Green Manure Crops for Management of *Meloidogyne javanica* and *Pythium aphanidermatum*

Anthony M. Ortiz and Brent S. Sipes

Department of Plant and Environmental Protection Sciences, University of Hawaii, 3190 Maile Way, Honolulu, HI 96822

Susan C. Miyasaka¹

Department of Tropical Plant and Soil Sciences, University of Hawaii, 875 Komohana Street, Hilo, HI 96720

Alton S. Arakaki

Department of Tropical Plant and Soil Sciences, University of Hawaii, P.O. Box 394, Hoolehua, HI 96729

Additional index words. cover crops, integrated pest management (IPM), root-knot nematode, oomycete pathogen

Abstract. To determine the potential to suppress root-knot nematode *Meloidogyne javanica*, 10 genotypes of seven green manure species were evaluated in a greenhouse study. These species were: black hollyhock (*Alcea rosea* L.); canola (*Brassica napus* L.); cabbage (*B. oleracea* L.); French marigold (*Tagetes patula* L.), sorghum–sudangrass [*Sorghum bicolor* (L.) Moench nothosubsp. *drummondii* (Steud.) de Wet ex Davidse]; sunn hemp (*Crotalaria juncea* L.); and yellow mustard (*Sinapis alba* L.). Plants were inoculated with eggs of *M. javanica* and after 6 weeks, nematode eggs and reproduction factor (Rf = final egg population density/initial egg population density) were determined. Marigolds were non-hosts to *M. javanica*; other crop species that were poor hosts to *M. javanica* included canola cv. Dwarf Essex, sorghum–sudangrass cvs. Piper and Sordan 79, black hollyhock cv. Nigra, and sunn hemp. Based on low Rf, four groups of species were selected for further evaluation in the greenhouse to determine the response to both *M. javanica* and another crop pathogen, *Pythium aphanidermatum*. These four groups of green manure crops were: 1) seven marigold genotypes; 2) four *Brassicaceae* genotypes; 3) seven sorghum–sudangrass hybrids; and 4) four other species [black hollyhock, sunn hemp, elecampane (*Inula helenium* L.), and black-eyed Susan (*Rudbeckia hirta* L.)]. Plants were inoculated with a factorial combination of *M. javanica* and *P. aphanidermatum* (none, each alone, and in combination) and repeated four times in a split-plot experimental design (whole plots were factorial treatments and subplots were green manure crop genotypes). Six weeks after inoculation, plants were harvested and measured for fresh and dry weights of shoots and roots and Rf of *M. javanica*. Adverse effects of *P. aphanidermatum* were characterized by dead or dying roots and measured by reduced plant biomass. Negative synergistic effects were observed in several marigold and *Brassicaceae* genotypes, in which the combined effects of *M. javanica* and *P. aphanidermatum* reduced shoot and root growth more severely than either treatment alone. Marigold *T. erecta* cv. Orangeade, sorghum–sudangrass cvs. Graze-All, Piper, and Sordan 79, and sunn hemp appeared to be resistant to *M. javanica* and *P. aphanidermatum*, either alone or in combination. Based on results of greenhouse trials, eight green manure crops (yellow mustard cv. Ida Gold, French marigolds cvs. Nema-gone and Golden Guardian, sorghum–sudangrass cvs. Sordan 79 and Tastemaker, sunn hemp, unplanted plot, and a control plot with weed mat) were selected and grown for 3 months in a field trial in Pepeekeo, HI. Each treatment was replicated four times in a randomized complete block design. Shoot biomass was sampled at 1, 2, and 3 months after planting. Plant–parasitic nematodes were counted before planting and at 4 months after planting. Dry weight biomass averaged across three sampling dates was greatest for the two sorghum–sudangrass hybrids followed by those of two marigold cultivars that did not differ from them. No significant differences in populations of root-knot nematodes were found. Based on this field trial as well as greenhouse trials, marigold cultivars, sorghum–sudangrass hybrids, and sunn hemp appeared to be non-hosts or poor hosts to reniform (*Rotylenchulus reniformis*) as well as root-knot nematodes and well adapted to the environmental conditions found along the Hamakua Coast of the Hawaii Island.

A common, sustainable disease management tactic is the use of green manure cropping systems. The primary benefits of green manure crops are to: 1) protect the soil

from erosion; 2) increase soil nutrients; 3) improve soil properties (e.g., water-holding capacity); and 4) provide an energy source for microbes, contributing to soil activity and

biodiversity (Brady and Wiel, 1999; McSorley, 2011; Thoden et al., 2011). Secondary benefits of green manure crops include the control of soilborne pathogens through several possible mechanisms: 1) non-host or poor host status for plant–parasitic nematodes; 2) production of phytochemicals that are toxic and/or antagonistic toward pathogens; and 3) stimulation of antagonists of plant–parasitic nematodes (Chitwood, 2002; Hooks et al., 2010; Wang et al., 2002).

To control pathogens that could affect the subsequent crop in a rotation, green manure genotypes should be non-hosts or poor hosts to these pathogens. In addition, an effective green manure crop could produce phytochemicals that control these pathogens as well as large amounts of biomass containing these phytochemicals. Marigolds (*Tagetes* sp.) produce polythienyls that suppress nematode populations (Chitwood, 2002; Hooks et al., 2010; Reynolds et al., 2000). Sorghum–sudangrass hybrids contain the cyanogenic glycoside dhurrin that, when hydrolyzed, yields cyanide, a nematicidal compound (Widmer and Abawi, 2002). *Brassicaceae* species contain glucosinolates that react with myrosinase when tissues are disrupted, forming isothiocyanates that are nematicidal (Chitwood, 2002; McLeod and Steel, 1999; Oka, 2010). Aqueous extracts of *Crotalaria* species were reported to protect tomato (*Solanum lycopersicum* L.) roots from *Meloidogyne incognita*, and the authors (Jourand et al., 2004) speculated that this effect could be the result of nematicidal alkaloids.

Recent literature reviews, however, have shown that effects of green manure crops on plant–parasitic nematodes can range from positive to negative (Hooks et al., 2010; McSorley, 2011; Oka, 2010; Thoden et al., 2011). Factors involved in this variability include: 1) species and cultivars of green manure crops; 2) species and races of target plant–parasitic nematodes; and 3) soil and environmental conditions. To determine effective green manure species and cultivars, it is necessary to conduct research on the particular pathogens to be controlled in both greenhouse and field.

The tropical root crop taro (*Colocasia esculenta*) when grown under upland conditions (i.e., non-flooded) can be infected by the root-knot nematode (*Meloidogyne javanica*), resulting in substantial reductions in yields (Sipes et al., 1995). Little naturally occurring resistance to *M. javanica* has been found within the taro germplasm (Ortiz et al., 2008). To reduce populations of *M. javanica* before the planting of taro, crop rotations with green manure crops that are non-hosts or poor hosts provide alternative means of controlling this pest (Sipes et al., 1995).

Soil is rarely infested with a single pathogen but more often with multiple pathogens that may infect a crop. In addition to *M. javanica*, upland-grown taro can be infected by several *Pythium* species (*P. aphanidermatum*, *P. graminicola*, *P. splendens*, *P. irregulare*, *P. myriotylum*, *P. carolinianum*, and *P. ultimum*) (Ooka, 1994). Bridge and Jatala

(2005) have speculated that nematode infection of taro tissues may allow a secondary invasion by pathogens, causing a corm rot. The combined effects of root-knot nematodes and *Pythium* are hypothesized to be negatively synergistic in taro, contributing to a reduction in growth and corm quality (Sipes et al., 1995). In chrysanthemum (*Chrysanthemum × morifolium* Ramat.), Johnson and Littrell (1970) found that combined effects of *P. aphanidermatum* and *M. incognita* reduced plant height and root weight to a greater extent than either pathogen alone. Similarly, Gracia et al. (1991) showed an additive interaction between *Pythium tracheiphilum* and *Meloidogyne hapla* in lettuce (*Lactuca sativa* L.). Consequently, effective green manure crops for upland-grown taro systems must control both pests.

