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Influence of Nonhost Plants on Population Decline of *Rotylenchulus reniformis*[1]

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Abstract: The influence of *Chloris gayana, Crotalaria juncea, Digitaria decumbens, Tagetes patula,* and a chitin-based soil amendment on Hawaiian populations of *Rotylenchulus reniformis* was examined. *Chloris gayana* was a nonhost for *R. reniformis.* The nematode did not penetrate the roots, and in greenhouse and field experiments, *C. gayana* reduced reniform nematode numbers at least as well as fallow. *Tagetes patula* was a poor host for reniform nematode and reduced reniform nematode numbers in soil better than did fallow. *Crotalaria juncea* was a poor host for *R. reniformis,* and only a small fraction of the nematode population penetrated the roots. *Crotalaria juncea* and *D. decumbens* reduced reniform nematode populations at least as well as fallow. A chitin-based soil amendment, applied at 2.24 t/ha to fallow soil, did not affect the population decline of reniform nematode.

Key words: allelopathy, chitin, *Chloris gayana,* cover crop, *Crotalaria juncea, Digitaria decumbens,* Hawaii, marigold, nematode management, nematode population decline, pangola grass, reniform nematode, rhodes grass, soil amendment, sunn hemp, *Tagetes patula.*

The reniform nematode, *Rotylenchulus reniformis* Linford & Oliveira, is a limiting factor in Hawaiian pineapple production. Management depends primarily on nematicides and fallow (4). Interest in alternative nematode management practices has increased because of environmental and health problems associated with pesticide use. Promising alternative management strategies include the use of rotation or cover crops and soil amendments (8,10,12,13).

The myriad effects plants exert on nematodes is well documented (29). Certain plants, including marigold (*Tagetes patula* L. and *T. erecta* L.) (10,11), pangola grass (*Digitaria decumbens* Stent.) (12,13), rhodes grass (*Chloris gayana* Kunth) (8), and sunn hemp (*Crotalaria juncea* L.), reduce soil populations of several plant-parasitic nematode species (21,22).

Tagetes erecta and *T. patula* have been reported as hosts or nonhosts for reniform nematodes (1,15,20). Toxic thiophenes have been recovered from marigold root extracts (10) and from undisturbed rhizospheres (27). Thiophenes may be directly toxic to soil nematodes, although their mode of action against plant-parasitic nematodes is not established (10).

Some *Crotalaria* spp. reduce numbers of reniform nematode (21), but *C. juncea* has been reported as a host for reniform nematode (19). The host status of *T. patula, D. decumbens, Chloris gayana,* and *Crotalaria juncea* for reniform nematode is, therefore, equivocal. *Chloris gayana* and *D. decumbens* have been shown to reduce numbers of root-knot nematode (7).

Chitin-based soil amendments may be directly nematicidal and stimulate chitinolytic microflora (25). Some efficacy in controlling several species of plant-parasitic nematodes has been reported (18,25,26).

The present research was conducted to assess the influence of *C. gayana, C. juncea, D. decumbens, T. patula,* and a chitin-based soil amendment on the population density of *R. reniformis* in Hawaiian pineapple soils. Multiple experiments were conducted to determine if observed effects were repeatable. Preliminary results have been published (3).

Materials and Methods

Experiments were conducted in the greenhouse, field, and laboratory. The

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plants assessed were cucumber (*Cucumis sativus* L.), tomato (*Lycopersicon esculentum* Mill. cv. Tropic), rhodes grass (*Chloris gayana* cvs. Katambora or Mbarara), marigold (*T. patula* cv. French Dwarf Double), sunn hemp (*Crotalaria juncea* cv. Tropic Sunn), and pangola grass (*D. decumbens*). Greenhouse experiments were conducted using *R. reniformis*-infested field soil in 15.2-cm-d black plastic pots arranged in completely randomized designs and maintained at 23–28 C. Treatments were randomly assigned to pots as soil was added. As soil was added, samples were taken to determine initial nematode population densities (Pi). Test plant seeds were planted in the pots and were watered and fertilized as necessary. Pots were kept free of weeds. Root balls were removed from pots and final densities (Pf) were assessed from soil shaken from roots.

