RESEARCH/INVESTIGACIÓN

VEGETABLE PLANT VIGOR AND SUPPRESSION OF *MELOIDOGYNE INCOGNITA* WITH VETIVER SHOOT AMENDMENTS IN SOIL

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ABSTRACT

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Vetiver grass (*Vetiveria zizanioides*) is widely planted in tropical areas, and has many uses, including application of shoots as a mulch or soil amendment. Vetiver produces compounds that are active against nematodes and various cultivars are resistant to *Meloidogyne* spp. (root-knot nematodes). The commercially available vetiver cv. Sierra was tested for host status to *Meloidogyne incognita* and found to be resistant. To determine effects of vetiver soil amendments on vegetable crops, we conducted greenhouse trials with seedlings of cucumber, pepper, and tomato transplanted into soil that had been mixed with chopped, fresh vetiver shoots at 0%, 3%, and 5% g fresh vetiver/g dry soil (weight/weight). Results varied with time of amendment, amount of vetiver green manure, and plant species. Cucumber seedling response varied from no significant effect to some phytotoxicity. Tomato seedlings had lower shoot heights and root fresh weights in higher vetiver amendment rates. Pepper roots tended to be smallest when seedlings were transplanted into amended soil 3-4 wk after vetiver amendment, as opposed to transplanting soon after amendment application. Vetiver soil amendments were also tested in the greenhouse for suppression of *M. incognita* on cucumber at 0%, 1%, 3%, 5%, and 10% g fresh vetiver/g dry soil (weight/weight). Only the highest amendment rate consistently suppressed nematodes on cucumber roots with eggs per gram root reduced by 46% to 67% compared with the controls without vetiver amendment. Further studies would indicate whether amending soil with vetiver at practical application rates, if incorporated as part of a broader strategy for nematode suppression, could potentially contribute to root-knot nematode management.

Key words: Green manure, host resistance, management, *Meloidogyne*, nematode, phytotoxicity, plant vigor, root-knot nematode, soil amendment, vetiver

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RESUMEN

Jindapunnapat, K., S. L. F. Meyer, M. H. MacDonald, N. D. Reetz, D. J. Chitwood, E. P. Masler, N. Soonthornchareonnon, M. J. Camp, A. Sasnarukkit, y B. Chinnasri. 2019. El vigor de la planta vegetal y la supresión de *Meloidogyne incognita* con enmiendas de vetiver en el suelo. Nematropica 49:208-219.

El pasto vetiver (*Vetiveria zizanioides*) se planta ampliamente en áreas tropicales y tiene muchos usos, incluida la aplicación de brotes como mulch o enmienda del suelo. Vetiver produce compuestos que son activos contra los nematodos y varios cultivares son resistentes a *Meloidogyne* spp. (nematodos de la raíz-nudo). El vetiver cv disponible comercialmente. Sierra se sometió a pruebas de estado de acogida a *Meloidogyne incognita* y se encontró que era resistente. Para determinar los efectos de las enmiendas del suelo del vetiver en los cultivos de hortalizas, realizamos pruebas en invernaderos con plántulas de pepino, pimiento y tomate transplantadas en el suelo que se había mezclado con brotes de vetiver frescos picados. Los resultados variaron con el tiempo de la enmienda, la cantidad de abono verde vetiver y las especies de plantas. La respuesta de las plántulas de pepino varió de ningún efecto significativo a cierta fitotoxicidad. Las plántulas de tomate tuvieron alturas de brotes más bajas y pesos frescos de raíz en tasas de enmienda de vetiver más altas. Las raíces de la pimienta tendieron a ser más pequeñas cuando las plántulas fueron trasplantadas a un suelo enmendado 3-4 semanas después de la modificación del vetiver, en lugar de trasplantar antes de la aplicación de la enmienda. Las enmiendas del suelo de vetiver también se probaron en el invernadero para la supresión de *M. incognita* en pepino al 1%, 3%, 5% y 10% de vetiver fresco/g de suelo (peso/peso). Solo la tasa de enmienda más alta suprimió sistemáticamente los nematodos en las raíces de pepino con huevos/g de raíz reducido en un 46% a 67% en comparación con los controles sin la enmienda del vetiver. Estudios adicionales indicarían si la modificación del suelo con vetiver a tasas de aplicación práctica, si se incorpora como parte de una estrategia más amplia para la supresión de nematodos, podría contribuir potencialmente al manejo de nematodos de nudo de raíz.

