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Management of *Rotylenchulus reniformis* in Pineapple, *Ananas comosus,* by Intercycle Cover Crops[1]

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Abstract: The effects of intercycle cover crops on *Rotylenchulus reniformis* population densities in pineapple were evaluated in one greenhouse and two field experiments. In the greenhouse, *Crotalaria juncea, Brassica napus,* and *Tagetes erecta* were planted for 3 months and then incorporated. These treatments were compared to weedy fallow with or without 1,3-dichloropropene (1,3-D) in three soils (Makawao fallow, Wahiawa fallow, and Wahiawa pineapple) naturally infested with *R. reniformis.* All cover crop incorporation suppressed *R. reniformis* numbers in cowpea more than did the weedy treatment in the Makawao ($P < 0.05$) but not in the Wahiawa soils. *Crotalaria juncea* treatment increased bacterivorous nematodes and nematode-trapping fungal population densities more than the other treatments in Makawao fallow and Wahiawa pineapple-planted soils. The field trials included the same plants as well as *Sinapis alba.* Treatments with *Crotalaria juncea* and 1,3-D maintained lower *R. reniformis* population densities on pineapple longer than other cover crops or weedy fallow treatments. *Crotalaria juncea* could have suppressed *R. reniformis* because it is a poor host and because it enhances nematode-trapping fungi when incorporated into soil. Treatment with 1,3-D reduced microbial activities but produced the greatest pineapple yield.

Key words: Brassica napus, cover crop, *Crotalaria juncea,* management, marigold, nematode, pineapple, rapeseed, *Rotylenchulus reniformis, Sinapis alba,* soil, sunn hemp *Tagetes erecta,* yellow mustard.

Nematicides are currently the key control tactic for *Rotylenchulus reniformis* in pineapple in Hawaii. This nematode can suppress plant crop yield by 60% to 74% and ratoon crop yield by 40% to 45% (Sipes, 1996). Due to environmental concerns, alternatives to chemical pesticides are highly desired.

Cover cropping is one such alternative. Cover crops are grown between the planting of cash crops to enhance soil fertility and soil structure, reduce soil erosion, and suppress plant pathogens or pests (Davis et al., 1991; Evenson and El-Swaify, 1997; Hooks et al., 1998). Lower plant-parasitic nematode abundance in cover cropping have been attributed to (i) poor host status (Rodríguez-Kábana et al., 1988), (ii) production of allelochemicals (Halbrendt, 1996), or (iii) enhancement of nematode-antagonistic flora and fauna (Kloepper et al., 1991; Linford, 1937).

Cover crops that decrease population densities of *R. reniformis* include *Secale cereale* (rye) (Guertal et al., 1998), *Crotalaria juncea* (sunn hemp) (Caswell et al., 1991a), *Tagetes patula* (French marigold) (Ko and Schmitt, 1996), *Chloris gayana,* (rhodegrass) (Caswell et al., 1991b), and *Digitaria decumbens* (pangolagrass) (Caswell et al., 1991a). In the Caribbean, incorporation of *Mucuna deeringiana* into the soil suppressed *R. reniformis* in pineapple and increased pineapple fruit yield (Chavarria-Carvajal et al., 2000).

Four cover crops that are known to produce nematicidal compounds were selected for this research. These crops include *C. juncea,* which produces monocrotaline (Fassuliotis and Skucas, 1969); *Brassica napus* (rapeseed), which produces glucosinolates (Brown et al., 1991); *Sinapis alba* (yellow mustard), which produces sinalbin, a component that hydrolyzes to acrinyl isothiocyanate with potential bio-activity (Brown et al., 1991); and *Tagetes erecta* (African marigold), which produces α-terthienyl (Gommers and Bakker, 1988). These cover crops are also nonhosts or poor hosts to *R. reniformis* (Birchfield and Brister, 1962; Caswell et al., 1991a; Robinson et al., 1998). In addition, these cover crops or their related species had potential in enhancing nematode-antagonistic fungi against other plant-parasitic nematodes (Cooke and Godfrey, 1964; Ko and Schmitt, 1996; Rodríguez-Kábana, 1998).

Management of plant-parasitic nematodes by manipulation of cropping sequences varies according to field history, nematode genotypes, and other biological components (McSorley, 2001). Many cover crops suppress *R. reniformis,* although the mechanisms of suppression are not understood. Pineapple cropping systems require longer-term nematode suppressiveness compared to short-term crops because pineapple is essentially a perennial crop. Thus, evaluation of changes in nematodes and potential nematode-antagonistic microorganisms such as nematode-trapping fungi over time is necessary. Here, the effects of cover crops on *R. reniformis* population growth were evaluated in three pineapple soils in the greenhouse, and were also followed through the cover crop-pineapple cropping cycle in the field. Our objective was to elucidate the factors (host status, and enhancement of nematode-trapping fungi) responsible for control of *R. reniformis* in pineapple soils by cover crops.

Materials and Methods

Effect of soil (greenhouse experiment): A greenhouse experiment was conducted to test cover crop effects on *R. reniformis* population density in three different pineapple field soils. A Makawao silty clay (MF) from Maui had been cropped with pineapple and then fallowed

