus P_i treatments were 0 and 1,000 zoospores per pot. Papaya age treatments were both pathogens inoculated at each 4 and 8 wk after seeding, and nematodes inoculated at 4 wk and fungus inoculated at 8 wk after seeding. The factorial design was 3 papaya ages × 2 fungus P_i levels × 3 nematode P_i levels (18 treatments total). Plants were harvested 15 weeks after seeding. *R. reniformis* P_f values and papaya fresh and dry weights of shoot and roots, stem height, canopy volume (canopy width × height), root ratings (1 = healthy, no necrosis, 2 = little necrosis, 3 = moderate necrosis, 4 = high levels of necrosis, but still many roots attached, and 5 = high levels of necrosis with most roots decayed), and percentage mortality data were collected. Additionally, papaya stem height at 8 wk was measured to determine how quickly after inoculation at 4 wk the pathogens affected papaya growth.

Experiment 4: Fungus P_i and Interaction with Nematode:

The fourth experiment evaluated the interaction of nematode P_i with a greater range of fungus P_i levels (sublethal and moderately lethal) on papaya growth and survival. Papaya plants were inoculated with both pathogens at 5 wk after seeding and harvested at 11 wk after seeding. *R. reniformis* inoculum was obtained directly from pineapple field soil from Whitmore, Oahu. *R. reniformis* vermiform stages were extracted by elutriation (Byrd *et al.*, 1976) and sugar flotation (Hussey and Barker, 1973). Vermiform stages were surface sterilized with streptomycin sulfate and spectomycin (each at 0.01 g per 10 ml) and live individuals collected after 15 hr at 25°C. Nematode P_i treatments were 0, 1,500 and 8,000 juvenile stages per pot. Fungus P_i treatments were 0, 10, 100, and 1,000 zoospores per pot. Data on papaya plant growth, root ratings and percentage mortality were collected as in Experiment 3. Nematode P_f data was not collected.

RESULTS

Effects of nematode and fungus P_i on papaya growth at harvest (3-4 months age) were similar for fresh and dry weight, stem height and canopy volume measurements. Strongest effects were more frequently observed for plant dry weight. Most experimental hypotheses were supported, at least to a degree. The exception was that no obvious sublethal effects of *P. palmivora* P_i levels below 1,000 zoospores per pot (moderately lethal P_i) were observed.

Effects of R. reniformis P_i on papaya survival and growth:

Papaya growth varied greatly between experiments, and may have been partly attributable to differences in greenhouse temperatures at different times of the year. *R. reniformis* alone did not cause papaya mortality. *R. reniformis* significantly reduced papaya growth in Experiments 2-4, but not in Experiment 1 where P_i levels were lower (Table 1). Negative impacts of *R. reniformis* on papaya growth were observed for plant dry weight (Experiments 2 and 3), stem height (Experiment 3) and canopy volume (Experiments 3 and 4) (Table 1). In Experiment 2, mean plant dry weight was greater in zero and low P_i (1,000 eggs and juveniles per pot) treatments than in higher P_i (5,000 and 20,000 eggs and juveniles per pot) treatments (p = 0.05). In Experiment 3, mean plant growth responses were

Table 1. Mean papaya plant growth as influenced by *Rotylenchulus reniformis* initial population density (P_i).

Experiment[a]	*R. reniformis* P_i[b]	Dry weight (g) at harvest[c]	Stem height (cm) at harvest[c]	Canopy volume (cm^3) at harvest[c]
1	0	25.5	—	—
	500	26.0	—	—
	1,000	24.2	—	—
	3,000	24.1	—	—
	P > F[d]	0.97		
2	0	3.3 a	—	—
	1,000	3.7 a	—	—
	5,000	2.9 ab	—	—
	20,000	2.7 b	—	—
	P > F	0.05		
3	0	14.0 a	33.4 a	13,184 a
	5,000	6.3 b	22.7 b	11,647 b
	20,000	5.4 b	22.2 b	10,573 b
	P > F	0.0003	0.03	0.06
4	0	4.1	18.8	2,956 a
	1,500	3.7	17.8	2,139 b
	8,000	3.1	16.2	1,776 b
	P > F	0.06	0.25	0.02

[a]N = 16, 32, 90, and 60 for Experiments 1-4, respectively.
[b]Number of *R. reniformis* eggs and juveniles inoculated into pots.
[c]Papaya growth measurements were taken at harvest (15, 14, 15, and 11 weeks after planting for Experiments 1-4, respectively).
[d]Means within a column followed by different letters are significantly different using the Waller-Duncan *k*-ratio *t*-test.

reduced by nematodes (5,000 and 20,000 eggs and juveniles per pot) as compared to no nematodes ($p = 0.05$), but there was no difference between nematode population densities. And likewise in Experiment 4, canopy volume was greater without nematodes than in treatments with nematodes (1,500 and 8,000 juveniles per pot).

