## Nematicide Efficacy, Root Growth, and Fruit Yield in Drip-irrigated Pineapple Parasitized by Rotylenchulus reniformis<sup>1</sup>

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Abstract: A 3-year field trial near Kunia, Oahu, Hawaii, was conducted to evaluate four nematicide treatments for efficacy against Rotylenchulus reniformis in drip-irrigated pineapple (Ananas comosus L. (Merr.)). The treatments were (A) preplant fumigation with 1,3-dichloropropene (1,3-D) (336 liter/ha) and postplant drip application of fenamiphos (3.4 kg/ha) with restricted irrigation, (B) preplant 1,3-D only, weekly irrigation. (C) 1,3-D fenamiphos, weekly irrigation, and (D) postplant fenamiphos only, weekly irrigation. Fenamiphos was applied at 3-month intervals for 1 year after planting in three treatments. Although nematode populations increased in all treatments 1 year after planting, no differences in fruit yield were detected among treatments in the first (plant crop) harvest 19 months after planting. In the second (ratoon) crop (33 months after planting) significant yield differences, larger fruit size, and greater root biomass were obtained in the dual nematicide treatments. Root biomass increased continuously throughout the crop cycle, was greatest near the drip line, and showed a shallow depth distribution (30-40 cm). Rotylenchulus reniformis populations and fenamiphos applications, nematode numbers were positively correlated with root biomass.

Key words: Ananas comosus, 1,3-dichloropropene, drip irrigation, fenamiphos, nematicide, nematode, pineapple, reniform nematode, root development, Rotylenchulus reniformis.

The reniform nematode (Rotylenchulus reniformis Linford & Oliveira) has been a serious pest of pineapple in Hawaii for several decades (2,6,7,18,19). The perennial nature of pineapple, with three fruit crops produced from a single planting, requires nematode control over a 4- to 5-year period. Recent changes in commercial pineapple culture in Hawaii include increased use of drip irrigation and a transition from cannery operations to fresh fruit production. The most prevalent chemical control strategy is preplant fumigation (1,3dichloropropene [1,3-D] or methyl bromide) followed by postplant systemic nematicides, usually fenamiphos (ethyl 3-methyl-4-(methylthio)phenyl (1methylethyl)phosphoramidate) by drip irrigation. A fallow cycle of 6 to 12 months is now used on some plantations to augment chemical control of nematodes. Preplant fumigation is usually sufficient to produce a successful plant crop (2,19).

This research was part of an integrated effort to evaluate nematicide efficacy by characterizing plant and root development, nematode population dynamics, and fenamiphos concentrations in the soil. We used a combination of nematicides, preplant 1,3-D fumigation, and postplant fenamiphos application, and compared the effect of weekly and restricted irrigation on fenamiphos movement and efficacy.

## MATERIALS AND METHODS

*Experimental site:* The experimental plot (0.2 ha) was located near Kunia, Oahu, Hawaii, within a commercial field. Wahiawa silty clay (Tropeptic Eutrustox; sand 7%, silt 40%, clay 53%), is a well-aggregated oxisol that is representative of much of the pineapple acreage on the island of Oahu. Four treatments were replicated three times in a completely random-

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ized design: (A) preplant fumigation with 1,3-D (336 liter/ha), trimonthly fenamiphos applications (3.4 kg/ha), restricted irrigation (description follows); (B) preplant 1,3-D (336 liter/ha), weekly irrigation (13 mm); (C) preplant 1,3-D (336 liter/ha), postplant fenamiphos (3.4 kg/ha), weekly irrigation (13 mm); and (D) postplant fenamiphos (3.4 kg/ha), weekly irrigation (13 mm). A control treatment without nematicides was not included in the experiment. The results from the dual nematicide treatments, A and C, will be compared with the single nematicide treatments B and D. The plots  $(7 \text{ m} \times 12 \text{ m})$  consisted of six beds (bed spacing 1.1 m) with two parallel plant rows per bed, 40 cm apart; plant spacing was 25 cm. A single drip irrigation tube (double wall tubing, 2-cm diameter) was positioned in the center of the bed, with emitters spaced 30 cm apart. Water application was uniform along the plant row due to overlapping wetting patterns from adjacent emitters; the emitter flow rate was 1 liter/hour. The beds were covered with black polyethylene (1-mil thickness). Separate water flow meters, valves, and chemical injection systems were used for each treatment (2).

