CHANGES IN POPULATIONS OF MICROORGANISMS ASSOCIATED WITH ORGANIC AMENDMENTS AND BENZALDEHYDE TO CONTROL PLANT-PARASITIC NEMATODES

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ABSTRACT

Chavarría-Carvajal, J. A., R. Rodríguez-Kábana, J. W. Kloepper, and G. Morgan-Jones. 2001. Changes in populations of microorganisms associated with organic amendments and benzaldehyde to control plant-parasitic nematodes. Nematropica 31:165-180.

Organic amendments and naturally occurring aromatic compounds can effectively suppress numbers of plant-parasitic nematodes. However, little information is available on the mode of action of many of these materials, especially how they affect activities and populations of soil microorganisms. A study was conducted to determine the effects of combinations of organic amendments and benzaldehyde on plant-parasitic and non-parasitic nematode populations, soil microbial activity, and plant growth. Pine bark, velvetbean and kudzu were applied to soil at rates of 30 g/kg and paper waste at 40 g/kg alone and in combination with benzaldehyde (300 µl/kg), for control of plant-parasitic nematodes. Pre-plant and post-harvest soil and soybean root samples were analyzed, and the number of parasitic and non-parasitic nematodes associated with soil and roots were determined. Soil samples were taken at 0, 2, and 10 weeks after treatment to determine population densities of bacteria and fungi. Treatment effects on microbial composition of the soybean rhizosphere were also determined by identifying microorganisms. Bacteria strains were identified using fatty acid analysis, and fungus identification was done using standard morphological measurements and appropriate taxonomic keys. Results showed that most amendments alone or in combination with benzaldehyde reduced damage from plant parasitic nematodes. Benzaldehyde applied alone or in combination with the amendments exerted a selective action on the activity and composition of microbial populations in the soybean rhizosphere. In control soils the bacterial flora was predominantly Gram-negative, while in soils amended with velvetbean or kudzu alone or with benzaldehyde, Gram-positive bacteria were dominant. Mycoflora promoted by the different amendments or combinations with benzaldehyde included species of *Aspergillus*, *Myrothecium*, *Penicillium*, and *Trichoderma*.

Key words: benzaldehyde, biological control, organic amendments, plant-parasitic nematodes, soil microflora.

RESUMEN

Chavarría-Carvajal, J. A., R. Rodríguez-Kabana, J. W. Kloepper, and G. Morgan-Jones. 2001. Cambios en poblaciones de microorganismos asociados con enmiendas orgánicas y benzaldehído aplicados en el control de nematodos fitoparásitos. Nematrópica 31:165-180.

Enmiendas orgánicas y compuestos aromáticos de origen natural son efectivos controlando poblaciones de fitonematodos. Sin embargo, poca información se encuentra disponible sobre el modo de acción de estos materiales, en especial como afectan las poblaciones de microorganismos del suelo. Este estudio fue conducido para determinar los efectos de combinaciones de enmiendas orgánicas y benzaldehído sobre las poblaciones de nematodos fitoparásitos y no fitoparásitos, la actividad microbiológica del suelo y el desarrollo de la planta. Enmiendas a base de corteza de pino, haba de terciopelo y kudzú fueron aplicadas al suelo a una dosis de 30 g/kg. Además, una enmienda a base de desechos de papel de imprenta fue aplicada a razón de 40 g/kg. Todas las enmiendas fueron aplica-

das solas o en combinación con benzaldehído (300 µl/kg), para el control de nematodos fitoparásitos. Muestras de suelo pre-siembra y post-siembra y de tejido radical de soya fueron analizadas, para determinar el número de nematodos fitoparásitos y no fitoparásitos asociados al suelo y al tejido radical. Las muestras de suelo fueron tomadas a las 0, 2 y 10 semanas después del tratamiento, para determinar poblaciones de bacterias y hongos. El efecto de los tratamientos sobre la composición microbiana de la rizósfera de la soya fue también determinada por medio de la identificación de los microorganismos asociados. Las cepas bacterianas fueron identificadas utilizando análisis de ácidos grasos, mientras que la identificación de las colonias de hongos fue realizada utilizando medidas morfológicas y claves taxonómicas. Los resultados demuestran que la mayoría de las enmiendas solas o en combinación con benzaldehído redujeron el daño causado por los nematodos fitoparásitos. Benzaldehído, aplicado solo o en combinación con las enmiendas ejerció una acción selectiva sobre la actividad y composición de las poblaciones microbianas asociados a la rizósfera de la soya. En el suelo proveniente del tratamiento control, la flora bacteriana predominante fue Gram-negativa, mientras que en suelos tratados con el haba de terciopelo o kudzú, solos o en combinación con benzaldehído, la flora bacteriana predominante fue Gram-positiva. La microflora promovida por las distintas enmiendas o combinaciones con benzaldehído incluyeron especies de *Aspergillus, Myrothecium, Penicillium* y *Trichoderma.*

Palabras claves: benzaldehído, control biológico, enmiendas orgánicas, fitonematodos, microflora del suelo.

