SUPPRESSION OF *ROTYLENCHULUS RENIFORMIS* BY *CROTALARIA JUNCEA*, *BRASSICA NAPUS*, AND *TAGETES ERECTA*†

Koon-Hui Wang,[1,2] B. S. Sipes,[2] and D. P. Schmitt[2]

Department of Entomology and Nematology, University of Florida, P. O. Box 110620, Gainesville, FL 32611,[1] and Department of Plant and Environmental Protection Sciences, 3190 Maile Way, University of Hawaii, Honolulu, HI 96822-2279, U.S.A. E-mail: koonhui@hawaii.edu.[2]

ABSTRACT

Wang, K. H., B. S. Sipes, and D. P. Schmitt. 2001. Suppression of *Rotylenchulus reniformis* by *Crotalaria juncea*, *Brassica napus*, and *Tagetes erecta*. Nematropica 31:237-251.

The effects of *Crotalaria juncea*, *Brassica napus* and *Tagetes erecta* on resistance, allelopathic suppression, and enhancement of nematode antagonists against *Rotylenchulus reniformis* were examined in a series of greenhouse experiments. *Crotalaria juncea* and *B. napus* are poor hosts to *R. reniformis* as compared to *Vigna unguiculata*. *Tagetes erecta* was as good a host for *R. reniformis* as was *Ananas comosus*. *Crotalaria juncea* delayed the development of female nematodes compared to *V. unguiculata*. Allelopathic effects against *R. reniformis* were most pronounced in leaf leachate of *C. juncea* 2 days after incorporation where the viability of the nematode was suppressed to <0.5% as opposed to >60% when the *R. reniformis* were incubated overnight in leachates of *B. napus*, *T. erecta*, *A. comosus*, sand or distilled water. Amendment with *C. juncea* was most efficient in enhancing parasitic nematode-trapping fungi, *R. reniformis* egg-parasitic fungi, vermiform stage parasites, and bacterivorous nematodes compared to *B. napus*, and *T. erecta* leaf amendments. Bare soil and 1,3-Dichloropropene treatments suppressed nematode-trapping fungal population densities. Suppression of *R. reniformis* development on *V. unguiculata* by these crop amendments was inconclusive due to the short cowpea bioassay period. However, *C. juncea* amendments enhanced cowpea growth more than did the other soil amendment treatments. Among the crops tested, *C. juncea* is the most promising cover crop for *R. reniformis* management.

Key words: Allelopathy, *Brassica napus*, *Crotalaria juncea*, nematode-trapping fungi, *Rotylenchulus reniformis*, *Tagetes erecta*.

RESUMEN

Wang, K. H., B. S. Sipes y D. P. Schmitt. 2001. Supresión de *Rotylenchulus reniformis* por medio de *Crotalaria juncea*, *Brassica napus*, y *Tagetes erecta*. Nematrópica 31:237-251.

Los efectos de *Crotalaria juncea*, *Brassica napus* y *Tagetes erecta* sobre la resistencia, supresión alelopática, y aumento de nematodos antagonistas contra *Rotylenchulus reniformis* se evaluaron en una serie de experimentos realizados en invernaderos. *C. juncea* y *B. napus* no son hospederas apropiadas para *R. reniformis* al compararlas con *Vigna unguiculata*. *T. erecta* fue tan buena hospedera para *R. reniformis* como lo fue *Ananas comosus*. *C. juncea* retardó el desarrollo de las hembras al compararlas con *V. unguiculata*. Efectos alelopáticos contra *R. reniformis* fueron más acentuados dos días después de la incorporación de filtrados de hojas de *C. juncea*. Bajo este tratamiento la viabilidad del nematodo se suprimió a <0.5%, valores opuesto a >60% cuando *R. reniformis* se incubó de la noche a la mañana en filtrados de *B. napus*, *T. erecta*, *A. comosus*, arena o agua destilada. La emienda con *C. juncea* fue la más eficiente en aumentar los hongos como trampas de nematodos, huevos de *R. reniformis* parasitados

†This paper is a contribution from the College of Tropical Agriculture and Human Resources, University of Hawaii, Honolulu, Journal Series No. 4558.

por hongos, parásitos en estado larval y nematodos bacterivorous al compararla con emiendas de hojas de *B. napus*, y *T. erecta*. Suelo sin vegetación y 1,3-Dicloropropano suprimieron la densidades poblacionales de hongos trampas de nematodos. La supresión del desarrollo de *R. reniformis* sobre *V. unguiculata* por las emiendas de estos cultivos fue inconclusiva debido al corto periodo del bio-ensayo de frijol. Sin embargo, las emiendas con *C. juncea* mejoraron el crecimiento del frijol al compararlo con los otros tratamientos emiendas. Entre los cultivos evaluados, *C. juncea* es el cultivo de cobertura más promisorio para el manejo de *R. reniformis*.

Palabras claves: Alelopatía, *Brassica napus*, *Crotalaria juncea*, hongos trampas de nematodos, *Rotylenchulus reniformis*, *Tagetes erecta*.

INTRODUCTION

Rotylenchulus reniformis Linford & Oliveira is one of the most important pineapple pathogens in Hawaii (Rohrbach and Apt, 1986) with an economic damage threshold between 300 to 1 000 vermiform stages/250 cm^3 of soil (Sipes and Wang, 2000). Frequently *R. reniformis* population densities exceed 10 000/250 cm^3 soil after plant crop harvest (unpublished data). Although preplant soil fumigation increases pineapple yield (Sipes, 1996), nematicides create a potential hazard to the environment. An alternative nematode management strategy such as rotating cover crops with pineapple may be beneficial to the industry. To manage *R. reniformis* below the economic threshold level, one approach is to select cover crops possessing multiple nematode suppressive mechanisms.

Modes of nematode suppression by cover crops can be categorized as providing a nonhost or a poor host environment for nematodes (Rodriguez-Kabana *et al.*, 1988), producing allelochemicals (Halbrendt, 1996), and enhancing nematode antagonistic flora and fauna (Linford, 1937), or acting as a trap crop to the nematode (Gardner and Caswell-Chen, 1994). These modes of action need not be mutually exclusive. An ideal cover crop should exhibit more than one mechanism involved in nematode suppression.

In this research, *Crotoalaria juncea* L., *Brassica napus* L., and *Tagetes erecta* L. were studied because these are among the few plants that are poor hosts to *R. reniformis* (Robinson *et al.*, 1997) which also produce allelopathic compounds toxic to plant-parasitic nematodes. An additional benefit of using *C. juncea* as a cover crop is its association with *Rhizobium* in the rhizosphere, allowing this crop to fix up to 150-165 kg N/ha if incorporated before flowering (Rotar and Joy, 1938).