The plots were fumigated with 1,3-D (Telone II, 92% a.i.) on 25 November 1986 and planted on 5 December 1986 with pineapple crowns (Smooth Cayenne cultivar). Fumigant was injected (4.8 ml/ injection) with hand-held fumiguns (N. A. MacLean Co., San Francisco, CA) to a depth of 20 cm at 25-cm intervals on both sides of the bed to correspond to a rate of 336 liter/ha. Beginning in February 1987, fenamiphos (Nemacur 3E, 36% a.i.) was applied at 3-month intervals until November 1987 at a rate of 3.4 kg/ha. Fenamiphos was prediluted in 26-liter polyethylene tanks (3,000 mg/liter) and pumped into the drip irrigation system to each treatment, followed by irrigation with 13 mm of water over a 3-hour period. All plots were irrigated weekly for 5 months after planting; irrigation of treatment A was then restricted for 7 months (May-November 1987), with 13 mm of water applied only during fenamiphos applications. Weekly and restricted irrigation were compared to evaluate persistence and leaching of fenamiphos below the root zone (20). Plant maintenance followed commercial plantation practice; macronutrients were applied by drip irrigation with micronutrients applied foliarly.

Fruiting was initiated (forced) on October 31, 1987, with a foliar application of ethephon. No irrigation or fertilizer was applied from November 1987 until harvest in June 1988. The first (plant) crop yield data were based on 70 fruit from two center beds in each plot. Fruit were sized and weighed in the field; fruit diameters corresponded to those adopted by the Hawaii pineapple industry for processing fruit into large (No. 2.5, >13.2 cm), and medium (No. 2, >10.8 cm) slices, chunks (No. 1, >9.5 cm), or juice (Sub No. 1, <9.5 cm).

After plant-crop harvest, all treatments were irrigated and fertilized according to normal plantation practice without additional nematicide treatments. Fruit from the second (ratoon) crop were harvested in August–September 1989, and yield data were based on 200 fruit from two center beds in each plot.

Plant and root sampling: Roots were sampled at 3-month intervals for 9 months after planting, using a monolith (pin board) method (15) to follow the growth of the plant and the development of the root system. Two additional samples were collected at 13 and 26 months after planting. At each sampling date, two plants from each treatment were randomly selected and their root systems excavated. Plant and root biomass measurements were taken from the same plant. To estimate the hemispherical distribution of roots in the soil, one root profile (91 cm wide  $\times$  64 cm deep  $\times$  10 cm thick) was collected perpendicular to the plant row, and the second profile was taken parallel to the row. Before sampling, one or two plants from the end of the plant row were removed to minimize border effects. A pit was dug to remove half of the root system and to accommodate a root board. Nails were driven into the soil to secure the roots. The monolith was excavated, and its surface was trimmed to yield a 10-cm-thick section. Monoliths were transported to a field station, where soil was rinsed from the boards with a fine water spray. Pin boards contained roots from the target plant as well as adjacent plants. Roots were sectioned on a grid into  $10 \times 10$ -cm subsamples (32 samples total). The dry root biomass from each grid cell was measured.

The plants corresponding to the excavated root systems were also measured for growth parameters. Green leaf area was obtained by separating leaves into dark green and light green plus white tissues. Green leaf area was measured with a LI-COR (LI 3100) area meter. The biomass of green and basal parts of leaves and stems was obtained after drying at 65 C in a forced-draft oven to a constant weight.

Soil profiles: In addition to the plant-root profiles, one soil monolith was collected per treatment in January 1988, 13 months after planting. These four monoliths were oriented perpendicular to the drip line to examine spatial variability in root distribution, nematode populations, and fenamiphos residues. The distance from the plant center axis to the drip tubing was 20 cm in all four profiles. The distance from the plant center to nearest drip emitter was measured; plants were directly opposite the drip emitter in monoliths from treatments C and D, and were spaced midway between two emitters in treatments A and B. The soil was not removed by rinsing, but instead was sectioned on a grid into 10-cm<sup>3</sup> samples. These samples were passed through a 4-mm sieve to separate roots and subsampled for nematode assay and pesticide analysis.

Nematode sampling and pesticide residue analysis: Soil samples were collected bimonthly to census nematode populations for 1 year in coordination with fenamiphos-soil-residue sampling. Ten core samples per plot were collected to a depth of 15 cm to form a composite sample for nematode counts. A single sample of 100 cm<sup>3</sup> per plot was processed by the centrifugal flotation method (3); nematodes were identified and counted using a stereomicroscope. Live motile nematodes were extracted from roots from the January 1988 soil profiles with the Baermann funnel method (3). Root wet weight from each grid cell was first determined; roots were then cut into smaller sections and incubated in the funnels for 72 hours.