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for 3 months. The MF is a very fine, parasesquic, isothermic, Ustic Palehumult (NRCS-USDA, 2000). Two Wahiawa silty clays were collected from Wahiawa, Oahu. One had been fallow for approximately 6 years (WF), whereas the second had been planted with pineapples for 7 months (WP). Wahiawa silty clay is a very fine, kaolinitic, isohyperthermic Rhodic soil (NRCS-USDA, 2000). Initial *R. reniformis* numbers were greater in MF and WP than in WF, and weeds included *Digitaria sanguinalis* in MF and *D. sanguinalis* and other species in WF and WP (Table 1). Soils were sieved through a 6-mm-pore screen and placed in 15-cm-diam. clay pots. Pots were planted with seeds of *C. juncea* 'Tropic Sun' (4 plants/pot), *B. napus* 'Dwarf Essex' (6 plants/pot), or *T. erecta* 'Cracker Jack' (4 plants/pot), or maintained without disturbance so that resident weeds could grow or were kept free of plants by removing germinating weeds. Soil amended with *C. juncea* was expected to have the lowest C: N among the treatments. The plants were grown for 3 months and then the entire plant was chopped into 1-cm pieces and the fresh material incorporated into the soil at 1% rate (dry weight plant material equivalent/dry soil weight). The bare soil treatment received 0.2 ml 1,3-dichloropene (1,3-D)/pot, equivalent to 263 kg a.i./ha). A 5-day-old *Vigna unguiculata* 'Black Eye' (cowpea) seedling was planted in each pot and grown for 2 months as an *R. reniformis* bioassay. The experiment had a 3 × 5 factorial arrangement (soil × preplant treatments) in a completely randomized design with four replications.

Rotylenchulus reniformis, bacterivorous, and fungivorous nematodes, and nematode-trapping fungal population densities in the soil and rhizosphere were determined before cover crop planting, 3 months after cover crop planting (3 days before cowpea planting), and 2 months after cowpea planting. Nematodes were extracted from the bulk soil and from the rhizosphere (most of the soil on the roots was removed by gentle shaking, leaving a rhizosphere sample consisting of roots with little adhering soil). A 50-cm^3 sample of soil was collected from each pot and nematodes were extracted using a mist chamber (Barker, 1985). Eggs and rhizosphere nematodes were extracted from root samples using an NaOCl method (Hussey and Barker, 1973), and eggs per gram root and vermiform stages per gram root were calculated.

Soil samples collected before cover crop planting, 5 days after cover crop incorporation, and 2 months after cowpea planting were assayed for nematode-trapping fungi. Soil (10 g) was suspended in 20 ml sterile, distilled water and processed through three 10-fold dilutions (Persmark and Jansson, 1997). A 100-µl aliquot of each dilution was plated on water agar with 100 mg streptomycin/L giving a 0.05, 0.005, and 0.0005 g soil/plate. Each dilution had three replicate plates. Three control plates without soil solution were used per sample. One hundred surface-sterilized *R. reniformis* eggs were added to each plate as bait. Eggs of *R. reniformis* were extracted from cowpea roots and centrifuged with sterile distilled water three times at 1,200 rpm for 3 minutes. The rinsed solution was resuspended with 1,000 mg/liter streptomycin sulfate solution and incubated overnight. Eggs were then rinsed three times in sterile distilled water and incubated in 3% H_2O_2 for 2 hours. Finally, the eggs were washed three times with sterile distilled water to remove the H_2O_2 before concentrating to the desired densities (Ko, pers. comm.). Nematode-trapping fungal population densities were estimated with a Most Probable Number program (Woomer et al., 1990).

Data were analyzed as a 3 × 5 factorial using the general linear model (SAS Institute, Cary, NC). For significant interactions between soil type and preplant treatment, treatment means within each soil type were compared using a Waller-Duncan k ratio ($k = 100$) t-test.

Intercycle cover crop system (field experiments): Two intercycle cover crop trials were conducted at a University of Hawaii research station (Whitmore, Oahu) in 1997 and 1999, respectively. The soil type, Wahiawa silty clay with pH of 5.0, is common in central Oahu where pineapple is grown.

In 1997, a field fallowed for 5 years with weeds and volunteer pineapples was blocked into 20 19 × 4.5-m plots. Five intercycle treatments were established before pineapple planting. The treatments were (i) *C. juncea* seeded at 37.2 kg/ha; (ii) *S. alba* seeded at 7.28 kg/ha; (iii) *T. erecta* seeded at 2 kg/ha; (iv) fallow with weeds including *Ipomoea alba, Richardia brasiliensis, Emilia sonchifolia,* and *Digitaria sanguinalis;* and (v) fallow with weeds followed by treatment with 1,3-D at 263 kg a.i./ha 2 weeks before pineapple planting.

Cover crops were grown for 3 months and incorporated into the soil. Planting beds were covered with plastic mulch, and 1,3-D was injected with a fumigun (N. A. MacLean Co., San Fransisco, CA.) to a depth of 30.5 cm through the plastic mulch on the planting

TABLE 1. Initial population of *Rotylenchulus reniformis* and weed species in three pineapple soils.

Soil	Soil type	History	*R. reniformis* /50 cm^3 soil	Weeds	pH	Organic matter (%)
MF	Makawao silty clay	3 months fallow	214	*Digitaria sanguinalis*	5.1	–
WF	Wahiawa silty clay	6 years fallow	6	*D. sanguinalis, Ipomoea obscura, Oxalis corniculata*	4.6	0.22
WP	Wahiawa silty clay	7 months pineapple	170	*D. sanguinalis, I. obscura, O. corniculata*	4.2	0.33

mark in treatment (v). Pineapple (*Ananas comosus* 'Smooth Cayenne' line F153) crowns were planted 1 month after cover crop incorporation. The experimental design was a randomized complete block with four replications.

Pineapple was fertilized monthly, irrigated according to plantation practice, and induced to flower 14 months after planting with ethephon (Ethrel®, Aventis, USA) at 500 mg/liter. Plants were treated with Diazinon 50W (Novartis, USA) at 2.24 kg a.i./ha upon detection of mealybugs 10 months after planting. D-leaf weight (the youngest, fully mature leaf), plant height, and damage index (1 = healthy, 2 = chlorotic, 3 = chlorotic and necrotic, 4 = heart die back) were measured 6, 12, and 18 months after planting from 40 plants per plot selected systematically in a Z-pattern. Fruits were harvested from the center two rows of each plot and weighed at 22 and 23 months after pineapple planting.