The relationship between papaya shoot and root dry weight and nematode P_i was described by quadratic functions in Experiments 1 ($y = 23.0 + 1.2x - 0.8x^2$; $R^2 = 0.91$; $p = 0.04$) and 2 ($y = 4.4 + 0.1x - 0.002x^2$; $R^2 = 0.86$; $p = 0.09$), and by linear functions in Experiments 3 ($y = 16.9 - 2.1x$; $R^2 = 0.82$; $p = 0.02$) and 4 ($y = 5.5 - 0.8x$; $R^2 = 0.68$; $p = 0.23$). Papaya growth was stimulated at the lower nematode P_i treatments included in Experiments 1 and 2 (500-1,000 eggs and juveniles per pot). When accounting for egg hatch of inoculum (35.6-49.3% after 7 days at 25°C), juvenile densities of approximately 200-500 per pot stimulated growth whereas juvenile densities of approximately 1,000-10,000 per pot reduced papaya growth.

R. reniformis required 10-11 weeks of feeding on papaya roots to cause consistent growth reductions. In Experiment 3, where plant stem height was measured at 8 weeks after planting, no effect of inoculation with *R. reniformis* 4 wk earlier was detected ($p = 0.92$). And in Experiment 4, where significant growth reductions from the nematode were found only for canopy volume, nematodes were present on plant roots for only 6 wk.

Effects of P. palmivora P_i on papaya survival and growth:

Lethal and sublethal effects of *P. palmivora* on papaya survival and growth were observed only for the P_i level of 1,000 zoospores per pot, and not for lower P_i levels included in Experiment 4 (Tables 2 and 3).

Table 2. Mean papaya plant growth as influenced by *Phytophthora palmivora* initial population density (P_i).

Experiment[a]	*R. reniformis* P_i[b]	Dry weight (g) at harvest[c]	Stem height (cm) at harvest[c]	Canopy volume (cm^3) at harvest[c]
2	0	4.1 a	—	—
	1,000	2.3 b	—	—
	P>F[d]	0.0001		
3	0	12.0 a	46.5 a	16,834 a
	1,000	5.1 b	19.2 b	7,435 b
	P>F	0.0001	0.0001	0.0001
4	0	3.8	18.7 a	2,589
	10	3.5	19.1 a	2,437
	100	3.7	19.4 a	2,664
	1,000	3.4	14.4 b	2,006
	P>F	0.54	0.02	0.13

[a]N = 32, 90, and 60 for Experiments 2-4, respectively.
[b]Number of *P. palmivora* zoospores inoculated into pots.
[c]Papaya growth measurements were taken at harvest (14, 15, and 11 weeks after planting for Experiments 2-4, respectively).
[d]Means within a column followed by different letters are significantly different using the Waller-Duncan *k*-ratio *t*-test.

Table 3. Percentage papaya mortality caused by *Phytophthora palmivora* initial population density (P_i).

Experiment[a]	Plant age at inoculation (weeks after seeding)	P_i (zoospores per pot)	% mortality
2	11	0	0 b
		1,000	43.8 a
		p[b]	0.003
3	4	0	0 b
		1,000	73.3 a
		p	0.002
	8	0	0 b
		1,000	53.3 a
		p	0.01
4	5	0	0 b
		10	0 b
		100	0 b
		1,000	26.7 a
		p	0.03

[a]N = 32, 90, and 60 for Experiments 2-4, respectively.
[b]Probability values for Fisher's Exact Test.

P. palmivora at 1,000 zoospores per pot significantly reduced all plant growth measurements collected in Experiments 2 and 3, whereas, in Experiment 4, only stem height was reduced (Table 2). The effect of *P. palmivora* on papaya growth occurred rapidly as in Experiment 3, papaya stem height at 8 weeks after planting was already reduced (p = 0.0001) for plants inoculated 4 weeks earlier with 1,000 zoospores per pot.

