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Changes in Plant-Parasitic Nematode Populations in Pineapple Fields Following Inter-Cycle Cover Crops[1]

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Abstract: The use of plant-covers oat (*Avena sativa* L.), rhodesgrass (*Chloris gayana* Kunth), soybean (*Glycine max* [L.] Merr.), and marigold (*Tagetes patula* L.) during pineapple inter-cycle planting periods was investigated at two sites (Kunia and Whitmore, Oahu, HI) as a potential means to reduce population densities of *Rotylenchulus reniformis, Helicotylenchus dihystera,* and *Paratylenchus* spp. Clean fallow and fallow covered with pineapple-plant residues (mulch) were the controls without plant-cover. Regardless of treatments, population densities of *R. reniformis* declined with time at both sites to low residue levels by the end of the 6-month period. Treatment means of *R. reniformis* population densities in the plant-cover treatments were lower than the controls' ($P = 0.05$). The plant-cover treatments also effected higher rates of R. reniformis population decline at both sites during the period, being 2.0 to 2.2 times that of the mulch control and 1.2 to 1.4 times that of the fallow control. Plant-covers' effect on *H. dihystera* during the same period at both sites was variable, resulting in decreased, unchanged, or increased population densities. The change was especially obvious in the oat-cover treatment, where *H. dihystera* population densities increased 9 to 15-fold at both sites. Population of *Paratylenchus* spp. was absent or present at low levels at the sites throughout the period. Biological activities antagonistic to *R. reniformis* at Kunia were estimated at the end of 6 months by comparing the extent of nematode's reproduction (on cowpea seedlings) in the treatment soils that had been subjected to autoclaving or freezing temperature. Although higher indices of antagonistic activities were observed in soils with prior plant-cover treatments than in soils from the controls, none of the treatments resulted in conferring soils the increased ability to suppress re-introduced *R. reniformis* populations or enhance subsequent pineapple-plant growth.

Key words: Ananas comosus, antagonistic plant, fallow, freezing soil, *Helicotylenchus dihystera,* marigold, nematode management, oat, *Paratylenchus,* plant-cover, rhodesgrass, *Rotylenchulus reniformis,* soybean.

Plant-parasitic nematodes are widespread in farms and plantations of Hawaii (10). The most prevalent species in pineapple (*Ananas comosus* (L.) Merr.) fields are *Rotylenchulus reniformis* Linford & Oliveira, *Helicotylenchus dihystera* (Cobb) Sher, *Paratylenchus* spp. Micoletzky, and *Meloidogyne javanica* (Treub) Chitwood (8,10). In particular, *R. reniformis* is one of the limiting factors in pineapple production (10). Current management of these nematodes in pineapple fields is primarily accomplished by fallow (up to 12 months), preplant fumigation, and post-plant nematicide application (10). Although fallow is used to reduce densities of *R. reniformis,* its effectiveness varies with soil moisture. The nematode can survive for as long as 1.5 years in dry fallow soils (2,36). Other concerns with clean fallow include energy requirement for cultivation, soil erosion, reduced soil fertility, and loss of beneficial micro-organisms such as mycorrhizae (35).

Nematicide provides the most effective nematode control in pineapple fields (8). Due to increasing concerns about the impact of these chemicals on the environment and human health, alternatives to nematicide are desirable. For pineapple these may include crop rotation, cover-crops during the inter-cycle period, or other practices resulting in reducing nematode numbers or enhancing the activities of resident nematode antagonists (16,25). Several plant species such as marigold (*Tagetes patula* L.) (17,18,28), rhodesgrass (*Chloris gayana* Kunth), and sunn hemp (*Crotalaria juncea* L.) (5,9,11) have been shown to reduce populations of several plant-parasitic nematode species, including *R. reniformis.* Information on their influence on population dynamics of

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nematodes as well as on the nematode's antagonists is needed in order to make wise use of plant-covers for nematode management.

The objectives of this research were to determine the effect of selected cover crops on the (i) temporal changes in population densities of *R. reniformis, H. dihystera,* and *Paratylenchus* spp. in pineapple fields during the growing phase of cover-crops, (ii) activities of nematode antagonist(s) in soil following each cover-crop treatment, and (iii) effect of cover-crops on re-introduced nematode populations and subsequent pineapple growth in soils following the cover-crop treatments. A preliminary account of this work has been reported (24).

Materials and Methods

Experimental sites, treatments, and design: The experiments were conducted at two sites on the island of Oahu, Hawaii, during November 1990 to June 1991. One site was located at the University of Hawaii Plant Pathology Pineapple Field facility at Whitmore (244 m elevation) and the other at a Del Monte plantation field at Kunia (304 m elevation). The sites had not been cropped with pineapple for 6 and 18 months, respectively, prior to these experiments. The soil at both sites is a fine kaolinitic, isothermic, Ustoxic, Humitropept, Inceptisol (14). Initial numbers of *R. reniformis, H. dihystera,* and *Paratylenchus* spp. averaged 4,000, 600, and 90/250 cm^3 soil at Kunia, and 600, 300, and 0/250 cm^3 soil at Whitmore, respectively. Each experimental plot was 3.1×3.7 m^2 at Whitmore and 1 m^2 at Kunia. Seven treatments each with five (Kunia) or six (Whitmore) replicates were arranged in a randomized completed block design. The treatments were: (i) mulch—fallow covered with pineapple-plant residues (a common plantation practice at Oahu), (ii) clean fallow—fallow without weeds, (iii) no-till—fallow with weeds, (iv) rhodesgrass (*Chloris gayana* Kunth) 'Katambora' seeded at 39 kg/ha, (v) marigold (*Tagetes patula* L.) 'Boy O Boy' seeded at 28 kg/ha, (vi) oat (*Avena sativa* L.) 'Hazel' seeded at 106 kg/ha, and (vii) soybean (*Glycine max* (L.) Merr.) 'Kirby' seeded at 196 kg/ha.