INTRODUCTION

Plant-parasitic nematodes are recognized in the United States and elsewhere as potentially serious constraints to crop production. According to Sasser and Freckman (1987), crop losses by nematodes range from 8 to 20% on major crops around the world. Traditional strategies for the management of problems caused by nematodes on many crops have been based on the use of pesticides. However, the use of some pesticides in agriculture has been questioned in recent years because of threats they can pose to wildlife, humans, and water resources. For these reasons, new management strategies for soil-borne plant pathogens are currently shifting toward increased dependence on biological and cultural methods that might eventually reduce the use of synthetic pesticides (Weller, 1988; Soler-Serratosa, 1993). Some of these methods have involved the use of cropping systems, crop rotations, resistant cultivars, microbial antagonists, naturally occurring volatile compounds and organic amendments (Rodríguez-Kábana *et al.*, 1987; Rodríguez-Kábana and Canullo, 1992; McSorley *et al.*, 1994; Vargas-Ayala, 1995).

Organic amendments have been studied for the management of plant parasitic nematodes in agronomic crops, and nematode populations have been positively or negatively correlated with organic matter content (Akhtar and Mahmood, 1994; Mannion *et al.*, 1994; McSorley and Gallaher, 1995). The efficacy of organic additives depends on their chemical composition and the type of microorganisms that develop during degradation (Rodríguez-Kábana *et al.*, 1987). Several nematicidal compounds (e.g., organic acids, hydrogen sulfide, nitrogenous ammonia, phenols, tannins) are released during degradation of organic amendments, or synthesized by microorganisms involved in such degradation (Rodríguez-Kábana *et al.*, 1995).

The exploitation of antagonistic activities by microorganisms decomposing organic amendments may be one of the more practical biocontrol tools that could be developed

to manage plant-parasitic nematodes (Mankau, 1981). Antagonistic interactions among microorganisms play an important role in determining the number and variety of organisms able to inhabit the rhizosphere (Stirling, 1991). Interactions between antagonistic microorganisms and plant pathogens are widespread in nature (Fridlender *et al.*, 1993), and organic amendments and naturally occurring aromatic compounds can be used to enhance naturally occurring biological control and reduce diseases caused by pathogens (Chavarría-Carvajal, 1997). The addition of organic matter to soil stimulates microbial populations of bacteria and fungi, some of which might be antagonistic to nematodes (Morgan-Jones and Rodríguez-Kábana, 1987).

In recent years, some naturally occurring volatile compounds with nematicidal and fungicidal properties have been reported (Bauske *et al.*, 1994; Soler-Serratosa *et al.*, 1996). Some of these compounds stimulated development of populations of fungi and bacteria antagonistic to soilborne pathogens (Canullo *et al.*, 1992; Soler-Serratosa, 1993) and increased parasitism to *Meloidogyne* spp. eggs (Chavarría-Carvajal *et al.*, 1994).

No information is available in the literature on the effectiveness of combinations of organic amendments and benzaldehyde for the management of phytonematodes, despite results showing that benzaldehyde applied alone is effective in reducing populations of plant-parasitic nematodes (Soler-Serratosa, 1993). Combinations of organic amendments and aromatic compounds could be useful to reduce the amounts of amendments needed by increasing the effectiveness of the treatment through synergistic or cumulative effects against nematodes. This study was conducted to determine the effects of combinations of selected organic amendments and benzaldehyde incorporated into soil on populations of plant-parasitic and non-parasitic nematode populations, on the microbial composition of soil, and on plant growth.

MATERIALS AND METHODS

The study was conducted under greenhouse conditions at the Department of Plant Pathology, Auburn University, Alabama, USA. Soil for the experiment was a Norfolk sandy loam (fine loamy, siliceous thermic, Typic Paleudults, pH 5.9, <1.0% organic matter) naturally infested with plant-parasitic and non-parasitic species of nematodes. Soil was screened (7 mm mesh) and mixed (1:1 by volume) with builders' river sand. This mixture, which will be called hereafter soil, had pH = 6.7, 0.6% organic matter, 0.02% nitrogen, with P = 10.8, K= 13.8, Mg = 47.4, and Ca = 246.8 ppm.

A greenhouse experiment was performed with four selected organic amendments (velvetbean, kudzu, pine bark, and paper waste) in combination with benzaldehyde. Green foliage and stems of velvetbean (*Mucuna deeringiana* [Bort.] Merr.) and kudzu (*Pueraria lobata* [Willd.] Maesen & S. Almeida) were collected and allowed to dry (25°C) for about a week and then ground into a powder (particle size ≈ 250 µm). Commercially available pine bark nuggets from slash pine (*Pinus elliottii* Engelm) and loblolly pine (*Pinus taeda* L.) were dried and ground as described for velvetbean and kudzu. Also, paper waste from cardboard (Tascon Inc., Houston, TX) was evaluated in this study. The experiment was arranged in a randomized complete block design with ten treatments and six replications per treatment. The treatments included the incorporation in soil of 30 g/kg of velvetbean, kudzu and pine bark, and 40 g/kg of paper waste, alone or in combination with benzaldehyde at 300 µl/kg, for a total of eight treatments. Also,

a treatment with benzaldehyde alone (300 µl/kg) and a treatment with non-amended soil were included to compare the effectiveness of the treatments.