Papaya mortality consistently occurred at a P_i of 1,000 zoospores per pot, and ranged from 26.7-73.3% (Table 3). No plant mortality was observed at lower P_i levels. Plant death occurred from 5 days to 3 weeks after inoculation with *P. palmivora.* Plant age at inoculation varied among experiments. Percentage mortality was not related to age at inoculation in Experiments 2 and 4, but mortality was greater in 4-wk than 8-wk plants in Experiment 3 (Table 3).

Interactive effects of nematode and fungus pest complex on papaya survival and growth:

Interaction of the two pathogens on papaya growth was observed in Experiment 2 for plant dry weight (p = 0.04) (Fig. 1), but not in Experiments 3 and 4 ($p >$ 0.05). In Experiment 2, the two higher *R. reniformis* P_i levels (5,000 and 20,000 eggs and juveniles per pot) caused synergistic reductions in plant dry weight following inoculation with the fungus as compared to the zero and low nematode P_i levels (Fig. 1). Although the growth reduction observed from the nematode alone wasn't substantial at the higher nematode P_i levels, the nematodes obviously predisposed papaya roots to attack by the fungus. Nematodes were inoculated 7 wk before the fungus in Experiment 2, and substantial feeding injury from *R. reniformis* may

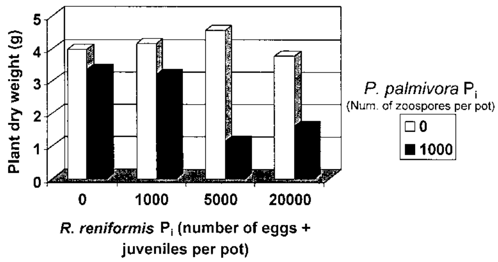

Fig. 1. Interactive effects of *Rotylenchulus reniformis* and *Phytophthora palmivora* initial population density (P_i) on papaya dry weight (g) (shoot + root) in Experiment 2. *R. reniformis* P_i levels: 0, 1000, 5000 and 20,000 eggs and juveniles per pot. *P. palmivora* P_i levels: 0 and 1000 zoospores per pot. Probability value (P > F) for the nematode x pathogen interaction was 0.04.

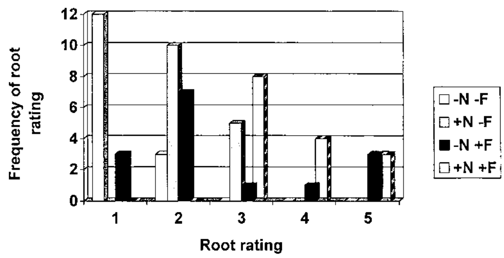

Fig. 3. The effects of inoculation with *Rotylenchulus reniformis* and *Phytophthora palmivora* on papaya root ratings at harvest (15 weeks after planting) in Experiment 3. Treatments: ±N = with and without *R. reniformis*; ±F = with and without *P. palmivora*. Root rating of 1 = healthy roots with no necrosis; root rating of 5 = roots with severe necrosis and rot. The Fisher's Exact Test probability value (p) for differences in root rating frequencies among nematode and fungus treatments was 0.0001.

have more strongly predisposed papaya roots to infection by *P. palmivora* as compared to Experiments 3 and 4 where nematodes were inoculated either simultaneously or 4 wk before the fungus.

Papaya mortality and root ratings were also influenced by an interaction of the two pests (Figs. 2 and 3). Significant effects of the pathogen interaction on

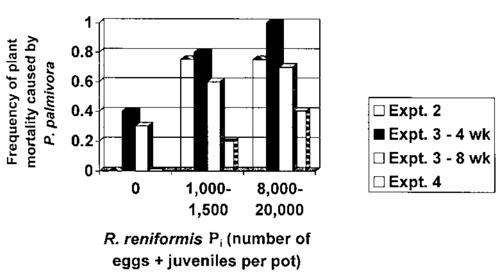

Fig. 2. The influence of *Rotylenchulus reniformis* P_i (0, 1,000-1,500 and 8,000-20,000 eggs and juveniles per pot) on frequency of papaya plant mortality caused by infection with *Phytophthora palmivora* (P_i = 1,000 zoospores per pot). For Experiment 3, means are presented for papaya inoculated with nematode and fungus on 4 and 8 weeks after planting. Probability values (p) for Fisher's Exact Test for comparison among nematode P_i levels were 0.008, 0.0001, and 0.156 for Experiment 2-4, respectively. The probability value (p) for comparison of plant age at inoculation (4 vs. 8 wk) in Experiment 3 was 0.008.