Allelopathy is a plant-plant or plant-microorganism biochemical interaction (Rice, 1984). *Tagetes* spp. produce α-terthienyl whereas *Crotalaria* spp. produce monocrotaline, both of which have nematicidal qualities (Fassuliotis and Skucas, 1969; Gommers and Bakker, 1988). *Brassica napus* produces glucosinolates that are nematicidal when reacted with myrosinase after crop incorporation (Brown *et al.*, 1991). Allelopathic effect of these cover crops against *R. reniformis* was tested in this research.

The ability to enhance nematode antagonisitic microorganisms by these cover crops was also tested. Since 1937, incorporation of organic amendments into the soil was reported to enhance antagonistic microorganisms (Linford, 1937; Linford *et al.*, 1938). One such group of antagonistic microorganisms is the nematode-trapping fungi. Cooke (1963) divided the nematode-trapping fungi into saprophytic and parasitic

groups. Saprophytic nematode-trapping fungi form three-dimensional-network traps in response to the presence of nematodes and are regarded as inefficient nematode-trappers. Parasitic nematode-trapping fungi have low sapro-phytic ability, but form traps spontaneously. This group consists of fungi that form constricting rings, adhesive knobs, or adhesive branches, and are more effective nematode-trappers than the saprophytic group (Jansson and Nordbring-Hertz, 1980). High organic matter and moisture increase the parasitic nematode-trapping fungal populations and may stimulate the trap formation of saprophytic nematode-trapping fungi (Gray, 1085).

The objectives of this study were to determine resistance of *C. juncea*, *B. napus*, and *T. erecta* to *R. reniformis,* assess the allelopathic effects of these plants towards reniform nematodes, and evaluate their effects on nematode-trapping fungi.

MATERIALS AND METHODS

Host Status: Crotalaria juncea L 'Tropic Sun' (sunn hemp), *Brassica napus* L. 'Dwarf Essex' (rapeseed), *Tagetes erecta* L. 'Cracker Jack' (African marigold), *T. polynema* L. (marigold hybrid), *Avena sativa* L. (oat), *Sorghum bicolor* (Desv.) Stapf. × *S. sudanense* (Staf.) *Hitchc.* 'DeKalb ST6E' (sorghum-sudangrass hybrid), *Pennisetum ciliare* L. (Link) 'T-4464' (buffel grass), and *Ageratum conyzoides* L. (ageratum) were tested for their susceptibility to *R. reniformis* and compared to *Vigna unguiculata* L. (cowpea) and *Ananas comosus* L. (Merr.) (pineapple). All the tested plants except *A. comosus* were seeded and transplanted 3 weeks later with a single plant per pot into 10-cm-diameter pots containing a sterile mix of silica sand and soil (1:1). *Ananas comosus* crowns were planted 7 weeks prior to the seeding of the other tested plants to establish the root system. One month after trans-planting into 10-cm-diameter pots, 1 000 streptomycine sulfate-sterilized (1 mg/ml), freshly hatched *R. reniformis* juveniles cultured on cowpea were inoculated per pot. Experiments were harvested 2 months after *R. reniformis* inoculation. This experiment was performed in the greenhouse using a randomized complete block design with 5 replications. The experiment was repeated once.

Rotylenchulus reniformis vermiform stages and eggs were extracted from the entire root system with a 0.5% NaOCl solution (Hussey and Barker, 1973) and from 250 cm^3 soil using elutriation and centrifugal flotation (Byrd *et al.*, 1976). Subsamples of 0.3 g of roots per plant were stained with acid fuchsin for the assessment of nematode infection (Daykin and Hussey, 1985). All roots were oven dried and weighed.

Female Development: *Rotylenchulus reniformis* female development in a poor host, *C. juncea*, was compared to that in *V. unguiculata*. *Crotalaria juncea* and *V. unguiculata* seeds were planted in 3-cm-diameter 12.5-cm-tall tubes filled with a 1:1 sand:soil mixture. One week after transplanting, seedlings were inoculated with 1 000 freshly hatched *R. reniformis* juveniles.

Four plants of each species were sampled at 21, 25, and 29 days after inoculation. The whole root system was stained with acid fuchsin (Daykin and Hussey, 1985). *Rotylenchulus reniformis* females were categorized as vermiform, slightly swollen, swollen, or kidney shaped (Fig. 1). Numbers of females in each stage were recorded and percentages were calculated.

Allelopathic Effects: Two sets of greenhouse experiments were conducted to test for toxicity to *R. reniformis* of root and leaf leachates of *C. juncea*, *B. napus*, *T. erecta*, and *A. comosus* compared to water or sand leachate. In the root leachate test, seedlings of *C. juncea*, *B. napus*, and *T. erecta* were grown in 10-cm-diameter clay pots

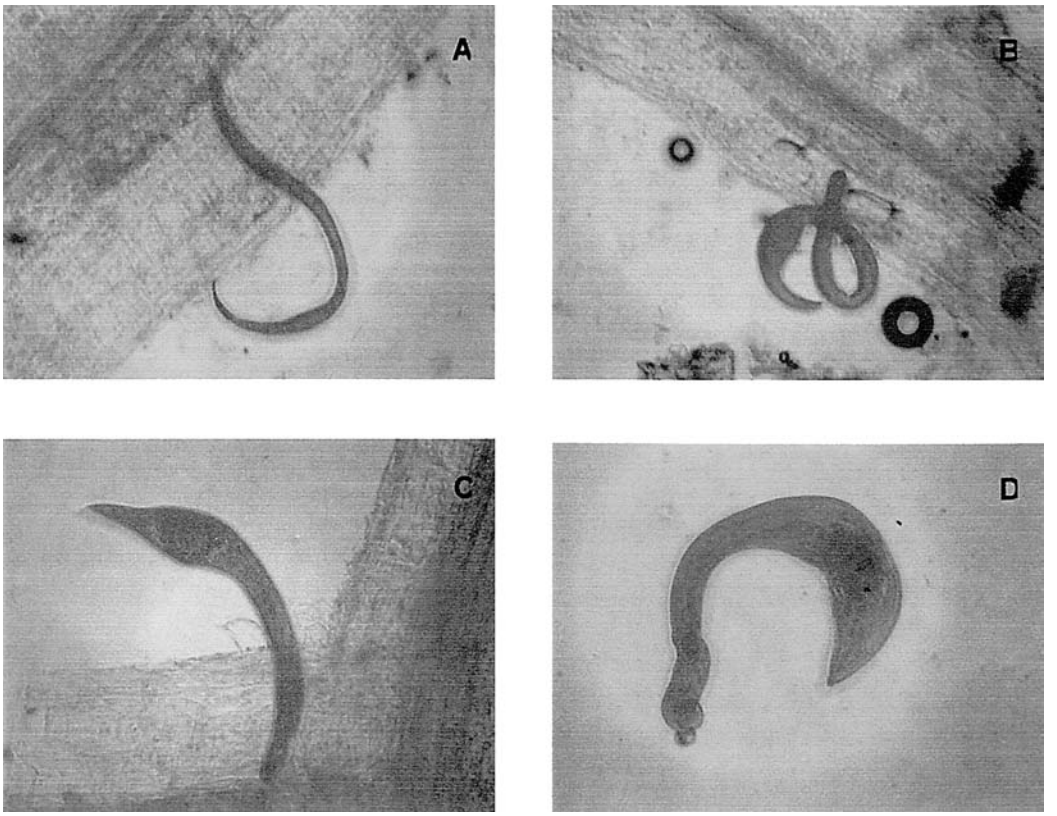

Fig. 1. Four development stages of *Rotylenchulus reniformis* female: A) vermiform, B) slightly swollen, C) swollen, and D) kidney shaped.