Soil samples were processed by modified centrifugal flotation (15) to extract motile stages of *R. reniformis*. Initial and final population density values included all motile stages, and Pf/Pi ratios were calculated at each sampling date for the individual treatment replicates. In some experiments roots were gently rinsed, weighed, and stained (2), and egg masses and sedentary females were counted with the aid of a stereomicroscope. Nematode counts were recorded as either numbers per 100 cm^3 soil or numbers per gram of fresh root weight. The normality of the data was assessed using the Shapiro-Wilk statistic (23), and data were square root or log transformed as necessary before analysis of variance. The Waller-Duncan k-ratio *t*-test (k-ratio = 100) or LSD *t*-test ($P = 0.05$) multiple comparison procedures were used to determine differences among treatments (5,23).

Experiment 1: Soil naturally infested with *R. reniformis* was obtained from Dole Co. field 4141 (Lahina silty clay) in Mililani, Oahu (9). The soil was screened through a 0.6-cm-mesh sieve to remove small pebbles and soil aggregates, mixed in a rotary cement mixer, and placed in pots.

Four treatments were replicated five times. The treatments were cucumber, Katambora rhodes grass, Mbarara rhodes grass, and marigold. Replicates of each treatment were destructively sampled at 86 and 114 days after planting to assess nematode numbers. At 86 days, roots were rinsed and stained, and egg mass production was qualitatively assessed.

Experiment 2: The same protocol as outlined for experiment 1 was used except the treatments were tomato, marigold, one tomato with three marigolds, and fallow. Treatments were replicated four times. Nematode population densities and number of egg masses on the roots were assessed at 41 and 63 days after planting. At 41 days all roots were examined for egg masses, and at 63 days 5% of the total fresh root weight was examined.

Experiment 3: Soil naturally infested with *R. reniformis* was obtained from Dole Co. field 4107 (Wahiawa silty clay) in Mililani, Oahu (9). The soil was screened through a 0.6-cm-mesh sieve before use. The six treatments were one tomato plant per pot, four marigold plants per pot, one tomato with four marigolds, approximately six Katambora rhodes grass plants per pot, tomato with Katambora rhodes grass, and fallow. Nematode numbers were assessed 40, 67, and 102 days after planting by destructive sampling of six replicates.

Experiment 4: The same soil source used in experiment 3 was used here. Treatments were Katambora rhodes grass, marigold, tomato, tomato with chitin, fallow, and fallow with chitin. Each treatment was replicated five times. Chitin amendment (chitin 15%, protein 30%, minerals 55%; supplier: Hosho-Somerset Corporation, Crisfield, MD 21817) was added to soil as the pots were filled (1.5 g/pot, a rate equivalent to 2.24 t/ha). Nematode numbers were assessed from replicates at 44 and 72 days after planting.

Experiment 5: This experiment was conducted in Dole Co. field 5425 (silty clay loam) on the island of Lanai (9). The field had previously been planted to pineapple but had received no nematicides for 3 years before the experiment, resulting in high reniform nematode population densities.

The experimental area was divided into plots 0.9 m wide by 6.4 m long. Plots were arranged in a complete randomized block design with five treatments and four replications. The treatments were rhodes grass, pangola grass, sunn hemp, fallow, and fallow with chitin amendment. Rhodes grass was hand seeded and raked into the soil at a rate of approximately 90 kg/ha. Pangola grass was hand planted from runners on 30-cm centers at a depth of 2.5 cm. Sunn hemp was planted by hand on 30-cm centers, 2 cm deep, with seeds spaced approximately 8 cm apart. The chitin amendment was spread on the soil surface at a rate of 2.24 t/ha and raked into the soil. All treatments received drip irrigation. Air temperatures at the site ranged from 17 to 26 C.

Soil samples were collected at 90, 144, 187, 239, and 342 days after planting. Soil samples were taken to a depth of 30 cm with an Oakfield tube, and five cores were combined per sample.