mortality were observed in Experiments 2 and 3 (p = 0.008 and 0.0001, respectively) where plant mortality caused by *P. palmivora* was substantially increased by the presence of *R. reniformis* (Fig. 2). In Experiment 4, plant mortality also increased in the presence of the nematode, but the effect was not significant (p > 0.05). Percentage plant mortality caused by *P. palmivora* when nematodes weren't present was zero in Experiments 2 and 4 and 30-40% in Experiment 3. When both pathogens were present, mortality ranged from 20-100%.

Root rating data was collected only in Experiments 3 and 4, and in both, there was an interactive effect of the pest complex on frequency of root ratings (p = 0.0001) (Fig. 3 shows data for Experiment 3; data for Experiment 4 are not shown, but results are similar to Experiment 3). The frequency of higher root ratings indicating more root necrosis and decay was greatest when both the fungus and nematode were present (Fig. 3). The next greatest frequency of higher root ratings were found in treatments with the fungus alone and then the nematode alone. Very few root ratings above the value 1, indicating healthy roots with no necrosis, were observed for plants without either pathogen (Fig. 3).

Effects of plant age at inoculation on susceptibility of papaya to pathogens:

Experiment 3 was the only trial to include direct evaluation of papaya age on the plant's susceptibility to the pathogen complex. The age of papaya at inoculation (4 or 8 wk after seeding) affected plant dry weight (p = 0.04), but not stem height or canopy volume (p > 0.05). Plant dry weight was reduced when papaya was inoculated with *P. palmivora* at 4 wk as compared to 8 wk after seeding (Fig. 4). The trend among age treatments was the same for stem height and canopy volume. The pathogen complex affected four-wk-old plants soon after inoculation, as an effect of plant age on stem height was already evident by 8 wk after planting (p = 0.0001) (data not shown). Age effects were also exhibited for root ratings. Root rating values were higher for plants inoculated with both pathogens at 4 wk than at 8 wk after seeding or sequentially with nematodes at 4 wk

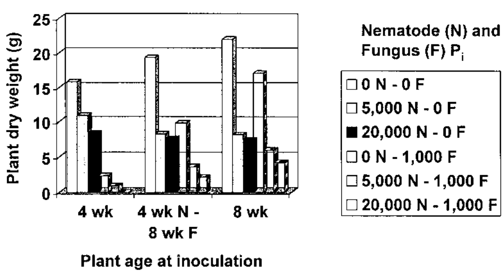

Fig. 4. Interactive effects of *Rotylenchulus reniformis* and *Phytophthora palmivora* initial population density (P_i) and plant age at inoculation on papaya dry weight (g) (shoot + root) in Experiment 3. Nematode P_i levels: 0, 5000 and 20,000 eggs and juveniles per pot. Fungus P_i levels: 0 and 1000 zoospores per pot. Plant ages at inoculation: 4 and 8 weeks after planting for nematode and fungus, and 4 weeks after planting for nematode and 8 weeks after planting for pathogen (4 wk N-8 wk F).

and the fungus at 8 wk (p = 0.006) (data not shown).

Papaya plant growth was influenced by interactions between each pathogen and plant age. The nematode × age interaction (p = 0.02) seemed to be an artifact caused by increasing dry weight of plants without pathogens from 4 wk to 8 wk of age at inoculation (Fig. 4). Without pathogens, the growth of these plants should have been similar across age treatments. The depression in dry weight caused by adding 5,000 and 20,000 nematodes per pot then intensified across age treatments from 4 to 8 wk, thus causing the nematode × age interaction (Fig. 4).

The fungus × age interaction was created because *P. palmivora* had the most negative effect on plant growth when inoculated at 4 versus 8 wk (Fig. 4). This interactive effect was seen for plant dry weight (p = 0.05) (Fig. 4), root ratings (Fisher's Exact Test, p = 0.003), and plant mortality (Fisher's Exact Test, p = 0.008) (Fig. 2). The impact of the sequential inoculation of pathogens (4 wk N-8 wk F) on plant dry weight was greater than simultaneous inoculation of both pathogens at 8 wk, but not greater than the effect of inoculating both pathogens at 4 wk of age (Fig. 4). There were no effects of the three-way interaction (nematode × fungus × age) on plant growth parameters.