Temporal changes in population densities of nematodes during cover-crop growing phase: Soil samples were collected in a Z- or X-pattern at 2-month intervals 5 to 20 cm deep, with seven cores of 150 cm^3 each from the large plots at Whitmore (October 1990 to April 1991) or five cores of the same volume from the small plots at Kunia (November 1990 to May 1991). Each sample was passed through a 1-cm-pore sieve, mixed thoroughly by hand coning and divided into three parts of 250 cm^3 each. One portion was processed immediately by elutriation (7) and centrifugal flotation (22). The second portion (AuS) was wrapped in cheesecloth, autoclaved for 30 minutes at 121 °C and 103.4×10^3 Pa, and then immediately aerated for 48 hours. The third portion (FzS) was placed in a plastic bag, moisture adjusted to 25% gravimetrically, and then frozen at −4 °C for 48 hours to kill most of the resident *R. reniformis* in the soil. Preliminary experiments have shown that freezing in this manner killed 96% of this resident *R. reniformis* population (Ko, unpubl.).

Effects of cover-crops on biological activity antagonistic to R. reniformis at end of cover-crop growing phase: The bioassay to determine the indices was modified from the one used to predict biotic replant problems in orchards (15). It was adopted here by comparing the extent of *R. reniformis* reproduction on cowpea grown in autoclaved soil (AuS) versus that in frozen soil (FzS). Specifically, the assay was conducted in the following manner: each AuS or FzS subsample was placed in a 7.6-cm-diam. clay pot, re-infested with 1,000 *R. reniformis* eggs, and then planted with a 5-day-old cowpea seedling (*Vigna unguiculata* (L.) Walpers). After 6 weeks, eggs or vermiform nematodes were recovered from each cowpea seedling using a modified hypochlorite technique (21) or from soil using the Baermann-funnel technique (4). Antagonistic activity index (Ix), Ieg, Ivm,

or Ifm, was defined as the number of eggs per gram root, number of vermiform per gram root, or number of kidney-shape females per plant in AuS divided by the corresponding numbers in FzS, respectively; that is, Ix = AuS/FzS. An Ix value of <1.0, 1.0, or >1.0 indicates the presence of biotic stimulation, absence of biotic effect, or presence of biotic inhibition on *R. reniformis* reproduction, where biotic refers to the influence exerted by soil organisms that survived the freezing.

Effect of cover-crops on subsequent pineapple growth and re-introduced nematode densities: An additional 1.5 kg of soil was collected from each treatment plot at 6 months after planting (May 1991 at Kunia and June 1991 at Whitmore, but only data from Kunia were shown here) to determine the residual effect of the plant-cover or control treatments on pineapple growth as well as on the re-introduced (inoculant) nematode populations. The soil samples from each treatment were first passed through a 1-cm-pore sieve to remove root debris, their moisture content adjusted to 25% by weight, and then subjected to −4 °C temperature for 48 hours to reduce the resident nematode populations. Each soil sample was then placed in 15-cm-diam. clay pots, re-infested with about 1,800 individuals of *R. reniformis* and 800 of *H. dihystera,* the same numbers as found in the infested field soil (IS) from the mulch control. IS soil was used as the nematode-positive control. The same soil frozen (FR) at −4 °C for 48 hours was used as the nematode-negative control. A single pineapple crown (cv. Smooth Cayenne) was transplanted into the center of each soil and allowed to grow for 7 months (June 1991–March 1992) in the greenhouse. Plant-growth parameters such as fresh or dry weights (measured after drying to constant weight at 70 °C) of root, shoot, and D-leaf, which is the longest leaf with terminating growth (32) that correlates highly with weight of the pineapple fruit (30), were measured. Eggs or vermiform stages of the R. *reniformis* were extracted from the pineapple roots or soil as described previously (4,21).

Correlation and data analysis: Pearson correlation coefficients were calculated between nematode population densities and parameters of pineapple growth, among Ieg, Ivm, or Ifm and nematode population densities, and among Ieg, Ivm, or Ifm and pineapple growth. Difference in treatments with respect to pineapple growth, nematode population densities, or antagonistic indices was tested by analysis of variance (ANOVA), and treatment means were separated by Waller-Duncan *k*-ratio *t* test ($P = 0.05$). In the regression analysis for temporal changes in nematode population under each cover-crop treatment, the nematode population densities (y) were transformed to y' by $\log_{10}(y + 1)$ to stabilize the variance before the ANOVA or regression analysis to examine the relationship between y' and time t (month after planting). Homogeneity of intercepts and slopes of the regression lines were tested to compare cover-crop effect by the general linear test (29). All analyses were performed with the help of SAS (SAS/STAT user's guide, release 6.03 ed., Cary, NC).

