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Development of Multi-Component Transplant Mixes for Suppression of *Meloidogyne incognita* on Tomato (*Lycopersicon esculentum*)

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Abstract: The effects of combinations of organic amendments, phytochemicals, and plant-growth promoting rhizobacteria on tomato (*Lycopersicon esculentum*) germination, transplant growth, and infectivity of *Meloidogyne incognita* were evaluated. Two phytochemicals (citral and benzaldehyde), three organic amendments (pine bark, chitin, and hemicellulose), and three bacteria (*Serratia marcescens, Brevibacterium iodinum,* and *Pseudomonas fluorescens*) were assessed. Increasing rates of benzaldehyde and citral reduced nematode egg viability in vitro. Benzaldehyde was 100% efficacious as a nematicide against juveniles, whereas citral reduced juvenile viability to less than 20% at all rates tested. Benzaldehyde increased tomato seed germination and root weight, whereas citral decreased both. High rates of pine bark or chitin reduced plant growth but not seed germination, whereas low rates of chitin increased shoot length, shoot weight, and root weight; improved root condition; and reduced galling. The combination of chitin and benzaldehyde significantly improved tomato transplant growth and reduced galling. While each of the bacterial isolates contributed to increased plant growth in combination treatments, only *Brevibacterium iodinum* applied alone significantly improved plant growth.

Key words: benzaldehyde, *Brevibacterium iodinum,* chitin, citral, hemicellulose, *Lycopersicon esculentum,* phytochemicals, pine bark, *Pseudomonas fluorescens,* rhizobacteria, root-knot nematode, *Serratia marcescens,* tomato, transplants.

The loss of methyl bromide for soil fumigation will result in serious disease problems for vegetable growers in the United States and elsewhere. Developing viable production practices that serve as replacements for soil fumigation in vegetable production systems will require an integrated approach, employing tactics that reduce pathogen inoculum potential and enhance host plant resistance. In transplanted crops, the transplant itself can provide the vehicle for delivery of low-risk chemicals, organic amendments, and plant growth-promoting rhizobacteria (PGPR) possessing these traits.

Naturally occurring phytochemicals and plant allelochemicals can be effective in reducing soilborne pathogens while minimizing environmental risks (Ferguson and Alford, 1985). Phytochemicals such as citral and benzaldehyde are known to alter the community structure of soil microbes, favor beneficial microorganisms, and be toxic to nematodes and fungi (Soler-Serratosa et al., 1996). Reports describing the decline in plant-parasitic nematodes in response to benzaldehyde and citral suggest that the compounds act as nematicides or ovicides (Soler-Serratosa et al., 1996). Benzaldehyde, a volatile, colorless liquid found in nature in several cyanogenic glucosides (Harborne and Baxter, 1993), has fungicidal and fungistatic properties that have long been recognized (Flor, 1926). Benzaldehyde and citral can be delivered by incorporation of plant tissue, crude extracts, processed active compounds, or active compound analogues.

Although application of organic amendments in the field for control of nematode pests is generally not practical for large-scale vegetable production, it is feasible to use these amendments in transplant mixes. Organic amendments that have demonstrated efficacy in reducing damage caused by root-knot and other nematodes include chitin, pine bark, and hemicellulose (Culbreath et al., 1985; Godoy et al., 1983; Kokalis-Burelle et al., 1994; Rodríguez-Kábana et al., 1983, 1987). Chitin and chitosan enhance plant growth and induce plant defense mechanisms when applied at low rates (Benhamou and Thériault, 1992; Benhamou et al., 1994). Chitosan applied to tomato plants prior to inoculation with *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL), the causal agent of Fusarium crown rot, reduced the number of root lesions, indicating that chitosan may elicit resistance in the plant (Benhamou and Thériault, 1992). In further studies, Benhamou et al. (1994) showed that a chitosan seed treatment in combination with a chitin-amended substrate increased resistance of tomato seedlings to FORL infection. Resistance was correlated with restricted fungal growth in root tissue, decreased pathogen viability, and accumulation of deposits in host cells. Chitin amendments also favor development of a chitinolytic microflora in soil (Rodríguez-Kábana et al., 1983) that can result in suppression of plant-parasitic nematodes and other soilborne pathogens (Rodríguez-Kábana et al., 1987).