In 1999, a similar experiment was initiated except that rapeseed, *Brassica napus,* replaced yellow mustard (*S. alba*). The field site had been bare fallowed for the previous 5 years. The soil was amended with coral lime at 1,371 kg/ha, gypsum at 9,462 kg/ha, and magnesium sulfate at 2,800 kg/ha. Soil analysis and fertilization recommendations were obtained from the College of Tropical Agriculture and Human Resources, Agricultural Diagnostic Service Center. Field practices were as described in the 1997 trial except that pineapples were planted 2 months after cover crop incorporation.

In both trials, soil samples were collected at time of cover crop planting, cover crop incorporation, pineapple planting, and at bimonthly intervals thereafter. In each plot, 15 soil cores from the top 20 cm were collected with a soil bucket auger from the cover crop, weed, or pineapple root zone and then mixed and sieved through a 1-cm mesh screen. Nematodes in vermiform stages were extracted from the soil, and nematode eggs were extracted from the roots as described for the greenhouse experiment. Root weight of each sample was measured to calculate eggs per gram root. Plant-parasitic nematodes were identified to genus, whereas other nematodes were identified to trophic level. Nematode numbers were monitored bimonthly, up to 9 months after planting.

In 1997, *R. reniformis* were quantified only in bulk soil. In the 1999 trial, host status of the three cover crops was compared to that of pineapple, *Erigeron canadensis* (fireweed), *Panicum maximum* (Guinea grass), and *Buddleja asiatica* (dogtail) from the weedy fallow plots by quantifying the *R. reniformis* population densities in the rhizosphere. Five plants of each species were sampled randomly from each plot and their roots shaken in 0.5% NaOCl followed by centrifugal flotation (Hussey and Barker, 1973) to extract vermiform stages and eggs of *R. reniformis* from the rhizosphere.

In the 1999 trial, nematode-trapping fungi were quantified in each sample by suspending 100 g bulk soil in 200 ml sterile distilled water followed by three 10-fold dilutions. A 100-µl aliquot of each dilution was plated on quarter-strength cornmeal agar (CMA/4) (Jaffee and Muldoon, 1995) with 100 mg of streptomycin/liter, giving 0.05, 0.005, or 0.0005 grams soil/plate. One hundred *Steinernema glaseri* were added to each plate as nematode-trapping fungus bait. The fungal population densities were estimated with a Most Probable Number program (Woomer et al., 1990) and were monitored bimonthly up to 9 months after pineapple planting. Nematode-trapping fungi in each plate were examined using a compound microscope and were identified to species according to trap and spore morphology in the keys of Cooke and Godfrey (1964).

Data on host status and pineapple growth and yield were subjected to General Linear Model analysis. Means were separated by Waller-Duncan *k*-ratio (k = 100) *t*-test (Steel and Torrie, 1981). Due to the fluctuation in population densities of *R. reniformis* and the nematode-trapping fungi, no geometric models fit the population data, so the data collected by sampling were subjected to repeated measure analysis (where treatments were the main plots and sampling time the subplot) using Proc Mixed Analysis (SAS Institute, Cary, NC). When the interaction between treatment and time was not significant ($P \geq 0.05$), treatment means were pooled across sampling times and least square means were calculated in the Proc Mixed Analysis to detect treatment main effects. Least square means of treatments were separated by Tukey test (P = 0.05) where appropriate.

Results

Effect of soil (greenhouse experiment): Response of *R. reniformis* to the cover crop incorporation differed among the soils ($P < 0.05$). *Rotylenchulus reniformis* reproductive factor (Rf = final population densities/initial population densities) was reduced by cover crop incorporation in the MF soil but not in WF and WP soils ($P < 0.05$) (Table 2). In MF soil, *R. reniformis* soil population den-

Table 2. Reproductive factor (Rf[a]) of *Rotylenchulus reniformis* in three pineapple soils (MF, WF, and WP) planted with cover crops *Crotalaria juncea, Brassica napus,* or *Tagetes erecta,* left fallow with weeds, or maintained bare.

	Soil		
Preplant treatments	MF	WF	WP
Crotalaria juncea	0.02 a[b]	7.67 a	3.23 ab
Brassica napus	0.03 a	8.67 a	4.29 ab
Tagetes erecta	0.05 a	10.00 a	8.16 a
Weedy fallow	0.04 a	7.00 a	1.27 b
Bare soil	0.02 a	9.33 a	1.80 b

[a] Rf = Population densities of *R. reniformis* in 50 cm^3 soil 3 months after crop planting/initial population densities of *R. reniformis* in 50 cm^3 soil.

[b] Values are means of four replicates. Means in a column followed by the same letters are not different according to a Waller-Duncan *k* ratio (k = 100) *t*-test (P = 0.05).

sities were lower in the cover crop treatments and in the 1,3-D treatment than in the weedy treatment (Fig. 1A). However, root population densities of *R. reniformis* and eggs were not lower in the cover crop treatments than in the weedy or 1,3-D treatments in all three soils (Fig. 1B,C).

Bacterivorous nematode densities were greater in WF than in the other two soils after the cover crop incorporation (Fig. 1D). Incorporation of *C. juncea* enhanced fungivorous nematodes in the two Wahiawa soils but not in the MF soil (Fig. 1E).