Results

Temporal changes in population densities of nematodes during the cover-crop growing phase: At Kunia, there was an interaction between time and treatment ($P = 0.05$). Treatment means of population density of either *R. reniformis* or *H. dihystera* (numbers transformed logarithmically) did not differ initially from one another but rather differed 3 or 6 months after the onset of the experiment ($P = 0.05$). Mean population of *R. reniformis* declined from initial densities of 3,300 to 5,300 vermiform stages to residual densities of 100 to 700 vermiform stages/250 cm^3 soil in the plant-cover treatments or controls over a 6-month period. The major decline occurred during the first 3 months. The plant-cover treatments also caused variable rates of *R. reniformis* population decline, some of which (e.g., oat from rhodesgrass) were different from one another ($P = 0.05$) (Table 1). The average rate of de-

TABLE 1. Linear regression of *Rotylenchulus reniformis* population densities (y) against month after planting (*t*) under various plant-covers. The nematode population densities *y* were transformed to *y'* with log10 (*y* + 1) before the regression analysis.

Location	Plant-cover	Slope[a]	Intercept[a]	R^2
Kunia	Mulch	−0.121 ± 0.016 a	3.549 ± 0.062	0.816**
	No-till	−0.159 ± 0.024 a	3.614 ± 0.093	0.771**
	Clean-fallow	−0.167 ± 0.015 ab	3.597 ± 0.059	0.902**
	Rhodesgrass	−0.204 ± 0.035 bc	3.467 ± 0.138	0.719**
	Marigold	−0.250 ± 0.085 cd	3.362 ± 0.329	0.400**
	Oat	−0.259 ± 0.042 d	3.394 ± 0.163	0.744**
	Soybean	−0.245 ± 0.020 cd	3.573 ± 0.076	0.922**
Whitmore	Mulch	−0.053 ± 0.044 a	2.426 ± 0.172	0.080(ns)
	No-till	−0.103 ± 0.032 ab	2.565 ± 0.122	0.400**
	Clean-fallow	−0.095 ± 0.024 ab	2.565 ± 0.092	0.497**
	Rhodesgrass	−0.137 ± 0.049 b	2.475 ± 0.189	0.330**
	Marigold	−0.097 ± 0.037 b	2.303 ± 0.143	0.299**
	Oat	−0.112 ± 0.026 b	2.562 ± 0.102	0.527**
	Soybean	−0.111 ± 0.031 b	2.576 ± 0.121	0.443**

[a] Parameter estimate ± SEM. Slopes with different letter in same location indicate significant difference ($\alpha = 0.05$) according to Neter and Wasserman general linear test (29).

cline (slope) caused by an incremental change in time was higher in plots covered with plants (oat, soybean, marigold, and rhodesgrass) than in plots not covered with plants (mulch or clean fallow), with the former rate being 2.0 times that of mulch and 1.2 times that of the fallow control ($P = 0.05$) (Table 1). At the end of 6 months, residual *R. reniformis* population in the mulch treatment was highest among all the treatments ($P = 0.05$). During the period, mean population density of *H. dihystera* decreased from initial densities of 500 to 875 to 100 to 240/250 cm^3 soil in clean fallow or marigold-cover treatment; increased by 9-fold in oat-cover treatment; and remained unchanged in no-till, mulch, rhodesgrass-, or soybean-cover treatment ($P = 0.05$). Mean population density of *Paratylenchus* spp. at this site was relatively low and tended to decline similarly (as *R. reniformis*) with time, decreasing from initial population densities of 30 to 180 to residual densities of 0 to 20/250 cm^3 soil. However, unlike *R. reniformis* or *H. dihystera*, there was no interaction between treatment and time, and *Paratylenchus* population densities differed little from one another throughout the whole period ($P = 0.05$).

At Whitmore, only *R. reniformis* and *H. dihystera* were detected and at one order of magnitude lower than the same nematodes at Kunia. At the end of 6 months, *R. reniformis* population density in the mulch treatment was again higher than any of the other treatment densities, which were not different from one another ($P = 0.05$). *R. reniformis* population densities in each of the treatments declined similarly but at slower rates than the respective treatment at Kunia (Table 1). Average rate of *R. reniformis* population decline in the plant-cover treatments was 2.2 times that of the mulch and 1.4 times that of the fallow control. *H. dihystera* population densities again increased 15-fold in the oat-cover treatment plots but remained unchanged in soybean, rhodesgrass, and mulch treatment plots, or decreased in marigold and clean fallow treatment plots ($P = 0.05$).