containing silica sand. Crowns of *A. comosus* were planted 3 months prior to the cover crop seeding. Pots with silica sand only were set up at the same time as controls and each treatment had 3 replicate pots. In the leaf leachate test, fresh leaves of *C. juncea*, *B. napus*, *T. erecta*, and *A. comosus* were chopped into approximately 5 mm^2 pieces and incorporated into sterile sand at 1% rate (w/w) in 10-cm-diameter pots. Each treatment was conducted in a single pot and a pot with sand only was used as a control. Sand was watered daily with 100 ml of sterile distilled water per pot without leaching. Plants were fertilized weekly (10 g of Peters solution N-P-K [20-20-20] fertilizer/3.79 L H_2O). Both experiments were arranged in a completely randomized design and repeated 3 times.

Root leachate was collected 1 month after cover crop planting by pouring 50 ml of sterile distilled water through the pots and collecting leachates that drained into a flask wrapped in aluminum foil in the greenhouse. Leaf leachates were collected in the same manner 24 and 48 h after leaf incorporation. Sterile distilled water and leachate collected from sand pots in each test were used as controls.

Leaf and root leachates were brought to the laboratory for bioassay. *Rotylenchulus reniformis* eggs from greenhouse cowpea cultures were surface sterilized by incubating in filter sterilized (0.22 μm) solution of

1 mg/ml streptomycin sulfate and spectomycin (1 mg/ml) for 12 hours followed by 2 hours incubation in filter sterilized 3% H_2O_2. The egg suspension was centrifuged and the supernatant was replaced with sterile distilled water before and after each change of incubating solution (Ko, unpublished). A portion of the eggs was hatched and juveniles were collected for leachate bioassay. For the leaf leachate test, 1 ml of leachate from each treatment was placed into a BPI (Bureau of Plant Industries) watch dish and 10 freshly hatched *R. reniformis* were added. Each treatment had 5 replicated dishes collected from one pot for each of the 3 time intervals. Viability of *R. reniformis* juvenile was assessed 24 hours after exposure by dental pick probing. Nematodes that did not respond to probing were considered dead. For the root leachate tests, only one BPI dish was used per pot per time to provide 9 observations (3 pots × 3 times) per treatment. In addition to the probing test, egg hatching during 10 days was also studied. Five surface sterilized eggs were placed in a 3-cm-diameter plastic cylinder with a 32-µm-pore screen secured to the bottom (hatching chamber). Eggs were then incubated in different root leachates. Root leachate was replaced daily. Each treatment had one hatching chamber per time.

Antagonistic Effect: Enhancement of microbial antagonistic effects against *R. reniformis* by incorporation of *C. juncea*, *B. napus*, *T. erecta*, and *A. comosus* was determined in the greenhouse. A Wahiawa silty clay soil was separated into two portions: one was heated to 60°C for 48 hours to kill all soil microorganisms; the other was frozen at -17°C for 1 week to kill only temperature sensitive organisms, including *R. reniformis* and other indigenous nematodes. Both soils were amended with finely chopped fresh biomass of *C. juncea*, *B. napus*, *T. erecta*, or *A. comosus* at 1% rate (plant dry matter weight equivalent/soil dry weight in a 10-cm-diameter pot). Additional portions of the same soils were either treated with 37 µl Telone II (equivalent to 211 kg a.i. 1,3-Dichloropropene/ha) or left unamended (hereafter referred to as bare soil) with and without *R. reniformis*. Each pot was inoculated with 1 000 surface sterilized eggs and 300 juveniles of *R. reniformis*. The experiment was a split-plot design with preplant treatments as the main plots and soil treatments (oven heated or frozen) as the subplots. The 7 preplant treatments were arranged in a completely randomized design with 4 replications. The experiment was repeated once. In the first test, 3 days after preplant treatment, 5-day-old cowpea seedlings were transplanted into each pot. Cowpeas exhibited slight phytotoxicity from the organic amendment, especially *B. napus*. Therefore, in the second test, cowpea seedlings were transplanted 1 week after preplant treatment. The experiment terminated 6 weeks after transplanting. Nematode vermiform stages and eggs were extracted from the cowpea roots using 0.5% NaOCl and centrifugal flotation methods (Hussey and Barker, 1973).

In the first test, 100 eggs from each sample were plated on 1% water agar for 2 weeks to observe fungal mycelia growth and record percentage of eggs parasitized. In the second test, alginate films containing surface sterilized *R. reniformis* eggs prepared as described by Rodriguez-Kabana *et al.* (1994) were used. Fiberglass screen of 2.5 cm × 5 cm was dipped into 2% sodium alginate (Sigma Chemical Co., St. Louis, MO, U.S.A.) solution containing *R. reniformis* eggs, transferred to 0.25M $CaCl_2$ for 3-5 seconds, followed by 3 successive washes of deionized water. Approximately 100 eggs were attached into each alginate film. One alginate film was placed into 10 g of soil from each pot in a 9-cm-diameter petri dish. The film was removed from the soil 2 days

later, washed gently to remove the soil and wrapped in a plastic film and incubated at 25°C for 1 week. Percentages of infected eggs and vermiform stages were recorded.

Nematode-trapping fungal population densities were quantified by a soil dilution method in combination with a most probable number estimation slightly modified after Jaffee *et al.* (1996). Subsamples of 10 g of soil from each sample were suspended in 20 ml sterile distilled water followed by two series of 10-fold dilutions. A 100 µl aliquot of each dilution was plated on 1% water agar amended with 0.1g/L streptomycin sulfate to give 0.05, 0.005 and 0.0005 dilution series. Three replicate plates were prepared for each dilution. One hundred *Steinernema glaseri* were added to each plate as nematode-trapping fungi bait. Nematode-trapping fungi were identified to species and grouped as parasitic- or saprophytic-nematode-trapping fungi (Cooke and Godfrey, 1964). Shoot fresh and root dry weights were recorded.

Statistical analysis: A test of variance was conducted to determine homogeneity between tests in each experiment (SAS, 2000). Data were combined between tests if the homogeneity test results indicated that $P > 0.05$. Data were transformed to log (x + 1) wherever necessary according to a normality test (PROC UNIVARIATE, SAS, 2000). Data were then subjected to analysis of variance according to the experimental design using the General Linear Model procedure (SAS, 2000). When the treatment effect was significant ($P < 0.05$), means were separated by Waller-Duncan *k*-ratio ($k = 100$) *t*-test except in the development experiment where means were separated by least significant difference (LSD).