Fenamiphos residues were measured at approximately bimonthly intervals during the first year after planting. At each of the five sampling dates, soil profiles were collected from two replicate plots of each treatment. Soil profiles were also collected 15 and 19 months after planting to evaluate fenamiphos persistence. Samples were collected in 15-cm depth increments, to a depth of 60 cm, from the center of the bed directly under a drip emitter using soil augers (10-cm diameter). The total toxic residue of fenamiphos (the parent nematicide and its two metabolites) was measured in the soil profiles (20).

## **RESULTS AND DISCUSSION**

Nematicide effects on nematode populations: Initial R. reniformis numbers in the experimental field were very low (2-22 per 100 cm<sup>3</sup> of soil) in September 1986. We attributed this low population to a 10-month fallow period. Nematode population densities remained low in all treatments for the first 6 months of the experiment (Fig. 1a). Rotylenchulus reniformis numbers in the nonfumigated plots (treatment D) increased markedly (P < 0.05) at 8 months after planting (Fig. 1a). By 10 months after planting, nematode levels had increased in all treatments. Mean values ranged widely between treatments, but these differences were not significant (P = 0.05) due to variability between replicate plots, as shown by the large error bars.

In the original experimental design for the Kunia site, fenamiphos applications were scheduled at 2-month intervals, beginning 27 February 1987. The soilresidue profiles collected on 20 April 1987 showed fenamiphos to be relatively persis-



FIG. 1. a) Change in numbers of Rotylenchulus reniformis during the first year after planting. Values are means of three replicate plots per treatment with standard error bars. b) Average total fenamiphos concentrations in the root zone (0-40 cm depth) for 1987 and 1988. Arrows denote the dates of the four fenamiphos applications: 27 February, 24 April, 24 July, and 6 November 1987. Pesticide sampling dates: 23 March, 20 April, 3 June, 14 July, 22 September 1987; 5 January, 31 March, and 27 July 1988. Treatment summary: A) preplant 1,3-D, postplant fenamiphos, restricted irrigation; B) preplant 1,3-D, weekly irrigation; C) preplant 1,3-D, postplant fenamiphos, weekly irrigation; D) postplant fenamiphos, weekly irrigation.

tent in the soil (averaging 4  $\mu$ g/g in the root zone) (Fig. 1b). Therefore, the application interval was lengthened to 3 months. Restricting irrigation from May to November 1987 resulted in higher average fenamiphos concentrations (P < 0.05) in the pineapple root zone (0–45 cm) in treatment A compared with treatments C and D as measured in June, July, and September 1987. Average concentrations ranged from 1 to 4  $\mu$ g/g in treatments C and D, and averaged 8  $\mu$ g/g in treatment A during June to September 1987 (Fig. 1b). In vitro toxicity studies (8,13,14) have demonstrated loss of motility, dispersion, and root penetration in a variety of nematode genera at fenamiphos levels of 1 to 3 µg/ ml. In soil,  $1.5 \,\mu g/g$  was sufficient to inhibit maturation in Heterodera glycines (22), and root galling by Meloidogyne incognita was inhibited by levels of 2 to 4  $\mu g/g$  (11). In March 1988, four months after fenamiphos applications and irrigation were suspended, fenamiphos soil residues had declined to  $1-2 \mu g/g$  in all three treatments (Fig. 1b) due to pesticide degradation and leaching from heavy rainfall (500 mm) in December 1987. Although the pineapple beds were covered with plastic, lateral movement of rainfall from the interbed region contributed to leaching of fenamiphos residues to 3 meters depth (20).

The low irrigation regime imposed on treatment A resulted in reduced leaching of fenamiphos and metabolites below the root zone (20), as well as increased fenamiphos persistence in the soil due to low soil moisture. From June to September 1987, the soil moisture tension in treatment A averaged 100 kPa, compared with 10 kPa measured in the weekly irrigation treatments (B, C, D). This moisture differential may have decreased fenamiphos degradation by a factor of two (16), thereby increasing its persistence in the root zone.