Palabras clave: Abono verde, resistencia del hospedador, manejo, *Meloidogyne*, nematodo, fitotoxicidad, planta vigor, nematodo de nudo de la raíz, enmienda del suelo, vetiver

INTRODUCTION

Concern about safety of synthetic agricultural pesticides has encouraged growers to reduce application of these chemicals in food production. The continuing need to mitigate damage caused by plant pathogens, including plant-parasitic nematodes, has resulted in work on developing or improving biobased management strategies. The many approaches employed for nematode management include application of plant parts as green manures/amendments or plant mulches, and identification of nematicidal secondary metabolites produced by these plants (Chitwood, 2002; Cherr *et al*., 2006; Dayan *et al.,* 2009).

Vetiver (*Vetiveria zizanioides* (L.) Nash (synonym: *Chrysopogon zizanioides* (L.) Roberty), a grass that is widely planted in tropical areas worldwide (Truong, 2000; Maffei, 2002; Lim, 2016), is a candidate for studies on nematode management. Vetiver is used for multiple purposes, including essential oil production, pharmaceuticals, construction materials, soil and water conservation, weed reduction, bioremediation, human food and animal feed, biofuel, and landscaping (Chomchalow, 2001; Belhassen *et al*., 2015; Lim, 2016). The shoots of vetiver are applied as mulches or green manures for weed control, water conservation, and improvement of soil physical qualities (Babalola *et al*., 2007; Are *et al*., 2018).

In addition to these numerous applications, vetiver produces compounds that are active against many organisms, including termites, ticks, fleas, and mosquitoes (Zhu *et al.,* 2001; Chauhan and Raina, 2006; Flor-Weiler *et al.,* 2011). Vetiver plants are also resistant to multiple *Meloidogyn*e spp., including *Meloidogyne incognita* (Kofoid &

White) Chitwood (West *et al*., 1996; Maffei, 2002; Fourie *et al*., 2007). Aqueous and ethanolic extracts from vetiver plants have variously been reported to be nontoxic, repellent, nematostatic, and/or lethal to plant-parasitic nematodes (Wiratno *et al*., 2009; Ahuja *et al*., 2014; Jindapunnapat *et al*., 2018). Nematode-antagonistic metabolites have the potential to suppress nematode populations when plant parts are incorporated as a soil amendment.

While vetiver amendment to soil may produce nematotoxic compounds, there is also the possibility for adverse effects on crop plants. Although vetiver oil has been reported to have “almost no” phytotoxicity (Belhassen *et al*., 2015), some constituents may be phytotoxic (Mao *et al*., 2004, 2006). Sensitivity to allelopathic compounds, including essential oils, can vary among plant species (Meyer *et al*., 2008). Consequently, when investigating effects of vetiver mulch for nematode suppression, effects on host crop plants should also be determined.

The research reported herein was conducted to investigate the potential for nematode management when vetiver shoots are applied as soil amendments. This study had three objectives. The vetiver cultivar used for the soil amendments was tested to determine resistance to *M. incognita*, because this nematode is a major plant pathogen, attacking numerous plant hosts. Vetiver soil amendments were tested for phytotoxic effects on cucumber, pepper, and tomato to determine positive or adverse effects on plant vigor. Vetiver amendments were investigated for management of *M. incognita* in the soil. These three objectives were chosen because resistance of vetiver to *M. incognita*, and determining effects of vetiver amendments on plant vigor and nematode populations, would indicate whether vetiver and vetiver-based amendments have potential as alternative methods for nematode management.

MATERIALS AND METHODS

Plant materials

Vetiver cv. Sierra was purchased from Agriflora Tropicals, Caguas, Puerto Rico, transplanted into Promix PGX (Premier Tech Horticulture, Quakertown, PA) in 3.8-L pots, and maintained in a greenhouse at 24 to 29°C, with natural and supplemental lighting combined for a 16-hr day length. The same greenhouse conditions were used for nematode cultures and greenhouse experiments.

Root-knot nematodes

Nematodes for greenhouse experiments were obtained with methods adapted from Meyer *et al*. (2016). Roots from 2- to 3-month-old pepper plants (*Capsicum annuum*) cv. PA-136 infected with *M. incognita* race 1 (originally isolated in Maryland) were collected from greenhouse pots and cleaned with tap water to remove soil. The root systems were cut, placed in 0.6% sodium hypochlorite and then rubbed by hand for 1.5 min. Egg suspensions were poured through nested sieves (250-µm pore size/63-µm pore size/25-µm pore size) and rinsed with tap water until cleaned. Eggs retained on the 25-µm pore sieve were rinsed into glass beakers using tap water. Suspensions contained eggs of various developmental stages, thus counts were adjusted so that each milliliter included 1,000 eggs that had either a first- or second-stage juvenile (J1 or J2, respectively). For greenhouse experiments with nematodes, each plant was inoculated with 5 ml of the egg suspension, applied to 2 holes in the soil near the base of the plant.