RESULTS

Host Status: *Avena sativa*, *S. bicolor* × *S. sudanense*, and *P. ciliare* were very poor hosts to *R. reniformis* (Table 1). These crops had less than 10 vermiform stages per gram of

Table 1. Number of *Rotylenchulus reniformis* recovered from ten plant species.

Plant	Rhizosphere vermiform stages/ g root	Vermiform stages/250 cm^3 soil[z]	Female/ 0.3 g root	Mobile vermiform stages/50 cm^3 soil[y]		Eggs/g root	
				Test 1	Test 2	Test 1	Test 2
Vigna unguiculata	263 a	2 276 a	39	877 a	2 559 a	1 082 a	5 844 a
Tagetes polynema	288 a	1 057 b	29	551 ab	1 920 ab	720 a	5 726 a
Tagetes erecta	73 b	386 bc	10	24 ab	1 120 ab	156 b	1 351 bc
Ananas comosus	64 b	460 bc	6	12 ab	1 172 ab	40 b	1 259 bc
Crotalaria juncea	25 b	66 c	8	37 ab	63 b	31 b	356 bc
Ageratum conyzoides	18 b	534 bc	11	1 b	1 605 ab	3 b	2 447 b
Brassica napus	18 b	250 bc	46	15 ab	360 ab	32 b	799 bc
Avena sativa	6 b	47 c	0	1 b	16 b	0 b	195 c
Sorghum bicolor × *S. sudanense*	4 b	16 c	1	2 b	6 b	0 b	14 c
Pennisetum ciliare	3 b	76 c	0	1 b	122 ab	2 b	71 c

[y] *R. reniformis* extracted from soil by mist chamber.
[z] *R. reniformis* extracted from soil by elutriation.
Values are average of 5 replicates. Means followed by the same letters were not differ among the plants tested according to Waller-Duncan *k* ratio ($k = 100$) t-test.

root, and lower numbers of vermiform stages in the soil than most of the other crops tested except *C. juncea*. Less than 2 females were detected in 0.3 g roots of these three plants resulting in least egg production ($P < 0.05$). Population density of *R. reniformis* in the soil and the rhizosphere, nematode mobility, and egg production were lower in *C. juncea* and *B. napus* than that in *V. unguiculata* ($P < 0.05$). *Tagetes erecta* was a moderate host to *R. reniformis*. None of the variables measured were different between *T. erecta* and *A. comosus* ($P > 0.05$). The number of mobile vermiform stages of *R. reniformis* in *T. erecta* was as high as that in *V. unguiculata*. *Tagetes polynema*, a crop reported to be resistant to *M. incognita*, was as good a host to *R. renifromis* as *V. unguiculata*. A common weed found in pineapple fields, *A. conyziodes*, had lower numbers of *R. reniformis* eggs and vermiform stages in the rhizosphere than those in *V. unguiculata* ($P < 0.05$), but the number of mobile vermiform stages in *A. conyziodes* was not different from that in *V. unguiculata* in test 2.

Female Development: Numbers of female *Rotylenchulus reniformis* did not differ between *C. juncea* and *V. unguiculata* ($P > 0.05$, data not shown). However, number and percentage of kidney-shaped females was higher in *V. unguiculata* 25 days after inoculation than in *C. juncea* (Fig. 2A). Number and percentage of swollen and slightly swollen females did not differ between the plants tested (Fig. 2B, C). Twenty-five days after inoculation, 41% of the *R. renifomis* remained vermiform in *C. juncea* (Fig. 2D). Kidney-shaped and swollen-females were not detected in *C. juncea* until 25 days after inoculation (Fig. 2A, B). Percentage and the number of vermiform females were higher in *C. juncea* than in *V. unguiculata* 25 and 29 days after inoculation (Fig. 2D).

Allelopathic Effects: Leaf leachate collected 1 day after cover crop incorporation

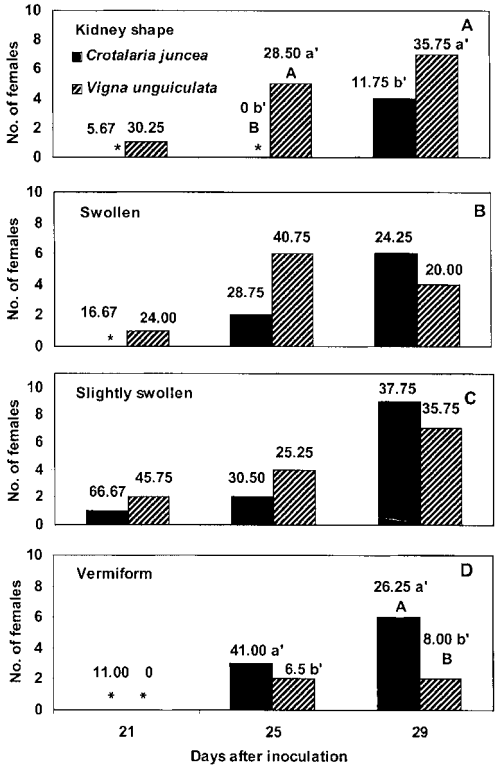

Fig. 2. Female development of *Rotylenchulus reniformis* in *Crotalaria juncea* and *Vigna unguiculata*. Value on top of each column is the percentage of each female stage in a sampling time. Means are an average of 4 replicates. * signifies a mean ≤0.25. Values in a column followed by the same letters (capital letters for number of females and lower case for percentage) are not different between *C. juncea* and *V. unguiculata* at each sampling time according to Least Significant Test ($P < 0.05$).

did not affect *R. reniformis* viability (data not shown). More than 80% of *R. reniformis* remained active in all treatments.

Crotalaria juncea leaf leachate collected 2 days after incorporation suppressed *R. reniformis* viability more than the other treatments (Fig. 3A). Less than 0.5% of *R. reniformis* remained active in the 2-day-old *C. juncea* leaf leachate. *Brassica napus* leaf leachate also suppressed *R. reniformis* viability more than water or sand leachate in test 3, but its effect varied with experiment.