Effects of cover-crops on biological activities antagonistic to R. reniformis: The trends in antagonistic activities against *R. reniformis*, after an adjustment for differences in root growth (i.e., based on per-gram root tissue), were found to be generally higher and greater than 1.0 in plant-cover treatments than in the controls without any plant-cover. The pooled averages of the indices of antagonistic activity (Ieg = 1.5, Ivm = 1.4, and Ifm = 1.3) in the plant-

cover treatments were 1.5-, 1.3-, 1.6- fold higher, respectively, than the corresponding indices in the controls (Fig. 1A,B). There was a trend that marigold-cover treatment had the highest indices at both sites, although these indices did not differ significantly from those of other treatments ($P = 0.05$). At time of cover-crop harvest at Kunia, the correlations between numbers of *R. reniformis* in soil and Ieg ($r = 0.5$, $P = 0.01$), Ivm ($r = -0.5$, $P = 0.01$), or Ifm ($r = -0.43$, $P = 0.01$) were negative and significant, but R^2 values were low. Similar relationships were observed between *Paratylenchus* population densities and Ieg ($r = -0.25$, $P = 0.05$) or

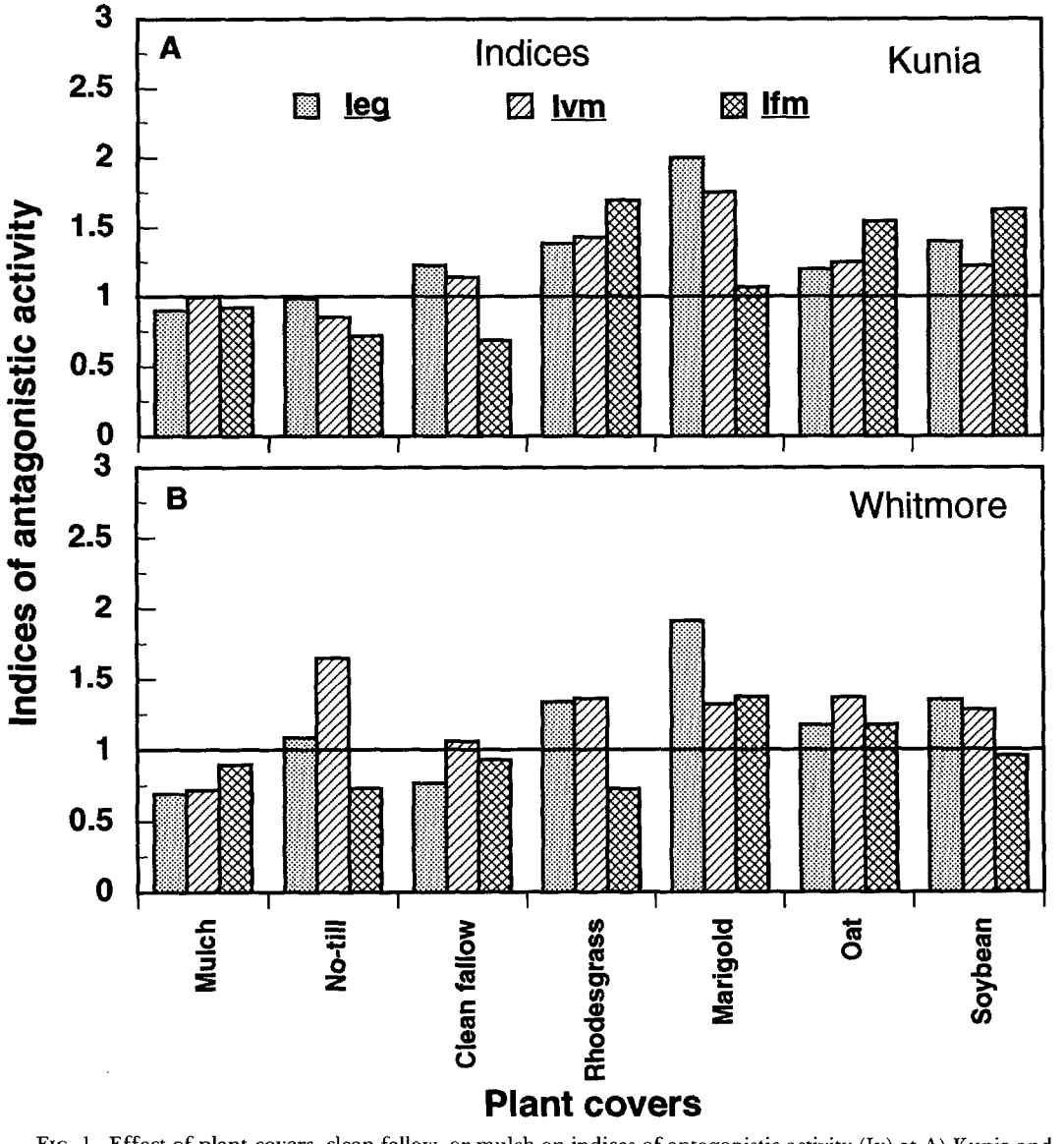

FIG. 1. Effect of plant-covers, clean fallow, or mulch on indices of antagonistic activity (Ix) at A) Kunia and B) Whitmore. Ix = *R. reniformis* reproduction on cowpea growing for 6 weeks (after inoculation) in autoclaved soil divided by its reproduction on cowpea growing similarly in same soil that was frozen for 48 hours at −4 °C. *R. reniformis* reproduction on cowpea was estimated by one of the following parameters: number of eggs per gram dry root (Ieg), number of vermiform nematode per gram dry root (Ivm), or number of kidney-shaped females per root system (Ifm). Mulch treatment was fallow-covered with pineapple-plant residues. Ix values of greater than 1.0 indicate antagonism.