The objectives of two greenhouse trials and one field trial reported here were to select promising, green manure crops for future field trials in rotation with taro based on: 1) non-host or poor host status of *M. javanica*; 2) disease resistance to the oomycete pathogen *P. aphanidermatum* alone and in the combination with *M. javanica*; and 3) adaptation to soil and environmental conditions of the Hamakua Coast of the Hawaii Island.

Materials and Methods

Nematode host status in the greenhouse trial. In the first greenhouse trial, 10 green manure crop genotypes [black hollyhock (cv. Nigra); canola (cvs. Dwarf Essex and Sunrise Canola); cabbage (cv. Capitata); French marigold (cvs. French Brocade and Pesche's Gold), sorghum–sudangrass (cvs. Piper and Sordan 79); sunn hemp; and yellow mustard (cv. Ida Gold)] were evaluated for host status to *M. javanica* (Table 1). The trial was conducted in the greenhouse during Jan. to Mar. 2004 at the University of Hawaii at Manoa (lat. 21.306488° N, long. –157.809892° W). The seeds were grown in vermiculite at 24 ± 2 °C and transplanted individually at the first true leaf stage into 7.5-cm-diameter clay pots containing sterilized 1:1 (v:v) soil:sand mix. Pots were arranged in a randomized complete block design with 10 treatments and five blocks.

Received for publication 22 Sept. 2014. Accepted for publication 11 Nov. 2014.

This research was supported in part by the Western Sustainable Agricultural Research & Education (Western SARE) program.

The authors thank Dr. Charles McCulloch at the University of California, San Francisco, Dept. of Epidemiology and Biostatistics, for his assistance with statistical evaluation of data. Also, we thank Dr. Janice Uchida at the University of Hawaii at Manoa, Department of Plant and Environmental Protection Sciences, for her assistance with the pathogen *Pythium aphanidermatum*.

The mention of a trademark, proprietary product, method, or vendor does not imply endorsement by the University of Hawaii nor its approval to the exclusion of other suitable products or vendors.

¹To whom reprint requests should be addressed; e-mail miyasaka@hawaii.edu.

Table 1. Ten species and cultivars evaluated under greenhouse conditions for reproductive factor (Rf) of *Meloidogyne javanica*.

Cultivar name	Rf ^{2,y}	SE	
<i>Alcea rosea</i> cv. Nigra	0.35	(0.22)	b
<i>Brassica napus</i> cv. Dwarf Essex	0.95	(0.14)	b
<i>Brassica napus</i> cv. Sunrise Canola	4.15	(2.61)	a
<i>Brassica oleracea</i> cv. Capitata	2.69	(0.85)	ab
<i>Crotalaria juncea</i>	0.21	(0.02)	b
<i>Sinapis alba</i> cv. Ida Gold	1.45	(0.85)	ab
<i>Sorghum nothosubsp. drummondii</i> cv. Piper	0.10	(0.08)	b
<i>Sorghum nothosubsp. drummondii</i> cv. Sordan 79	0.62	(0.61)	b
<i>Tagetes patula</i> cv. French Brocade	0.00	(0.00)	b
<i>Tagetes patula</i> cv. Pesche's Gold	0.02	(0.01)	b
ANOVA: <i>P</i> > <i>F</i>			
Cultivar	0.03		
Block	0.06		

²Means that are followed by the same letter in one column are not significantly different among genotypes (*P* < 0.05), according to the Waller–Duncan multiple range test.

^yMeans are followed by SE in parentheses.

ANOVA = analysis of variance.

They were fertilized weekly with 100 mL of a 0.4% dilution of a commercial liquid fertilizer (Miracle-Gro 24–8–16 formulation; Scots, Marysville, OH). Two weeks after transplanting, each pot was inoculated with 500 eggs of *M. javanica*, previously cultured on tomato cv. Orange Pixie, and extracted using the NaOCl method (Hussey and Barker, 1973).

At 6 weeks post-inoculation, plants were removed from pots, separated into shoots and roots, and the potting medium washed from roots. Nematode eggs were extracted from roots using the NaOCl-extraction method (Hussey and Barker, 1973). Nematode Rf was calculated as the final nematode population divided by the initial inoculum level. A green manure genotype was considered a good host for nematodes when Rf was greater than 1, a poor host when Rf was less than 1 but greater than 0.1, and a non-host when Rf was less than or equal to 0.1 (Mojtahedi et al., 1993; Seinhorst, 1967).

Analysis of variance using PROC GLM (Statistical Analysis System®, Version 9.2; SAS Institute Inc., Cary, NC) was conducted on Rf. The Waller–Duncan multiple range test was used to separate treatment means.

Effects of *Meloidogyne javanica* and *Pythium aphanidermatum* in greenhouse trials. In the second series of greenhouse trials, factorial experiments were conducted to determine effects of *M. javanica* and *P. aphanidermatum*, alone and in combination, on four different groups of green manure crops (marigolds, *Brassicaceae* species, sorghum–sudangrass hybrids, and other species). The marigold group included four cultivars of *T. patula* (cvs. French Brocade, Golden Guardian, Nema-gone, and Pesche's Gold) and three cultivars of *T. erecta* (cvs. Orangeade, Orange Deep, and Scarletade). The *Brassicaceae* group included *S. alba* cv. Ida Gold, *B. juncea* cv. Pacific Gold, and *B. napus* cvs. Dwarf Essex and Sunrise Canola. The sorghum–sudangrass group included *S. bicolor* nothosubsp. *drummondii* cvs. Bale All, Bale All III, Baler, Graze-All, Piper, Sordan 79, and Tastemaker. The other species group included black hollyhock, elecampane, black-eyed Susan, and sunn hemp.

Seeds were germinated in vermiculite and transplanted individually into 10-cm-diameter clay pots containing 1:1 (v:v) sterile soil:sand mixture. Transplants were grown for 4 weeks before inoculation. Plants were fertilized weekly as described in the first trial. This second trial was conducted in the greenhouse during May to Aug. 2004 with a greenhouse temperature of 28 ± 2 °C.

A split-plot design was installed with main plots of *M. javanica* and *P. aphanidermatum* treatments and subplots of genotypes. This design was used to minimize contamination by different pests. Each treatment was replicated five times. If contamination of *M. javanica* or *P. aphanidermatum* occurred in a control plant, then that pot was removed from the statistical analysis.

Eggs of *M. javanica* were applied in 3-mL aliquots containing a total of zero or 500 eggs. *Pythium aphanidermatum* inoculum was grown on 25 mL of a 10% V8 agar (Uchida and Aragaki, 1980) for 72 h in 100 × 15-mm petri plates. After 72 h, cultures were divided in half and transferred to new plates. A 20-mL aliquot of sterilized, distilled water was added to each plate. Agar was cut into 1-cm³ pieces and incubated for 24 h at 24 °C to induce sporangia. Zoospores from the germinated sporangia were collected and adjusted to 1 × 10⁴/mL. *Pythium aphanidermatum* was inoculated onto plants at the rate of 10 mL (1 × 10⁵ zoospores per pot). The zero *P. aphanidermatum* level was achieved with 10 mL of water collected from one plate of non-inoculated agar. After inoculation, all pots were covered with 1 cm of vermiculite to protect the spores from desiccation.

