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Intercropping Cover Crops with Pineapple for the Management of *Rotylenchulus reniformis*[1]

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Abstract: Effect of cover crops intercropped with pineapple (*Ananas comosus*) on *Rotylenchulus reniformis* population densities and activity of nematode-trapping fungi (NTF) were evaluated in two cycles of cover crop and pineapple. Sunn hemp (*Crotalaria juncea*), rapeseed (*Brassica napus*), African marigold (*Tagetes erecta*), or weeds were intercropped with pineapples. Beds planted with sunn hemp or rapeseed had lower population densities of *R. reniformis* than African marigold, weeds, or pineapple plots during cover crop growth, and the subsequent pineapple-growing periods. Rapeseed was a good host to *Meloidogyne javanica* and resulted in high population densities of *M. javanica* in the subsequent pineapple crop. Fireweed (*Erigeron canadensis*) occurred commonly and was a good host to *R. reniformis*. Bacterivorous nematode population densities increased ($P \leq 0.05$) most in sunn hemp, especially early after planting. Nematode-trapping fungi required a long period to develop measurable population densities. Population densities of NTF were higher in cover crops than weeds or pineapples during the first crop cycle ($P < 0.05$). Although pineapple produced heavier fruits following sunn hemp than in the other treatments ($P < 0.05$), commercial yields were not different among rapeseed, weed, and sunn hemp treatments.

Key words: Ananas comosus, Brassica napus, Crotalaria juncea, marigold, *Meloidogyne javanica,* nematode, nematode-trapping fungi, rapeseed, root-knot, reniform, sunn hemp, *Tagetes erecta,* weeds.

Current pineapple nematodes management in Hawaii relies heavily on the nematicide 1,3-dichloropropene (1,3-d) (Sipes and Schmitt, 1994). This chemical-based management is of high risk for the environment. It is important and urgent to develop alternatives to nematicides as the Food Quality Protection Act of 1996 may drastically affect the availability and registration of currently used nematicides (Huettel, 1997). Pineapple growers in many parts of the world are interested in alternatives to intensive pesticide application (Chavarria-Carvajal et al., 2000; Sipes and Wang, 2000). Recently, the pineapple industry in Hawaii has explored the production of organic pineapple to meet the rising health concerns of consumers (Fleisch, pers. comm.). Cover cropping offers one such alternative for nematode management.

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 and Johnson, 2002). Cover crops are traditionally used in some cropping systems to manage plant-parasitic nematodes (Johnson, 1982; Nusbaum and Ferris, 1973; Trivedi and Barker, 1986). A common practice of cover cropping is planting the cover crop during the inter-cycle period (Wang et al., 2002a). Targeted plant-parasitic nematodes can be suppressed by cover crops that are either poor hosts or produce allelopathic chemicals (Alam et al., 1990; Halbrendt, 1996; McSorley et al., 1994; Rodríguez-Kábana et al., 1994). However, the nematode-suppressive effect is normally operative only during the cover crop growing period, with plant-parasitic nematode populations likely to increase after the subsequent susceptible crop is planted (McSorley et al., 1994). Because cropping practices that produce conditions favorable for pest management are gaining acceptance, cover crops that suppress nematode populations by enhancing antagonistic microorganisms can help achieve the goal of sustainable agriculture. On the other hand, common plantation practices such as fumigation with 1,3-d have negative impacts on the nematode-antagonistic microorganisms in the soil (Wang, et al., 2002a). In one study, nematode-trapping fungi (NTF) were not recovered from soil following fumigation with 1,3-d even after organic matter was incorporated into the soil in an attempt to stimulate soil microbial activities (Wang et al., 2002b).

A cover crop can enhance nematode-antagonistic microorganisms by providing a more favorable environment for microbial activity (Kloepper et al., 1991), by increasing the soil organic matter content that favors the development of microorganisms, or by temporarily removing soil microbiostasis after residues are incorporated into the soil (Ho and Ko, 1986; Linford et al., 1938; Mankau and Minteer, 1962; Muller and Gooch, 1982).

Nematode-trapping fungi are among the many microorganisms in the soil that are antagonistic to nematodes. They are usually more abundant in the rhizosphere than root-free soil (Gaspard and Mankau, 1986; Persson and Jansson, 1999; Peterson and Katznelson, 1965). The population densities of nematophagous fungi were 19 times greater in a pea (*Pisum sativum*) rhizosphere than those in root-free soil (Persmark and Jansson, 1997). Some plant species appear to provide a

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better environment for NTF than others. For example, the number of species of nematophagous fungi was higher in the rhizosphere of pea than those in yellow mustard (*Sinapis alba*) or barley (*Hordeum* spp.) (Persmark and Jansson, 1997). Bacteria with nematode-antagonistic properties, and chitinolytic fungi, are important in the dynamics of nematode antagonism. These types of organisms were isolated at a higher rate from the rhizosphere of cover crops suppressive to *Heterodera glycines* and *Meloidogyne incognita* as compared to soybean (*Glycine max*), which is a host to both nematode species. These cover crops include velvetbean (*Mucuna deeringiana*), castor (*Ricinus communis*), sword bean (*Cannavalia ensiformis*), and 'Abruzzi' rye (*Secale sereale*) (Kloepper et al., 1991).