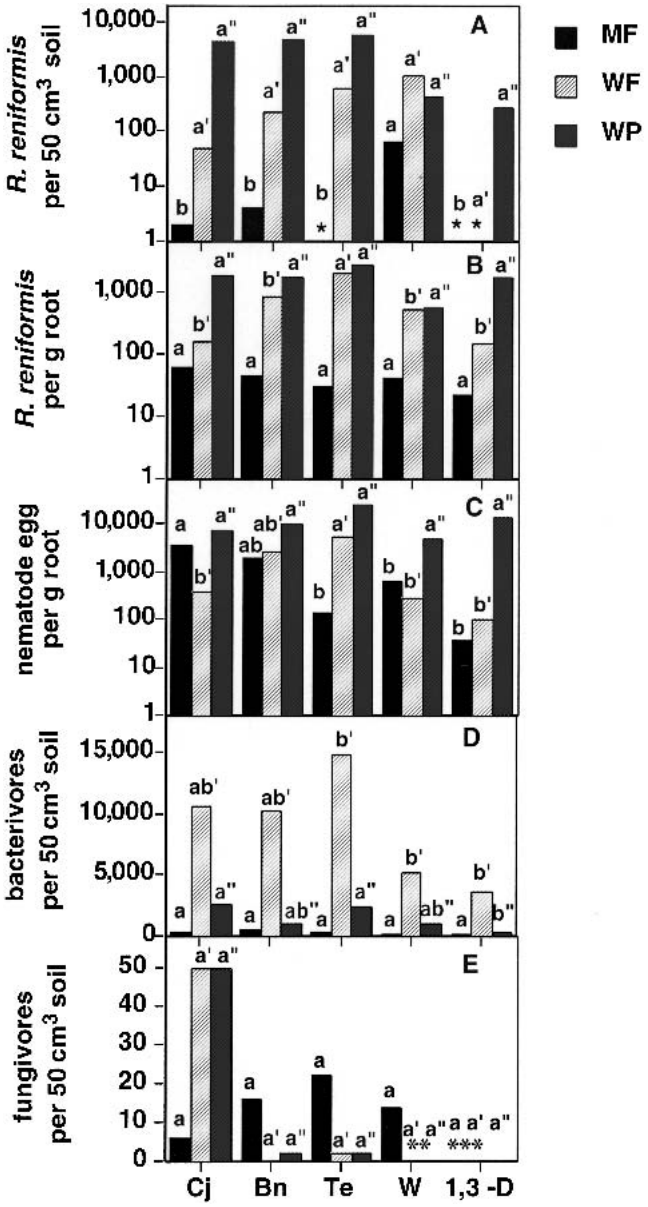

FIG. 1. *Rotylenchulus reniformis* vermiform stages in (A) soil and (B) rhizosphere, and (C) eggs; and (D) bacterivorous and (E) fungivorous nematodes in the greenhouse experiment with three soils: MF, WF, and WP. Treatments were *Crotalaria juncea* (Cj), *Brassica napus* (Bn), *Tagetes erecta* (Te), weeds (W), and bare soil followed by 1,3-dichloropropene (1,3-D) before cowpea bioassay. Bars followed by the same letters are not different among the treatments within a soil type according to Waller-Duncan *k* ratio ($k = 100$) *t*-test. *indicates zero observation.

Enhancement of nematode-trapping fungal population densities by the cover crops differed among the soils. Nematode-trapping fungi were generally more abundant in the soil previously planted to pineapple, WP, than the fallow soils, MF or WF (Fig. 2A). Five days after cover crop incorporation, numbers of nematode-trapping fungi were enhanced by *C. juncea* in MF (Fig. 2A,B). The nematode-trapping fungal population densities in WP soil dropped after biomass incorporation (Fig. 2A,B) but still remained highest in *C. juncea*-incorporated soil among the treatments (Fig. 2B) after incorporation. Two months after cowpea was planted in MF soil, fungal numbers were greater in *C. juncea* treated MF soil than in that treated with *B. napus* and 1,3-D (Fig. 2C). In WP soil, nematode-trapping fungi increased 2 months after cowpea bioassay compared to those 5 days after incorporation (Fig. 2B, C). However, the population density was not different among the treatments ($P > 0.1$) (Fig. 2C).

Intercycle cover crop system (field experiments): Rotylenchulus reniformis densities decreased in all the cover crop

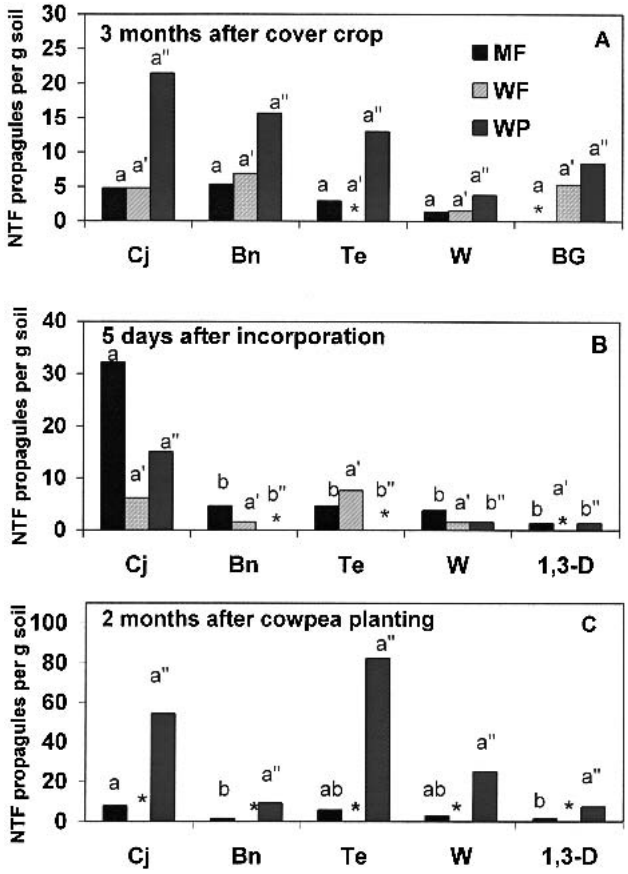

FIG. 2. Nematode-trapping fungal population densities (A) 3 months after cover crop planting, (B) 5 days after cover crop incorporation, and (C) 2 months after cowpea planting in three soils, MF, WF, and WP. Treatments were *Crotalaria juncea* (Cj), *Brassica napus* (Bn), *Tagetes erecta* (Te), weeds (W), and bare soil followed by 1,3-dichloropropene (1,3-D) in the greenhouse experiment. Means followed by the same letters are not different among the treatments within a soil type according to Waller-Duncan *k* ratio ($k = 100$) *t*-test.