Six weeks after inoculation, plants were removed, separated into shoots and roots, weighed followed by drying to a constant weight at 55 °C, and reweighing. Nematodes were extracted using the NaOCl method (Hussey and Barker, 1973) and counted. Nematode Rf was calculated as described previously. *Pythium aphanidermatum* was re-isolated from plants treated with *P. aphanidermatum* alone using the NaOCl-extraction method (Hussey and Barker, 1973), condensing the liquid and plating the concentrated

liquid on 10% V8 agar (Uchida and Aragaki, 1980). Fresh and dry weights of shoots and roots were recorded.

Analysis of variance of nested data was conducted using PROC MIXED (Statistical Analysis System©, Version 9.2). Where appropriate, least-square means of genotypes were compared using the Tukey-Kramer adjustment for multiple comparisons (PROC GLM, Statistical Analysis System©, Version 9.2).

Field trial of promising green manure genotypes. Based on greenhouse studies, eight green manure crops were selected and planted on 9 July 2004 at Pepeekeo, HI (lat. 19.835° N, long. 155.102° W). The treatments included six green manure genotypes (yellow mustard cv. Ida Gold), French marigolds cvs. Nema-gone and Golden Guardian, sorghum–sudangrass cvs. Sordan 79 and Tastemaker, and sunn hemp). Two additional treatments consisted of a weedy, unplanted plot and a control plot covered with weed mat. Plots were 3.0 × 3.7 m and each treatment was replicated four times in a randomized complete block design.

Yellow mustard seed was broadcast at 45 kg·ha⁻¹ using a mechanical seeder and then raked manually to cover seeds with soil. Marigold seeds were sowed into 96-cell trays in the greenhouse 6 weeks before transplanting into the field. Each tray contained peat-perlite medium (ProMix; Premier Tech Horticulture, Quakertown, PA) at 3 L per tray and fertilized with slow-release fertilizer (Osmocote 13.5–13.5–13.5; The Scotts Company, Marysville, OH) at 89 mL per tray. To control weeds, oxyfluorfen (Goal 2XL; Dow AgroSciences, Indianapolis, IN) was sprayed at 2.3 L·ha⁻¹ onto the marigold plots 1 week before transplanting. Marigold cultivars were transplanted at 0.3 × 0.3-m spacing. Sorghum–sudangrass hybrids were broadcast at 67 kg·ha⁻¹ and raked to cover seeds with soil. As a result of problems with pigeons (*Columba livia* ssp. *domestica*) consuming planted seeds, sorghum–sudangrass hybrids were re-sowed on 12 July 2004 and then re-sowed on 16 July 2004 after installation of bird netting. Sunn hemp seed was inoculated with peanut rhizobial inoculum (Becker Underwood, Ames, IA); then seeds were planted into furrows 0.3 m apart at a rate of 90 kg·ha⁻¹.

The soil series is Hilo (medial over hydrous, ferrihydritic, isohyperthermic acrodioxmic Hydrudands). Before planting, dolomitic lime and crushed coral were broadcast at 2242 kg·ha⁻¹ of CaCO₃ equivalents and then plowed and tilled. Fertilizer (16N–16P–16K analysis) was broadcast in all plots at planting and then at 1 month after planting to result in a rate of 45 kg nitrogen/ha. Rainfall was measured on a daily basis using a data logger (Watchdog data logging rain gauge; Spectrum Technologies, Aurora, IL) and summed per month during the growing period.

Plots of green manure treatments were sampled for biomass in a 100-cm² area at 1, 2, and 3 months after planting. Biomass was not sampled at 4 months after planting, because tops were cut back after 3 months to prevent seeding. Fresh weights were determined followed by drying to constant weight at 55 °C (≈1 week) and then re-weighing. Observations of disease on green manure crops were made along with culturing of pathogens through the University of Hawaii's Agricultural Diagnostic Service Center.

Before planting, two soil samples (0- to 15-cm depth) were taken from five locations each in the experimental field and populations of plant–parasitic nematodes determined per 50 cm³ soil (Byrd et al., 1976; Jenkins, 1964). In addition, the two soil samples were composited and analyzed at the University of Hawaii's Agricultural Diagnostic Service Center for pH, organic carbon (C; Heans, 1984), total nitrogen (N; Yeomans and Bremner, 1991), extractable phosphorus (P) by a modified Truog method (Ayers and Hagihara, 1952), and exchangeable potassium (K), calcium (Ca), and magnesium (Mg) by the ammonium acetate (pH 7) method (Thomas, 1982). On 9 Nov. 2004, soil was sampled at five locations per treatment plot in a zig-zag pattern, composited, and analyzed for final populations of plant–parasitic nematodes.

Analysis of variance was conducted on dry weights of green manure crops using PROC MIXED (SAS Institute©, Version 9.2), because there were repeated measures over time (1, 2, and 3 months). In addition, normalized counts of nematode species were calculated by adding one followed by conversion into natural logarithms; analysis of variance was conducted on this parameter

using PROC GLM (SAS Institute©, Version 9.2). The Waller–Duncan multiple range test was used to separate treatment means. A probability level of 0.05 or less was considered to be statistically significant.

Results

Nematode host status in the greenhouse trial

Significant differences in the Rf of *M. javanica* were found among the 10 green manure genotypes tested (Table 1). *Brassica napus* cv. Sunrise Canola had the highest Rf value, indicating that it served as an excellent host to *M. javanica*. *Tagetes patula* cvs. French Brocade and Pesche's Gold had nil or almost nil Rf values, indicating that they were non-hosts to *M. javanica*. Other green manure genotypes with Rf values lower than one (indicating that they were poor hosts to *M. javanica*) included *Alcea rosea* cv. Nigra, *B. napus* cv. Dwarf Essex, *Crotalaria juncea*, and *Sorghum nothosubsp. drummondii* cvs. Piper and Sordan 79.

Effects of *Meloidogyne javanica* and *Pythium aphanidermatum* in the greenhouse trial: All crops

Probabilities of whole plot main effects, subplot main effects, and their interactions on fresh weights of shoots and roots and Rf are shown in Table 2. Not surprisingly, whole plot main effects of inoculation with *M. javanica* on Rf were significantly positive for all green manure crops.

Effects of *Meloidogyne javanica* and *Pythium aphanidermatum* in the greenhouse trial: Marigolds

Shoots. As a result of significant interactions between marigold genotypes and *M. javanica* treatment as well as among genotype, *M. javanica*, and *P. aphanidermatum* treatments (Table 2), fresh weight of shoots of each marigold genotype as affected by treatments is shown in Figure 1 (top). In other words, effects of *M. javanica* and *P. aphanidermatum* treatments on fresh weight of shoots depended on the marigold genotype.

There appeared to be several patterns of interaction. First, fresh weight of shoots tended to increase compared with the control

Table 2. Probabilities of whole plot factorial treatments (*Meloidogyne javanica*, Mj, and *Pythium aphanidermatum*, Pa) and their interaction, subplot treatments (green manure genotypes, G), and their interactions (G × Mj, G × Pa, and G × Mj × Pa) for fresh weights of shoots or roots and Rf of *M. javanica* in four groups of green manure crops (marigolds, *Brassicaceae*, sorghum–sudangrass, and other species).