The moist soil (≈ 60% field capacity) was apportioned in 1.0-kg quantities, placed into 4-L capacity polyethylene bags and thoroughly mixed with the amendments and benzaldehyde, and transferred to 1-L capacity, 10-cm-diameter PVC pots. The pots with soil were placed in a greenhouse (25-30°C) and kept moist for two weeks before planting. Each pot was planted with five 'Davis' soybean (*Glycine max* [L.] Merr.) seeds. Ten days after planting, the percentage of germination was recorded; plants were allowed to grow for 8 weeks, and the experiment was terminated 10 weeks after treatment. Soil samples (20 g/pot) were taken at 0 (pre-plant), 2 weeks (planting) and 10 weeks (final) after treatment, to determine populations of bacteria and fungi. Pre-plant and final soil and root samples were analyzed by the "salad bowl" incubation method (Rodríguez-Kábana and Pope, 1981), and the number of parasitic and non-parasitic nematodes associated with soil and roots were determined.

Ten grams of moist soil from each replication of each treatment was mixed with 90-ml sterile demineralized water, and four serial ten-fold dilutions were prepared to 10^{-4}. For bacteria, 0.05 ml of suspension from 10^{-3} and 10^{-4} dilutions were plated on 5% (w/v) tryptic soy agar (Difco, Detroit, MI) using a spiral platter (Spiral Biotech, Bethesda, MD). The plates were incubated at 28°C for 48 h, when the number of bacterial colonies was enumerated using a laser colony counter with Bacterial Enumeration Software (Spiral Biotech). Bacterial populations were expressed as $\log_{10}$ cfu (colony forming units)/g of air-dried soil. A total of 40 bacterial colonies per replication were randomly selected from 5% TSA plates for each replication (240 bacterial colonies per treatment). Bacterial strains were individually transferred to Tryptic Soy Broth Medium (TSB; Difco, Detroit, MI) and incubated for 24 h at 28°C. Bacterial strains were identified using fatty acid analysis. Fatty acid analysis was done with a Hewlett-Packard Series II gas chromatograph model 5890 and processed for extraction of fatty acid methyl esters (FAMEs) using the procedure of Sasser (1990). Strains were identified with the Sherlock Microbial Identification System (MIS) aerobe method and TSBA library version 3.9 of MIDI (Microbial ID, Inc., Newark, DE). For isolation of fungal colonies 100 µl of suspension from the 10^{-2} and 10^{-3} dilutions were plated on Rose-Bengal Streptomycin Agar (Skipper *et al.*, 1986) using an adjustable serial micropipette (Wheaton, Millville, NJ) and a cell spreader (Fisher Scientific, Pittsburg, PA). Plates were incubated at 28°C for 3 days, when the number of colonies was enumerated by direct observation and expressed as $\log_{10}$ cfu/g of air-dried soil. A total of 30 fungal colonies per replication were randomly selected (180 fungal colonies per treatment) and reisolated in petri dishes containing appropriate media (e.g., potato dextrose agar, Czapek agar) for identification. Fungal identification was achieved using an Olympus compound microscope (Olympus Optical Co. Ltd., Japan), and pertinent taxonomic keys. Most isolates were identified to species level, while isolates that did not sporulate on selective media or did not produce distinguishing structures useful for identification, were classified as 'unknown'.

At the end of the experiment several plant variables were recorded including the shoot size and fresh weighs of shoots and roots. Other parameters recorded were the number of galls per gram of root, and the gall index value based on a scale of 0 to 10 in which 0 = no nematode infestation and 10 = maximum infestation (Zeck, 1971).

All data were analyzed using standard procedures for analysis of variance (ANOVA) (Steel and Torrie, 1980). Means were compared for significance using Least Significant Differences (LSD) when F values were significant at $P \leq 0.05$.

RESULTS

Pre-plant populations of parasitic nematodes were significantly reduced by benzaldehyde, paper waste, paper waste/benzaldehyde, and kudzu/benzaldehyde in comparison with the nonamended natural soil (Table 1). Final populations of parasitic nematodes in soil and roots were lower than those of the control ($P \leq 0.05$) for all organic amendments alone or in combination with benzaldehyde. Pre-plant populations of non-parasitic nematodes were higher in soils treated with pine bark, pine bark/benzaldehyde, velvetbean and kudzu alone or in combination with benzaldehyde than in the control soil. Postharvest populations of free-living nematodes in roots increased in response to all amendment treatments, but only kudzu and paper waste/benzaldehyde significantly increased soil populations of non-parasitic nematodes.