Months or years may be required for the establishment of nematode-antagonistic microorganisms. For example, 10 years of cereal monoculture were required to establish natural nematode-antagonistic microorganisms to a suppressive level against the cereal cyst nematode *Heterodera avenae* (Kerry et al., 1982). Therefore, our approach was to prolong the cover crop planting period to promote establishment of nematode-antagonistic microorganisms. Whereas long-term culture of cover crop during the intercycle period is not an economical practice, intercropping cover crops with pineapple might offer an approach to prolong the cover crop period. The cover crops were grown throughout the pineapple cycle, and in the next planting cycle, cover crops and pineapple planting beds were alternated. This intercropping practice would then serve as a nematode management treatment for the next planting cycle instead of a mixed cropping effect.

The objectives of this research were to: (i) determine the suppressive effects of cover crops intercropped with pineapple on *Rotylenchulus reniformis* during the cover crop growing period, (ii) determine the subsequent residual effects of the cover crops on population changes of *R. reniformis* after a susceptible host or pineapple was planted in the cover crop intercropped soil, and (iii) identify the microorganisms present in the cover crop rhizosphere and their population changes in response to cover crop treatments.

Materials and Methods

A pineapple-cover crop intercropping trial was conducted from February 1998 to November 1999 (cycle I) at the University of Hawaii Whitmore Experiment Station on Oahu Island, Hawaii. This experiment was repeated at the same site from January 2000 to September 2001 (cycle II). The soil type was a Wahiawa silty clay with pH of 4.9. The field site was left fallow for 5 years with several cultivations a year to suppress weeds, and was cultivated with a rotary tiller prior to the experiment.

Pineapple was intercropped with: (i) sunn hemp (*Crotalaria juncea* 'Tropic Sun', seeded at 47.0 kg/ha), (ii) rapeseed (*Brassica napus* 'Dwarf Essex', 7.2 kg/ha), (iii) African marigold (*Tagetes erecta* 'Cracker Jack', 2.3 kg/ha), or (iv) indigenous weeds. Experimental design was a randomized complete block with four replications. Experimental plots were two intercrop beds and two pineapple beds (Fig. 1). Each bed was 4 m × 8 m. Samples collected from pineapple beds were treated as the control treatment in cycle I. In cycle II, two independent sets of samples were collected: soil from the intercrop treatment was used to generate a repeated data set for cycle I, and soil from the pineapple beds was used to monitor population changes after cover crop treatments.

Prior to crop planting, beds for cover crop and weed were amended with coral lime (195 kg/ha), gypsum (7,178 kg/ha), and magnesium sulfate (2,397 kg/ha) to achieve pH 6.0. This is because previous experience showed that rapeseed could not grow in highly acidic soil. Pineapple beds, which were not limed because pineapple is very susceptible to *Phytophthora* spp. at pH > 5 (Rohrbach and Schmitt, 1998), were treated as the standard control for plantation practice in cycle I. Pineapple and cover crops were irrigated weekly with 252 m^3 water/ha. Cover crops were fertilized bimonthly with a 19-19-19 (N-P_2O_5-K_2O) fertilizer at a total weight of 753 kg/ha/year. Pineapples were fertilized according to standard plantation practice (400 kg/ha/year for N and K, and 5 kg/ha/year for Fe) and induced to flower 12 months after planting with ethephon (Ethrel, Aventis Environmental Science, Montvale, NJ) at 89 kg a.i./ha. Twenty-three months after planting pineapple in cycle I, plots were mowed, plowed, and replanted within 1 month of plowing by alternating the pineapple and cover crop beds (Fig. 1). In cycle II, pineapple and cover crops were cultivated and managed as described earlier. Pineapple fruits were harvested at 20 months after planting.