	Marigolds: <i>P</i> > <i>F</i>			<i>Brassicaceae</i> : <i>P</i> > <i>F</i>			Sorghum–sudangrass: <i>P</i> > <i>F</i>			Other sp.: <i>P</i> > <i>F</i>		
	FW shoots	FW roots	Rf	FW shoots	FW roots	Rf	FW shoots	FW roots	Rf	FW shoots	FW roots	Rf
Whole plot treatment												
Mj	0.6300	0.0008	0.014	<0.0001	0.0020	0.0496	0.3100	0.7600	<0.0001	0.1700	0.5200	0.039
Pa	0.0450	0.0900	0.330	0.0001	<0.0001	0.5400	0.1000	0.0700	0.2300	0.5500	0.4100	0.096
Mj × Pa	<0.0001	0.1000	0.330	0.0580	0.9900	0.5700	<0.0001	0.0005	0.2300	0.0270	0.3900	0.091
Subplot treatment												
G	0.1200	<0.0001	0.014	<0.0001	<0.0001	0.6000	<0.0001	0.0013	<0.0001	<0.0001	<0.0001	0.024
G × Mj	0.0490	0.6100	0.014	<0.0001	<0.0001	0.6000	0.8500	0.2400	<0.0001	0.3400	0.0040	0.026
G × Pa	0.6600	0.3900	0.001	0.1800	0.0001	0.3100	0.2200	0.4500	0.0820	0.4400	0.5900	0.087
G × Mj × Pa	0.0005	0.0002	0.001	0.0022	0.0100	0.3300	0.1400	0.1800	0.0820	0.1100	0.1400	0.081

FW = fresh weight; Rf = reproductive factor.

Fig. 1. Effect of inoculation of *Meloidogyne javanica* (Mj) alone, *Pythium aphanidermatum* (Pa) alone, or together on per-plant fresh weights of shoots (top) and roots (bottom) of seven *Tagetes* genotypes: *T. patula* cvs. French Brocade (F), Golden Guardian (G), Nema-gone (N), and Pesche's Gold (P); and *T. erecta* cvs. Scarletade (S), Orangeade (O), and Orange Deep (D). Bars indicate SE for all genotype-treatment combinations.

when inoculated with *M. javanica* or *P. aphanidermatum* alone, but when both treatments were combined, a severe decrease occurred. This pattern, indicating negative synergistic effect, was found in *T. patula* cvs. Nema-gone and Pesche's Gold and *T. erecta* cv. Scarletade (Fig. 1, top).

Second, fresh weight of shoots tended to increase compared with the control as a result of *M. javanica* or *P. aphanidermatum* treatment alone with similar shoot fresh weights relative to controls when inoculated with both treatments. This second pattern indicated tolerance to *M. javanica* and *P. aphanidermatum*, alone or in combination, and was exhibited by *T. patula* cv. Golden Guardian and *T. erecta* cv. Orangeade.

Similar results were found for dry weight of shoots (data not shown). We decided to report on results for fresh weight of shoots, because these data integrate information on both dry matter and percent moisture.

Roots. As a result of significant interaction among marigold genotypes and *M. javanica*

and *P. aphanidermatum* treatments (Table 2), fresh weight of roots was plotted for each marigold genotype as affected by treatments (Fig. 1, bottom). Fresh weight of roots exhibited two patterns that were similar to those for fresh weight of shoots. First, cvs. Nema-gone, Pesche's Gold, and Scarletade showed little effect of *M. javanica* or *P. aphanidermatum* treatments alone on fresh weight of roots compared with controls but a severe reduction when both treatments were applied together. Second, cvs. Golden Guardian and Orangeade had similar or increased fresh weight of roots compared with controls in the presence of *M. javanica* or *P. aphanidermatum* treatments alone and a similar weight relative to controls for the combined treatment. This pattern indicated tolerance to *M. javanica* or *P. aphanidermatum* treatments alone or in combination.

In contrast to fresh weight of shoots, another pattern was found for fresh weight of roots in which all three treatments reduced root growth about equally. Cultivars French

Brocade and Orange Deep exhibited this pattern.

Similar results were found for dry weight of roots (data not shown). The data on fresh weight of roots were reported here, because they are indicative of root volume as well as dry matter.

Nematode host status. Significant differences in Rf were found among genotypes as well as significant interactions between genotypes and *M. javanica* treatments, between genotypes and *P. aphanidermatum* treatments, and among genotypes and *M. javanica* and *P. aphanidermatum* treatments (Table 2). However, mean Rf among marigold genotypes inoculated with *M. javanica* ranged from 0 to 0.03, indicating that all marigold genotypes tested were non-hosts to *M. javanica*.

Effects of *Meloidogyne javanica* and *Pythium aphanidermatum* in the greenhouse trial: *Brassicaceae* species

Shoots. As a result of significant interactions between genotypes and *M. javanica* treatments, and among genotypes and *M. javanica* and *P. aphanidermatum* treatments (Table 2), fresh weight of shoots was plotted for individual *Brassicaceae* species for all treatments (Fig. 2, top). In other words, the response of fresh weight of shoots to *M. javanica* or *P. aphanidermatum* treatments depended on the particular genotype.

In the first pattern of interaction, shoot fresh weight of *S. alba* was dramatically reduced by all pathogen treatments. In the second pattern, *B. juncea* cv. Pacific Gold had fresh weight of shoots in the presence of *M. javanica* that was similar to the control. However, it exhibited depressed shoot fresh weights when inoculated with *P. aphanidermatum* alone or in combination, indicating intolerance of this pathogen.

In the third pattern of interaction, *B. napus* cvs. Dwarf Essex and Sunshine Canola had fresh weight of shoots similar to controls when inoculated with *P. aphanidermatum* alone. Shoots of these two cultivars both decreased when inoculated with *M. javanica*. However, when inoculated with both *M. javanica* and *P. aphanidermatum* together, shoot fresh weights decreased dramatically, indicating a negative synergistic effect. These same three patterns of treatment effects were found for dry weights of shoots (data not shown).

Roots. Significant interactions on fresh weight of roots were found between *Brassicaceae* genotypes and *M. javanica* treatments, between genotypes and *P. aphanidermatum* treatment, and among genotypes, *M. javanica*, and *P. aphanidermatum* treatments (Table 2). As a result, fresh weight of roots of individual *Brassicaceae* genotypes were plotted for each treatment (Fig. 2, bottom).

Each genotype appeared to have a different root growth response to the various pathogen treatments. In the case of *S. alba*, all treatments severely depressed root fresh weight. In the case of *B. juncea*, treatment with the *M. javanica* alone appeared to increase root fresh weights, whereas *P. aphanidermatum*

Fig. 2. Effect of inoculation of *Meloidogyne javanica* (Mj) alone, *Pythium aphanidermatum* (Pa) alone, or together on per-plant fresh weights of shoots (top) and roots (bottom) of four *Brassicaceae* genotypes: *Sinapis alba* cv. Ida Gold, *Brassica juncea* cv. Pacific Gold, *B. napus* cvs. Dwarf Essex (D) and Sunshine Canola (S). Bars indicate SE for all genotype-treatment combinations.

or combination treatments reduced root fresh weights. For *B. napus* cv. Dwarf Essex, *M. javanica* treatments alone or in combination resulted in decreased root fresh weights; *P. aphanidermatum* treatment alone had no effect on root fresh weight. For *B. napus* cv. Sunshine Canola, *M. javanica* treatment alone had little effect on root fresh weight; however, *P. aphanidermatum* treatment alone reduced root fresh weight, and the combination of treatments severely depressed root fresh weight, indicating a negative synergistic effect. Similar results were found for dry weights of roots (data not shown).

Nematode host status. There were no significant effects on Rf resulting from *P. aphanidermatum* treatment or genotype or interactions (Table 2). For genotypes inoculated with *M. javanica* alone, Rf were 0.003, 0.52, 0.14, and 0.30 for *S. alba*, *B. juncea*, and *B. napus* cvs. Dwarf Essex and Sunshine Canola, respectively. These Rf values indicated that *S. alba* was a non-host and the other three *Brassicaceae* genotypes were poor hosts for *M. javanica*.