Ivm ($r = -0.25$, $P = 0.05$) at Kunia, and between numbers of *R. reniformis* and Ieg ($r = -0.39$, $P = 0.05$)or Ivm ($r = -0.452$, $P = 0.01$) at Whitmore. There was no correlation between numbers of *H. dihystera* and any of the indices at either site.

Effect of cover-crops on subsequent pineapple growth and on re-introduced nematode densities: After 7 months, pineapple growth in terms of D-leaf dry weight was highest in FR (nematode-negative control) and lowest in IS (nematode-positive control) ($P = 0.05$). However, the D-leaf weight in soil previously cropped with oats, soybean, rhodesgrass, or marigold did not differ from that in the clean fallow or mulch ($P = 0.05$) (Fig. 2). Root dry weight was also significantly the highest in FR, but the weight in IS, or again in each of the other plant-cover treatments, did not differ from that in the fallow or mulch ($P = 0.05$) (Fig. 2). The correlation between the indices and subsequent pineapple growth was poor, with the exception that Ifm and root growth were weakly correlated ($r = -0.341$, $P = 0.05$).

Re-introduced nematode populations increased by various degrees in all the treatments during the 7-month bioassay period (Fig. 3). Specifically, *R. reniformis* increased by 12- to 125-fold from the inoculum density (Pi) of 1,800/250 cm^3 soil (Fig. 3A), and *H. dihystera* by 450- to 1,000-fold from the Pi of 800/250 cm^3 soil (Fig. 3B). Mean *R. reniformis* population density (both in terms of number of vermiform stages per 250 cm^3 soil or per gram root tissue) in each of the plant-cover treatments, FR, fallow, or mulch was lower than that in the originally infested field soil IS ($P = 0.05$). In particular, number of vermiform stage per 250 cm^3 soil ranged only one-sixth (oat-cover treatment) to one-tenth (soybean-cover treatment) times as much as that in IS (Fig. 3A). However, this nematode's population density in any of the cover-crop treatments did not differ from that in the fallow or mulch treatment

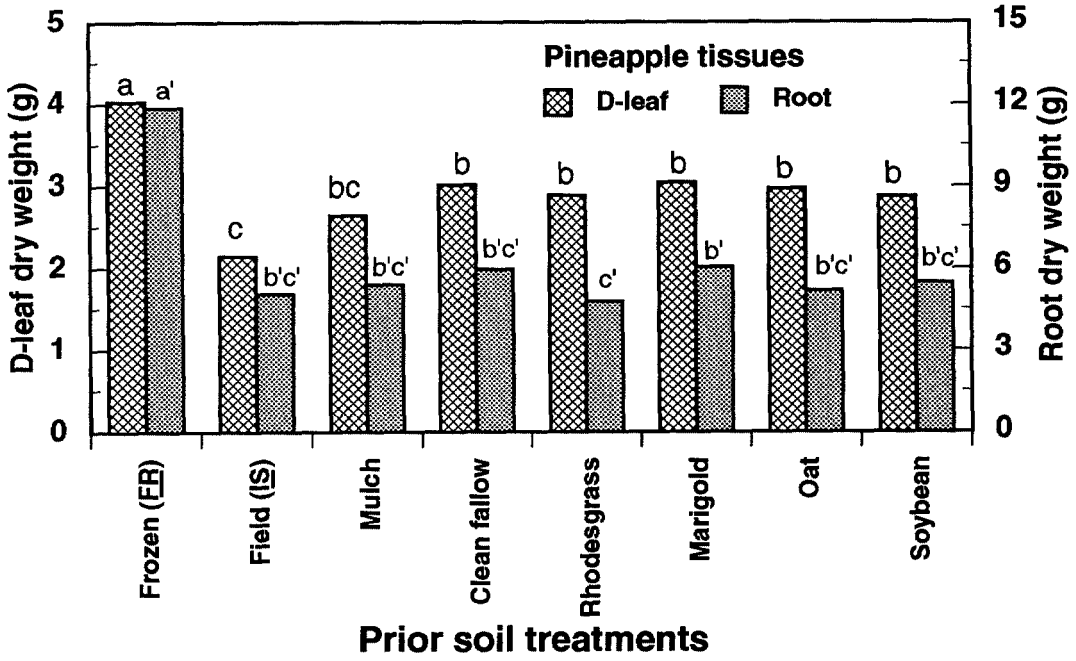

FIG. 2. Residual effect of plant-covers, clean fallow, or mulch on subsequent pineapple growth in terms of D-leaf dry weight and root dry weight. FR, soil previously frozen at −4 °C for 48 hours but not re-infested with *R. reniformis* and *H. dihystera;* IS, soil naturally infested with *R. reniformis* and *H. dihystera.* Mulch treatment was fallow-covered with pineapple-plant residues. Comparisons apply to bars of same fill pattern. Bars with same letters are not significantly different according to the Waller-Duncan *k*-ratio *t* test ($k = 100$).