At treatment time, the combination of benzaldehyde with pine bark, velvetbean and kudzu significantly reduced bacterial populations compared with the control soil (Table 2). Two weeks later, most treatments, except the control and benzaldehyde resulted in increased bacterial populations.

Table 1. Effect of organic amendments and benzaldehyde on pre-plant and post-harvest soil and root populations of parasitic and non- parasitic nematodes.[y]

	Pre-plant		Post-harvest			
Treatment	$\log_{10}$ parasitic/ 100 cm^3 soil	$\log_{10}$ non-parasitic/ 100 cm^3 soil	$\log_{10}$ parasitic/ 100 cm^3 soil	$\log_{10}$ non-parasic/ 100 cm^3 soil	$\log_{10}$ parasitic/ g root	$\log_{10}$ non-parasitic/ g root
NS [z]	2.52	2.46	2.23	2.15	2.75	0.68
B	0.32	1.63	0.68	2.62	1.86	2.08
PB	2.38	3.58	1.26	1.93	1.20	2.15
PB/B	1.87	3.00	0.32	2.60	1.08	1.78
V	2.22	3.65	0.31	2.83	0.45	1.48
V/B	2.35	3.80	0.00	2.83	0.00	1.78
PW	1.32	2.58	0.95	1.62	1.88	1.92
PW/B	1.00	2.35	0.32	3.02	1.13	2.32
K	2.10	3.58	0.63	2.95	1.50	2.10
K/B	1.42	3.57	0.00	2.82	0.90	1.58
LSD ($P \leq 0.05$)	0.85	0.41	0.88	0.72	0.74	0.79

[y]Parasitic nematodes include: *Meloidogyne incognita*, *Heterodera* spp., *Helycotylenchus* spp., *Hoplolaimus* spp., and *Pratylenchus* spp. Non-parasitic nematodes include: Dorylaimida, Rhabditida, and Mononchida. Mean of six replicates.
[z]NS = natural soil, B = benzaldehyde, PB = pine bark, PB/B = pine bark/benzaldehyde, V = velvetbean, V/B = velvetbean/benzaldehyde, PW = paper waste, PW/B = paper waste/benzaldehyde, K = kudzu, K/B = kudzu/benzaldehyde.

Table 2. Effect of organic amendments and benzaldehyde on population densities of bacteria in the rhizosphere of soybean (*Glycine max*).[y]

Treatment	0 weeks $\log_{10}$ cfu/g soil	2 weeks $\log_{10}$ cfu/g soil	10 weeks $\log_{10}$ cfu/g soil
NS[z]	5.81	5.76	6.02
B	5.78	5.55	6.19
PB	5.59	6.00	6.38
PB/B	5.52	6.19	6.37
V	5.73	7.00	7.00
V/B	5.53	6.50	6.52
PW	5.59	5.70	6.59
PW/B	5.71	5.98	6.27
K	5.82	6.98	6.94
K/B	5.56	6.19	6.48
LSD ($P \leq 0.05$)	0.24	0.11	0.06

[y]Mean population of six replicates ($\log_{10}$ cfu/g dry soil) of bacteria from dilution 10^{-3} on 5% TSA.
[z]NS = natural soil, B = benzaldehyde, PB = pine bark, PB/B = pine bark/benzaldehyde, V = velvetbean, V/B = velvetbean/benzaldehyde, PW = paper waste, PW/B = paper waste/benzaldehyde, K = kudzu, K/B = kudzu/benzaldehyde.

At the end of the experiment all treatments with organic amendments and benzaldehyde showed higher bacterial populations than the control.

A total of 2 400 bacterial isolates belonging to sixty-eight bacterial species were identified using fatty acid methyl-ester (FAME) analysis. *Bacillus* was the dominant genus in the rhizosphere of soybean, comprising 20.3% in the control and 47.1 and 72.9% in soils amended with velvetbean and kudzu, respectively. *Burkholderia* was the next most dominant genus with 5.7% in control soil, and 30.8, 33.7, 12.5 and 12.5% in soils treated with benzaldehyde, pine bark/benzaldehyde, velvetbean/benzaldehyde, and paper/benzaldehyde, respectively. Tables 3 and 4 present results from the statistical analysis ($P \leq 0.05$), of the genera commonly associated with each specific treatment. Benzaldehyde alone significantly increased *Burkholderia* spp. while not affecting numbers of *Bacillus* spp., *Cytophaga* spp., *Micrococcus* spp. or *Clavibacter* spp. Soil treatment with velvetbean and kudzu, alone or combined with benzaldehyde tended to increase the abundance of *Bacillus* spp. in the rhizosphere of soybean 10 weeks after treatment (Table 3). *Burkholderia* spp. was most abundant in treatments with benzaldehyde, pine bark, or benzaldehyde combined with pine bark, velvetbean or paper (Table 3). The percentage and number of isolates of *Cytophaga* spp., and *Micrococcus* spp. were increased by velvetbean alone or in combination with benzaldehyde. The percentage and number of isolates of *Clavibacter* spp. were increased by pine bark alone or in combination with benzaldehyde (Table 3). There were no differences among treatments for the number of 'oligotrophic' or 'heterotrophic' bacterial groups (Table 4). Benzaldehyde combined with velvetbean or paper