plots as well as the weedy plots during the cover crop growing period (i.e., between C and I in Figs. 3 and 4) in both 1997 and 1999 trials (Figs. 3A and 4A). *Erigeron canadensis,* a common weed in the 1999 trial, was as susceptible a host for *R. reniformis* as pineapple (Table 3). In the 1997 trial, *R. reniformis* reproductive factor was lowest in *S. alba* plots (Table 4); in the 1999 trial, plots with *C. juncea, T. erecta,* and weedy fallow had lower *R. reniformis* reproductive factors than *B. napus* plots ($P < 0.05$) (Table 5).

Between biomass incorporation and pineapple planting (P), numbers of *R. reniformis* increased in all the treatments in the 1997 trial (Fig. 3A) but increased only in the weedy fallow plots in the 1999 trial (Fig. 4A).

Crotalaria juncea (in 1997 and 1999) and 1,3-D treatments (in 1999) maintained lower *R. reniformis* population densities than the other treatments until 6 months after pineapple planting (Figs. 3A,4A). Population densities of *R. reniformis* continued to increase in 1,3-D-treated plots after fruit harvest (18 months after planting, Fig. 3A) but remained at a moderate level in *C. juncea* treated plots. Repeated measure analysis showed that interaction between treatment and time effects for all the parameters tested were not significant ($P > 0.05$) in either trial. Thus, analysis revealed that *R. reniformis* population densities over the 22 months in 1997 were lowest in the *C. juncea* treatment and highest in the *T. erecta* treatment ($P < 0.05$) (Table 4). However, repeated measure analysis showed that in the 1999 trial, *R. reniformis* population densities for up to 9 months after pineapple planting were not different among the treatments ($P > 0.05$) (Table 5). Pineapple planted in the *C. juncea*-treated plots had the lowest eggs per gram root, whereas weedy fallow and *T. erecta* treatments had the highest numbers of eggs per gram root in the 1997 trial based on repeated measure analysis (Table 4). However, in the 1999 trial, eggs per gram root were not

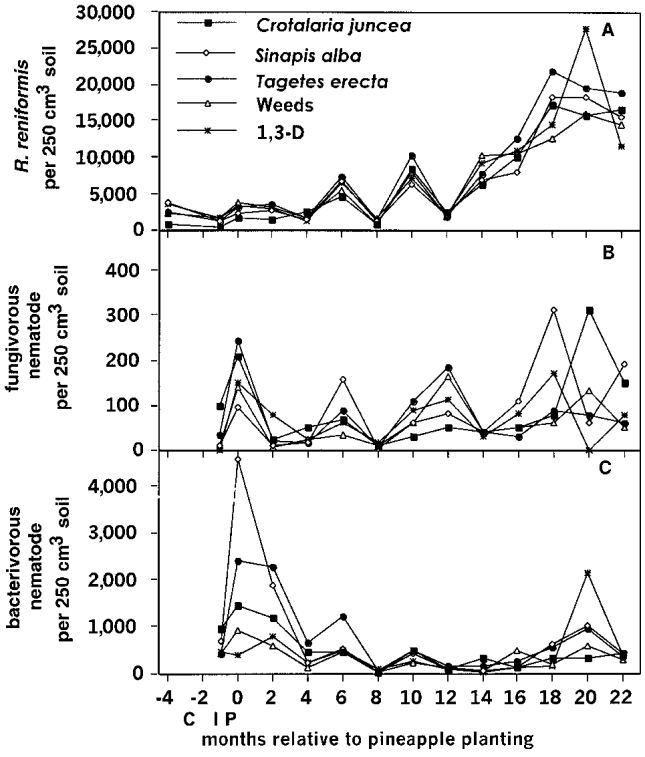

FIG. 3. (A) *Rotylenchulus reniformis,* (B) fungivorous, and (C) bacterivorous nematode numbers in soil in 1997 intercycle cover crop trial. C = cover crop planting, I = cover crop incorporation into soil, P = pineapple planting. 1,3-dichloropropene (1,3-D) was applied 2 weeks prior to pineapple planting. Values are means of 4 replications.