Effects of *Meloidogyne javanica* and *Pythium aphanidermatum* in the greenhouse trial: Sorghum-sudangrass

Shoots. A significant whole plot interaction was found for fresh weight of shoots of sorghum-sudangrass genotypes between *M. javanica* and *P. aphanidermatum* treatments (Tables 2 and 3). Inoculation with *M. javanica* in the absence of *P. aphanidermatum* increased mean fresh weight of shoots (Table 3); however, in the presence of *P. aphanidermatum*, inoculation with *M. javanica* decreased mean fresh weight of shoots.

Subplot main effect of sorghum-sudangrass genotypes on fresh weight of shoots was significant (Table 2). The cultivar with the lowest fresh weight of shoots was cv. Piper; all other genotypes had significantly greater fresh weight of shoots and did not differ from each other (Table 3). Statistical analysis of dry weight of shoots was not conducted as a result of too many missing values caused by an oven malfunction.

Roots. Similar to fresh weight of shoots, a significant interaction was found between

M. javanica and *P. aphanidermatum* treatments on fresh weight of roots of sorghum-sudangrass genotypes (Table 2). Inoculation with *M. javanica* in the absence of *P. aphanidermatum* increased mean fresh weight of roots; however, inoculation with *M. javanica* in the presence of *P. aphanidermatum* decreased fresh weight of roots.

The subplot main effect of sorghum-sudangrass genotypes was significant (Table 2). Cultivar Tastemaker had the highest fresh weight of roots averaged across all treatments (Table 3); it differed from cvs. Piper and Sordan 79, which had the lowest fresh weight of roots. The other four cultivars had fresh weight of roots that did not differ from the highest weight. Similar results were found for dry weight of roots (data not shown).

Nematode host status. Subplot genotype treatments differed significantly in Rf as well as the interaction between genotype and *M. javanica* (Table 2). This interaction was the result of some genotypes responding to inoculation with *M. javanica* by a greater increase in Rf compared with others; in other words, some genotypes are more susceptible to *M. javanica* than others. Cultivar Bale All had the greatest Rf (Table 3). Cultivars Graze-All, Baler, Piper, and Sordan 79 all had significantly lower Rf than cv. Bale All. Cultivars Bale All and Tastemaker were the only good hosts.

Effects of *Meloidogyne javanica* and *Pythium aphanidermatum* in the greenhouse trial: Other species

Shoots. As a result of accidental contamination of controls by *M. javanica*, data for *A. rosea* were removed. For the three remaining species, there were no significant whole plot main effects of *M. javanica* or *P. aphanidermatum* treatments on fresh weight of shoots; however, a significant interaction was found between the two treatments (Table 2). In other words, the effect of *M. javanica* treatment on fresh weight of shoots depended on the presence or absence of the *P. aphanidermatum* treatment. Treatment with *M. javanica* in the absence of *P. aphanidermatum* increased mean fresh weights of shoots compared with the uninoculated control; however, treatment with *M. javanica* in combination with *P. aphanidermatum* treatment tended to decrease fresh weight of shoots (Table 4).

The subplot main effect of genotypes on fresh weight of shoots was significant, and there were no significant interactions between subplot treatments and whole plot treatments (Table 2). Mean fresh weight of shoots averaged across all treatments was lowest for *I. helenium* (Table 4). *Crotalaria juncea* and *R. hirta* had similar fresh weights of shoots, not differing from each other but both differing from *I. helenium*.

Roots. A significant subplot main effect of genotypes was found on fresh weight of roots with *R. hirta* having significantly greater root fresh weight than the other two species. In addition, a significant interaction was found between *M. javanica* treatments and genotypes

Table 3. Fresh weights of shoots and roots of sorghum–sudangrass genotypes and reproductive factor (Rf) as affected by whole plot treatment of *Meloidogyne javanica* (without, Mj 0, and with, Mj 1), interaction effects of *M. javanica* and *Pythium aphanidermatum* (without, Pa 0, and with, Pa 1), and subplot treatment of genotypes (cvs. Bale All, Bale All III, Baler, Graze-All, Piper, Sordan 79, Tastemaker).

	FW shoots (g) ^{a,y}	SE		FW roots (g)	SE		Rf	SE	
Whole plot treatment									
Mj treatment means									
Mj 0	27.9	(1.24)		14.8	(0.72)		0.0001	(0.0001)	
Mj 1	29.9	(1.22)		14.6	(0.67)		1.2900	(0.1700)	
Interaction means									
Mj 0 × Pa 0	21.9	(1.22)		12.0	(4.2)		0.0000	(0.0000)	
Mj 1 × Pa 0	33.8	(1.91)		16.1	(1.04)		1.1500	(0.2000)	
Mj 0 × Pa 1	33.8	(1.62)		17.5	(1.04)		0.0002	(0.0002)	
Mj 1 × Pa 1	26.2	(1.26)		13.3	(0.79)		1.4300	(0.2700)	
Subplot treatment									
Sorghum means									
Bale All	32.1	(1.63)	a	13.8	(0.88)	ab	1.3900	(0.1700)	a
Bale All III	33.1	(2.51)	a	16.1	(1.68)	ab	0.8700	(0.1700)	abc
Baler	33.4	(2.31)	a	15.7	(1.04)	ab	0.6300	(0.1700)	bcd
Graze-All	28.5	(2.17)	a	15.6	(1.21)	ab	0.1000	(0.1700)	de
Piper	17.5	(1.32)	b	11.9	(1.26)	b	0.1900	(0.1900)	cde
Sordan 79	28.0	(2.04)	a	11.9	(0.63)	b	0.2400	(0.1800)	cde
Tastemaker	28.5	(2.07)	a	17.6	(1.57)	a	1.0000	(0.1700)	abc

^aMeans that are followed by the same letter in one column are not significantly different among genotypes ($P < 0.05$), according to the Tukey-Kramer adjustment for multiple comparisons.

^yMeans are followed by SE in parentheses.

FW = fresh weight.

Table 4. Fresh weight (FW) of shoots and roots of other species and nematode reproductive factor (Rf) as affected by whole plot treatment of *Meloidogyne javanica* (without, Mj 0, and with, Mj 1), interaction effect of by *Pythium aphanidermatum* (without, Pa 0, and with, Pa 1), subplot treatment of genotypes (G), and interaction effects of genotype (*Crotalaria juncea*, C, *Inula helenium*, I; and *Rudbeckia hirta*, R) by *M. javanica*.

	FW shoots (g) ^{a,y}	SE		FW roots (g)	SE		Rf	SE	
Whole plot treatment									
Nematode treatment means									
Mj 0	10.6	(1.10)		14.4	(2.53)		0.24	(8.44)	
Mj 1	12.8	(1.03)		12.1	(2.46)		26.2	(7.93)	
Interaction means									
Mj 0 × Pa 0	9.2	(1.52)		11.3	(3.56)		0.41	(11.50)	
Mj 1 × Pa 0	15.2	(1.31)		12.2	(3.24)		5.86	(9.90)	
Mj 0 × Pa 1	12.1	(1.59)		17.5	(3.60)		0.07	(11.97)	
Mj 1 × Pa 1	10.5	(1.59)		12	(3.71)		46.5	(12.00)	
Subplot treatment									
Genotype means									
<i>C. juncea</i>	15.2	(1.13)	a	12.6	(2.18)	bc	0.3	(8.64)	a
<i>I. helenium</i>	4.14	(1.48)	b	6.64	(2.73)	c	39.2	(11.17)	a
<i>R. hirta</i>	15.9	(1.28)	a	20.5	(2.42)	a	0.11	(9.77)	a
G × Mj interaction means									
C × Mj 0	12.8	(1.48)		9.64	(2.88)		0	(11.16)	
C × Mj 1	17.6	(1.71)		15.5	(3.27)		0.6	(12.86)	
I × Mj 0	3.06	(2.14)		5.46	(3.94)		0.62	(16.00)	
I × Mj 1	5.24	(2.03)		7.83	(3.77)		77.8	(15.23)	
R × Mj 0	16.1	(2.00)		28.2	(3.78)		0.09	(15.20)	
R × Mj 1	15.7	(1.57)		12.9	(3.00)		0.13	(11.83)	

^aMeans that are followed by the same letter in one column are not significantly different among genotypes ($P < 0.05$), according to the Tukey-Kramer adjustment for multiple comparisons.