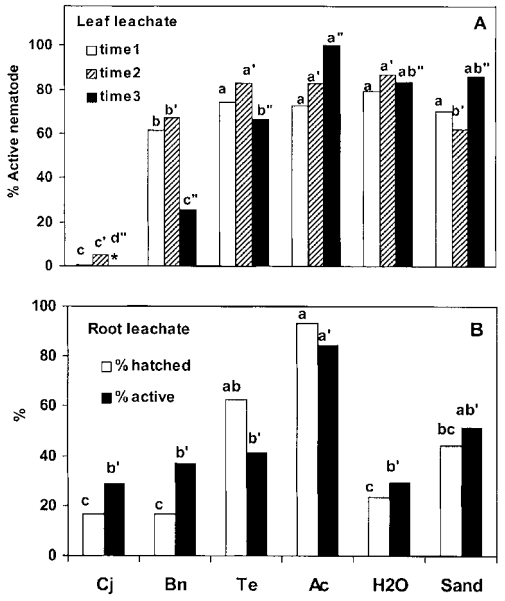

Fig. 3. Effects of leaf (A) and root (B) leachate of *Crotalaria juncea* (Cj), *Brassica napus* (Bn), *Tagetes erecta* (Te), or *Ananas comosus* (Ac) on activity and hatching rate of *Rotylenchulus reniformis* as compared to sterile distilled water (H_2O) or sand leachate (Sand). Columns in A represent means of 5 replicates. Data from 3 experiments were pooled in B. Columns followed by the same letters were not different according to Waller-Duncan *k*-ratio ($k = 100$) *t*-test ($P < 0.05$).

Ananas comosus and *T. erecta* maintained the same percentage of active nematodes as those in the sand leachate and water.

Suppression of viability of *R. reniformis* by *C. juncea* root leachate (29% remained viable) was not as effective as that in the leaf leachate (<5% viable). The percentage of *R. reniformis* active in three of the cover crop root leachates was not different from those incubated in water or sand leachate (Fig. 3B). However, *A. comosus* root leachate maintained the highest *R. reniformis* viability among the treatments (Fig. 3B). Hatching rate of *R. reniformis* was lowest in root leachate of *C. juncea* and *B. napus* as compared to other root leachates (Fig. 3B), but was not lower than that in water or sand leachate.

Antagonistic Effects: Oven heating failed to eliminate all soil microbial activities in the two antagonism tests and total numbers of nematode-trapping fungi were not different between oven heated and frozen soils 6 weeks after planting cowpea (Table 2). However, oven heating did affect recovery of either parasitic or saprophytic nematode-trapping fungi in test 1 or test 2, respectively. Nematode-trapping fungi data from the two soil treatments (frozen and oven heated) were combined when the analysis of variance showed that soil treatment factor was not significant ($P > 0.05$). Soil amendments had significant effects on nematode-trapping fungi in both tests ($P < 0.05$, Table 2). Very low or no nematode-trapping fungi were detected in the absence of cover crop amendment in either frozen or heated soil (Fig. 4 A, B).

Numbers of total nematode-trapping fungal propagules/g soil were not different among the organic amended soils ($P > 0.05$, data not shown). The majority of nematode-trapping fungi in *C. juncea* and *B. napus* amended soil in test 1 were parasitic forms (Fig. 4A). *Ananas comosus* amendment enhanced parasitic nematode-trapping fungi population densities in the oven heated soil as compared to unamended soils, but did not enhance those in the frozen soil in test 1 (Fig. 4A). In test 2, no organic-amended soils increased parasitic nematode-trapping fungi higher than unamended soils. Only *C. juncea* and *A. comosus* amended soil had higher population densities of saprophytic nematode-trapping fungi than the non-amended soils in the oven heated treatment (Fig. 4B).

Parasitized eggs in test 1 were only detected in *C. juncea* amended soil when eggs were plated on water agar and incubated for 2 weeks (Fig. 4C). The percentage of eggs parasitized in test 2 quantified by incubating eggs in alginate film for 1 week was not different among the treatments (Fig.

Table 2. Analysis of variance for effects of soil treatment (freezing or oven heating), and amendment (Amdt.) on nematodes, nematode-trapping fungi, plant weight and percentage of nematode eggs and vermiform stages parasitized.

		Nematode-trapping fungi propagules/g soil		% Parasitized		*R. reniformis*					
Source	Total	Saprophytic	Parasitic	Egg	J2	Mobile vermiform stages/ 250 cm^3 soil	Vermiform stages/g root	Eggs /g root	Bacteri-vorous nematodes/ 250 cm^3 soil	Shoot fresh weight	Shoot dry weight
Test 1											
Soil[y]	NS	NS	NS	NS	-	0.0154	NS	0.0275	NS	NS	NS
Soil × Amdt	NS	NS	NS	NS	-	NS	NS	NS	NS	NS	0.0402
Amdt.	0.0465[z]	NS	0.0423	0.0032	-	NS	NS	0.0229	NS	NS	NS
Test 2											
Soil	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Soil × Amdt.	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Amdt.	0.0015	NS	NS	NS	0.0103	0.0007	0.008	NS	0.001	0.0003	0.0137

[y]Frozen or heated.
[z]P-value of each factor from split-plot analysis of variance. NS signifies the treatments in a factor did not differ significantly.

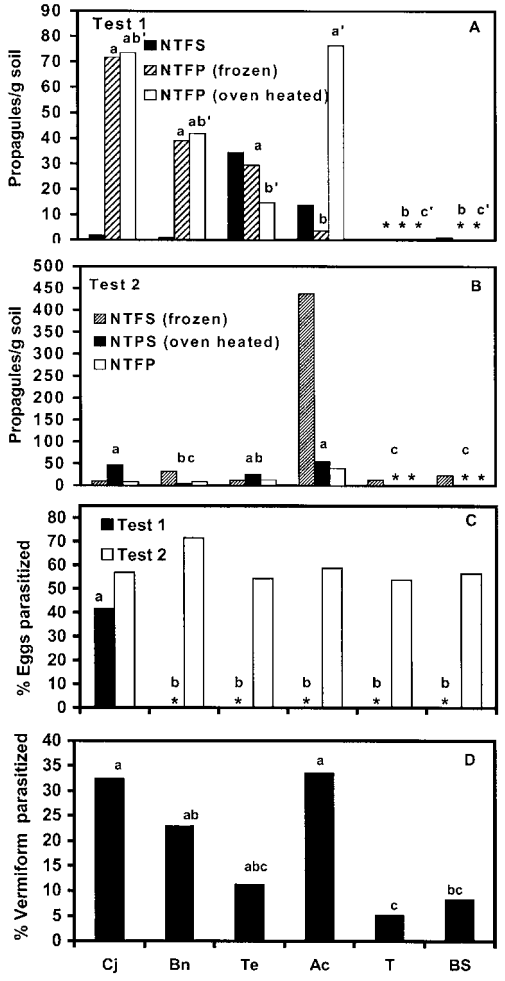

Fig. 4. Effects of amendments on nematode-trapping fungal population densities and percent *Rotylenchulus reniformis* eggs and vermiform stages parasitized 6 weeks after cowpea planting. Cj = *Crotalaria juncea*, Bn = *Brassica napus*, Te = *Tagetes erecta*, Ac = *Ananas comosus*, T = Telone II, BS = Bare soil, NTFS = saprophytic nematode-trapping fungi, and NTFP = parasitic nematode-trapping fungi. * signifies a zero value. Columns followed by the same letters were not different among the treatments according to Least Square Means analysis on log (x + 1) transformation ($P < 0.05$).