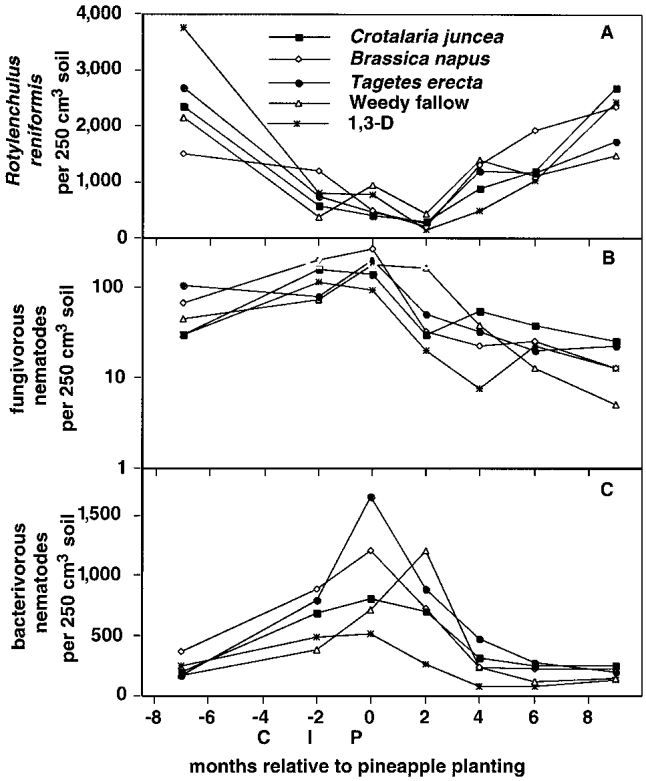

FIG. 4. (A) *Rotylenchulus reniformis,* (B) fungivorous, and (C) bacterivorous nematode numbers in soil in 1999 intercycle cover crop trial. C = cover crop planting, I = cover crop incorporation into soil, P = pineapple planting. 1,3-Dichloropropene (1,3-D) was treated 2 weeks prior to pineapple planting. Values are means of 4 replications.

TABLE 3. Host status of pineapple, cover crops, and weeds to *Rotylenchulus reniformis* in the 1999 trial.

Plant	*R. reniformis* Vermiform stages /g roots	*R. reniformis* Eggs/g roots
Ananas comosus	4,319 a[a]	14,872 a
Erigeron canadensis	1,461 ab	5,233 a
Tagetes erecta	159 bc	102 b
Buddleja asiatica	47 c	44 b
Brassica napus	78 c	73 b
Crotalaria juncea	78 c	52 b
Digitaria violascens	88 c	139 b
Panicum maximum	143 c	24 b

[a] Means of numbers of vermiform stages and eggs of *R. reniformis* extracted from 5 plants in 4 plots in the 1999 trial by sodium hypochlorite method. Values in a column followed by the same letters were not different, after transformation by log $(x + 1)$, according to Waller-Duncan k ratio ($k = 100$) t-test.

TABLE 4. *Rotylenchus reniformis* reproductive factor (Rf), numbers of vermiform stages and eggs, bacterivorous and fungivorous nematodes in a 1997 intercycle cover crop field trial.

Treatment	*Rotylenchulus reniformis*			Bacterivorous nematode /250 cm^3 soil[b]	Fungivorous nematode /250 cm^3 soil[b]
	Rf[a]	250 cm^3 soil[b]	Eggs/g root[b]		
Crotalaria juncea	−3.8 a	6,375 b	572 b	504 a	87 a
Sinapis alba	−22.4 a	6,763 ab	685 ab	818 a	83 ab
Tagetes erecta	−10.5 a	8,184 a	933 a	767 a	64 ab
Weedy fallow	−5.3 a	6,624 ab	941 a	348 a	47 b
1,3-D	−16.6 a	7,386 ab	753 ab	432 a	60 ab

[a] Reproductive factor, Rf = *R. reniformis* population densities in 250 cm^3 soil at 3 months after cover crop planting/*R. reniformis* population densities in 250 cm^3 soil prior to cover crop planting.
[b] Values are least square means of 4 replications obtained by Proc Mixed Analysis over a 22-month period. Least square means in a column followed by the same letters are not different according to Tukey test ($P = 0.05$).

different among the treatments for up to 9 months after planting (Table 5).

Bacterivorous and fungivorous nematode population densities generally increased 1 month after biomass incorporation in all the treatments except in 1,3-D treatment in the 1997 trial, and in 1,3-D and *C. juncea* treatments in the 1999 trial (Figs. 3B, C; 4B, C). Fungivorous nematodes included *Aphelenchoides* spp. and *Tylenchus* spp. Repeated measure analysis revealed that the number of fungivorous nematodes in the 1997 trial was higher in the *C. juncea* treatment than in the 1,3-D treatment 22 months after pineapple planting ($P < 0.05$) (Table 4), whereas in the 1999 trial, the number in the *C. juncea* treatment was not different from that in the 1,3-D treatment 9 months after planting ($P > 0.05$) (Table 5). Population densities of bacterivorous nematodes, mainly the Rhabditoids, were not different among the treatments in the 1997 trial (Table 4) but were higher in the cover crop-treated plots than the nematicide-treated plots in the 1999 trial (Table 5).

Nematode-trapping fungal population densities did not increase during cover crop planting but increased after biomass incorporation in the 1999 trial (Fig. 5). Numbers then crashed, except in the *C. juncea*-treated plots where the nematode-trapping fungal population densities increased to 43 propagules/gram soil at 4 months after planting (Fig. 5). Repeated measure analysis indicated that the number of nematode-trapping fungal propagules/gram soil was highest in the *C. juncea* treatment and lowest in 1,3-D-treated plots (Table 5). The fungi were identified as *Arthrobotrys eudermata* and *Dactylellina ellipsospora* (Cooke and Godfrey, 1964; Scholler et al., 1999), with *A. eudermata* being the most abundant.

Among the intercycle treatments, pineapple height was greater in the *C. juncea* and 1,3-D treatments than in the other treatments in 1997 (Table 6). Six months after planting, most of the pineapples in all the treatments exhibited brownish and papery leaf tips; a few plants showed butt rot symptoms. All of these symptoms were due to *Chalara paradoxa* infection. Mealy bug wilt symptoms also were observed in the trial. *Sinapis alba*-treated plots had the highest damage index among the treatments, whereas those in 1,3-D and *T. erecta*-treated plots had the lowest ($P < 0.05$) (Table 6). The cover crop effect on pineapple growth in the 1999 experiment could not be determined because the experiment was terminated too early to draw conclusions. D-leaf weight was highest in 1,3-D and *T. erecta*-treated plots 6 months after planting ($P < 0.05$); however, the largest plant height difference was only 2 cm, and D-leaf weight difference of 40 leaves was less than 70 g (Table 6).