Effects of pathogens on nematode reproduction:

Not surprisingly, *R. reniformis* P_i had a significant effect on *R. reniformis* final population density (P_f) and population increase (P_f/P_i) in Experiments 1-3 where nematode P_f was measured (Table 4). In these experiments, nematode P_f increased with increasing P_i. P_f/P_i was similar among treatments with nematodes (Experiments 1 and 2) or greater in the moderate than high nematode P_i levels (Experiment 3) (Table 4). Nematode population increase ranged from 0.058 to 0.672 in treatments with nematodes.

Table 4. Mean reniform nematode final population densities (P_f) and population increase (P_f/P_i) per pot as influenced by *Rotylenchulus reniformis* (Rr) and *Phytophthora palmivora* (Pp) initial population densities (P_i) and plant age at inoculation.

Experiment[a]	Effect	Levels	P_fb	P_f/P_i
1	Rr[c]	0	0 c	0 b
		500	29 bc	0.058 ab
		1,000	617 ab	0.617 a
		3,000	1,471 a	0.490 a
		P > F[d]	0.02	0.04
2	Rr	0	0 d	0 b
		1000	650 c	0.650 a
		5000	3,360 b	0.672 a
		20,000	11,643 a	0.582 a
		P > F	0.0001	0.001
	Pp	0	4,046	0.541
		1,000	3,780	0.360
		P > F	0.50	0.19
3	Rr	0	0 c	0 c
		5,000	2,623 b	0.525 a
		20,000	6,790 a	0.340 b
		P > F	0.001	0.001
	Pp	0	4,541 a	0.403 a
		1,000	1,735 b	0.173 b
		P > F	0.001	0.001
	Age	4 wk	3,376 a	0.274 ab
		4 wk N-8 wk F	3,761 a	0.360 a
		8 wk	2,276 b	0.230 b
		P > F	0.04	0.02

[a]N = 16, 32, and 90 for Experiments 1-3, respectively.
[b]Final nematode population densities (eggs and vermiform) per pot were determined at harvest (15, 14 and 15 weeks after planting for Experiments 1-3, respectively).
[c]*R. reniformis* was inoculated at 5, 4, and 4 and 8 weeks after planting for Experiments 1-3, respectively. *P. palmivora* was inoculated at 11, and 4 and 8 weeks after planting for Experiments 2 and 3, respectively.
[d]Means within a column followed by different letters are significantly different using the Waller-Duncan *k*-ratio *t*-test.

The time period available for nematode population increase was 10 wk (Experiments 1 and 2) or 11 wk (Experiment 3). *R. reniformis* populations did not replace themselves, but only achieved 6-67% replacement. If egg hatch of nematode inoculum is considered, then mean P_f/P_i values ranged from 0.079 to 1.003, and in the treatments with highest reproduction, nematode populations just replaced themselves.

The presence of *P. palmivora* decreased nematode reproduction in Experiment 3, but not in Experiment 2 (Table 4). This difference may be explained by the later inoculation of *P. palmivora* in Experiment 2 as compared to Experiment 3. In Experiment 2, the fungus had only 3 wk to affect nematode reproduction before harvest versus 7 and 11 wk in Experiment 3. The greatest nematode population growth occurred in Experiments 1 and 2, where the influence of the fungus on nematode reproduction was absent or minimized. The decline in root health associated with infection by *P. palmivora* likely reduced suitable root feeding sites for *R. reniformis.*

In Experiment 3, the age of papaya at inoculation influenced *R. reniformis* population growth (Table 4). P_f and P_f/P_i values were greater on plants inoculated with nematodes at 4 wk (4 wk and 4 wk N-8 wk F treatments) than on plants inoculated at 8 wk. The greater length of time for the nematode to reproduce on plant roots allowed greater population increase.

The only fungus interaction effects on *R. reniformis* P_f and P_f/P_i was observed for nematode × fungus in Experiment 3. The fungus had a greater impact on reducing nematode population increase in the highest nematode P_i (20,000 *R. reniformis* per pot) than in the moderate nematode P_i (5,000 *R. reniformis* per pot) ($p = 0.002$ and 0.03 for P_i and P_f/P_i, respectively). There was no interactive effect of the two pathogens in Experiment 2 because of the minimal effect of *P. palmivora* on nematode reproduction and similar reproduction among treatments with nematodes (1,000, 5,000 and 20,000 *R. reniformis* per pot) (Table 4).