Host resistance

Two-month-old vetiver plant divisions (small sections with shoots and roots) and 6-wk-old pepper seedlings (PA-136; used as a known susceptible plant for comparison) were removed from pots and the roots shaken in water to remove the Promix PGX media. Vetiver and pepper plants were transplanted into 10-cm-diam. plastic pots (one plant per pot) containing a loamy sand soil enriched with compost (16 parts sand to 9 parts compost, v/v; 85.1% sand, 7.2% silt, 7.6% clay, pH 6.9; 0.6% organic matter) that had been steamed 6 hr and dried in sunlight. The same enriched soil was used for all greenhouse experiments. After 10 days, each plant was inoculated with 5 ml of egg suspension, applied to 2 holes in the soil near the base of the plant. Pots were arranged in a randomized complete block design. Six weeks after inoculation, plants were harvested to determine root fresh weights and the numbers of galls and eggs per root system. Roots were gently rinsed in water, and galls were counted. To extract eggs, roots were cut into pieces, blended for 1 min in 130 ml of 0.6% sodium hypochlorite, and then rinsed in tap water. The egg suspension was poured through nested sieves (250-µm pore size/25-µm pore size) and eggs collected from the 25-µm pore size sieve. Eggs were resuspended in 40 ml tap water, and eggs in 1 ml of a 1:10 aqueous dilution of the suspension were counted to estimate the number of eggs per root system. Two trials were conducted, with eight vetiver plants in each trial. Four pepper seedlings were included in the second trial to confirm nematode reproduction on a known susceptible host.

Phytotoxicity of vetiver amendments to vegetable seedlings

Studies on phytotoxicity of vetiver shoot soil amendments were conducted in the greenhouse with cucumber (*Cucumis sativus*) cv. Sweet Slice, tomato (*Solanum lycopersicum*) cv. BHN 589, and pepper cv. PA 136, with procedures adapted from Meyer *et al*. (2008). Seeds were planted in Promix PGX, and seedlings were allowed to grow to the first true leaf stage. For soil amendments, shoots from greenhouse-grown vetiver (trimmed 2 months prior to the experiments) were cut at ca. 20-cm above soil level, further cut into 1-cm pieces, and mixed with steamed, dried soil at three amendment rates: 0% (no vetiver amendment control), and 3% and 5% g fresh vetiver shoots/g dry soil (weight/weight). These treatments were placed into 7.6-cm diam. pots (ca. 280 g per pot). All treatments were set up at each of five times: 4 wk, 3 wk, 2 wk, and 1 wk prior to transplant, and 0 wk (on the day of transplant). At transplant, each pot received 1 seedling of each plant type. This resulted in a total of 45 combinations: 3 seedling types × 3 amendment rates × 5 application times. The plants were arranged in a randomized complete block design and harvested 9 days after transplanting. At harvest, viability (plant alive or dead), shoot heights (from soil to growing tip), and shoot fresh weights were recorded. Root fresh weights were recorded following a water rinse to remove soil. The experiment was conducted twice, with five seedlings of each plant type for each amendment rate x application time combination in Trial 1, and four (pepper and tomato) or five (cucumber) seedlings in Trial 2.

Suppression of M. incognita *on cucumber with vetiver amendment*

Shoots were cut from vetiver plants as described above, mixed with steamed, dried soil at ratios of 0%, 1%, 3%, 5%, and 10% w/w, and placed into 10-cm diam. plastic pots. Within three days, soil in half of the pots was infested with an egg suspension (5 ml per pot) as described above. Cucumber seedlings (9-10 days old) were removed from Promix PGX, the roots were dipped in water to remove the potting mixture, and one seedling was transplanted into each pot 9-10 days after soil amendment. Pots were arranged in a randomized complete block design. Six weeks after transplant, the cucumber seedlings were harvested, and fresh shoot and root weights were determined. Eggs were extracted from roots and counted as described above. Trials were conducted with five (Trial 1) or six (Trial 2) replicate plants per treatment.