Soil profiles: The spatial distribution of nematodes, roots, and fenamiphos concentrations from the four soil monoliths collected 13 months after planting were evaluated using correlation analysis, and the data for profiles from treatments B and D are shown in Figure 2. In all four profiles, motile R. reniformis extracted from roots were highly correlated with soil nematodes (P = 0.001), so only soil nematode values are reported here. Nematode numbers were highly correlated (P = 0.001) with root biomass in treatment B (Fig. 2a,b), which received preplant fumigation only. Both roots and nematodes were concentrated in the 0- to 10-cm soil layer. When postplant fenamiphos was used (treatments A, C, and D), R. reniformis numbers and fenamiphos concentrations were neg-



FIG. 2. Nematode, root, and fenamiphos distributions from two-dimensional soil profiles from treatment B (preplant 1,3-D, weekly irrigation) and treatment D (postplant fenamiphos, weekly irrigation), collected in January 1988, 13 months after planting. Soil profiles were oriented with respect to the plant center and the location of the drip irrigation tube as shown on the x-axes. Values represent measurements from each 10-cm<sup>3</sup> grid cell. A) Treatment B *Rotylenchulus reniformis* numbers and B) total root biomass. C) Treatment D *Rotylenchulus reniformis* numbers, D) total root biomass, and E) total fenamiphos concentration.

atively correlated (P < 0.05). This inverse relationship is illustrated for treatment D in Figure 2c and e. The two-row planting pattern, with a single drip tube in the bed center, resulted in only half of the root system receiving nematicidal levels of fenamiphos. Most of the root biomass occurred on the drip line side of the plant, resulting in a positive correlation with pesticide concentration (P < 0.05) for treatments A, C, and D (e.g., Fig. 2d,e). This pattern of root development was most likely due to gradients in water, fertilizer, and fenamiphos distribution in the plant row. Similar root growth was observed in drip-irrigated sugarcane and green peppers where gradients in soil water potential and nutrient concentration resulted from drip application (4,12). The shallow distribution of the pineapple roots in this study (0-40 cm) was due to adequate soil moisture in the root zone and a tillage-compacted soil layer at approximately 40 cm depth.

In an efficacy study of oxamyl in pineapple, Hylin et al. (9) reported an inverse relationship between *R. reniformis* numbers and oxamyl residues in two-dimensional soil profiles. In their untreated control, the nematode populations were 10 times greater than in the oxamyl treatment. For the soil profiles reported here, with fumigation alone, nematode levels in treatment B were three- to fourfold greater than in the fenamiphos treatments A, C, and D (e.g., Fig. 2a vs. Fig. 2c).

Due to the major effort involved in field

collection and sample analysis, these twodimensional soil profiles were not replicated. Spatial comparison of nematicide concentrations with nematode numbers is a useful method of assessing nematicide efficacy in the root zone. Fenamiphos treatments were applied over a 10-month period prior to collecting the soil profiles, and nematode spatial distribution was most likely a response to the integrated pesticide concentration in soil over that time period (Fig. 2c,e). This concentration × time relationship for fenamiphos efficacy was demonstrated by simultaneously monitoring fenamiphos soil residues and root-gall indices for M. incognita (10). With a perennial crop such as citrus or pineapple, it is useful to measure efficacy long before yield data become available.

Plant growth, root system development, and fruit yield: Plant and root biomass increased steadily over the 2-year experimental period in all treatments. The growth data from 14 and 26 months after planting are summarized in Table 1. There were no treatment differences in plant and root biomass during the first year (March, June, August 1987 sample collections). Leaf-area expansion closely paralleled plant-dry matter accumulation; leaves represent 90% of above-ground plant biomass during vegetative growth (17). Table 1 shows no significant treatment effects on plant biomass. This negative result is most likely due to the small sample size (two plants per treatment) and the large variability between plants. Between June 1988 and February 1989, suckers were produced on the mother-plant stems, which then produced ratoon fruit; suckers do not produce separate root systems (21).

Root biomass increased in all treatments between plant and ratoon crop development (Table 1). Bonzon (5) found similar pineapple root system development over two crop cycles in the Ivory Coast but reported a decrease in the growth rate at the time of fruit initiation. There were no clear-cut treatment effects on root biomass in 1988. In February 1989, treatments A and C, the dual nematicide treatments, had greater root biomass than treatments B and D, the single nematicide treatments (Table 1). An analysis of variance comparing the combined root biomass data from treatments A and C against measurements for B and D showed a significant difference (P < 0.01).

Average rooting depth reached 30 cm (range 30–40 cm) 1 year after planting and was probably limited by tillage compaction (e.g., Fig. 3b). Rooting depth in all treatments remained constant throughout the two crop cycles. Horizontal root extension in treatments A, C, and D increased over a 2-year period and averaged 70 cm in February 1989 compared with treatment B, which peaked at 65 cm in August 1987 and

TABLE 1. Effects of management treatments on average plant and root-dry-biomass measurements of pineapple parasitized with *Rotylenchulus reniformis* from Kunia experiment sampled 14 and 26 months after planting.