^yMeans are followed by SE in parentheses.

for fresh weight of roots (Table 2). In other words, the effect of *M. javanica* on fresh weight of roots depended on the green manure genotype. For both *C. juncea* and *I. helenium*, inoculation with *M. javanica* increased mean fresh weight of roots compared with the uninoculated control (Table 4). In contrast, for *R. hirta*, inoculation with *M. javanica* depressed mean fresh weights of roots.

Results for dry weight of roots were similar (data not shown) with one exception. Dry weight of roots was greatest for *C. juncea*; it was significantly greater than that for *I. helenium* or *R. hirta*.

Nematode host status. A subplot main effect of genotype on Rf was significant; also, a significant interaction was found

between *M. javanica* and genotype, indicating that genotypes differed in host status to *M. javanica* (Table 2). Reproductive factor of *I. helenium* inoculated with *M. javanica* was the greatest (Table 4).

Field trial of promising green manure genotypes

Preplant plant–parasitic nematodes populations varied. Reniform nematodes (*Rotylenchulus reniformis*) ranged from 670 to 2270 per 50 cm³. Root-knot nematodes ranged from 30 to 120 per 50 cm³; and spiral nematodes (*Helicotylenchus* sp.) ranged from 0 to 20 per 50 cm³.

Analysis of a composited soil sample before planting showed a pH of 6.2, organic C of 7.6%, and total N of 0.5%. Extractable P

and exchangeable K, Ca, and Mg were (mg·kg⁻¹): 60, 164, 1304, and 374, respectively. Rainfall for the months of July, August, September, and Oct. 2004 were 95, 359, 282, and 682 mm, respectively.

On 21 July 2004, leaf spots were observed on 25% of the sorghum–sudangrass hybrids and diagnosed as caused by *Colletotrichum* spp., an opportunist fungal pathogen. A few marigolds were observed to die back on 3 Aug. 2004. *Sclerotium rolfsii*, a soilborne pathogen with a wide host range, was isolated from these diseased plants. Over 50% of yellow mustard plants were observed to be dying back on 23 Aug. 2004. Two possible pathogens isolated from roots were *Rhizoctonia solani* and *Pythium splendens*; both pathogens are favored by poor root drainage

and poor soil aeration. We decided to remove all yellow mustard plants during Oct. 2004, because they were severely affected by this disease and *P. splendens* is a pathogen that could affect a subsequent taro crop.

Significant effects resulting from sampling time (months) and green manure treatments were found for dry weights of plants in a 100-cm² area. Mean shoot dry weight of sorghum–sudangrass (*S. nothosubsp. drummondii*) cv. Sordan 79 was the greatest (Table 5). Dry weights of both marigold (*T. patula*) cultivars, sorghum–sudangrass cv. Tastemaker, and sunn hemp (*C. juncea*) did not differ from each other nor from the maximum dry weight. Yellow mustard (*S. alba*) cv. Ida Gold had the lowest dry weight, which was less than all other treatments except for sunn hemp and weedy, unplanted plots.

No significant differences among green manure treatments were found in populations of root-knot nematodes with values ranging from 0 to 80 per 50 cm³ (data not shown). Green manure crops significantly affected populations of reniform nematodes with the lowest counts found in both marigold plots (Table 5). Next lowest were sorghum–sudangrass cv. Tastemaker and sunn hemp, which did not differ from each other nor from the marigolds. Interestingly, populations of reniform nematodes in these four green manure genotypes were lower than those of clean fallow (weed mat). Initial, normalized population counts of reniform nematodes ranged between 6.50 and 7.73, levels similar to the weedy, unplanted plots and those containing yellow mustard.

Discussion

In several recently published literature reviews (McSorley, 2011; Thoden et al., 2011), it was shown that effects of green manure crops on plant–parasitic nematodes depend on the specific species and cultivars of crops, the specific species and races of target pathogens, and environmental conditions. In this series of greenhouse and field

experiments, we examined various green manure crop species and cultivars under relevant soil and environmental conditions.

The two greenhouse studies involved many different plant genotypes, and the decision was made to use Rf as the most objective, quantitative parameter to measure for comparison of nematode host status across genotypes. A green manure genotype was considered a good host for *M. javanica* when Rf was greater than 1, a poor host when Rf was less than 1 but greater than 0.1, and a non-host when Rf was less than or equal to 0.1 (Mojtahedi et al., 1993; Seinhorst, 1967). In the first, preliminary study, marigold *T. patula* cvs. French Brocade and Pesche's Gold were considered to be non-hosts of *M. javanica*. Poor hosts of *M. javanica* were canola cv. Dwarf Essex, sorghum–sudangrass cvs. Piper and Sordan 79, and sunn hemp.

Temperatures have been shown in earlier studies to affect the suppressiveness of green manure crops to nematodes. In the two greenhouse trials, Rf of *M. javanica* in *B. napus* cv. Sunrise Canola ranged from 4.15 in the first study to 0.04 in the second one. Reproductive factor of *M. javanica* in *S. alba* cv. Ida Gold ranged from 1.45 in the first study to 0.003 in the second one. Different Rf ranges of *M. javanica* between the two trials could be the result of seasonal effects. The first trial was conducted during the winter, and the second trial was conducted during the summer with mean air temperatures of 24 and 28 °C, respectively. Similar results were found in Australia, where increased populations of *M. javanica* were found for *Brassica juncea* and *B. napus* grown during high temperatures; however, under cooler temperatures, the plants prevented proliferation of *M. javanica* (Oka, 2010). Also, *Meloidogyne incognita* was able to complete its life cycle on a marigold *Tagetes* hybrid at a soil temperature of 30 °C but not at lower temperatures (Ploeg and Maris, 1999). Apparently, there are optimum growth temperatures for particular green manure plant species at which they are most healthy and able to resist or tolerate plant–parasitic nematodes.

Evaluating green manure crops for multiple pathogens that are likely to be found in certain crop rotations is important. However, there are very few studies (Gracia et al., 1991; Johnson and Littrell, 1970) that examine the interaction between plant–parasitic nematodes and other pathogens on green manure crops. In the second series of greenhouse studies, we examined the interaction of *M. javanica* and *P. aphanidermatum* on four groups of green manure genotypes and found that the effectiveness of particular green manure genotypes depends on their resistance to or tolerance of multiple pathogens.

The usefulness of marigolds as green manures in an upland taro cropping system will depend on the species and cultivar selected. Although all genotypes tested did not support *M. javanica* reproduction, *T. patula* cv. French Brocade and *T. erecta* cv. Orange Deep were susceptible to *P. aphanidermatum* based on reduced shoot and root weights. Although *T. patula* cvs. Nema-gone and Pesche's Gold and *T. erecta* cv. Scarletade were not susceptible to nematode or *Pythium* treatment alone, they were severely suppressed by the combination of these two pathogens. Such a negative synergistic effect is thought to be the result of wounding by the endoparasitic *Meloidogyne* spp. that allows entry of *Pythium*. Gracia et al. (1991) showed an additive interaction between *Meloidogyne hapla* and *Pythium tracheiphilum* on lettuce. Similarly, in chrysanthemum, it was reported that combined inoculations of *P. aphanidermatum* and *M. incognita* reduced height and root growth to a greater extent than inoculation of either pathogen alone (Johnson and Littrell, 1970).