Statistical analyses

For the studies on phytotoxicity of vetiver soil amendment to cucumber, pepper and tomato, analyses were conducted with SAS (SAS Institute Inc., 2017). Shoot height, shoot fresh weight, and root fresh weight were analyzed separately by plant species as three-factor mixed models using PROC MIXED (SAS), where treatment and week were the fixed factors and trial was the random factor. The variance grouping technique was used to correct for any variance heterogeneity. If the treatment (amendment rate) × week two-way interaction was statistically significant, means comparisons were conducted across rate within a week and across weeks within a rate. In some cases, the two-way interaction was not statistically significant but amendment rate was significant, so just the amendment rate means were compared. No week means were statistically significant without the two-way interaction being significant. The mean comparisons were conducted with Sidak adjusted p-values so that the experiment-wise error was 0.05. Analyses of studies on nematotoxicity of vetiver amendment to *M. incognita* on cucumber were conducted with the statistical package JMP Version 12.1.0 (SAS Institute Inc., 2017).

Differences among treatments were determined by analysis of variance (ANOVA), and means were compared using Tukey-Kramer's adjustment for multiple comparisons ($P \leq 0.05$).

For the greenhouse trials on host resistance of vetiver to *M. incognita*, the variables shoot fresh weight, root fresh weight, galls per gram of root, and eggs per gram of root from the trials were analyzed separately using Proc Ttest (SAS Institute Inc., 2018). The assumption of equal variances was checked. When the variance equality test was statistically significant, the Satterthwaite adjustment method was used for the T-test ($P \leq 0.05$).

RESULTS

Host resistance

Greenhouse trials demonstrated that vetiver cv. Sierra is resistant to *M. incognita* (Table 1). In Trial 1, no galls or eggs were observed on the vetiver roots. In Trial 2, few galls or eggs were observed on vetiver. No significant differences were observed between trials in shoot or root fresh weights, galls per gram of root, or eggs per gram of root. Egg and gall numbers on pepper, a known susceptible plant, demonstrated that the nematode cultures were viable and active.

Phytotoxicity of vetiver amendments to vegetable seedlings

All seedlings transplanted into soil amended with chopped, fresh vetiver shoots were viable at harvest. With cucumber and pepper, shoot fresh weight was unaffected by any treatment × week interaction. In tomato, shoot height and root fresh weight were unaffected as well (Tables 2, 3, and 4).

Cucumber shoot heights decreased only in Week 1 and Week 2 plants (in 3% and 5% vetiver, respectively; Table 2). Overall, shoots were shortest (strongest interactions) from plants that had been transplanted Week 2 into 5% vetiver. Shoot fresh weights did not show significant differences for vetiver treatments within transplant week, nor for weeks within a treatment. However, amendment rates differed across time. Mean cucumber shoot fresh weights were significantly lowest in the 5% vetiver amendment rate. Root fresh weights were lowest in 5% vetiver applied Week 4 prior to transplant. Thus, the application time of 5% vetiver had different effects on cucumber shoots vs. roots.

With pepper, shoot heights did not differ among weeks for any vetiver amendment rate, or among most vetiver treatments within any week (Table 3). However, Week 3 shoot heights were lowest with the 3% vetiver amendment rate. Shoot fresh weights did not show any effects of vetiver amendment rate within any week, effect of week for any vetiver amendment rate, nor of treatment. Root fresh weights decreased only during Week 3 of amendment, with smaller roots in the presence of both 3% and 5% vetiver. This was somewhat similar to effects of 3% vetiver on shoot heights. For 5% vetiver, roots were smaller when pepper seedlings were transplanted 3 and 4 wk after vetiver amendment to soil.

In contrast with cucumber and pepper, there was a week × vetiver amendment rate effect on tomato shoot fresh weights, but not on shoot heights or root fresh weights (Table 4). However, shoot heights and root fresh weights were affected by vetiver treatments across time. Mean tomato shoot heights were lowest in 3% and 5% vetiver treatments. Root fresh weights were lowest in 5% vetiver compared with the 0% vetiver control.

Table 1. Plant vigor and host resistance of vetiver (*Vetiveria zizanioides*) cv. Sierra and susceptible pepper (*Capsicum annuum*) cv. PA-136 inoculated with the root-knot nematode *Meloidogyne incognita*.

Plant	Shoot fresh weight (g)[y]		Root fresh weight (g)		Galls/g of root		Eggs/g of root	
	Trial 1[z]	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Vetiver	25.4	19.1 b	13.4	16.8 a	0.0	0.02 b	0.0	10 b
Pepper	-	47.1 a	-	20.7 a	-	172.30 a	-	8,241 a

[y]Similar letters indicate that means are not significantly different within a column (between vetiver and pepper in Trial 2); $P \leq 0.05$.