Soil was sampled before planting and at 3-month intervals thereafter. Ten soil cores were collected from the top 20 cm with an 8-cm-diam. soil bucket auger in

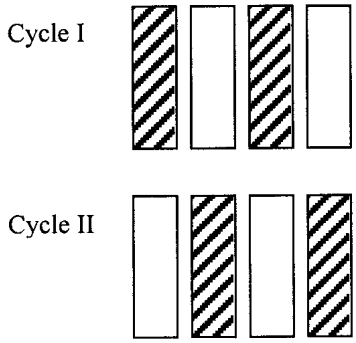

Fig. 1. Planting bed arrangement in a treatment plot in pineapple intercropping trial. Pineapples, , were intercropped with cover crop, , in the first cycle (cycle I), and the planting beds were reversed in the second cycle (cycle II).

a Z pattern, mixed, and sieved through a 4-mm opening (No. 5 mesh) screen. Roots collected on the screen were bagged separately from the soil sample and were the rhizosphere sample. Nematodes were extracted from a subsample of 250 cm^3 soil by elutriation and centrifugal flotation (Byrd et al., 1976). Nematode eggs and vermiform stages in the rhizosphere samples were extracted using NaOCI and centrifugal flotation (Hussey and Barker, 1973). Plant-parasitic nematodes were identified to species, and bacterivorous and fungivorous nematodes were identified at the trophic level. Because *R. reniformis* and *M. javanica* were the dominant species of plant-parasitic nematodes, only data for these species will be reported.

A cowpea bioassay was conducted for samples collected from cycle I to test the viability of *R. reniformis*. Cowpea was planted into a 300-cm^3 subsample of soil from each plot and grown for 6 weeks in a greenhouse. Vermiform nematodes and eggs were extracted as described.

Nematode-trapping fungal population densities were quantified by soil dilution in combination with a most probable number estimation (Persmark and Jansson, 1997). 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 containing 100 mg streptomycin/liter, giving 0.05, 0.005, and 0.0005 g soil per plate. Each dilution had three replicate plates. Three control plates without soil solution were used per sample. For samples collected from 0 to 23 months after pineapple planting, 100 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 420 *g* for 3 minutes. The rinsed solution was then suspended with 1,000 mg/liter streptomycin sulfate solution and incubated overnight. Eggs were 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 before concentrating to the desired densities (Ko, pers. comm.). Species of NTF from each plate were identified 3 weeks after plating (Cooke and Godfrey, 1964). These fungi were categorized into two groups according to their ecological behavior: saprophytic or parasitic. The saprophytic group consists of NTF characterized by sticky three-dimensional networks and nonspontaneous trap formation (Cooke, 1963). Trap formation is induced in the presence of nematodes, or even exudates and homogenates of nematodes (Nordbring-Hertz, 1973). The parasitic group consists of NTF that form constricting rings, adhesive knobs, or adhesive branches. These fungi form traps spontaneously, and thus are more effective trappers (Cooke, 1963). The fungal population densities were estimated with a most probable number (MPN) program (Woomer et al., 1990).

One problem encountered in this procedure was the failure of the eggs to hatch in the dishes. Therefore, a modification of the previous method was adopted from Jaffee and Muldoon (1995) for samples collected 17 to 35 months after the first pineapple planting. A 100-g soil sample was suspended in 200 ml sterile distilled water followed by 2 series of 10-fold dilutions. A 100-µl aliquot of each dilution was plated on 1/4-strength corn meal agar amended with 100 µg/liter streptomycin, giving 0.05, 0.005, and 0.0005 g soil per plate. Approximately 100 axenically cultivated *Steinernema glaseri* were added to each plate as bait. The NTF species were determined 3 weeks after plating as described earlier. An MPN-table for 10-fold dilutions with 5 plates per dilution was used to quantify the number of NTF propagules per g of soil (Woomer et al., 1990).

Species and percent coverage of each weed were determined at 11 months after cycle I began. A 0.25-m^2 square quadrate was randomly placed at 10 different areas within a weed plot and the percentage of area covered by each weed within the quadrate recorded. Weeds commonly present were fireweed (*Erigeron canadensis*), goosegrass (*Eleusine indica*), ageratum (*Ageratum conyzoides*), emilia (*Emilia sonchifolia*), and violet crabgrass (*Digitaria violascens*) along with the three cover crops and pineapple. Five plants of each weed and crop, collected randomly, were used to extract *R. reniformis* vermiform stages and eggs (Hussey and Barker, 1973).

Rotylenchulus reniformis population densities and pineapple growth and yield were subjected to analysis of variance using SAS (general linear model procedure, SAS Institute, Cary, NC). 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 NTF, no geometric models fit the population progressive curves over time. Therefore, data from all sampling dates were subjected to repeated measure analysis, treatments were the main plots and sampling time the subplot (Campbell and Madden, 1990) 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 to detect treatment effects. Least-square means of treatments were separated by adjusted Tukey test ($P = 0.05$), where appropriate.

Results

During the first pineapple intercrop cycle (cycle I), repeated measures analysis indicated that population densities of *R. reniformis* were lower in sunn hemp and rapeseed planted beds as compared to pineapple ($P < 0.05$; Table 1). Rapeseed maintained the lowest *R. reniformis* population density throughout the cycle, with the final population density similar to the initial density

TABLE 1. Population densities of *Rotylenchulus reniformis,* nematode eggs, *Meloidogyne javanica,* and bacterivorous nematodes on first (cycle I) and second (cycle II) pineapple intercrop cycles.