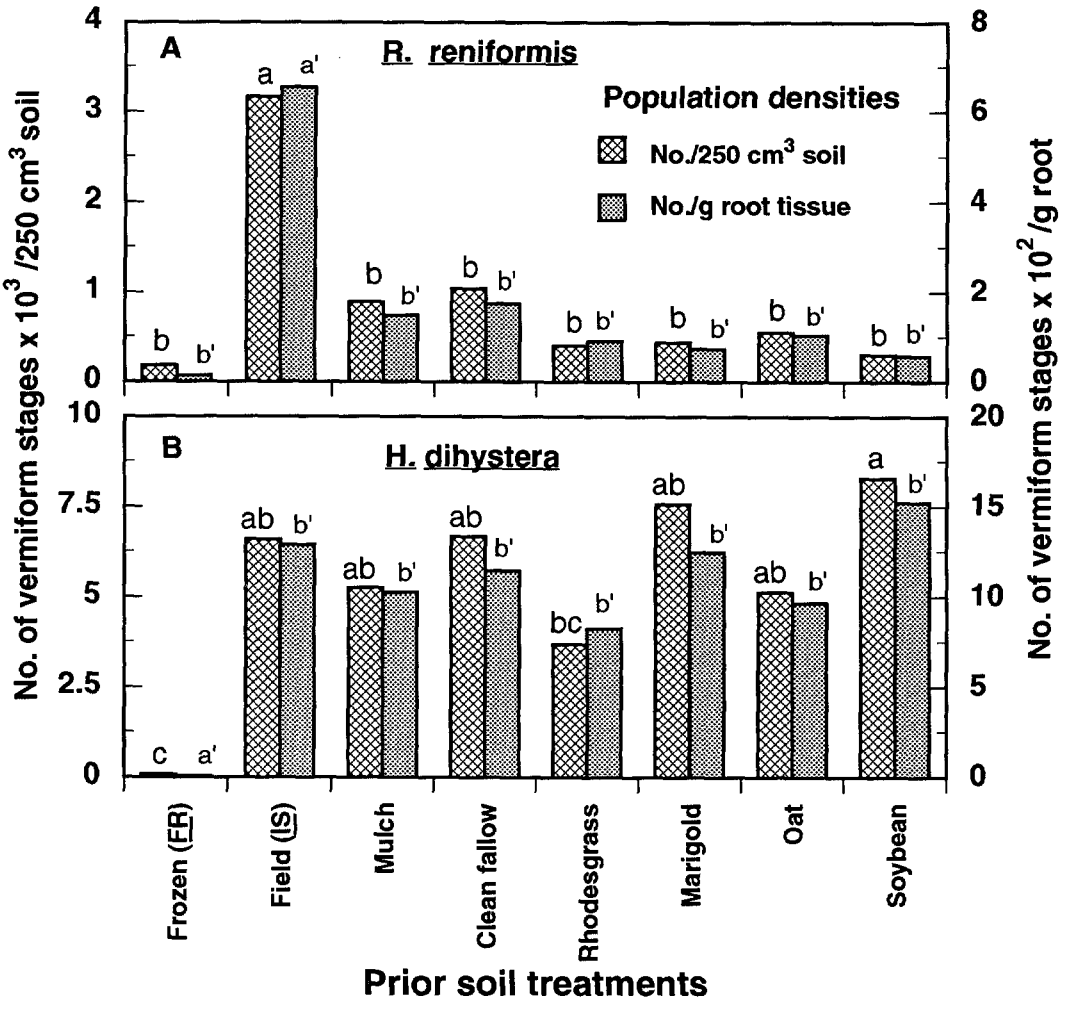

FIG. 3. Residual effect of plant-covers, clean fallow, or mulch on subsequent *Rotylenchulus reniformis* (A) and *Helicotylenchus dihystera* (B) population densities in terms of number of juveniles per 250 cm^3 soil or number of juveniles per gram of dry root tissue. FR, soil previously frozen at −4 °C for 48 hours but not re-infested with *R. reniformis* and *H. dihystera;* IS, soil naturally infested with *R. reniformis* and *H. dihystera.* Mulch treatment was fallow-covered with pineapple-plant residues. Comparisons apply to bars of same fill pattern. Bars with same letters are not significantly different according to the Waller-Duncan *k*-ratio *t* test (k = 100).

(Fig. 3A). Treatment means of *H. dihystera* population density measured in terms of number of individuals per 250 cm^3 soil were different between certain treatments (e.g., soybean from rhodesgrass) (P = 0.05), with the density in FR being the lowest (Fig. 3B). However, the number of individuals (adjusted to per-gram-root basis) in all the plant-cover treatments did not differ from one another or from the fallow or mulch treatment (P = 0.05) (Fig. 3B). There was also no correlation between the indices and number of *R. reniformis* or *H. dihystera* at the termination of the pineapple bioassay.