[z]Each Trial had 8 vetiver plants; Trials 1 and 2 had 0 and 4 pepper plants, respectively.

Table 2. Cucumber (*Cucumis sativus*) seedling vigor 9 days after transplant into soil amended with vetiver (*Vetiveria zizanioides*) cv. Sierra. Fresh, chopped vetiver shoots were mixed into soil at 0, 3, or 5% (w:w) and cucumber seedlings were transplanted into the treatments 0, 1, 2, 3, or 4 wk later. Mean values are shown for: 1) data with a week × treatment effect (shoot height and root fresh weight), or 2) data with an amendment rate across all times effect (shoot fresh weight).

Treatment[w]	Week					Amendment rates across time[y]
	0[x]	1	2	3	4	
			Shoot height – cm[z]			
0% vetiver	11.1 aA	11.1 aA	10.5 abA	12.0 aA	11.0 aA	-
3% vetiver	12.2 aA	10.1 aB	11.6 aAB	10.9 aAB	10.8 aAB	-
5% vetiver	11.7 aA	10.4 aAB	9.9 bB	11.2 aAB	10.6 aAB	-
			Shoot fresh weight – g			
0% vetiver	5.3	5.1	4.5	5.0	5.0	5.0 a
3% vetiver	5.1	5.0	5.3	4.9	4.8	5.0 a
5% vetiver	4.6	4.8	4.5	4.2	3.7	4.4 b
			Root fresh weight – g[z]			
0% vetiver	3.1 aA	2.8 aA	2.5 aA	2.9 aA	3.0 aA	-
3% vetiver	3.2 aA	3.2 aA	3.0 aA	2.9 aA	2.6 aA	-
5% vetiver	2.6 aAB	3.2 aA	2.7 aAB	2.5 aB	1.4 bC	-

[w]Treatment = fresh vetiver shoot weight to dry soil weight (w/w).
[x]On Week 0, seedlings were transplanted the same day that the amendments were mixed into the soil.
[y]Similar significance letters within this column indicate that means are not significantly different for amendment rate across time (data for all times combined within each amendment rate). Mean comparisons were done with Sidak adjusted p-values; experiment-wise error = 0.05. Data for week × treatment means are also shown, but without letters because the two-way interactions were not statistically significant.
[z]Significance letters indicate differences in week × treatment analyses. Mean comparisons were done with Sidak adjusted p-values; experiment-wise error = 0.05. For each plant part, similar lower case letters indicate that means are not significantly different within a column (differences among treatments within a week). Similar upper case letters indicate that means are not significantly different within a row (differences among weeks within a treatment).

Shoot fresh weights decreased when seedlings were transplanted at either 0 or 4 wk after amendment of soil with 5% vetiver.

Suppression of M. incognita *on cucumber with vetiver amendment*

Shoot weights were not affected by the vetiver soil amendment or the presence of *M. incognita.* Root weights were unaffected by soil amendment (Table 5). At each amendment rate, root fresh weights were generally higher in plants inoculated with *M. incognita* than in plants without *M. incognita* due to gall formation. Also, although means were not different, uninoculated plants trended towards lower root weights with higher amendment rates. This trend did not occur in plants inoculated with *M. incognita*.

The numbers of eggs/g root were significantly reduced by the 10% vetiver amendment in both trials, and by the 5% vetiver amendment in Trial 1 (Fig. 1). In Trial 1, *M. incognita* without amendment resulted in 2.3 to 3.0 times more eggs per gram of root than were found with 5% and 10% vetiver amendment, respectively. In Trial 2, the 10% vetiver amendment resulted in almost half as many eggs per gram of root as the 0% amendment. Between the two trials, this was a 46% to 67% reduction in eggs per gram of root in the 10% amendment rate, compared with the controls without amendment.

DISCUSSION

Our greenhouse studies indicated that vetiver cv. Sierra is resistant to the tested isolate of *M. incognita* race 1. There was little or no gall

Table 3. Pepper (*Capsicum annuum*) seedling vigor 9 days after transplant into soil amended with vetiver (*Vetiveria zizanioides*) cv. Sierra. Fresh, chopped vetiver shoots were mixed into soil at 0, 3, or 5% (w:w) and pepper seedlings were transplanted into the treatments 0, 1, 2, 3, or 4 wk later. Mean values are shown for data with a week × treatment effect (shoot height and root fresh weight).