The *R. reniformis* suppressive effects of *C. juncea* were not reflected in the pineapple yield in 1997. Pineapple yields were greater ($P \leq 0.05$) in the 1,3-D-treated plots than in the other treatments (Table 7).

TABLE 5. *Rotylenchus reniformis* reproductive factor (Rf), numbers of vermiform stage and eggs, bacterivorous and fungivorous nematodes, and nematode-trapping fungi in a 1999 intercycle cover crop field trial.

Treatment	*Rotylenchulus reniformis*			Bacterivores /250 cm^3 soil	Fungivores /250 cm^3 soil	Nematode-trapping fungal propagules/g soil
	Rf[a]	/250 cm^3 soil[b]	Eggs/g root			
Crotalaria juncea	0.3 b	1,193 a	2,452 a	458 a	68 ab	10 a
Brassica napus	0.9 a	1,280 a	1,822 a	555 a	89 a	2 ab
Tagetes erecta	0.3 b	1,171 a	1,831 a	637 a	72 a	3 ab
Weedy fallow	0.3 b	1,120 a	2,053 a	425 ab	73 a	2 ab
1,3-D	0.5 ab	1,341 a	1,940 a	255 b	42 b	1 b

[a] Reproductive factor, Rf = *R. reniformis* population densities in 250 cm^3 soil at 3 months after cover crop planting/*R. reniformis* population densities in 250 cm^3 soil prior to cover crop planting.
[b] Values are least square means of 4 replications obtained by Proc Mixed Analysis over a 9-month period. Least square means in a columnn followed by the same letters are not different according to Tukey test ($P = 0.05$).

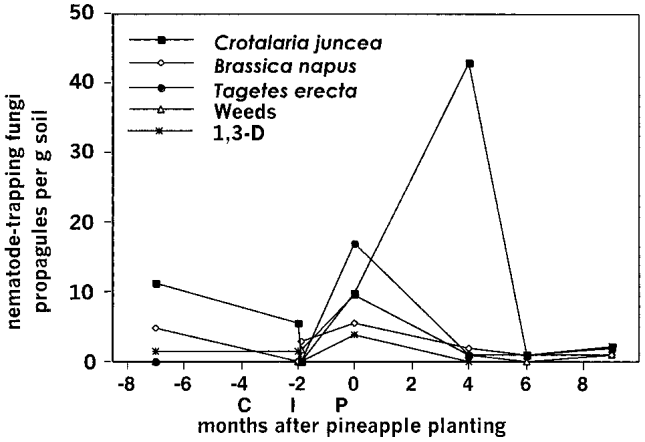

FIG. 5. Nematode-trapping fungal propagules in 1999 intercycle cover crop trial. C = cover crop planting, I = cover crop incorporation, P = pineapple planting. Values are means of 4 replications.

DISCUSSION

The reduction of *R. reniformis* population densities by the three cover crops studied varied among soils. These variations could be due to differences in reproduction of *R. reniformis,* or soil microbial activities, or both. Seven-month-old pineapple planted soil, WP, had higher *R. reniformis* reproductive factor, likely due to the presence of host roots prior to the experiment. Therefore, suppression of *R. reniformis* by *C. juncea* was difficult in this soil. Moreover, this soil also had been treated with herbicides. The weeds that could support population development of *R. reniformis* were mostly killed, leading to the lower densities of *R. reniformis* in the weedy treatment in WP. In fallow soil such as MF, reproductive ability of *R. reniformis* was less; this could lead to more efficient suppression of *R. reniformis* by cover crops. However, this phenomenon was not observed in 6-year-fallow WF soil. This is because *C. juncea* incorporation enhanced nematode-trapping fungi the most in MF soil, which might have contributed to the low *R. reniformis* reproduction in this soil. Enhancement of nematode-trapping fungi by *C. juncea* was less in long-term fallow soil, such as that observed in the 6-year-fallow WF soil. This effect may be due to nematode-trapping fungal activities that are reduced in the fallow soil with lower organic content (Table 1). Soil from 7-month-old pineapple planted field in Wahiawa had higher organic matter content than the fallow soils (Table 1), thus maintaining a higher density of nematode-trapping fungi even before green matter incorporation. However, the high population density of nematode-trapping fungi did not suppress *R. reniformis* densities in this field.

The poor host status of *C. juncea* to *R. reniformis* could be a primary factor responsible for the decrease of *R. reniformis* population densities during the intercycle period. *Crotalaria juncea* allows only a small fraction of the nematode population to penetrate the roots, thereby suppressing reproduction (Caswell et al., 1991a). However, the ability of *C. juncea* treatment to maintain lower *R. reniformis* population densities in the pineapple crop for a longer period than the other treatments may be associated with its ability to enhance nematode-trapping fungi. As a legume, *C. juncea* enhanced nematode-trapping fungi better than the two non-legume cover crops. This is similar to Persmark and Jansson (1997), who demonstrated that pea, a legume, increased the densities of nematode-trapping fungi better than did non-legumes. Linford (1937) suggested that incorporation of organic matter increased the activities of free-living nematodes and thus increased nematode-trapping fungi. Cooke (1968) suggested that the addition of organic amendments to soil enhanced nematode-trapping fungal densities for less than 2 months. However, in our experiment, numbers of nematode-trapping fungi remained high for up to 6 months after *C. juncea* incorporation.

Sinapis alba and *B. napus* suppressed *R. reniformis* by being poor hosts (Stoyanov, 1967) and by producing glucosinolates that decompose to compounds including isothiocyanates, thiocyanates, and nitriles (Donkin et al., 1995) that are toxic to nematodes (Halbrendt, 1996). *Sinapis alba* senesced early in the 1997 intercycle trial; thus, its suppression of *R. reniformis* was not obvious. *Brassica napus* established well and produced the greatest biomass among the treatments. Suppression of *R. reniformis* by *B. napus* after incorporation was greater

TABLE 6. Pineapple plant growth in intercycle cover crop field trials.