Table 3. Effect of organic amendments and benzaldehyde on abundance of *Bacillus* spp., in the rhizosphere of soybean (*Glycine max*) 10 weeks after treatment.[u]

	Bacillus spp.[w]		*Burkholderia* spp.[x]		*Cytophaga* spp.		*Micrococcus* spp.		*Clavibacter* spp.[y]	
Treatment	# isolates	%[v]	# isolates	%	# isolates	%	# isolates	%	# isolates	%
NS [z]	8.2	20.4	2.3	5.8	0	0	0.7	1.7	0.2	0.4
B	8.8	22.1	12.3	30.8	0.2	0.4	0.8	2.1	0	0
PB	11.3	28.3	7.8	19.6	0.2	0.4	0.2	0.4	2.7	6.7
PB/B	7.3	18.3	13.5	33.8	0	0	0.5	1.2	1.8	4.6
V	18.8	47.1	0.8	2.1	5.5	13.8	1.7	4.2	0.2	0.4
V/B	17.0	42.5	5.0	12.5	2.7	6.7	2.7	6.7	0.3	0.8
PW	6.0	15.0	1.8	4.6	0	0	0.2	0.4	0.5	1.2
PW/B	5.7	14.2	5.0	12.5	0	0	0	0	0.3	0.8
K	29.2	72.9	0.2	0.4	1.3	3.3	1.0	2.5	0	0
K/B	19.5	48.8	2.2	5.4	0.5	1.2	1.7	4.2	0	0
LSD ($P \leq 0.05$)	3.7	9.4	2.3	5.8	1.4	3.5	1.2	2.9	1.5	3.8

[u]Values represent the number of isolates of 40 tested per replicate which were identified to each taxon using fatty acid methyl-ester (FAME) analysis.
[v]Percentage based on a total of 40 isolates per replicate.
[w]*Bacillus* spp. includes: *B. brevis, B. cereus, B. laterosporus, B. megaterium* and *B. thuringiensis.*
[x]*Burkholderia* spp. includes: *B. cepacia, B. gladioli, B. picketii,* and *B. solanacearum.*
[y]*Clavibacter* spp. includes: *C. michiganense.*
[z]NS = natural soil, B = benzaldehyde, PB = pine bark, PB/B = pine bark/benzaldehyde, V = velvetbean, V/B = velvetbean/benzaldehyde, PW = paper waste, PW/B = paper waste/benzaldehyde, K = kudzu, K/B = kudzu/benzaldehyde.

Table 4. Effect of organic amendments and benzaldehyde on abundance of bacterial groups in the rhizosphere of soybean (*Glycine max*) 10 weeks after treatment.[y]

	Unknown		Oligotrophic		Heterotrophic		Gram-positive		Gram-negative	
Treatment	# isolates	%	# isolates	%	# isolates	%	# isolates	%	# isolates	%
NS [z]	4.5	11.3	4.2	10.4	35.8	89.6	12.0	30.0	19.3	48.3
B	2.3	5.8	2.2	5.4	37.8	94.6	11.7	29.2	23.8	9.6
PB	2.5	6.2	4.7	11.7	35.3	88.3	16.2	40.4	16.7	41.7
PB/B	3.8	9.6	6.3	15.8	33.7	84.2	12.5	31.2	17.3	43.3
V	3.2	7.9	2.2	5.4	37.8	94.6	22.8	57.1	11.8	29.6
V/B	1.7	4.2	2.3	5.8	37.7	94.2	23.2	57.9	12.8	32.1
PW	7.0	17.5	4.7	11.7	35.3	88.3	8.5	21.2	19.8	49.6
PW/B	1.7	4.2	3.8	9.6	36.2	90.4	8.7	21.7	25.8	64.6
K	2.5	6.2	1.7	4.2	38.3	95.8	32.7	81.7	3.2	7.9
K/B	4.7	11.7	2.0	5.0	38.0	95.0	24.8	62.1	8.5	21.2
LSD ($P \leq 0.05$)	2.4	6.0	2.6	6.5	2.6	6.5	3.8	9.6	4.2	10.4

[y]Values represent the number of isolates of 40 tested per replicate which were identified to each taxon using fatty acid methyl-ester (FAME) analysis.
[z]NS = natural soil, B = benzaldehyde, PB = pine bark, PB/B = pine bark/benzaldehyde, V = velvetbean, V/B = velvetbean/benzaldehyde, PW = paper waste, PW/B = paper waste/benzaldehyde, K = kudzu, K/B = kudzu/benzaldehyde.

resulted in a lower number and percentage of 'unknown' bacterial isolates; while paper alone increased 'unknown' bacterial groups. Pine bark, velvetbean and kudzu or benzaldehyde plus velvetbean or kudzu increased gram-positive bacteria. Benzaldehyde and benzaldehyde plus paper waste increased Gram-negative bacteria, while Kudzu and velvetbean with and without benzaldehyde reduced numbers of Gram-negative bacteria.