Treatment[x]	Week				
	0[y]	1	2	3	4
			Shoot height – cm[z]		
0% vetiver	10.9 aA	11.6 aA	10.1 aA	12.0 aA	11.7 aA
3% vetiver	11.7 aA	11.6 aA	10.2 aA	9.5 bA	10.7 aA
5% vetiver	11.8 aA	11.0 aA	11.5 aA	10.7 abA	10.7 aA
			Root fresh weight – g[z]		
0% vetiver	1.3 aA	1.6 aA	1.2 aA	1.4 aA	1.2 aA
3% vetiver	1.6 aA	1.2 aA	1.0 aAB	0.7 bB	1.0 aAB
5% vetiver	1.5 aA	1.1 aAB	1.3 aAB	0.8 bB	0.8 aB

[x]Treatment = fresh vetiver shoot weight to dry soil weight (w/w).
[y]On Week 0, seedlings were transplanted the same day that the amendments were mixed into the soil.
[z]Significance letters indicate differences in week × treatment analyses. Mean comparisons were done with Sidak adjusted p-values; experiment-wise error = 0.05. For each plant part, similar lower case letters indicate that means are not significantly different within a column (differences among treatments within a week). Similar upper case letters indicate that means are not significantly different within a row (differences among weeks within a treatment). No differences were found with shoot fresh weight means.

Table 4. Tomato (*Solanum lycopersicum*) seedling vigor 9 days after transplant into soil amended with vetiver (*Vetiveria zizanioides*) cv. Sierra. Fresh, chopped vetiver shoots were mixed into soil at 0, 3, or 5% (w:w) and tomato seedlings were transplanted into the treatments 0, 1, 2, 3, or 4 wk later. Mean values are shown for: 1) data with a week × treatment effect (shoot fresh weight), or 2) data with an amendment rate across all times effect (shoot height and root fresh weight).

Treatment[w]	Week					Amendment rates across time[y]
	0[x]	1	2	3	4	
			Shoot height – cm			
0% vetiver	9.6	10.4	9.9	9.5	10.3	10.0 a
3% vetiver	9.3	9.3	9.5	8.7	9.4	9.2 b
5% vetiver	8.8	10.1	9.6	9.2	8.2	9.2 b
			Shoot fresh weight – g[Z]			
0% vetiver	3.3 aA	3.5 aA	2.6 aA	3.0 aA	2.9 aA	-
3% vetiver	2.6 abA	2.5 aA	2.8 aA	2.3 aA	2.6 aA	-
5% vetiver	2.2 bAB	2.9 aA	3.0 aA	2.6 aAB	1.7 bB	-
			Root fresh weight – g			
0% vetiver	1.2	1.1	1.0	1.3	1.2	1.2 a
3% vetiver	1.1	1.0	1.1	1.0	1.1	1.1 ab
5% vetiver	0.9	1.2	1.0	1.1	0.8	1.0 b

[w]Treatment = fresh vetiver shoot weight to dry soil weight (w/w).
[x]On Week 0, seedlings were transplanted the same day that the amendments were mixed into the soil.
[y]For each plant part, similar significance letters within this column indicate that means are not significantly different for amendment rate means across time (data for all times combined within each amendment rate). Letters are not comparable between plant parts within a column. Mean comparisons were done with Sidak adjusted p-values; experiment-wise error = 0.05. Data for week × treatment means are also shown, but without letters because the two-way interactions were not statistically significant.
[z]Significance letters indicate differences in week × treatment analyses. Mean comparisons were done with Sidak adjusted p-values; experiment-wise error = 0.05. For each plant part, similar lower case letters indicate that means are not significantly different within a column (differences among treatments within a week). Similar upper case letters indicate that means are not significantly different within a row (differences among weeks within a treatment).

Table 5. Effect of soil amendment with fresh shoots of vetiver (*Vetiveria zizanioides*) cv. Sierra on cucumber (*Cucumis sativus*) plant vigor, with and without inoculation of the root-knot nematode (RKN) *Meloidogyne incognita*. Amendments were applied 9-10 days prior to seedling transplant. Cucumber plants were harvested 6 wk after transplant.