Of the seven marigold genotypes tested in the greenhouse, *T. patula* cv. Golden Guardian and *T. erecta* cv. Orangeade exhibited the least reduction in shoot and root fresh weights in the presence of both pathogens and could be considered the most promising marigold cultivars. In the field, *T. patula* cv. Golden Guardian grew well and supported the lowest populations of reniform nematodes.

Table 5. Mean dry weight of shoots sampled in a 100-cm² area averaged across three sampling dates (1, 2, and 3 months after planting) and normalized counts of reniform nematodes (per 50 cm³ of soil) sampled at 4 months after planting in green manure treatments (yellow mustard, *Sinapis alba* cv. Ida Gold; marigold, *Tagetes patula* cvs. Nema-gone and Golden Guardian; sorghum–sudangrass, *Sorghum nothosubsp. drummondii* cvs. Sordan 79 and Tastemaker; sunn hemp, *Crotalaria juncea*; weedy unplanted; and clean fallow) planted in a field trial at Pepekeo, HI.

Green manure crops	Shoot dry wt (g) ^{a,y,x}			Reniform counts ^w		
<i>Sinapis alba</i> cv. Ida Gold	3.7	(1.79)	c	7.57	(0.30)	a
<i>Tagetes patula</i> cv. Nema-gone	47.2	(14.4)	a	2.45	(0.82)	e
<i>Tagetes patula</i> cv. Golden Guardian	43.0	(10.8)	ab	2.46	(0.21)	e
<i>Sorghum nothosubsp. drummondii</i> cv. Sordan 79	50.3	(11.6)	a	3.77	(0.49)	cd
<i>Sorghum nothosubsp. drummondii</i> cv. Tastemaker	46.8	(8.2)	a	2.95	(0.38)	de
<i>Crotalaria juncea</i>	26.0	(5.6)	abc	3.13	(0.50)	de
Weedy, unplanted	20.6	(9.7)	bc	6.09	(0.52)	b
Clean fallow (weed mat)				4.81	(0.36)	c
ANOVA: <i>P</i> > <i>F</i>						
Month	0.003			—		
Block	0.990			0.13		
Treatment	0.012			<0.0001		
Treatment × month	0.180			—		

^aDry weights were measured repeatedly at 1, 2, and 3 months.

^yMeans are followed by SE in parentheses.

^xMeans in a column with the same letters are not significantly different based on the Waller–Duncan multiple range test.

^wNematode counts were normalized by the addition of 1 followed by conversion to the natural logarithm.

ANOVA = analysis of variance.

However, both marigold cultivars in the field were occasionally infected with *Sclerotium rolfsii*, and this pathogen is known to attack taro also (Ooka, 1994).

One economic limitation to the use of marigolds in an integrated pest management (IPM) system is that they were planted first in trays in the nursery and then transplanted to the field, resulting in an extra step and added cost to the establishment of a rotational green manure crop. Another limitation is the high cost of marigold seed (Hooks et al., 2010). Seed of *T. patula* cv. Golden Guardian was \$1.95 per packet of 800 seeds (\$0.0024 per seed); similarly, seed of *T. patula* cv. Nema-Gone was \$6.95 per packet of 2000 seeds (\$0.0035 per seed). These marigold cultivars are sold to backyard gardeners rather than to commercial farmers. As a more cost-effective alternative to French marigolds, African marigold (*T. erecta*) cultivars are grown commercially for poultry feed (Taylor, 2010), and the cost of their seeds was 10-fold less than that of French marigold cultivars. The cost of *T. erecta* seed was \$100/kg with 300 seeds/g (\$0.0003 per seed).

The *Brassicaceae* species screened in the greenhouse are considered to be unacceptable as green manures for an upland taro cropping system as a result of their high susceptibility to *M. javanica* and/ or *P. aphanidermatum* treatments (Fig. 2). The field trial of yellow mustard cv. Ida Gold demonstrated that this genotype was not well adapted to the humid environment of the Hawaii Island, dying back as a result of several root pathogens. In particular, yellow mustard would not be suitable for an IPM system with taro, because it was infected with *Pythium splendans*, a pathogen that is known to infect taro.

Sorghum–sudangrass hybrids tested in both the greenhouse and field are promising green manure crops for rotation with upland taro production. These hybrids were not only resistant and tolerant to *M. javanica*, undamaged by *P. aphanidermatum*, or the combination of both pathogens, but they also produced large amounts of biomass (Table 3). Cultivars Graze-All and Sordan 79 were least susceptible to *M. javanica* and their shoot fresh weights were not different from other hybrids tested. An additional advantage of cv. Graze-All is that as a male-sterile hybrid it will not set seed, providing that other sorghum–sudangrass cultivars with viable pollen are not grown nearby. This trait will prevent it emerging as a weed during the next crop rotation. In the field, cvs. Sordan 79 and Tastemaker grew well and produced the copious amounts of biomass (Table 5). In addition, cv. Tastemaker did not differ in the population of reniform nematode from marigold genotypes.

Consumption of planted sorghum–sudangrass seeds by pigeons was a major problem during planting of these small plots; however, it is likely that a commercial-scale planting will exhibit reduced impact of bird feeding. If birds continue to be a problem at a larger field scale, perhaps, then planting of seedlings similar to marigolds might be more

cost-effective than establishment of bird netting. Alternatively, the use of a compound that deters feeding by birds would be useful if it were registered for use in Hawaii.

Sunn hemp (*C. juncea*) is another promising rotation crop, because in the greenhouse, it is a poor host for *M. javanica* with a low Rf. In addition, its biomass was not adversely affected by *M. javanica* or *P. aphanidermatum* alone or in combination (Tables 2 and 4). However, in the field, sunn hemp was slower in getting established than sorghum–sudangrass hybrids as shown by its lower mean biomass (Table 5). Also, in another field trial, sunn hemp was affected by powdery mildew (*Oidium* spp.) as a result of the very moist conditions found along the Hamakua Coast of the Hawaii Island.

Sunn hemp has been used in several tropical cropping systems for control of several nematode species and weeds (Valenzuela and Smith, 2002; Wang et al., 2002, 2008), including a taro intercropping system (Oliveira et al., 2007). An additional benefit from sunn hemp is that it is a legume and can fix atmospheric N₂, making it a sustainable alternative to synthetic N fertilizer inputs. According to Rotar and Joy (1983), 150 to 165 kg N/ha was added to the soil when sunn hemp was incorporated after 60 d of growth.

In the field trial, both marigold genotypes, sorghum–sudangrass cv. Tastemaker, and sunn hemp had populations of reniform nematodes that were lower than clean fallow (weed mat), perhaps indicating that allelopathic compounds could be effective in addition to their non-host status to nematodes. Interestingly, in a review of cover crops in the southeastern United States, McSorley (2011) stated that effective rotation crops were not able to reduce nematode numbers below those achieved during clean fallow. Our results will need to be confirmed in future field studies; however, environmental conditions in Hawaii differ from those in the southeastern United States and could affect production of allelopathic compounds in green manure genotypes.