Experiment 6: The soil used in experiment 3 was placed in pots and planted to pineapple for 1 year. Four treatments were investigated: one tomato plant per pot, two marigold plants per pot, three rhodes grass plants per pot, and one sunn hemp plant per pot. Treatments were replicated five times and destructively sampled at 15 and 26 days after planting. Roots were stained and stages of reniform nematode were counted as in experiment 2. A root penetration index (RPI) was calculated by dividing the number of sedentary females per gram of fresh root by the corresponding Pi value.

Experiment 7: Three treatments were investigated: tomato, rhodes grass, and sunn hemp. Each treatment was replicated five times. Seeds were germinated on moist filter paper. After germination individual seedlings were transferred to petri dishes (100 × 15 mm), each dish having a 5-mm square hole in the side. Seedlings were positioned so that the stems and leaves grew out the opening of the dish, while the roots grew onto White's medium (minus sucrose and organics) (27). The dishes were wrapped with foil and placed in a growth chamber at 24 C (14 hours light, 10 hours dark). Two days after transferring the seedlings to the dishes, each dish was inoculated with 75–100 motile reniform nematodes. Roots were stained and the sedentary females were counted 24 days after inoculation.

RESULTS

Experiment 1: The marigold treatment had a significantly higher initial population density than did the other treatments. Soil populations and Pf/Pi ratios were significantly lower in marigold and both rhodes grass cultivars than in cucumber at days 86 and 114 (Table 1). Soil populations (days 86 and 114) and Pf/Pi ratios (day 86) of both rhodes grass cultivars were significantly lower than those of marigold (Table 1). A visual, qualitative inspection of stained roots revealed many egg masses on cucumber, a few on marigold, and no egg masses on rhodes grass.

Experiment 2: Initial nematode numbers were significantly higher in fallow than in remaining treatments (n = 8) (Table 1). At 41 days there were significantly fewer nematodes in the marigold than in the other treatments. Marigold yielded a significantly lower Pf/Pi ratio than did tomato or tomato with marigold (Table 1). At day 41 there were significantly fewer egg masses per gram of root in marigold than in tomato growing with marigold or tomato alone (Table 1).

At 63 days tomato yielded significantly higher soil population densities than did the remaining treatments. Marigold yielded significantly fewer nematodes than did all other treatments and a Pf/Pi ratio lower than tomato or tomato with marigold (Table 1). There were fewer egg masses per gram of root in marigold and marigold planted with tomato than in tomato alone at 63 days (Table 1).

Experiment 3: Differences were observed among initial population densities when a multiple comparison was performed using all replicates (n = 18 per treatment) (Table 2). However, when Pi values were grouped

TABLE 1. Changes in numbers of *Rotylenchulus reniformis* and Pf/Pi ratios during the growth of different plants over time.

Plant	Pi	Sample 1†			Sample 2‡		
		Pf	Pf/Pi	Egg/g	Pf	Pf/Pi	Egg/g
		Experiment 1					
Cucumber	722 b	1,256 a	1.8 a	—	1,258 a	2.0 a	—
Marigold	1,354 a	248 b	0.2 b	—	350 b	0.2 b	—
Rhodes grass (cv. Mbarara)	826 b	32 c	0 c	—	84 c	0.1 b	—
Rhodes grass (cv. Katambora)	704 b	19 c	0 c	—	93 c	0.2 b	—
		Experiment 2					
Tomato	381 b	835 a	2.3 ab	18 a	2,227 a	6.6 a	38 a
Marigold	335 b	77 b	0.2 c	0 b	77 d	0.4 b	0 b
Marigold + tomato	285 b	757 a	3.3 a	36 a	877 b	3.6 a	8 b
Fallow	506 a	560 a	1.1 c	—	268 c	0.5 b	—

Values are means of four (experiment 2) or five (experiment 1) replicates. Within experiments means followed by the same letter in vertical columns are not significantly different as judged by the Waller-Duncan k-ratio *t*-test (k-ratio = 100). Dashes indicate data not collected. Pi = initial nematode population density (#/100 cm³ soil). Pf = nematode population density at sampling date. Egg/g = number of egg masses per gram (fresh weight) of root.
† Experiment 1 = 86 days; experiment 2 = 41 days.
‡ Experiment 1 = 114 days; experiment 2 = 63 days.

by subsequent sampling date and compared (n = 6 per sampling period per treatment), no significant differences were detected.