Plant	*R. reniformis*/250 cm^3 soil		Nematode eggs/g root		*M. javanica*/250 cm^3 soil		Bacterivorous nematode/250 cm^3 soil	
	Cycle I							
Sunn hemp	781 bc		1,078 b		2 c		885 a	
Rapeseed	261 c		31,614 a		1,346 a		394 b	
Marigold	1,718 ab		5,626 b		6 bc		123 b	
Weeds	1,707 ab		12,737 ab		104 b		226 b	
Pineapple	2,951 a		824 b		25 bc		221 b	
	Cycle II							
	I	P	I	P	I	P	I	P
Sunn hemp	1,262 b	2,464 b	154 b	4,614 a	65 b	19 b	242 a	391 a
Rapeseed	1,336 b	293 c	1,888 a	10,786 a	408 a	595 a	194 a	401 a
Marigold	2,408 ab	5,162 a	93 b	8,460 a	32 b	6 b	54 b	108 a
Weeds	4,234 a	4,512 a	38 b	8,943 a	46 b	40 b	248 a	230 a

Data were collected only from intercrop plots in cycle I, whereas data were collected from intercrop (I) and pineapple plots in cycle II.

Values are least-square means over 23 and 15 months in cycles I and II, respectively. Means in the same column followed by same letters are not different according to repeated measure tests followed by adjusted Tukey test ($P = 0.05$) for the corresponding log ($x + 1$) value.

(Fig. 2A). However, rapeseed supported the highest population densities of *M. javanica* (Table 1) and had the highest number of nematode eggs in the rhizosphere (Table 1). Population densities of *R. reniformis* on sunn hemp gradually increased to a moderate level in cycle I (23 months after planting) (Fig. 2A). Weeds and African marigold maintained similar population densities of *R. reniformis* as the pineapple (Table 1; Fig. 2A). Similar results were obtained in cycle II.

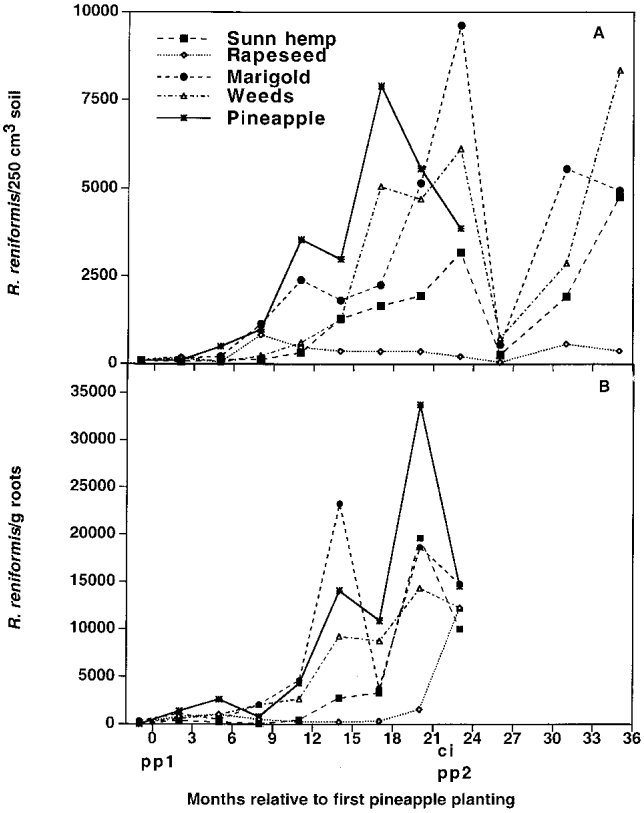

FIG. 2. Population progressive curve for *Rotylenchulus reniformis* (in 250 cm^3 soil) in A) pineapple intercrop trial; B) cowpea bioassay of soil collected from sunn hemp, rapeseed, marigold, weed, and pineapple in a pineapple intercrop trial. pp1 = first pineapple planting; pp2 = second pineapple planting, ci = crop incorporation.

During cycle II, when the cover crops were planted on plots previously planted with pineapple, population densities of *R. reniformis* in general were higher than those in cycle I (maximum of 2,951 in cycle I, and 4,234 in cycle II; Table 1). According to the *R. reniformis* reproductive factor (Rf = population densities at 3 months after planting in cycle II / population densities after cycle I), plots previously planted to sunn hemp (Rf = 0.17) suppressed *R. reniformis* more effectively than those grown with weeds (Rf = 1.59; $P < 0.05$).