Treatment[y]	Shoot fresh weight (g)[x]		Root fresh weight (g)	
	Trial 1[z]	Trial 2	Trial 1	Trial 2
0%, no RKN	26.6 a	23.0 ab	11.8 bcd	17.3 bcde
1%, no RKN	17.7 a	29.9 a	6.5 cd	15.5 cde
3%, no RKN	18.3 a	27.5 ab	6.4 d	11.3 de
5%, no RKN	16.4 a	25.4 ab	6.2 d	11.0 de
10%, no RKN	22.3 a	22.3 ab	6.4 d	8.7 e
0%, + RKN	19.0 a	21.2 b	18.4 ab	26.7 a
1%, + RKN	23.2 a	23.1 ab	24.4 a	26.5 a
3%, + RKN	22.7 a	22.7 ab	25.3 a	25.8 ab
5%, + RKN	17.2 a	21.7 ab	17.0 abc	22.8 abc
10%, + RKN	21.1 a	22.2 ab	17.1 ab	18.1 abcd

[x]For each plant part and trial, means within a column followed by the same letter are not significantly different according to Tukey's adjustment for multiple comparisons ($P \leq 0.05$). Means are not comparable among columns.
[y]Treatment = fresh vetiver shoot weight to dry soil weight (w/w).
[z]Trials 1 and 2 had 5 and 6 plants per treatment, respectively.

formation or egg production. Previous studies demonstrated that vetiver cv. Sunshine was resistant to *M. incognita* race 2 and *Meloidogyne javanica* (Fourie *et al*, 2007). West *et al*. (1996) reported that the vetiver cv. Monto and a non-sterile vetiver from western Australia were resistant to *M. arenaria*, *M. hapla*, *M. incognita* (populations B1 and B2), and *M. javanica*. Vetiver was also reported as resistant to mixed populations of *M. incognita* race 1 and *M. javanica* with no galls, parasitic forms of the nematode, or symptoms reported (de Moura *et al*., 1990). Unlike results with *Meloidogyne* spp., vetiver was reported as a host for *Heterodera zeae* and *Bilobodera mesoangusta* (syn. *Verutus mesoangustus*) (Lal and Mathur, 1982; Bajaj and Dalal, 1997). Vetiver resistance to root-knot nematodes, combined with reports of vetiver as a host for other nematode taxa, suggests that planting vetiver could aid in reducing root-knot nematode populations in a field, but would not contribute to management of all plant-parasitic nematode taxa.

When vetiver is cut and applied to fields, it is possible that phytotoxic chemical constituents could affect crop plants. Vetiver oil inhibited seed germination of the weeds common lambsquarters, giant ragweed, pitted morningglory, redroot pigweed, and velvetleaf, but not sicklepod. Vetiver oil was also phytotoxic to seedlings of common lambsquarters and redroot pigweed (Mao *et al*., 2004). Vetiver oil reduced pea plant growth but not growth of citrus trees (*Citrus unshiu*) (Mao *et al*., 2006). A constituent of vetiver oil, eugenol, can be phytotoxic to cucumber, muskmelon, pepper, and tomato seedlings (Meyer *et al*., 2008).

In our greenhouse study, some indications of phytotoxicity of chopped vetiver shoot amendments were apparent. Phytotoxic effects varied with vegetable plant species, time from amendment to transplanting, and application rate. When plants were harvested 9 days after transplanting, higher vetiver amendment rates were more likely than lower rates to result in reduced cucumber shoot fresh weights and tomato shoot heights and root fresh weights. Increased time from transplant to harvest resulted in reduced phytotoxic effects, as indicated by cucumber plant vigor when plants were harvested 6 wk after transplant. Despite potentially phytotoxic effects of vetiver, field studies with vetiver treatments did not report negative effects on seedlings. Vetiver mulches and green manures were generally beneficial. Dried shoots of vetiver applied to soil as a green manure at 2.25 and 4.45 T/ha increased soil organic matter, total porosity, total N and P, and available N and P, and resulted in higher corn yields (Xu *et al*., 2003). Vetiver mulch improved maize yields and soil quality ratings (Are *et al*., 2012). Growth and yield of a corn hybrid improved when treatment with a chemical fertilizer was combined with a fresh