Sampling date	Treatment <sup>†</sup>	Plant weight (g)		Root Weight (g)	
		Mean	(SD)‡	Mean	(SD)‡
February 3, 1988	Α	437 a	(46)	37 a	(3)
	В	372 a	(82)	21 ab	(7)
	С	334 a	(12)	24 ab	(12)
	D	460 a	(113)	16 b	(4)
February 6, 1989	Α	572 a	(156)	56 a	$\dot{(17)}$
	В	709 a	(285)	25 a	( <u>9</u> )
	С	698 a	(244)	65 a	(9)
	D	574 a	(180)	34 ab	(5)

<sup>†</sup> Treatment summary: A) preplant 1,3-D, postplant fenamiphos, restricted irrigation; B) preplant 1,3-D, weekly irrigation; C) preplant 1,3-D, postplant fenamiphos, weekly irrigation; D) postplant fenamiphos, weekly irrigation.

 $\ddagger$  Values are means of replicates. Treatment means with the same letters are not significantly different (P = 0.05) based on Duncan's multiple-range test. SD = standard deivation of treatment means.



FIG. 3. Pineapple root systems from *Rotylenchulus reniformis*-infested plots as collected by the pin-board method in February 1989, 26 months after planting, oriented parallel to the drip line. Root-board dimensions: 91 cm (width)  $\times$  64 cm (height). A) Treatment B (preplant 1,3-D, weekly irrigation), B) Treatment C (preplant 1,3-D, postplant fenamiphos, weekly irrigation).

declined to an average of 40 cm in February 1989 (e.g., Fig. 3a,b). This decline in the lateral extent of the root system in treatment B was associated with a resurgence of nematodes after fumigation, as shown by the high levels present in January 1988 (Fig. 2a). Fumigation controls nematodes effectively for 6–12 months in pineapple without additional nematicide treatments (2,19).

In the 1988 fruit yields (Fig. 4a), there were no differences between treatments B, C, and D, but we found a significant yield loss in treatment A (P < 0.05). Treatment A also showed a corresponding smaller fruit size, with fewer size No. 2.5 fruit (P <0.01), and more size No. 2 fruit (P < 0.05) compared with the other three treatments. This yield decline and fruit-size effect were probably due to the restricted irrigation and nutrient regime imposed on treatment A during May-November 1987. In the 1989 ratoon crop (Fig. 4b), treatment A produced the highest yield (P < 0.05) and treatment B the lowest. Overall fruit size was smaller in 1989 across all treatments, but treatment B (fumigation only) produced more small fruit, size No. 1, (P <(0.05) than the other three treatments. Apt (1) reported an increase in fruit size (compared to an irrigated control) in the ratoon harvest when fenamiphos was applied monthly for 16 months after planting. We attribute the surprising 1989 yield increase in treatment A to the nematicidal effect of high fenamiphos concentrations measured



FIG. 4. Fresh fruit yield and fruit-size distribution from two pineapple crop harvests as related to management treatments including control of *Rotylenchulus reniformis*. A) The plant crop (1988) harvest and B) ratoon crop (1989) harvest. Fruit diameter: No. 2.5 > 13.2 cm; No. 2 = 13.2-10.8 cm; No. 1 = 10.8-9.5 cm; Sub No. 1 = <9.5 cm). Treatment summary: A) 1,3-D + fenamiphos + restricted irrigation, B) 1,3-D + weekly irrigation, C) 1,3-D + fenamiphos + weekly irrigation, D) fenamiphos + weekly irrigation. Values plotted over treatment bars are means of three replicate plots per treatment. Means with the same letter are not significantly different (Duncan's multiple-range test, P = 0.05).

in the root zone during the first year when irrigation was restricted for 7 months (May–November 1987) (Fig. 1b).

Combining preplant 1,3-D fumigation with postplant fenamiphos applications provided the best control of R. reniformis. Fumigation alone provided sufficient nematode control in the first year to produce good pineapple yields in the plant crop. Fumigation allows a healthy root system to develop, and the length of the initial control period determines the yield response (2,17,19). A healthy root system can better tolerate the increases in nematode numbers compared with an initially stunted root system. In commercial fresh fruit pineapple production, preplant fumigation and postplant nematicides are needed throughout the crop cycle to sustain high yields.

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