Powdered pine bark has reduced numbers of root-knot nematodes in soil while increasing numbers of nonparasitic nematodes (Kokalis-Burelle et al., 1994). Rates of amendments are also important, as levels above 1% with chitin (Godoy et al., 1983; Mian et al., 1982) and 5% with pine bark (Kokalis-Burelle et al.,

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1994) are phytotoxic to certain crops including summer squash and soybean, respectively. Hemicellulosic waste, a product of the paper pulp industry generated by alkaline and bisulfite wood treatments that release cellulose, has been investigated as a soil amendment for nematode control (Huebner et al., 1983). This material is composed primarily of lignins, xylans, and other hemicellulosic components (Culbreath et al., 1985). Hemicellulosic waste alone did not eliminate all plant-parasitic nematodes but became significantly more effective when combined with urea (Huebner et al., 1983).

Rhizobacteria that improve plant growth and root health are referred to as plant growth-promoting rhizobacteria (PGPR) (Kloepper and Schroth, 1978). The beneficial effects of PGPR, including increased plant growth, have been attributed to shifts in the microbial ecology of the rhizosphere (Kloepper and Schroth, 1981). Production of iron-chelating siderophores, antibiotics, and hydrogen cyanide by PGPR also has been implicated in control of plant pathogens and increases in plant growth (Weller, 1988). *Bacillus subtilis* has been the most successful PGPR that exhibits disease-reducing capabilities and is well known for its antibiotic production capabilities (Brannen and Backman, 1993, 1994). Other PGPR strains control disease by mechanisms that do not involve production of toxic compounds. These mechanisms include substrate or site competition and induced systemic resistance in the host (Van Loon et al., 1998). Some researchers (Gagné et al., 1993; Nemec et al., 1996) have shown the potential to introduce biocontrol agents such as PGPR into commercial peat-based substrates for vegetable production to control root diseases and increase fruit yields. Also, Zehnder et al. (1997) found that PGPR-induced resistance against cucumber beetle feeding involved a change in the metabolic pathway for cucurbitacin synthesis, illustrating the wide array of mechanisms operative in pest suppression by PGPR.

The objectives of this research were to evaluate combinations of phytochemicals, organic amendments, and PGPR for levels of phytotoxicity, growth promotion, and efficacy in reducing disease caused by root-knot nematodes. One goal of the research was to develop a biologically based product that, when added to vegetable transplant growing mixes, would enhance plant growth and yield and provide protection against early invasion by root-knot nematodes in the field.

Materials and Methods

Effects of phytochemicals on nematode viability and plant growth: Experiments were performed to determine the direct effects of benzaldehyde (benzoic aldehyde, Aldrich Chemical, Allentown, PA) and citral (3,7-dimethyl-2,6-octadienal) (Aldrich Chemical, Allentown, PA) on nematode egg viability under saturated conditions. The phytochemicals were first evaluated in vitro. Three rates of citral and benzaldehyde (5, 10, and 20% [v/v]) were tested and compared to a water control. Experiments were conducted using alginate films (Rodríguez-Kábana et al., 1994) containing approximately the same number of *Meloidogyne incognita* eggs extracted from 8-week-old tomato plants. Films were suspended in plastic chambers containing the solutions of citral or benzaldehyde, incubated in the dark for 24 hours at 25 °C, and rinsed with water. To determine viability, eggs were stained using 0.25% rose bengal in 2.5% ETOH at 25 °C for 2.5 hours and destained with several water rinses. Films were then placed directly on microscope slides and evaluated. Eggs exhibiting staining were determined to be viable. Treatments were replicated eight times and arranged in a completely randomized design.