At treatment time, benzaldehyde, pine bark, and paper or the combinations of benzaldehyde with pine bark or paper reduced fungal densities (Table 5). Two weeks after treatment, pine bark, velvetbean, kudzu, and velvetbean/benzaldehyde significantly increased fungal populations. At the end of the experiment, fungal populations were significantly higher in soils treated with pine bark, velvetbean, kudzu, and the combinations of benzaldehyde with those materials.

A total of 1 800 fungal isolates belonging to thirty-six species were found during the experiment. *Aspergillus* was one of the most common genera representing 10.6% of the isolates from control soil and 23.3, 70.6, 36.1, and 28.9% in soils treated with benzaldehyde, pine bark/benzaldehyde, velvetbean/benzaldehyde, and kudzu/benzaldehyde, respectively (Table 6). *Penicillium* was also a dominant genus, representing 8.8% of isolates from untreated soil, and 60.6, 52.8, 61.1, 47.8, and 43.3 in soils with pine bark, paper, paper/benzaldehyde, kudzu, and kudzu/benzaldehyde, respectively. Abundance of *Acremonium* spp. was reduced by most treatments. Paper/benzaldehyde stimulated populations of *Aspergillus* spp. Populations of *C. elegans* were most commonly associated with velvetbean/

Table 5. Effect of organic amendments and benzaldehyde on population densities of fungi in the rhizosphere of soybean (*Glycine max*).[y]

Treatment	0 weeks $\log_{10}$ cfu/g soil	2 weeks $\log_{10}$ cfu/g soil	10 weeks $\log_{10}$ cfu/g soil
NS[z]	2.69	2.67	2.60
B	2.52	1.80	2.63
PB	2.45	3.04	3.03
PB/B	2.29	2.59	2.81
V	2.63	2.96	3.16
V/B	2.58	2.88	3.14
PW	2.38	2.37	2.46
PW/B	2.38	1.57	2.31
K	2.66	3.20	3.07
K/B	2.61	2.53	3.26
LSD ($P \leq 0.05$)	0.12	0.17	0.12

[y]Mean population of six replicates ($\log_{10}$ cfu/g dry soil) of fungi from dilution 10^{-2} on Rose-Bengal Streptomycin Agar.

[z]NS = natural soil, B = benzaldehyde, PB = pine bark, PB/B = pine bark/benzaldehyde, V = velvetbean, V/B = velvetbean/benzaldehyde, PW = paper waste, PW/B = paper waste/benzaldehyde, K = kudzu, K/B = kudzu/benzaldehyde.

Table 6. Effect of organic amendments and benzaldehyde on abundance of fungal species in the rhizosphere of soybean (*Glycine max*) 10 weeks after treatment.[vw]

	Acremonium spp.[x]		*Aspergillus* spp.[y]		*Cunninghamella elegans*		*M. verrucaria*	
Treatment	# isolates	%	# isolates	%	# isolates	%	# isolates	%
NS[5]	2.33	7.78	3.17	10.56	8.83	29.44	0.50	1.67
B	0.50	1.67	7.00	23.33	0.83	2.78	0.33	1.11
PB	0.17	0.56	4.33	14.44	0.17	0.56	0.33	1.11
PB/B	0.33	1.11	21.17	70.56	0.17	0.56	0.50	1.67
V	0.17	0.56	0.83	2.78	4.00	13.33	7.17	23.89
V/B	0.00	0.00	10.83	36.11	16.50	55.00	0.00	0.00
PW	2.00	6.67	1.83	6.11	3.00	10.00	0.67	2.22
PW/B	0.00	0.00	0.00	0.00	2.00	6.67	0.00	0.00
K	0.33	1.11	1.50	5.00	6.17	20.56	5.17	17.22
K/B	0.00	0.00	8.67	28.89	8.33	27.78	0.00	0.00
LSD ($P \leq 0.05$)	1.48	4.95	12.42	41.38	6.58	21.92	5.24	17.48

	Paecilomyces spp.[w]		*Penicillium* spp.[x]		*Trichoderma* spp.[y]	
Treatment	# isolates	%	# isolates	%	# isolates	%
NS[z]	0.50	1.67	5.67	8.89	1.17	3.89
B	0.17	0.56	2.00	6.67	0.50	1.17
PB	0.33	1.11	18.17	60.56	0.83	2.78
PB/B	2.83	9.44	1.67	5.56	0.17	0.56
V	4.50	15.00	0.33	1.11	0.00	0.00
V/B	0.00	0.00	0.00	0.00	2.33	7.78
PW	0.50	1.67	15.83	52.78	2.83	9.44