DISCUSSION

Both *Rotylenchulus reniformis* and *Phytophthora palmivora* significantly reduced papaya growth under greenhouse conditions for a 3-4 month growth period. *P. palmivora* was the more virulent pathogen at an inoculum density of 1,000 zoospores per pot, and caused substantial plant mortality (27-73%). Below 1,000 zoospores per pot no detectable effects were observed on plant survival and growth, however, this study included only one trial with lower *P. palmivora* P_i levels. No P_i levels above 1,000 zoospores per pot were investigated, but we would anticipate high initial plant mortality and significantly reduced growth of surviving plants.

R. reniformis was not a highly virulent pathogen in these greenhouse studies. Suppression of papaya growth was likely limited by the pot environment and relatively short time frame of the greenhouse studies. *R. reniformis* stimulated papaya growth at P_i levels below approximately 500 juveniles per pot and caused modest growth reductions at higher P_i levels (1,000-10,000 juveniles per pot). In the linear portion of the nematode damage functions described above, slopes ranged from –0.8 to –2.1, except for Experiment 2 in which it was only –0.002. A loss of approximately 1-2 g of plant dry weight occurred for every $\log_{10}$ increase in nematode P_i. In pineapple, lower initial population densities caused greater yield reduction as compared to higher initial nematode population densities (Schenck, 1990; Sipes and Schmitt, 1998). Similar responses have been noted in snap bean (McSorley, 1980).

R. reniformis soil densities causing modest papaya growth reductions in this study (763-7,043 juveniles per 250 cm^3 soil when converting from per pot to per 250 cm^3 soil) are in line with densities found in field surveys during or immediately following papaya production (1,000-8,000 juveniles per 250 cm^3 soil) (Alston *et al.*, unpublished). We can infer that *R. reniformis* population densities typically found in papaya fields in Hawaii are high enough to induce at least moderate plant growth reductions.

The low rates of population increase (8-100%) exhibited by *R. reniformis* was somewhat surprising. Densities of 1,000-8,000 *R. reniformis* juveniles per 250 cm^3 soil recovered from papaya field soil in limited surveys on Oahu and Maui indicate that papaya is a "good" host for reniform nematode. In previous tests, *R. reniformis* has more than doubled its population on a good host in 8 wk (Wang *et al.*, 2001). After 16 wk on cowpea, *R. reniformis* populations increased 428% in one test (Inserra *et al.*, 1994). Yet in the 10-11 wk period in these greenhouse studies, *R. reniformis* populations only replaced themselves at best. The inclusion of *P. palmivora* in the factorial experiments is a likely reason as this fungus was shown to have a negative effect on *R. reniformis* reproduction.

There were definite synergistic interactions between the two pathogens. The interactions were negative on plant survival and growth and both positive and negative of one pathogen upon the other. Co-occurrence of the two pathogens caused a synergistic increase in plant mortality, root ratings, and loss in plant dry weight in some experiments. The presence of *R. reniformis* predisposed papaya to increased mortality and poorer root growth caused by *P. palmivora*. *P. palmivora* had indirect negative effects on the nematode by reducing *R. reniformis* population growth through reductions in quantity and quality of host roots.

Papaya was sensitive to age of attack by the two pathogens. Very young plants, 4 wk after seeding, were more susceptible to growth reductions, especially by *P. palmivora*. However, papaya up to 11 wk after planting was still susceptible to *P. palmivora* attack and plant mortality reached 44%. In these studies, papaya was inoculated with *R. reniformis* and *P. palmivora* at 4-8 wk after seeding. Papaya transplants placed in the field would be of a similar age and so we can infer their susceptibility to these soil pathogens would be similar to study findings.

We can deduce from these results that co-occurrence of *Phytophthora* and reniform nematode in a field at time of transplanting papaya seedlings could substantially reduce plant stands and plant growth. Crop rotation, fallowing, or using virgin soil in replant holes are current options for management of this pathogen complex. The exploration of more management alternatives should be pursued. One alternative under current study is the use of inter-cycle crops that suppress both pathogens, during the initial growth of papaya.

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