Populations of *R. reniformis* on cowpea in the bioassay were lower when planted in soil collected from sunn hemp and rapeseed beds than those planted in soil from pineapple and African marigold beds (Table 2; Fig. 2B). The number of eggs was lower from cowpea planted in soil collected from sunn hemp beds than those in soil collected from pineapple, marigold, and weed beds ($P < 0.05$; Table 2). Resurgence of *R. reniformis* and *M. javanica* in cycle I also can be evaluated from the repeated measure analysis of the nematode population densities over the 15 months in cycle II.

TABLE 2. Repeated measure analysis of the population densities of *Rotylenchulus reniformis* and nematode eggs in the cowpea bioassay of the first intercrop cycle.

Plants in cycle I	*R. reniformis*/250 cm^3 soil	Nematode eggs/g roots
Sunn hemp	1,566 bc	29,574 b
Rapeseed	356 c	66,123 ab
Marigold	2,710 a	95,232 a
Weeds	2,347 ab	65,164 a
Pineapple	5,276 a	86,764 a

Least-square means over 20-month period. Values in the same column followed by same letters are not different according to adjusted Tukey test ($P = 0.05$) for the corresponding log ($x + 1$) value.

Results consistent with the cowpea bioassay were obtained. Population densities of *R. reniformis* were lower on pineapple planted in plots that previously contained rapeseed and sunn hemp than those that previously contained marigold and weed ($P < 0.05$; Table 1). Numbers of *M. javanica* remained highest in pineapple planted in plots that previously contained rapeseed ($P < 0.05$; Table 1).

Among the weed species, fireweed and goose grass maintained populations of *R. reniformis* similar to those of pineapple (Table 3). Ageratum, fireweed, and goose grass had the highest number of nematode eggs per g root among the weeds. Emilia also had a relatively high number of nematode eggs per g root among the plants tested. Fireweed, goose grass, ageratum, and emilia covered 32.75% of the weedy beds (Table 3). African marigold supported intermediate population densities of *R. reniformis,* whereas sunn hemp and rapeseed were poorer hosts of *R. reniformis* than pineapple (Table 3). However, a high number of nematode eggs per g roots was recovered from rapeseed. Although the proportion of eggs of *R. reniformis* and *M. javanica* was not determined, data from Table 1 indicated that population of *M. javanica* was higher in rapeseed followed by weed plots. Pineapple, violet crabgrass, and sunn hemp had lower numbers of nematode eggs in their roots (Table 3).

Bacterivorous nematode populations were higher on sunn hemp than any other crop in cycle I (Table 1; Fig. 3), but this effect was lost when the plot was replanted to pineapple (Table 1). The number of bacterivorous nematodes was low in the cover crop plots in cycle II and was not different among treatments with the exception of a lower value in the marigold plots (Table 1), which had poor crop establishment (>50% of the marigold senesced). Fungivorous nematode numbers were higher in the weeds (112/250 cm^3 soil) than the pineapple (41/250cm^3 soil) ($P < 0.05$), but were not different among the other cover crop treatments.

The three cover crops tested maintain higher densities of total NTF (either saprophytic or parasitic) than pineapple or weeds during cycle I ($P < 0.05$; Table 4). Sunn hemp and African marigold had higher saprophytic NTF population densities relative to rapeseed ($P < 0.05$), but rapeseed had higher numbers of parasitic NTF relative to other treatments ($P < 0.05$). Nematode-trapping fungal propagules in sunn hemp beds increased to a higher level earlier (14 months after planting) than the other treatments (Fig. 4A). The fungal populations decreased sharply but increased again during month 23. Nematode-trapping fungal populations in rapeseed and African marigold beds increased only at 20 months after first planting (Fig. 4A). Although the data for NTF assayed with *S. glaseri* were not collected early in cycle I, the maximum number of NTF propagules measured in the assay with *S. glaseri* was 12.5 times greater than that recovered from the assay without *S. glaseri* (200 vs. 16 propagules/g soil, respectively; Fig. 4). Repeated measure analysis of total NTF population quantified with *S. glaseri* was higher in the cover crop plots than weed and pineapple plots in cycle I (Table 4). Fungi recovered included the saprophytic

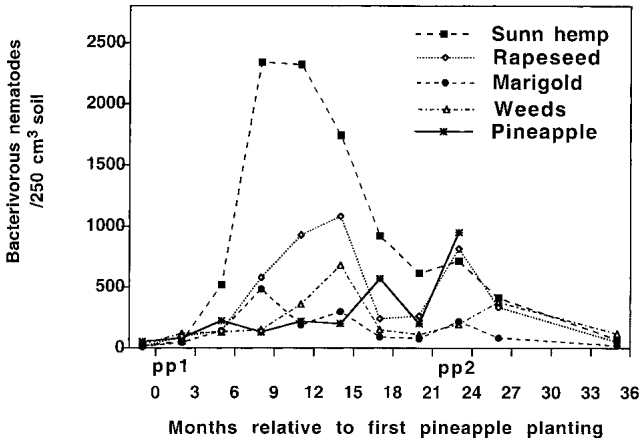

FIG. 3. Population progressive curve for bacterivorous nematodes (in 250 cm^3 soil) on sunn hemp, rapeseed, marigold, weed, and pineapple in a pineapple intercrop trial. pp1 = first pineapple plating; pp2 = second pineapple planting, ci = crop incorporation.