In conclusion, based on resistance and/or tolerance to *M. javanica* or *P. aphanidermatum*, alone or in combination in the greenhouse, and/or adaptation to the field environment on the Hawaii Island, the most promising green manure crops evaluated were marigold cv. Orangeade, sorghum–sudangrass cvs. Graze-All and Sordan 79, and sunn hemp. Evaluations of several of these promising green manure crops in rotation with taro are in progress. It must be remembered, however, that green manure crops are only one aspect needed for sustainable management of plant–parasitic nematodes (Collange et al., 2011; McSorley, 2011).

Literature Cited

- Ayers, A.S. and H.H. Hagihara. 1952. Available phosphorus in Hawaiian soil profiles. Hawaiian Planter's Record. 54:81–99.
- Brady, N. and R. Weil. 1999. Elements of the nature and properties of soils. Prentice-Hall, Upper Saddle River, NJ.

- Bridge, J. and P. Jatala. 2005. Nematode parasites of root and tuber crops, p. 137–180. In: Luc, M., R.A. Sikora and J. Bridge (eds.), Plant parasitic nematodes in subtropical and tropical agriculture. CAB International, Oxfordshire, UK.
- Byrd, D.W., Jr., K.R. Barker, H. Ferris, C.J. Nusbaum, W.E. Griffin, R.H. Small, and C.A. Stone. 1976. Two semi-automatic elutriators for extracting nematodes and certain fungi from the soil. J. Nematol. 8:206–213.
- Chitwood, D.J. 2002. Phytochemical based strategies for nematode control. Annu. Rev. Phytopathol. 40:221–249.
- Collange, B., M. Navarrete, G. Peyre, T. Mateille, and M. Tchamitchian. 2011. Root-knot nematode (*Meloidogyne*) management in vegetable crop production: The challenge of an agro-nomic system analysis. Crop Prot. 30:1251–1262.
- Gracia, J.A., R.D. Reeleder, and G. Belair. 1991. Interactions between *Pythium tracheiphilum*, *Meloidogyne hapla*, and *Pratylenchus penetrans* on lettuce. Phytoprotection 72:105–114.
- Heans, D.L. 1984. Determination of total organic C in soils by an improved chromic acid digestion and spectrophotometric procedure. Commun. Soil Sci. Plant Anal. 15:1191–1213.
- Hooks, C.R.R., K.-H. Wang, A. Ploeg, and R. McSorley. 2010. Using marigold (*Tagetes* spp.) as a cover crop to protect crops from plant–parasitic nematodes. Appl. Soil Ecol. 46:307–320.
- Hussey, R.S. and K.R. Barker. 1973. A comparison of methods of collecting inocula for *Meloidogyne* spp., including a new technique. Plant Dis. Rptr. 57:1025–1028.
- Jenkins, W. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Dis. Rptr. 48:692.
- Johnson, A.W. and R.H. Littrell. 1970. Pathogenicity of *Pythium aphanidermatum* to chrysanthemum in combined inoculations with *Belonolaimus longicaudatus* or *Meloidogyne incognita*. J. Nematol. 2:255–259.
- Jourand, P., S. Rapior, M. Fargette, and T. Mateille. 2004. Nematostatic effects of a leaf extract from *Crotalaria virgulata* subsp. *grantiana* on *Meloidogyne incognita* and its use to protect tomato roots. Nematol. 6:79–84.
- McLeod, R.W. and C.C. Steel. 1999. Effects of brassica-leaf green manures and crops on activity and reproduction of *Meloidogyne javanica*. Nematol. 1:613–624.
- McSorley, R. 2011. Overview of organic amendments for management of plant–parasitic nematodes, with case studies from Florida. J. Nematol. 43:69–81.
- Mojtahedi, H., G.S. Santo, and R.E. Ingham. 1993. Suppression of *Meloidogyne chitwoodi* with sudangrass cultivars as green manure. J. Nematol. 25:303–311.
- Oka, Y. 2010. Mechanisms of nematode suppression by organic soil amendments—A review. Appl. Soil Ecol. 44:101–115.
- Oliveira, F.L., J.G.M. Guerra, R.L.D. Ribeiro, D.L. Almeida, E.E. Silva, S. Urquiza, and J.A.A. Espindola. 2007. The use of sunn hemp as green manure intercropped with taro. Hort. Brasileira 25:562–566.
- Ooka, J.J. 1994. Taro diseases a guide for field identification. Univ. of Hawaii, College of Trop. Agr. and Human Resources, Honolulu, HI, Research Extension Series 148.
- Ortiz, A.M., S.C. Miyasaka, J.J. Cho, and B.S. Sipes. 2008. Resistance and tolerance to *Meloidogyne javanica* in *Colocasia esculenta* from Thailand, Vietnam, and Nepal. Intl. J. Nematol. 18:66–70.

- Ploeg, A.T. and P.C. Maris. 1999. Effects of temperature on the duration of the life cycle of a *Meloidogyne incognita* population. *Nematol.* 1:389–393.
- Reynolds, L.B., J.W. Potter, and B.R. Ball-Coelho. 2000. Crop-rotation with *Tagetes* sp. is an alternative to chemical fumigation for control of root-lesion nematodes. *Agron. J.* 92:957–966.
- Rotar, P.P. and R.J. Joy. 1983. ‘Tropic Sun’ sunn hemp *Crotalaria juncea* L. Univ. of Hawaii, College of Trop. Agr. and Human Resources, Honolulu, HI, Res. Ext. Series 036. 21 Aug. 2012. <<http://www.ctahr.hawaii.edu/oc/freepubs/pdf/RES-036.pdf>>.
- Seinhorst, J.W. 1967. The relationships between population increase and population density in plant parasitic nematodes. III. Definition of the terms host, host status and resistance. IV. The influence of external conditions on the regulation of population density. *Nematologica* 13: 429–442.
- Sipes, B., S. Nelson, and A. Arakaki. 1995. *Meloidogyne javanica* damage to dryland taro cultivars. *Afro-Asian J. of Nematol.* 5:141–147.
- Taylor, J. 2010. The marigold of California. *Pacific Hort.* 20 Dec. 2012. <<http://www.pacifichorticulture.org/articles/the-marigold-of-california/>>.
- Thoden, T.C., G.W. Korthals, and A.J. Termorshuizen. 2011. Organic amendments and their influences on plant-parasitic and free-living nematodes: A promising method for nematode management? *Nematol.* 13:133–153.
- Thomas, G.W. 1982. Exchangeable cations, p. 159–165. In: Page, A.L., R.H. Miller, and D.R. Keeney (eds.). *Chemical and microbiological properties*. Amer. Soc. of Agron., Madison, WI.
- Uchida, J.Y. and M. Aragaki. 1980. Chemical stimulation of oospore formation in *Phytophthora capsici*. *Mycologia* 72:1103–1108.
- Valenzuela, H. and J. Smith. 2002. ‘Tropic Sun’ Sunnhemp. Univ. of Hawaii, Coll. of Trop. Agr. and Human Resources, Honolulu, HI, Publication SA-GM-11.
- Wang, K.-H., R. McSorley, R.N. Gallaher, and N. Kokalis-Burelle. 2008. Cover crops and organic mulches for nematode, weed and plant health management. *Nematol.* 10:231–242.
- Wang, K.-H., B.S. Sipes, and D.P. Schmitt. 2002. *Crotalaria* as a cover crop for nematode management: A review. *Nematropica* 32:35–57.
- Widmer, T. and G.S. Abawi. 2002. Relationship between levels of cyanide in sudangrass hybrids incorporated into soil and suppression of *Meloidogyne hapla*. *J. Nematol.* 34:16–22.
- Yeomans, J.C. and J.M. Bremner. 1991. Carbon and nitrogen analysis of soils by automated combustion techniques. *Commun. Soil Plant Anal.* 22:843–850.