4C). Percent of *R. reniformis* vermiform stage parasitism was higher in *C. juncea* and *A. comosus* amended soil than unamended soils ($P < 0.05$, Fig. 4D). Soil treated with 1,3-D or left bare suppressed the activities of nematode-trapping fungi as well as fungal parasitism of eggs and vermiform stages of *R. reniformis* (Fig. 4A, B, C, D).

Numbers of *R. reniformis* vermiform stages from roots in test 1 and 2, and eggs in test 2 were not different between oven heated and frozen soil (Table 2). Thus, the data for frozen and heated soils were pooled. Effect of soil amendment on *R. reniformis* population densities differed between the tests. Nematode reproduction rate and population densities were higher in test 2 than in test 1 (Fig. 5). In general, bare soil inoculated with *R. reniformis* maintained the highest numbers of vermiform stages and eggs in the cowpea rhizosphere in both tests (Fig. 5A-D). None of the cover crop amended soils suppressed *R. reniformis* vermiform stages in the cowpea roots as compared to the bare soil treatment except *B. napus* amended soil in test 1 (Fig. 5A, B). All the cover crop amended soils suppressed *R. reniformis* egg production on cowpeas in the frozen but not in oven heated soils in test 1; despite the same trend in test 2, significant suppression was only observed in *B. napus* amended soil (Fig. 5C, D).

Bacterivorous nematode numbers, as an indirect measure of microbial activities, were greater in all the organic amended soils in test 1, and in *C. juncea* and *A. comosus* amended soils in test 2 than in the 1,3-D and the bare soil treatments ($P < 0.05$) (Fig. 5E, F).

Cowpea growth was not different among the soil amendments in test 1 but was enhanced in *C. juncea*-amended soil in test 2 (Fig. 6). In general, plant biomass was less in test 1 compared to test 2, reflecting the phytotoxicity occurring in the early stages of test 1. Infection by *R. reniformis* did not affect cowpea growth in these two tests as cowpea shoot fresh and root dry weights were not different between nematode inoculated and non-inoculated soil treatments (Fig. 6).

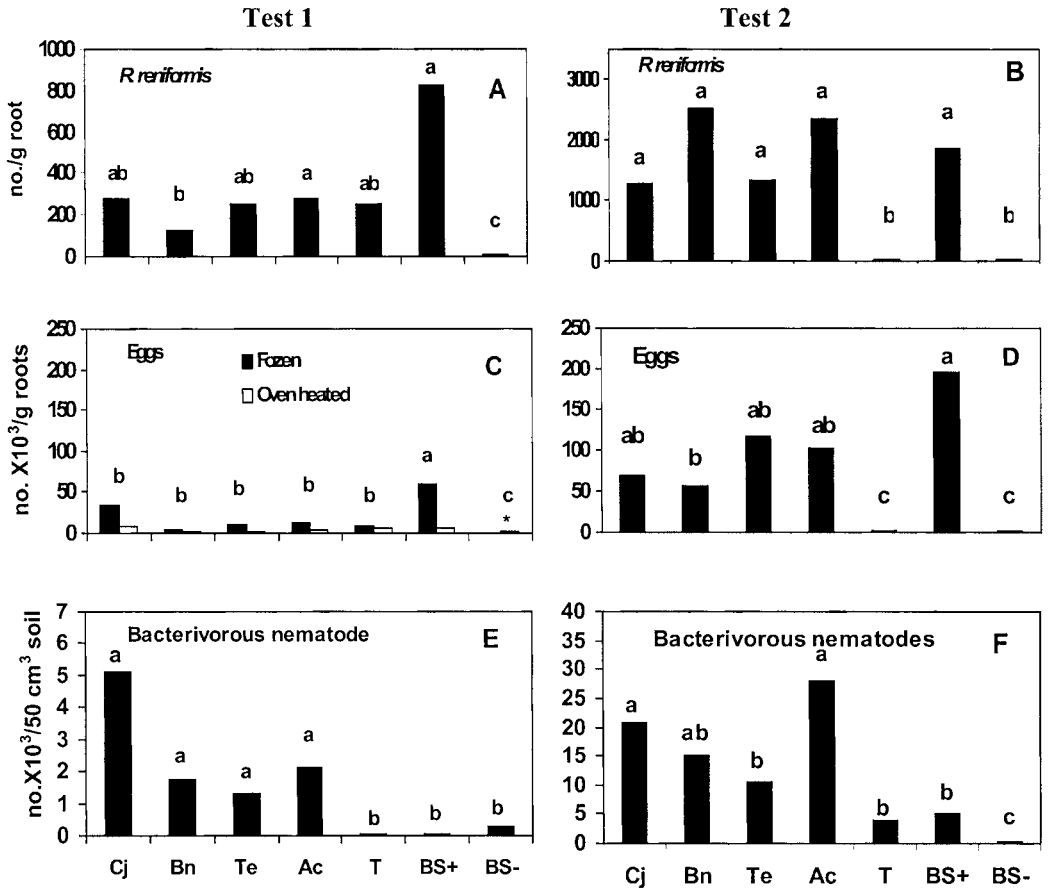

Fig. 5. Effects of soil amendments on *Rotylenchulus reniformis* vermiform stages and eggs in root, and bacterivorous nematode in soil. Cj = *Crotalaria juncea*, Bn = *Brassica napus*, Te = *Tagetes erecta*, Ac = *Ananas comosus*, T = Telone II, BS = bare soil. BS+ was inoculated with nematodes whereas BS- was not. * signifies a zero value. Means are averages of 8 samples. Columns followed by the same letters were not different among the soil amendments according to Least Square Means analysis on log (x + 1) transformation ($P < 0.05$).

DISCUSSION

Crotalaria juncea exhibited multiple mechanisms in the suppression of *R. reniformis*. It is a poor host to *R. reniformis*, as reflected by a low nematode reproduction rate and reduced female development. In addition, *C. juncea* produced allelopathic compounds following decomposition time of 48 hours that suppressed *R. reniformis* viability and hatch. Soil amended with *C. juncea* leaves enhanced parasitic nematode-trapping fungi. Our data conform with those of Caswell *et al.* (1991), who found that *C. juncea* supports low levels of *R. reniformis* reproduction. This characteristic may be advantageous for nematode control as it will maintain *R. reniformis* activity and discourage anhydrobiosis, with the result that the nematodes may be more susceptible to environmental stress. When *C. juncea* is incorporated into the soil, the tissues release monocrotalin which are lethal to *R. reniformis* and further suppress the nematode.