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

Plant	*R. reniformis*/ g root	Nematode eggs/ g root	Dry root weight (g)	% coverage
Fire weed	1,794 a[z]	16,040 a[y]	1.57 bcd	13.25
Pineapple	821 a	38 bc	3.52 ab	—
Goose grass	291 ab	6,036 a	1.05 cd	4.00
African marigold	351 ab	1,602 a	4.35 a	—
Rapeseed	100 bc	16,593 a	4.70 a	—
Ageratum	44 bc	17,661 a	3.20 abc	12.00
Emilia	30 bc	560 ab	0.80 d	3.50
Violet crabgrass	26 c	64 bc	0.65 d	32.12
Sunn hemp	15 c	16 c	4.15 a	—

Means followed by the same letter were not different according to Duncan's Multiple *k*-ratio ($k = 100$) *t*-test. Data were transformed logarithmically, $\log_{10}(x + 1)$, before statistical analysis.

TABLE 4. Repeated measure analysis of population densities of nematode-trapping fungi (NTF) on sunn hemp, rapeseed, marigold, weed, and pineapple plots during the first and second intercropping cycles.

Plant	NTF propagules/g soil					
	Saprophytic	Parasitic	Total	Saprophytic	Parasitic	Total
	Cycle I			Cycle II		
Sunn hemp	52 a	1 b	53 a	8 a	1.11 a	9 a
Rapeweed	4 b	100 a	104 a	12 a	0 a	12 a
Marigold	78 a	0 b	78 a	9 a	0 a	9 a
Weeds	14 ab	6 b	20 b	5 a	0 a	5 a
Pineapple	7 ab	0 b	7 b	—	—	—

Values are least-square means over 20- and 15-month periods in cycles I and II, respectively. Values in the same column followed by the same letters are not different according to adjusted Tukey test ($P = 0.05$) for the corresponding log $(x + 1)$ value.

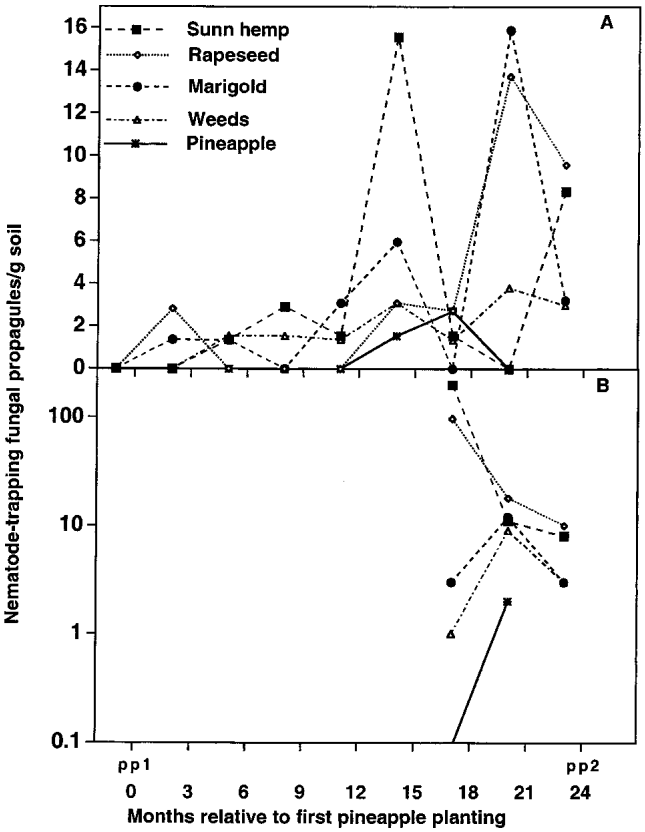

FIG. 4. Population progressive curve of nematode-trapping fungi (propagules/g of soil) in the first cycle of pineapple intercrop trial. Fungi were quantified on A) 1% water agar with *Rotylenchulus reniformis* eggs, and B) quarter-strength corn meal agar baited with *Steinernema glaseri*. pp1 = first pineapple planting; pp2 = second pineapple planting; ci = crop incorporation.

NTF *Arthrobotrys oligospora* and *A. eudermata* that form adhesive networks, and the parasitic NTF *Dactylellina ellipsospora* and *Dactylaria sclerohypha* that form adhesive knobs.