At 40 days soil populations of *R. reniformis* and Pf/Pi ratios in Katambora rhodes grass and fallow were significantly higher than all other treatments (Table 2). At 67 days marigold and rhodes grass yielded significantly lower soil population densities and Pf/Pi ratios than did all other treatments. The soil population density in mari-

TABLE 2. Changes in soil populations of *Rotylenchulus reniformis* and Pf/Pi ratios during growth of different plants or addition of chitin soil amendment (added at a rate of 2.24 t/ha).

Plant or treatment	Pi	Sample 1†		Sample 2‡		Sample 3§	
		Pf	Pf/Pi	Pf	Pf/Pi	Pf	Pf/Pi
		Experiment 3					
Tomato	617 c	503 bc	0.8 b	1,115 a	1.9 ab	663 a	1.4 a
Marigold	797 abc	618 b	0.8 b	75 c	0.1 c	206 c	0.3 c
Marigold + tomato	960 a	577 bc	0.6 bc	1,460 a	2.2 ab	338 ab	0.4 bc
Rhodes grass	807 ab	1,442 a	1.8 a	88 b	0.1 c	31 d	0.04 d
Rhodes grass + tomato	841 ab	407 c	0.4 c	618 a	0.7 b	235 bc	0.3 bc
Fallow	693 bc	1,480 a	2.0 a	1,135 a	1.8 a	528 ab	0.9 ab
		Experiment 4					
Tomato	1,428 ab	138 c	0.1 cd	1,490 a	1.1 a	—	—
Tomato + chitin	1,712 a	206 bc	0.1 bc	1,418 a	0.8 a	—	—
Marigold	1,442 a	44 d	0.03 d	48 c	0.03 d	—	—
Rhodes grass	1,526 ab	314 ab	0.2 ab	312 b	0.2 c	—	—
Fallow	1,136 b	280 ab	0.3 a	400 b	0.4 b	—	—
Fallow + chitin	1,425 ab	486 a	0.3 a	420 b	0.3 bc	—	—

Values are means of six (experiment 3) or five (experiment 4) replicates. Within experiments means followed by the same letter in vertical columns are not significantly different as judged by the Waller-Duncan k-ratio *t*-test (k-ratio = 100). Dashes indicate data not collected. Pi = initial nematode population density #/100 cm³ soil). Pf = nematode population density at sampling date.
† Experiment 3 = 40 days; experiment 4 = 44 days.
‡ Experiment 3 = 67 days; termination of experiment 4 = 72 days.
§ Termination of experiment 3 = 102 days.

TABLE 3. Influence of nonhost plants and a chitin soil amendment (added at a rate of 2.24 t/ha) on soil populations of *Rotylenchulus reniformis* over time.

Plant or treatment	Pi	Day 90	Day 144	Day 187	Day 239	Day 342
Sunn hemp	2,075 b	937 c	1,142 a	237 b	965 a	2,513 a
Rhodes grass	3,133 ab	1,497 b	802 a	1,245 a	857 a	625 a
Pangola grass	5,070 a	2,477 a	2,082 a	1,307 a	1,187 a	720 a
Fallow + chitin	3,258 ab	2,550 a	1,640 a	1,232 a	1,480 a	2,203 a
Fallow	4,675 ab	2,777 a	2,397 a	2,882 a	2,187 a	2,163 a

Values are means of four replicates. Means followed by the same letter in vertical columns are not significantly different as judged by the Waller-Duncan k-ratio *t*-test (k-ratio = 100). Pi = initial nematode population density (#/100 cm^3 soil).

gold was significantly lower than that in rhodes grass (Table 2).