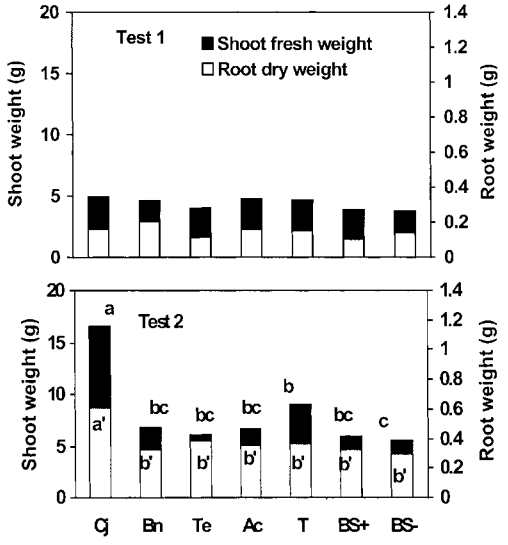

Fig. 6. Effects of amendments on shoot fresh and root dry weight of cowpea. Cj = *Crotalaria juncea*, Bn = *Brassica napus*, Te = *Tagetes erecta*, Ac = *Ananas comosus*, T = Telone II, BS = bare soil. BS+ was inoculated with nematodes whereas BS- was not. Means are averages of 8 samples. Columns followed by the same letters were not different among the soil amendments according to least square means analysis ($P < 0.05$).

During the *C. juncea* growing period and after *C. juncea* incorporation, a niche was created that favored free-living nematodes. This is similar to results from Jaffee *et al.* (1998) that showed bacterivorous nematodes were more abundant in organic as compared to conventional plots. As postulated by Linford *et al.* (1938), and demonstrated by Nordbring-Hertz (1973), in the presence of nematodes, or even exudates and homogenates of nematodes, trap formation is induced in nematode-trapping fungi. High bacterivorous nematode densities induced by *C. juncea* are favorable for both endoparasitic fungi and nematode-trapping fungi to form constricting rings and adhesive knobs (Gray, 1985; Jaffee *et al.*, 1993). Moreover, in test 1 of the antagonistic effect experiment, where parasitic nematode-trapping fungi were more abundant, *C. juncea* enhanced parasitic nematode-trapping fungi rather than saprophytic nematode-trapping fungi. Among the most commonly found nematode-trapping fungi detected in *C. juncea* amended soil were *Monocosporium ellipsospora* and *Arthrobotrys dactyloides*. Both of these fungi were found to be effective against *M. javanica* and were formulated for nematode biocontrol (Jaffee and Muldoon, 1995; Stirling and Smith, 1998). These two nematode-trapping fungi are consistently associated with roots, and thus in a favorable position to prey upon economically important nematodes (Mankau, 1980). It is also noteworthy that nematode egg parasites were detected only in *C. juncea* amended soil. The presence of parasitic nematode-trapping fungi and egg parasites might explain the longer period of *R. reniformis* suppression in intercycle and intercrop field trials (Wang, 2000).

Brassica napus was also a poor host to *R. reniformis; however*, allelopathic effects of *B. napus* were not as strong as those of *C. juncea*. Although, glucosinolate compounds in cruciferous crops are known for their fungicidal effect on pathogens such as *Gaeumannomyces graminis* and *Rhizoctonia solani* (Kirkegaard *et al.*, 1996), this property did not affect parasitic nematode-trapping fungi in *B. napus* amended soil.

Unlike previous reports regarding the nonhost status of *T. erecta* to *R. reniformis*, *T. erecta* 'Cracker Jack' was a relatively good host to the population of *R. reniformis* used in these tests. Allelopathic effects of leaf and root leachates of *T. erecta* were low against *R. reniformis*. Marles *et al.* (1992) found that toxicity of α-terthienyl from *T. erecta* results from photoactivation, but the compounds are completely devoid of nematicidal activity when mixed in soil. This is consistent with results from the current research where nematicidal activity of *T. erecta* was only detected in the root

leachate. Although the total nematode-trapping fungi population densities were enhanced by *T. erecta* amendment compared to those in bare soil, a large portion of these fungi were saprophytes.

Numbers of females per gram of pineapple roots 6 weeks after nematode inoculation were relatively low. Pineapple roots may have been difficult to stain for *R. reniformis* as females were dislodged easily. Sipes and Paul (pers. comm.) have speculated that protease inhibitors produced by young pineapple might affect nematode development. Sipes reported that high population densities of *R. reniformis* were not detected in pineapple fields until 9 months after planting (Sipes and Schmitt, 1994). At the termination of these host status tests, pineapples were only 6 months old. The low surface/biomass ratio of pineapple roots might also account for lower numbers of nematodes in pineapple. Although pineapple residues also enhanced nematode-trapping fungi, most of these fungi were saprophytic and less efficient nematode trappers. Nematode-trapping fungal populations were highest in *A. comosus* amended soil in test 2, but this treatment did not effectively suppress *R. reniformis*. A high number of saprophytic nematode-trapping fungi do not equate to efficient nematode trapping.

The oven heated treatment in the antagonism tests failed to eliminate all soil microbial activity. Therefore, microbial antagonistic and allelopathic effects could not be partitioned. Autoclaving the soil resulted in phytotoxicity to the assay plants. Therefore, antagonism activities were demonstrated by quantifying the nematode-trapping fungi, parasitized eggs and vermiform stages, and bacterivorous nematode population densities. The alginate film assay was a good measure of soil microbial activity (Rodriguez-Kabana *et al.*, 1994). The difficulty faced was ensuring a constant amount of nematode bait in the alginate film and only the percentage of nematodes parasitized could be quantified with this technique. No difference in percentage egg parasitism among the treatments was detected in this test. However, there were differences among the treatments in the percentage of parasitism of the vermiform stages hatched from the eggs. Vermiform stage parasites were possibly more abundant than egg parasites in the soil in the antagonism test.

The duration of the cowpea bioassay should be extended. Cowpea plants in test 1 suffered from phytotoxicity at the early stage of the experiment, resulting in similar *R. reniformis* suppression in all the organic amended soils compared to bare soil. The low population densities of *R. reniformis* in *B. napus* treated pots in test 1 could result from the severe phytotoxicity on cowpea after *B. napus* leaf incorporation. Phytotoxicity was prevented in test 2, thus, the *R. reniformis* population developed to a higher level 6 weeks after transplanting. More discernable differences in *R. reniformis* suppression and associated plant growth may have been observed if the cowpea bioassay period was extended.

Crotalaria juncea was a poor host to *R. reniformis*, produced leaf leachate that suppressed *R. reniformis* viability, and enhanced several nematode antagonistic fungi including parasitic nematode-trapping fungi and egg parasites of *R. reniformis*. Other modes of suppression could be involved as *C. juncea* also enhances bacterivores. This research demonstrates that incorporation of *C. juncea* could enhance cowpea plant growth and is a promising cover crop for *R. reniformis* management.

ACKNOWLEDGMENTS

This research was supported in part by Hawaii Department of Agriculture Grant #43181.

LITERATURE CITED

BROWN, P. D., M. J. MORRA, J. P. McCAFFREY, D. L. AULD, and L. WILLIAMS III. 1991. Allelochemicals produced during glucosinolate degradation in soil. Journal of Chemical Ecology 17:2021-2034.