Treatment Months after pineapple planting	1997			1999		
	Height (cm) 18	D-leaf[a] (g) 12	Damage[b] index 18	Height (cm) 6	D-leaf (g) 6	Damage index 6
Crotalaria juncea	61 a[c]	975 a	1.17 ab	28.2 bc	536 b	1.09 a
Sinapis alba	54 c	873 a	1.23 a	29.1 ab	544 b	1.04 a
Tagetes erecta	57 bc	894 a	1.06 c	29.8 a	560 ab	1.05 a
Weedy fallow	58 b	963 a	1.17 ab	27.7 c	570 ab	1.09 a
1,3-D	61 a	907 a	1.12 bc	29.4 a	601 a	1.05 a

[a] D-leaf is the newest, fully matured pineapple leaf. Values are total of 40 leaves sampled systemically from 40 pineapple plants in each treatment plot.
[b] Damage index: 1 = healthy, 2 = chlorotic, 3 = chlorotic and necrotic, 4 = heart die back.
[c] Values in a column followed by the same letter are not different according to Waller-Duncan *k*-ratio ($k = 100$) *t*-test.

TABLE 7. Pineapple fruit yield in intercycle field trial of 1997.

Treatment	Average fruit weight (g)	Marketable fruit yield (mt/ha)
Crotalaria juncea	851 b[a]	1,155 bc
Sinapis alba	777 c	1,117 c
Tagetes erecta	875 b	1,153 bc
Weeds	852 b	1,171 b
1,3-D	921 a	1,228 a

[a] Means are average of 4 replicates. Values in each column followed by the same letters are not different according to a Waller-Duncan *k* ratio ($k = 100$) *t*-test ($P > 0.05$).

than during the intercycle period. However, glucosinolates also have fungistatic effects (Davis et al., 1991), so it is not surprising to observe a low nematode-trapping fungal population in *B. napus*-treated plots and a corresponding *R. reniformis* rebound to higher numbers 4 months after pineapple planting in *B. napus* plots (in contrast to the 8-month rebound after pineapple planting in *C. juncea* plots).

Tagetes erecta was a better host to *R. reniformis* than *C. juncea* and *B. napus*. Birchfield and Brister (1962) found *T. erecta* to be resistant to *R. reniformis;* however, the cultivar tested was not specified and may have been different than *T. erecta* 'Cracker Jack' used in this research, or their *R. reniformis* populations may have differed in host range from ours. *Tagetes erecta* did not suppress *R. reniformis* efficiently during the 1997 and 1999 intercycle periods. However, *T. erecta* increased nematode-trapping fungal population densities 1 month after incorporation. *Tagetes patula* has been found to enhance activity of nematode-antagonistic microbes (Ko and Schmitt, 1996). The enhancement of nematode-trapping fungal population densities by *T. erecta* in 1999 ceased after pineapple planting.

Weedy plots were among the poorest treatments for suppression of *R. reniformis*. Although *R. reniformis* populations decreased in weedy plots during the intercycle period, nematode population densities increased on pineapple after weed incorporation. *Rotylenchulus reniformis* may have undergone anhydrobiosis during the intercycle period when nonhosts were present, but revived after pineapple planting and irrigation.

1,3-Dichloropene reduced *R. reniformis* numbers for up to 8 months after pineapple planting but decreased nematode-trapping fungi. The continued increase of *R. reniformis* populations after pineapple fruit harvest indicates a need for additional nematicide treatment to protect the ratoon crop.

Except for 1,3-D, intercycle treatments did not affect pineapple yield. Pineapple growth in *C. juncea*-treated plots was as good as those in the 1,3-D-treated plots in 1997, but this growth did not correspond to improved yield. The presence of other diseases in *C. juncea*-treated plots early in the 1997 cycle and the irregular fruit setting might have confounded crop yield. Additional crop yield data are needed to determine the intercycle effect on pineapple yield.

Results from the greenhouse studies using different soil types suggest that reducing *R. reniformis* reproduction before *C. juncea* treatment could strengthen the nematode-suppressive effect of the plant. This reduction could be achieved by fallowing the soil for 2 months before planting *C. juncea*. Planting pineapple 2 months after *C. juncea* incorporation might also prolong nematode-trapping fungi establishment.

In addition to *R. reniformis* suppression, *C. juncea* provides additional benefits in a pineapple cropping system. It suppressed *M. incognita* when rotated with cotton (Robinson et al., 1998) and *M. javanica* when rotated with taro (Sipes and Arakaki, 1997) and was reported as a very poor host for *M. arenaria, M. incogita,* and *M. javanica* (McSorley, 1999). All of these *Meloidogyne* species are pathogens of pineapple. However, because *C. juncea* is a host for *Pratylenchus* spp. (Robinson et al., 1998), pineapple fields infested with *Pratylenchus* spp. are not suitable for rotation with *C. juncea*. Using *C. juncea* as green manure can supply 150 to 165 kg N/ha if incorporated before it flowers (Rotar and Joy, 1983). Growing *C. juncea* as an intercycle crop with pineapple also increases the percentage of vitamin C in the pineapple fruit (Chavarria-Carvajal et al., 2000). Because *C. juncea* is a fast-growing plant, it can outcompete weeds and decrease soil erosion. *Crotalaria juncea* is drought tolerant, producing 1,120 kg/ha of green matter in 6 weeks with 50 mm of irrigation (Rotar and Joy, 1983). Incorporating *C. juncea* prior to seed formation limits its potential as a weed problem.

In conclusion, *C. juncea* is a poor host to *R. reniformis* and can increase populations of nematode-trapping fungi. When planted as an intercycle cover crop in pineapple production, *C. juncea* can suppress *R. reniformis* as efficiently as 1,3-D.

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