Effect of previous cover crop on pineapple yield was inconclusive. Although average fruit weight was higher following sunn hemp than following other treatments ($P < 0.05$, data not shown), number of commercial fruits (fruit weight >1 kg) and commercial fruit weight were not different between plots previously cropped to sunn hemp and weeds (data not shown).

DISCUSSION

Among the cover crops tested, sunn hemp and rapeseed were most effective in suppressing *R. reniformis* populations. Rapeseed was not a host for *R. reniformis,* which supports the report by Stoynov (1967). *Rotylenchulus reniformis* failed to reproduce on rapeseed throughout the intercropping cycle. Sunn hemp is a poor host to *R. reniformis,* allowing the nematode to penetrate the roots but restricting development and reproduction (Caswell et al., 1991). Silva et al. (1990) could not find females of *R. reniformis* on sunn hemp roots, indicating incomplete or no development. Our results confirm these previous reports (Caswell et al., 1991; Silva et al., 1990) because *R. reniformis* produced few progeny on sunn hemp and rapeseed. *Rotylenchulus reniformis* populations increased slowly on sunn hemp, but not on rapeseed, at the end of the pineapple cycle. The fact that rapeseed is a host of *M. javanica,* competition between *M. javanica* and *R. reniformis* could have resulted in lower *R. reniformis* population levels on this crop. The susceptibility of rapeseed to *M. javanica* would make it unsuitable as an intercrop for pineapple because this nematode is a pathogen of pineapple (Caswell et al., 1990), often occurs at low levels in Hawaiian pineapple fields, and could increase to damaging population densities.

Previous research indicates that using cover crops as intercrops seldom suppresses plant-parasitic nematodes sufficiently. Powers et al. (1993) demonstrated that intercropping cucurbits with alfalfa (*Medicago sativa*) or French marigold (*Tagetes patula*) had no influence on densities of various nematode genera or trophic groups as compared to monocropped cucurbits. Intercropping raspberry (*Rubus idaeus*) with oat (*Avena sativa*), creeping red fescue (*Festuca rubra*), or redtop (*Agrostis alba*)—all resistant to the lesion nematode *Pratylenchus penetrans*—suppressed population densities of this nematode in the cover crops, but raspberry plant vigor was not increased (Vrain et al., 1996). Our research demonstrated that an intercropping system could suppress *R. reniformis* effectively by planting pineapple on the beds previously planted with cover crop.

Unlike the findings of Linford and Yap (1940) and Birchfield and Brister (1962), African marigold employed in this experiment was a host to *R. reniformis.* Resistance of African marigold to *R. reniformis* varies among cultivars and could be temperature dependent, as is its susceptibility to *M. incognita* (Ploeg and Maris, 1999). The lower number of *R. reniformis* on African marigold planted in cycle II was due to poor crop establishment caused by a heavy infestation of marigold thrips (*Neohydatothrips pseudoannulipes*).

Some of the weeds commonly present in this test plot are hosts to both *R. reniformis* and *M. javanica.* Although numbers of vermiform stages of *M. javanica* from the weeds were not recorded, high numbers of nematode eggs and low numbers of vermiform stages of *R. reniformis* recovered from ageratum and emilia suggested that the nematode eggs could be eggs of *M. javanica.* Higher numbers of *M. javanica* collected from the rhizosphere of rapeseed and weed plots also supported this finding.

Rapid buildup of *R. reniformis* population levels in the pineapple beds demonstrated potential nematode problems in continuous monoculture of pineapple. The common practice of fallow with weeds also poses a potential problem because some weeds are hosts of *R. reniformis* and *M. javanica.* When a host is absent, *R. reniformis* can undergo anhydrobiosis, a survival strategy

(Womersley and Ching, 1989). The purpose of the cowpea bioassay was to test the viability of *R. reniformis* and ensure that nematodes that have undergone anhydrobiosis in the cover crop planted plots are detected. The results from the cowpea bioassay were consistent with *R. reniformis* population resurgence after pineapples were planted in cycle II, thus providing an earlier detection method for *R. reniformis* population changes for subsequent pineapple planting.

The long-term culture of sunn hemp in this intercropping system elevated bacterivorous nematode population densities relative to other treatments, indicating higher microbial activities under sunn hemp cultivation. This is consistent with the result of Venette et al. (1997) in which bacterivorous nematodes, specifically *Acrobeloides bodenheimeri,* were stimulated in the rhizosphere of sunn hemp. Baath et al. (1981) suggested that the abundance of bacterivorous nematodes should be considered as an indicator of previous bacterial biomass. Using bacterivorous nematodes as indicators could provide more information than direct measures of bacterial populations (Clarholm et al., 1981). It has been proposed that increased bacterial growth enhances bacterial grazers, including bacterivorous nematodes, which leads to the development of nematophagous fungi and bacteria that may in turn regulate non-bacterivorous nematodes, including plant-parasitic nematodes (Cooke, 1962, 1963; Cooke and Godfrey, 1964; Linford et al., 1938). Recently, van den Boogert et al. (1994) determined that the enhancement of *Drechmeria coniospora* (an NTF) population densities by lucerne meal (ground alfalfa tissue) relied upon a concomitant population of bacterivorous nematodes. An additional benefit of the presence of bacterivorous nematodes is the enhancement of nitrogen mineralization due to nematode grazing of bacterial biomass (Bouwman et al., 1994).