Table 6 (Continued). Effect of organic amendments and benzaldehyde on abundance of fungal species in the rhizosphere of soybean (*Glycine max*) 10 weeks after treatment.[v,w]

	Paecilomyces spp.[w]		*Penicillium* spp.[x]		*Trichoderma* spp.[y]	
Treatment	# isolates	%	# isolates	%	# isolates	%
PW/B	0.00	0.00	18.33	61.11	0.00	0.00
K	0.67	2.22	14.33	47.78	0.00	0.00
K/B	0.00	0.00	13.00	43.33	0.00	0.00
LSD ($P \leq 0.05$)	4.85	16.18	7.42	24.73	2.35	7.84

[v]Values represent the number of isolates of 30 tested per replicate.
[w]Percentage based on a total of 30 isolates per replicate.
[x]*Acremonium* spp. includes: *A. roseolum* and *A. strictum*.
[y]*Aspergillus* spp. includes: *A. flavus, A. fumigatus, A. niger, A. terricola, A. terreus* and *A. ustus*.
[z]NS = natural soil, B = benzaldehyde, PB = pine bark, PB/B = pine bark/benzaldehyde, V = velvetbean, V/B = velvetbean/benzaldehyde, PW = paper waste, PW/B = paper waste/benzaldehyde, K = kudzu, K/B = kudzu/benzaldehyde.

benzaldehyde, but were reduced by benzaldehyde, pine bark, pine bark/benzaldehyde and paper/benzaldehyde. Soils treated with velvetbean contained a higher percentage and number of isolates of *M. verrucaria* than in control soil. Abundance of *Paecilomyces* spp. was not affected by the treatments while pine bark, paper, paper/benzaldehyde, kudzu, and kudzu/benzaldehyde stimulated *Penicillium* spp. Abundance of *Trichoderma* spp. was highest in soils treated with paper waste.

All treatments, except paper and velvetbean/benzaldehyde, increased soybean germination (Table 7). Also, shoot and root weights were increased by velvetbean, kudzu, and the combinations of these amendments with benzaldehyde. Velvetbean and velvetbean/benzaldehyde, increased the shoot length. All organic amendments and benzaldehyde significantly reduced root gall formation by *M. incognita* and gall index values 10 weeks after treatment (Table 8).

DISCUSSION

Combinations of organic amendments and benzaldehyde were effective in reducing *M. incognita* and other plant-parasitic nematodes at the end of the experiment. Conversely, populations of non-parasitic nematodes in roots were significantly higher in soil treated with organic matter and benzaldehyde at the end of the experiment. Non-parasitic nematodes feed on different soil microorganisms and may also compete with phytonematodes for ecological niches both in the soil and roots (Eisenback and Griffin, 1987; Stirling, 1991). Also, non-parasitic nematodes in the orders Dorylaimida and Mononchida have been reported as antagonistic to plant-parasitic nematodes (Yeates and Coleman, 1982; Rodríguez-Kábana, 1991).

Benzaldehyde applied at 300 µl/kg soil showed a wide-spectrum biocidal activity between 0 and 2 weeks, reducing popula-

Table 7. Effect of organic amendments and benzaldehyde on plant growth of soybean (*Glycine max*).[y]

Treatment	Germination (%)	Shoot length (cm)	Shoot weight (g)	Root weight (g)
NS[z]	61.67	28.82	3.40	3.65
B	80.00	26.92	2.95	4.33
PB	86.67	30.87	3.87	4.68
PB/B	78.33	30.45	3.92	4.90
V	85.00	37.50	8.37	6.05
V/B	71.67	36.22	7.95	6.37
PW	71.67	20.02	1.75	5.17
PW/B	86.67	24.22	2.60	7.35
K	81.67	32.43	5.60	6.42
K/B	73.33	29.08	4.70	5.37
LSD ($P \leq 0.05$)	13.99	5.37	0.92	1.56

[y]Mean of eight replicates.

[z]NS = natural soil, B = benzaldehyde, PB = pine bark, PB/B = pine bark/benzaldehyde, V = velvetbean, V/B = velvetbean/benzaldehyde, PW = paper waste, PW/B = paper waste/benzaldehyde, K = kudzu, K/B = kudzu/benzaldehyde.