At 102 days marigold and rhodes grass had significantly lower population densities than did tomato, marigold with tomato, or fallow. The Pf/Pi ratio for marigold was significantly lower than for tomato or fallow. The population density and Pf/Pi ratio for rhodes grass were significantly lower than those for all other treatments (Table 2). At 102 days the Pf/Pi ratios for marigold growing with tomato and rhodes grass growing with tomato were significantly lower than the Pf/Pi ratio for tomato alone (Table 2).

Experiment 4: Differences were observed among initial population densities when a multiple comparison was performed on all replicates (n = 10 per treatment) (Table 2). Initial population densities were grouped by sampling dates and compared (n = 5 per treatment), and no significant differences were detected. At 44 days there were significantly lower nematode numbers in marigold than in remaining treatments, and the Pf/Pi ratio for marigold was lower than those for all other treatments except tomato (Table 2). Numbers of *R. reniformis* were higher in rhodes grass than in tomato or marigold (Table 2). The Pf/Pi ratio for rhodes grass was significantly higher than those for marigold or tomato.

Nematode numbers and the Pf/Pi ratio for marigold were significantly lower than those for all other treatments at 72 days (Table 2). The Pf/Pi ratio for rhodes grass was significantly lower than those for all other treatments except marigold and fallow with chitin (Table 2). At 72 days tomato and tomato with chitin had significantly higher nematode numbers and Pf/Pi ratios than did the remaining treatments (Table 2).

Experiment 5: The Pi for pangola grass was significantly higher than for sunn hemp (Table 3). At 90 days nematode numbers in the sunn hemp treatment were significantly lower than those in all other treatments (Table 3). The population density in the rhodes grass treatment was significantly lower than in pangola grass, fallow, or fallow with chitin (Table 3). There were no differences among treatment Pf/Pi ratios.

At 187 days nematode densities in sunn hemp were significantly lower than in all other treatments (Table 3). Comparisons of Pf/Pi ratios at 187 days (data not shown) were in agreement with comparisons of population densities. There were no significant differences among treatment population densities at 144, 239, and 342 days. At 342 days the Pf/Pi ratio for pangola grass was significantly lower than for fallow with chitin or sunn hemp (data not shown).

Experiment 6: There were no significant differences among treatment initial population densities. At 15 days there were significantly greater numbers of sedentary females in roots of sunn hemp and tomato than in rhodes grass or marigold (Table 4). The RPI for sunn hemp was significantly higher than that for marigold or rhodes grass. The RPI for rhodes grass was significantly lower than tomato or sunn hemp at 15 days (Table 4).

At 25 days there were significantly fewer

TABLE 4. Penetration and development of *Rotylenchulus reniformis* in different plants.

Plant	Pi	Day 15		Day 25		
		Total/g	RPI	Vermiform	Total/g	RPI
Tomato	111 a	66 a	63.4 ab	238 a	1,038 a	1,265 a
Marigold	158 a	7 b	7.8 bc	21 c	47 c	47 c
Sunn hemp	129 a	68 a	121.8 a	116 b	204 b	296 b
Rhodes grass (cv. Katambora)	184 a	0 b	0 c	1 c	1 d	1 d

Values are means of five replicates. Means followed by the same letter in vertical columns are not significantly different as judged by the Waller-Duncan k-ratio *t*-test (k-ratio = 100). Pi = initial soil population density (number/100 cm^3 soil). Total/g = total number of vermiform and swollen females per gram of root (fresh weight). Vermiform = number of vermiform females observed in roots. Root penetration index (RPI) = ([total/g]/init) × 100.

vermiform females in marigold and rhodes grass roots than in tomato or sunn hemp, with sunn hemp having fewer vermiform females than tomato (Table 4). The total numbers of females (swollen + vermiform) per gram of root were significantly different among all treatments (Table 4). The RPI values were significantly different among all treatments (Table 4).

Experiment 7: No females were observed in rhodes grass. Swollen females were observed in roots of both tomato and sunn hemp. There was not a significant difference in the numbers of females in tomato vs. sunn hemp (data not presented). The females observed in sunn hemp were only slightly swollen, compared with those in tomato that appeared to be normally swollen females.