The greater number of NTF propagules in the cover crops compared to weeds or pineapple beds indicates that the cover crops were more efficient in enhancing NTF activities. One concern in our experimental design is that, due to the poor growth of rapeseed in low pH soil, pH of all the cover crops and weed plots were adjusted to 6.0, whereas pH in pineapple plots remained 4.9 to avoid *Phytophthora* rot (Rohrbach and Schmitt, 1998). Therefore, we might have created an uneven pH among the treatments for NTF evaluation. However, nematode-endoparasitic and NTF were detected more frequently in acidic than in neutral soils of Ireland as well as vineyard soil in California (Gray, 1985; Jaffee and Zasoski, 2000), and the activity of *Hirsutella rhossiliensis* declined to near zero as the pH dropped below 4.0 (Jaffee and Zasoski, 2000). Pineapple plots in this experiment with pH 4.9 would be expected to have a better pH for NTF, but instead the NTF population densities were lower than cover crops. Previously, we had determined that sunn hemp amendment enhanced higher parasitic NTF propagules than pineapple amendment without pH adjustment in typical acidic pineapple soil (Wang et al., 2001). In addition, the cover crops still had higher population densities of NTF than the weed control with pH adjusted to 6.0. If sunn hemp were to be recommended as a cover crop in Hawaiian pineapple production, soil pH need not be adjusted and thus would provide a more favorable environment for NTF establishment. Previous experiments demonstrated that the rhizosphere of legumes increased NTF population densities compared to those of non-legumes (Persmark and Jansson, 1997). In this intercrop experiment, the NTF assay was conducted on soil adjacent to the crop rhizosphere to examine cover crop effects on root-free soil. Although no differences in NTF populations accumulated over time between the leguminous and non-leguminous cover crops, the density of NTF in sunn hemp, a legume, increased sooner than the other crops.

The NTF population progressive curve observed in cycle I demonstrated that the fungal population established slowly. In fact, the first peak of the NTF population did not occur until 14 months after cover crop planting. This supported our hypothesis that cover crops need to be grown for a long period (14 months) to affect NTF populations. Since the NTF data collection for cycle II was terminated at 15 months after planting, an extensive increase in NTF population levels might have been missed. However, the fungal population decreased sharply after sunn hemp was incorporated. It is most probable that the NTF population will increase again after a period of establishment as occurred in the intercycle cover-cropping system (Wang et al., 2002b).

Effect of the cover crops in the intercrop system on pineapple yield was inconclusive due to several confounding factors. Although sunn hemp-cropped plots produced higher average pineapple fruit weight than the weed-treated plots, their commercial yields were not different. The field was severely infested with pineapple mealybug-associated virus. The diseased plants were scattered among the plots and thus created a high variation in the treatment effect. Sunn hemp in the intercropped bed shaded the pineapple grown beside it and perhaps reduced the pineapple photosynthesis to a rate below optimum for commercial grade production. This is one disadvantage of using sunn hemp as an intercrop treatment. Low yield in plots previously planted with African marigold was due to heavy infestation of marigold thrips (*Neohydatothrips pseudoannulipes*) on the marigold and resulted in senesces and lower cover crop biomass production in these plots.

We conclude that, although both sunn hemp and rapeseed can reduce *R. reniformis* population densities, neither may be good candidates to intercrop with pineapples. Although sunn hemp suppressed *R. reniformis* effectively when intercropped with pineapple, it is more

suitable as an intercycle crop due to its shading effect on the pineapple during intercropping. Other plant species that will not shade the pineapple should be investigated as potential intercrop candidates. On the other hand, planting rapeseed will increase *M. javanica* population densities. As we had expected, prolonged culture of cover crops enhances NTF populations over time. Therefore, growers will benefit from planting sunn hemp at a longer period while shortening or replacing the fallowing period, which commonly ranges from 6 to 12 months for Hawaiian pineapple production (Caswell et al., 1990). While establishment of nematode-antagonistic microorganisms will take time, cover cropping will be compatible with environmentally sound nematode management approaches or biological-based nematicides that are currently under investigation in our pineapple projects (Sipes and Wang, 2000). Thus, this result will provide more opportunities for the development of an integrated pest management strategy for nematode management in pineapple production.

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