Table 8. Effect of organic amendments and benzaldehyde on gall formation caused by *M. incognita* 10 weeks after treatment.[x]

Treatment	Galls/g root	Gall index value[y]
NS[z]	15.83	5.50
B	5.00	3.00
PB	4.17	2.83
PB/B	3.33	2.33
V	4.00	3.33
V/B	4.50	3.67
PW	4.17	3.50
PW/B	4.33	3.50
K	5.00	3.67
K/B	4.00	3.00
LSD ($P \leq 0.05$)	1.73	0.92

[x]Mean of six replicates.
[y]Scale 0 to 10 (0 = no nematode infestation and 10 = maximum infestation) (Zeck, 1971).
[z]NS = natural soil, B = benzaldehyde, PB = pine bark, PB/B = pine bark/benzaldehyde, V = velvetbean, V/B = velvetbean/benzaldehyde, PW = paper waste, PW/B = paper waste/benzaldehyde, K = kudzu, K/B = kudzu/benzaldehyde.

tion sizes of parasitic and non-parasitic nematodes and fungi. Also, bacterial populations were significantly reduced by this terpene 2 weeks after application. Kim *et al.* (1995) attributed the antimicrobial effects of aromatic compounds to different mechanisms such as: a) increased permeability and loss of cellular constituents as a result of interference with cell membranes, b) impairing of enzyme systems, including those involved in the production of cellular energy and synthesis of structural components, and c) the inactivation or destruction of genetic material. Benzaldehyde is a less toxic compound to mammals when compared with some commercially available soil fumigants and nematicides. The oral LD_{50} of benzaldehyde for rats is 1 300 mg/kg, while those for commercial nematicides such as aldicarb, carbofuran, and phenamiphos are 1, 2, and 10 mg/kg, respectively (Budavari, 1996).

Benzaldehyde alone or in combination with organic amendments exerted a selective action on bacteria and fungal populations. At the end of the experiment population sizes of bacteria were increased by benzaldehyde or its combinations with organic amendments, and selectivity was evident by increases in Gram-negative bacteria including *Burkholderia* spp. The results with bacteria agree with research conducted by Soler-Serratosa (1993) and Canullo *et al.* (1992) who reported selective pressure of natural aromatic compounds including benzaldehyde, toward Gram-negative bacteria. *Burkholderia* comprises a large and diverse genus which includes species which have been associated with biological control of several soilborne pathogens (Fridlender *et al.*, 1993; Chen *et al.*, 1995; King and Parke, 1996). In the unamended soil treatment, the bacterial flora was predominantly Gram-negative. However, in soils amended with velvetbean or kudzu, both alone or combined with benzaldehyde, Gram-positive bacteria were dominant. Results with velvetbean contrast with a previous report in which predominantly Gram-negative genera were found on velvetbean roots, when this legume was used in crop rotation (Kloepper *et al.*, 1992).

Most treatments, except benzaldehyde, paper and paper/benzaldehyde increased populations of soil fungi. Some fungal taxa promoted by the different amendments or combinations with benzaldehyde included *Aspergillus*, *M. verrucaria*, *Penicillium* and *Trichoderma*. Interactions between fungal populations antagonistic to plant-parasitic nematodes have been reported in agricultural soils (Morgan-Jones and Rodríguez-Kábana,

1987; Mankau, 1980). Some organic amendments have been associated with increases in soil fungi that may play a role in the reduction of nematode populations (Mankau, 1972; Kokalis-Burelle, 1993). Reductions in the number of galls caused by *Meloidogyne* spp. have been reported after soil addition of substrates colonized by *Paecilomyces* spp., *Verticillium* spp., and *Gliocladium* spp. (Rodríguez-Kábana *et al.*, 1984). It has been shown that root colonization by fungi, including mycorrhizal species, increases plant tolerance to nematode parasitism and reduces the reproduction of plant-parasitic nematodes (Hussey and Roncadori, 1982; Smith *et al.*, 1986).

Results from this experiment illustrated the ability exerted by some organic materials and benzaldehyde to change the composition of a particular soil. It has been shown that some amendments and aromatic compounds may stimulate antagonistic activities against soil-borne plant pathogens by inducing changes in taxonomic composition and physiological activities of soil microflora (Soler-Serratosa, 1993; Canullo *et al.*, 1992; Rodríguez-Kábana, 1986).

The rates of organic amendments and benzaldehyde used in this study may be practical for use in agriculture as alternatives to chemical nematicides. Considering that the weight of soil in a hectare to a 15-cm depth (cultivable zone) is 2.242×10^6 kg (Rodríguez-Kabana *et al.*, 1993), the rate of benzaldehyde used in this study represents 660 kg/ha. This rate fall into the range of recommended dosages for several commercial nematicides and soil fumigants, i.e., 60 to 1,200 kg/ha including methyl bromide, methyl isothiocyanate and chloropicrin (Alabama Cooperative Extension Service, 1988). The organic amendment rates used in our study were equivalent to 74 and 98 ton/ha, respectively, and may be feasible for use in small-plot agriculture or perhaps on high value crops where the use of synthetic pesticides is limited by higher cost, environmental constraints, or adoption of organic farming practices. Thus, organic amendments and naturally-occurring aromatic compounds may be useful in reducing the need for chemical-based pesticides, increasing desirable soil properties, enhancing natural antagonism, and providing for the proper disposal of agro-industrial wastes.

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