BYRD, 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 8:206-212.

CASWELL, E. P., J. DEFRANK, W. J. APT, and C.-S. TANG. 1991. Influence of nonhost plants on population decline of *Rotylenchulus reniformis*. Journal of Nematology 23:91-98.

COOKE, R. C. 1963. Ecological characteristics of nematode-trapping fungi Hyphomycetes. Annual Review of Applied Biology 52:431-437.

COOKE, R. C., and B. E. S. GODFREY. 1964. A key to the nematode-destroying fungi. Transactions British Mycological Society 47:61-74.

DAYKIN, M. E., and R. S. HUSSEY. 1985. Staining and histopathological techniques in nematology. Pp. 39-48 *in* K. R. Barker, C. C. Carter and J. N. Sasser, eds. An Advanced Treatise on *Meloidogyne*, Vol. II. North Carolina State University Graphics, Raliegh, NC, U.S.A.

FASSULIOTIS, G., and G. P. SKUCAS. 1969. The effect of pyrrolizidine alkaloid ester and plants containing pyrrolizidine on *Meloidogyne incognita acrita*. Journal of Nematology 1:287-288.

GARDNER, J., and E. P. CASWELL-CHEN. 1994. *Rhaphanus sativus. Sinapis alba Fagopyrum esculentum* as hosts to *Meloidogyne incognita, Meloidogyne javanica,* and *Plasmodiophora brassicae*. Supplement to Journal of Nematology 26:756-760.

GOMMERS, F. J., and J. BAKKER. 1988. Physiological diseases induced by plant responses or products. Pp. 3-22 *in* G. O. Poinar and H.-B. Jansson, eds. Diseases of Nematodes., Vol. I. CRC Press, Boca Raton, CA, U.S.A.

GRAY, N. F. 1985. Ecology of nematophagous fungi: distribution and habitat. Annual Review of Applied Biology 102:501-509.

HALBRENDT, J. M. 1996. Allelopathy in the management of plant-parasitic nematodes. Journal of Nematology 28:8-14.

HUSSEY, R. S., and K. R. BARKER. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp. including a new technique. Plant Disease Reporter 57:1025-1028.

JAFFEE, B. A., H. FERRIS, and K. M. SCOW. 1998. Nematode-trapping fungi in organic and conventional cropping systems. Phytopathology 88:344-350.

JAFFEE, B. A., and A. E. MULDOON. 1995. Susceptibility of root-knot nematode and cyst nematodes to the nematode trapping fungi *Monacrosporum ellipsosporum* and *M. cionopagum*. Soil Biology and Biochemistry 27:1083-1090.

JAFFEE, B. A., D. R. STRONG, A.E. MULDOON. 1996. Nematode-trapping fungi of a natural shrubland: tests for rood chain involvement. Mycologia 88: 554-564.

JAFFEE, B. A., E. C. TEDFORD, and A. E. MULDOON. 1993. Tests for density-dependent parasitism of nematodes by nematode-trapping and endoparasitic fungi. Biological control 3:329-336.

JANSSON, H. B., and B. NORDBRING-HERTZ. 1980. Interaction between nematophagous fungi and plant-parasitic nematodes: attraction, induction of trap formation and capture. Nematologica 26:383-389.

KIRKEGAARD, J. A., P. T. W. WONG, and J. M. DESMARCHELIER. 1996. *In vitro* suppression of fungal root pathogens of cereals by *Brassica* tissues. Plant Pathology 45:593-603.

LINFORD, M. B. 1937. Stimulated activity of natural enemies of nematodes. Science 85:123-124.

LINFORD, M. B., F. YAP, and J. M. OLIVEIRA. 1938. Reduction of soil populations of root-knot nematode during decomposition of organic matter. Soil Science 45:127-141.

MANKAU, R. 1980. Biocontrol: Fungi as nematode control agents. Journal of Nematology 12:244-252.

MARLES, R. J., J. B. HUDSON, E. A. GRAHAM, C. S. BREAU, P. MORAND, R. L. COMPADRE, C. M. COMPADRE, G. H. N. TOWERS, and J. T. ARNASON. 1992. Structure-activity studies of photoactivated antiviral and cytotoxic tricyclic thiophenes. Phytochemistry and Phytobiology 56:479-487.

NORDBRING-HERTZ, B. 1973. Peptide-induced morphogenisis in the nematode-trapping fungus *Arthrobotrys oligospora*. Physiology of Plant 29:223-233.

RICE, E. L. 1984. Allelopathy. Academic Press, Orlando, FL, U.S.A.

ROHRBACH, K. G., and W. J. APT. 1986. Nematode and disease problems of pineapple. Plant Disease 70:81-87.

ROBINSON, A. F., R. N. INSERRA, E. P. CASWELL-CHEN, N. VOVLAS, and A. TROCCOLI. 1997. *Rotylenchulus* species: Identification, distribution, host ranges, and crop plant resistance. Nematropica 27:127-180.

RODRIGUEZ-KABANA, R., N. KOKALIS-BURELLE, S. KIEWNICK, R.-P. SCHUSTER, and R. A. SIKORA. 1994. Alginate films for delivery of root-knot nematode inoculum and evaluation of microhial interactions. Plant and Soil 164:147-154.

RODRIGUEZ-KABANA, R., C. F. WEAVER, D. G. ROBERTSON, and H. IVEY. 1988. Bahiagrass for the management of *Meloidogyne arenaria* in peanut. Annals of Applied Nematology 2: 110-114.

ROTAR, P. P., and R. J. JOY. 1983. 'Tropic Sun' sunn hemp, *Crotalaria juncea* L. Research Extension Series 036. HITAHR. 7 pp.

SAS INSTITUTE. 2000. SAS OnlineDoc®, Version 8, HTML Format. HTTP://www.sas.com. Cary, NC, U.S.A.

SIPES, B. S. 1996. Control of *Rotylenchulus reniformis* in pineapple with fosthiozate. Fruits 51:173-177.

SIPES, B. S., and D. P. SCHMITT. 1994. Population fluctuations of *Rotylenchulus reniformis* in pineapple fields and the effect of the nematode on fruit yield. Plant Disease 78:895-898.

SIPES, B. S., and K.-H. WANG. 2000. Sustainable nematode control in Hawaii Pineapple. Nematropica 30:149.

STIRLING, G. R., and L. J. SMITH. 1998. Field tests of formulated products containing either *Verticillum chlamydosporum* or *Arthrobotrys dactyloides* for biological control of root-knot nematodes. Biological control 11:231-239

WANG, K.-H. 2000. Management of *Rotylenchulus reniformis* in pineapple with tropical cover crops. Dissertation. University of Hawaii at Manoa, Honolulu, HI, U.S.A.

Received: *26.I.2001*
Recibido:

Accepted for publication: *15.V.2001*
Aceptado para publicación:

BLANK PAGE USED IN PAGE COUNT