About three to seven times more eggs were found in FR than in any of the plant-cover treatments (P = 0.05) (Fig. 4). Egg number in each of the plant-cover treatments or in IS was relatively low and again did not differ from one another or from the number in the fallow or mulch (P = 0.05) (Fig. 4). However, after the egg numbers were adjusted to a per-gram-root basis, there were no treatment differences (P = 0.05) (Fig. 4).

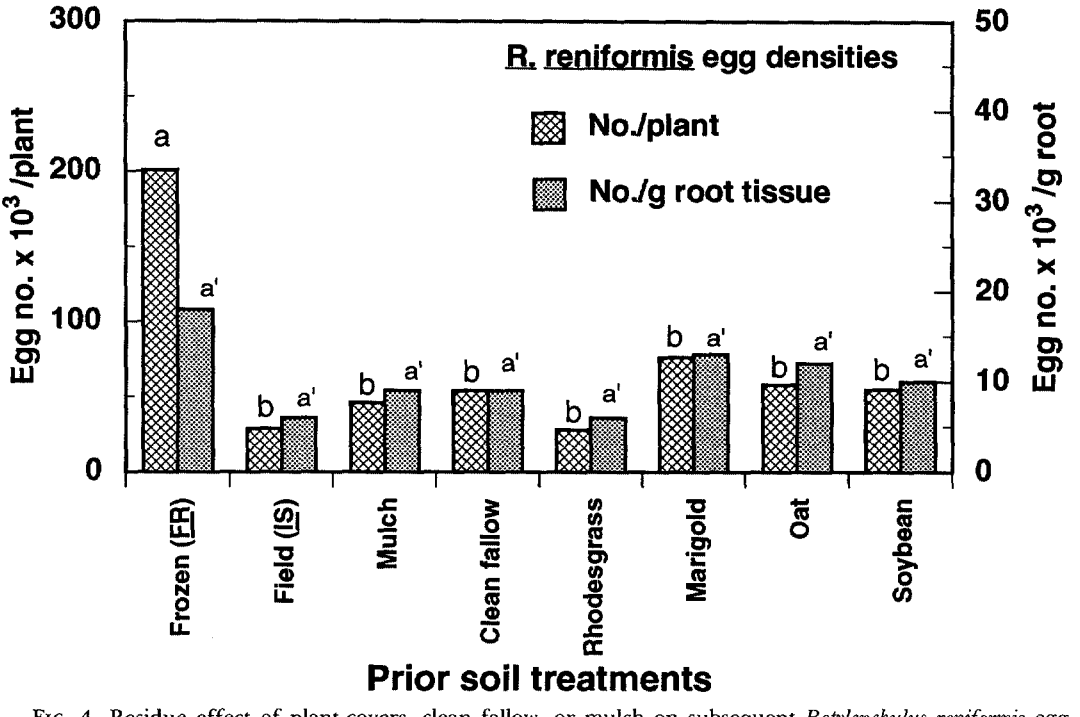

FIG. 4. Residue effect of plant-covers, clean fallow, or mulch on subsequent *Rotylenchulus reniformis* egg production in terms of number of eggs per plant or number of eggs per gram dry root tissue. Mulch treatment was fallow-covered with pineapple-plant residues. Comparisons apply to bars of same fill pattern. Bars with same letters are not significantly different according to the Waller-Duncan *k*-ratio *t* test ($k = 100$).

Correlation between various nematode population densities and pineapple growth: Of all the plant-growth parameters measured, only pineapple D-leaf weight was correlated with numbers of *R. reniformis* ($r = -0.45$, $P = 0.05$) (Table 2). However, all pineaplle growth parameters correlated negatively with *H. dihystera* population densities in the soil. *R. reniformis* egg numbers per plant correlated positively with pineapple growth (Table 2). There was no correlation between the numbers of the two nematode species.

DISCUSSION

Reduction of *R. reniformis* numbers by marigold, rhodesgrass, resistant soybean, and oat in our experiment is in agreement with reports that these plants are poor hosts or nonhosts (5,9,26). However, the magnitude of reduction was similar to that

TABLE 2. Correlation coefficients between pineapple growth parameters and nematode population densities in Kunia soil replanted with pineapple. The soil was placed in 15-cm-diam. pots, re-infested with 1,000 *Rotylenchulus reniformis* and 800 *Helicotylenchus dihystera,* and planted with pineapple crowns that were allowed to grow for 7 months in the greenhouse.

Nematode density parameter	Pineapple growth parameter			
	Shoot fresh weight (g)	Root fresh weight (g)	D-leaf dry[b] weight (g)	Total plant fresh weight (g)
R. reniformis/g root[a]	ns	ns	−0.449**	ns
H. dihystera/g root[a]	−0.513**	−0.516**	−0.444**	−0.515**
R. reniformis egg no./plant	+0.328*	+0.550**	ns	+0.334*

[a] Number of vermiform stages of nematode per gram root fresh weight; ns = not significant at $P = 0.05$.
[b] D-leaf is the longest leaf with terminating growth on a pineapple plant (32).

attained by the clean fallow treatment. Thus, the reduction would not be sufficient to prevent subsequent pineapple crop damage (10). However, the increased rate in nematode population decline caused by the selected plant-covers (e.g., marigold) may help shorten the pineapple inter-cycle fallow period, which generally lasts 6 to 12 months (10). Fallow covered with pineapple residues was least effective in hastening the rate of *R. reniformis* population decline. The slower rate of population decline in these mulch plots was probably due, at least partially, to the slower rate of moisture loss. The rate, rather than the extent, of drying is the most important factor determining survival of *R. reniformis* in the soil (2,36).