DISCUSSION

Marigold and rhodes grass reduced Hawaiian reniform nematode populations at least as well as did fallow. Sunn hemp is a poor host for reniform nematode and pangola grass is a nonhost. The capacities of marigold, rhodes grass, and sunn hemp for supporting reniform nematode penetration and development can be evaluated. The root penetration index allows discrimination of relative host status among these plants, with tomato >>> sunn hemp >> marigold > rhodes grass in order of decreasing host suitability. Nematode population reduction by sunn hemp, marigold, rhodes grass, and pangola grass was not consistently superior to fallow. However, these plants do have potential use in reniform nematode management programs in Hawaii, particularly between cropping cycles.

Rhodes grass is a nonhost and is immune to penetration by the reniform nematode. In greenhouse experiments rhodes grass reduced nematode numbers at least as well as did fallow. After 90 days in the field, rhodes grass was no better at reducing nematode numbers than was fallow. However, the general trend of reduced nematode numbers in the field combined with our greenhouse results allows that rhodes grass reduces nematode numbers better than does fallow. Our results do not indicate allelopathy by rhodes grass. The gradual decline of nematode populations in rhodes grass treatments confirms the ability of reniform nematode to survive extended periods without a host (24).

The grass *Digitaria decumbens* was investigated only in the field trial. The grass did not support reproduction of reniform nematode in the field as determined by comparison with fallow.

Marigold is an extremely poor host for reniform nematode, allowing only a few nematodes to penetrate and develop. Marigold has a RPI much lower than tomato, and in greenhouse experiments marigold was more effective than fallow in reducing nematode numbers.

Christie (6) suggested that root diffusates from *Tagetes* spp. might function as nematistats or neutralize or mask host root diffusates and thus interfere with infection. Reniform nematode can survive for extended periods without a host (24); in the absence of a host, nematode numbers will slowly decline. The rapid nematode population decline under marigold combined with the low RPI observed in these experiments supports the hypothesis of nematode population reduction by allelopathy, possibly by toxic thiophenes (10,27).

Sunn hemp is a moderately poor host for the reniform nematode examined in these studies, but it does not reduce nematode numbers better than does fallow. After 342 days in the field, nematode numbers were not significantly lower in sunn hemp than in fallow. The generation time of reniform nematode is approximately 20 days under favorable conditions (20), and 342 days is sufficient time for the completion of many generations. The lack of increase in nematode numbers during the field experiment indicates that sunn hemp does not support reniform nematode reproduction. Other greenhouse experiments (data not presented) yielded similar results.

Sunn hemp had as many parasitic females within the roots as did tomato in experiment 8. However, the inoculum level used in experiment 8 was very low, and in the remaining experiments dealing with penetration and development only a small fraction of the nematode population penetrated and initiated development in sunn hemp. Additionally, nematode development in sunn hemp seemed less rapid than in tomato. The low frequency of penetration and slow development could result in a slight population increase over time. The potential for population increase on sunn hemp in the field is low, as it is typically grown for short periods as a nitrogen source or to limit erosion (22).

High initial population densities were used in most experiments and might be responsible for the low Pf/Pi ratios on tomato, less than 1 in some cases. Tomato root leachates stimulate reniform nematode hatch (14) and if nematode survival is poor because of intraspecific competition, Pf/Pi ratios would be lowered. In addition, Pf/Pi ratios decrease with increasing Pi for reniform nematode infecting tomato (17).

Field experiment Pf/Pi ratios are not presented because of difficulties in obtaining Pf values corresponding to particular Pi values in large plots. Cool field temperatures may have influenced nematode activity (20) and thus the capacity of the experimental plants to enhance nematode population decline.

The addition of chitin to fallow soil or soil in which a tomato was growing did not influence nematode population densities or Pf/Pi ratios. Other formulations, rates, and application strategies may enhance the activity of the chitin amendment (18,25,26). The rate and method of application chosen for this study was based on potential economic acceptability to the Hawaiian pineapple industry.

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