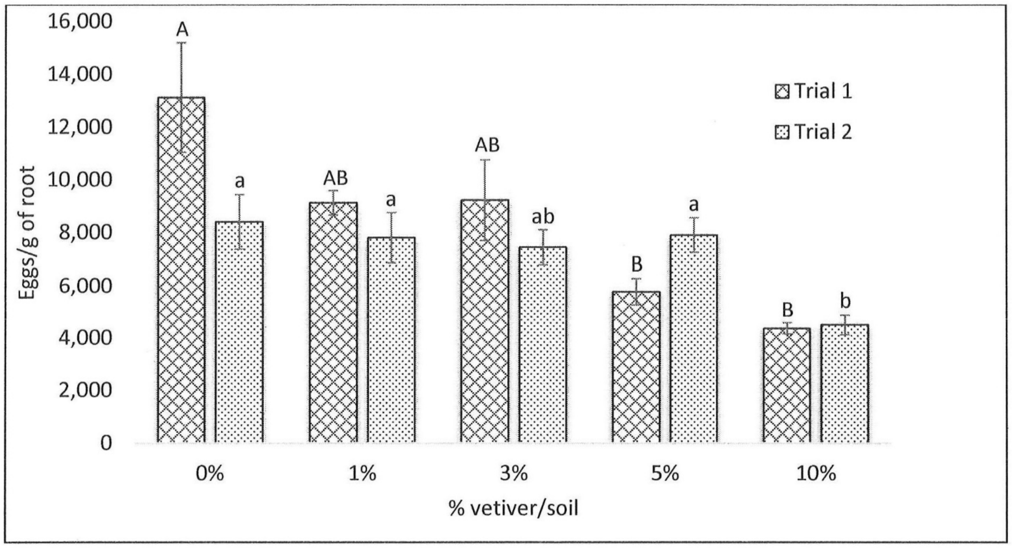

Figure 1. Effect of soil amendment with fresh shoots of vetiver (*Vetiveria zizanioides*) cv. Sierra on *Meloidogyne incognita* population densities on cucumber (*Cucumis sativus*) roots. Treatments were amendments of fresh vetiver shoot weight to dry soil weight (% vetiver/soil, w/w). Amendments were applied 9-10 days prior to seedling transplant. Cucumber plants were harvested 6 weeks after transplant. Trials 1 and 2 had 5 and 6 plants per treatment, respectively. Similar upper case letters (Trial 1) and lower case letters (Trial 2) indicate that means are not significantly different within a trial; $P \leq 0.05$. Significance letters are not comparable between trials.

vetiver grass mulch at 31.25 T/ha (Roongtanakiat *et al*., 2000). Phytotoxicity to seedlings would have been noted had it been an issue.

Vetiver produces compounds active against nematodes, including borneol, bornyl acetate, *trans*-cinnamic acid, *p*-coumaric acid, eugenol, geraniol, linalool, α-terpinene, terpinen-4-ol, and α-terpineol (Sangwan *et al*., 1990; Al-Banna *et al*., 2003; Echeverrigaray *et al*., 2010; Ntalli *et al*., 2010; Būda and Čepulytė-Rakauskienė, 2011; López-Martínez *et al*., 2011; Aoudia *et al*., 2012). Additionally, our earlier studies indicated that aqueous vetiver shoot and root extracts were lethal and repellent to *M. incognita* (Jindapunnapat *et al*., 2018). In addition, preliminary results of *M. incognita* J2 inactivity in bioassays using reverse-phase HPLC fractions indicated that highly nematotoxic materials (resulting in 100% nonviable *M. incognita* J2 in an initial bioassay) extracted from vetiver shoots and roots were highly polar and stable at low pH (Masler and Jindapunnapat, unpublished). However, presence of nematotoxic compounds does not always lead to effects on nematode populations when plant material is incorporated into a field (Timper *et al*., 2011). Suppression of *M. incognita* in our greenhouse study was only consistent at the high amendment rate corresponding to 200 T/ha. As noted by McSorley (2011), greenhouse rates can equate to a substantial amount of plant material in the field. By comparison, a recommendation for hay mulch (used to suppress weed seedlings) was 12-25 T/ha (Schonbeck, 2012). Only the lowest vetiver amendment rate, which did not reduce nematodes in our greenhouse study, is comparable to this recommendation.

This study determined that the previously untested vetiver cv. Sierra is resistant to *M. incognita* and might therefore be useful if planted for suppressing root-knot nematodes. When fresh shoots of this cultivar were cut and amended into soil, no strong phytotoxic or beneficial effects on vegetable seedling growth were observed, but there was some variation in response among the three types of plants. In association with the host status results with *M. incognita*, our work indicates that vetiver applied at high rates as a soil amendment has the potential to suppress nematode populations in the field while having minimal effects on crop plants. Further studies are needed to determine if practical application rates of vetiver amendments, combined with other nematode management strategies, could improve nematode management in fields, or if vetiver applications over several years could eventually contribute to reduced nematode populations and improved crop vigor and yields.

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