Our results stress the importance of knowing the host status of the plant-covers so that the target nematodes can be controlled. For example, marigold would be preferred to oat (although oat is a more practical and valuable crop economically) since it suppressed all the nematodes in the pineapple field. Oat, on the other hand, though suppressive to *R. reniformis,* encouraged build-up of *H. dihystera.* A similar phenomenon is observed with planting of pangola grass (*Digitaria decumbens* Stent), which reduces population densities of *M. incognita, Criconemella* spp., and *Helicotylenchus* spp. (20) but not *Pratylenchus brachyurus* (3).

It would be beneficial to identify a cover crop that would not only suppress all the nematode species in a targeted pineapple field but at the same time promote resident nematode antagonists to sustain suppression. Unfortunately, none of the plant-covers tested by our bioassay procedure indicated any more current or subsequent reduction of *R. reniformis* population than the fallow or mulch control. Using the ratio of degree of *R. reniformis* reproduction in autoclaved soil to that in frozen soil as a means of detection, no antagonist to *R. reniformis* in the plant-cover treated soils was evident. The indices of antagonistic activities, though greater than 1.0 for most of the plant-cover treated soil correlated only weakly and negatively with *R. reniformis* population densities under plant-covers. The indices also did not correlate with *R. reniformis* population densities on subsequent pineapple plants, nor were they correlated positively with pineapple growth, as would be expected if antagonists of *R. reniformis* were present in the soil. The 6- to 10-fold lower *R. reniformis* population density in the cover-crop treated soils than in the nematode-infested field soil was attributed more to initial mortality faced by introduced nematode species than to the antagonists. This was supported by the evidence that *R. reniformis* densities in the mulch and fallow soils also had low nematode densities.

In the determination of biological activities antagonistic to *R. reniformis,* there was an assumption that freezing, used to minimize the number of resident nematodes in the soil and thus their confounding effect on the experimental data, would affect the nematodes to a greater degree than the soil microbial antagonists. How well this assumption holds has not been investigated. The indices of antagonistic activity may therefore fall short of reflecting the true natural conditions. However, most elements of the soil microbial community would be expected to survive a freezing process (31), even though recovery after freezing is highly variable and is a function of the species (1). For instance, even though most invertebrates including nematodes are not able to tolerate freezing (6), bacteria are reduced by only 40% after soils have been frozen; some species even recover rapidly to pre-frozen levels (34). Therefore, although effect of freezing to nematode antagonists is unknown, some of them are expected to survive or recover during the long (7-month) incubation period. However, none of these was detected in pineapple field soils. The selected cover-crops, therefore, failed to stimulate any antagonistic microflora that were resistant or tolerant to freezing as measured by our present assay methodology. Alternatively, the assay methodology of measuring nematode reproduction, itself a lengthy

and cumbersome process, may not be sufficiently sensitive to detect antagonists, if they were indeed present but at low levels. Other methods of detecting antagonists (12,27) and the effects of other plant-covers (13) are being investigated in our laboratory with soils collected from various regions of Hawaii where nematode antagonists were once found (25). These antagonists reduced *R. reniformis* numbers, although not as effectively as soil fumigation (25).

Generally, pineapple growth correlated negatively with numbers of *R. reniformis* in the field (33). The lack of correlation between pineapple growth and *R. reniformis* densities in our pot experiments may be due to the relatively low number of nematodes present, whereas in the field experiments their numbers were high (33). On the other hand, the negative correlations of numbers of *H. dihystera* and plant growth in our studies underscore the need to re-examine the role of *H. dihystera* in damaging pineapple plants.

The high numbers of *R. reniformis* eggs found in soil (FR) that had been subjected to freezing temperature might be due to multiplication by the relatively few *R. reniformis* (4%) that survived the freezing temperature. The build-up of *Heterodera trifolii* and *M. hapla* in white clover in a previously frozen soil was similarly observed and attributed to the survival of eggs, from which juveniles hatched to invade the clover roots (31). *Rotylenchulus reniformis* population density, low in pineapple plantation soil following fumigation, also increased exponentially after 6 to 8 months (33), attributed in part to the lack of competition among the few surviving individuals or to the larger food substrates available as a result of better host plant growth in the fumigated soil.

A good plant-cover has many advantages over bare fallow. It reduces plant-parasitic nematode populations more rapidly than fallow or mulch plots, competes with weeds, decreases erosion, maintains or enhances soil fertility, and provides a niche for nematode-antagonistic fauna (10,13) or flora (23). Some plant-covers may promote indigenous mycorrhizae populations (23) or produce alleochemicals in root exudates (17,19) that are actively toxic or inhibitory to the nematodes. Plant-cover(s) may also provide some added economic value, such as hay for livestock. The continued research on cover crops for the pineapple inter-cycle periods may provide a viable means to reduce or replace the use of preplant nematicide.

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