Greenhouse experiments were conducted in soil-less potting mix (Fafard's #2 loose potting mix: 55% peat, 25% perlite, 20% vermiculite, plus wetting agent, limestone, and gypsum) (Fafard Inc., Apopka, FL) treated with increasing rates of benzaldehyde and citral (0.10, 0.25, 0.50, and 1.00 ml/kg of mix), untreated potting mix, and a water control. A water control was included to determine baseline egg and juvenile viability while the reduction in egg and juvenile viability attributable to the peat-based mix alone was assessed in potting mix not receiving any phytochemical treatment. Water-control films were placed in sterile water in petri dishes and randomized on the greenhouse bench with the other treatments. A group of four attached cells from plastic flats containing 72 cells (TLC Polyform, Inc., Morrow, GA) were planted with two 'Rutgers' tomato seed (Asgrow Seed Co., Homestead, FL) 24 hours after treatment with phytochemicals. Treatments were replicated six times with four cells per replicate and arranged in randomized complete blocks on the greenhouse bench. One cell from each treatment and replication received alginate films containing root-knot nematode inoculum prepared as previously described (Rodríguez-Kábana et al., 1994). Films were placed in the potting mix at seeding. All films were removed after 48 hours, placed in petri dishes containing sterile water, and incubated at 25 °C for 5 days. Alginate films and incubation water were then evaluated for egg and juvenile viability. Egg viability was determined by using 0.25% rose bengal in 2.5% ETOH as a vital stain, as described previously. Juveniles were determined to be viable if they exhibited characteristic body curvature and movement as opposed to dead juveniles, which exhibited rigid, straight bodies with vacuolated or atypical internal morphology.

Germination was assessed for 2 weeks, and additional plants were removed from cells containing more than one germinated seedling by pinching them off at the base of the stem. Four weeks after seeding, two tomato plants from each treatment were transplanted into 10-

cm-diam. pots containing sand infested with approximately 1,000 root-knot nematode (*M. incognita*) eggs extracted from 8-week-old nematode culture tomato plants (Hussey and Barker, 1973). Plants from cells receiving alginate films were discarded. One plant that did not receive alginate films was sampled from each treatment and replication and evaluated for growth by measuring shoot length and weight, and root weight at transplanting. Plant-growth measurements and disease evaluations were performed 4 weeks after inoculation. The total number of galls were counted and root condition was evaluated using a 1-to-5 scale, where 1 = white, firm, healthy roots and 5 = fully lesioned, discolored, deteriorated roots. Root galling also was assessed using a root-gall index based on a scale of 1 to 10, where 1 = no galls and 10 = severe galling (Zeck, 1971).

Effects of organic amendments on nematode viability and plant growth: Experiments were conducted to evaluate the effects of pine bark (Union Camp Pulp Mill Plant, Opelika, AL), chitin (Sigma Chemical Co., St. Louis, MO), and hemicellulose (Georgia Pacific, Pittsfield, AL) at increasing rates on nematode viability and plant growth. Rates were composted or powdered pine bark at 50, 100, and 200 g/kg peat-based potting medium (Fafard's #2 peat medium); chitin at 25, 50, and 100 g/kg peat-based medium; and hemicellulose at 50, 100, and 200 g/kg peat-based medium. Also included were an untreated potting mix control and a water control as previously described. Amendments were thoroughly mixed in a cement mixer, and two 'Rutgers' tomato seeds were planted after 24 hours. Treatments were replicated six times and arranged in randomized complete blocks on the greenhouse bench. One of the four cells from each treatment and replication received alginate films as previously described. Films were placed in the potting medium after 24 hours at the same time as seeding. Viability of eggs and juveniles from alginate films and incubation water, seed germination, and plant growth were performed as previously described. Four weeks after seeding, tomatoes were transplanted, grown an additional 4 weeks in nematode infested soil, and evaluated for growth and disease as previously described.

Effects of combinations of phytochemicals and organic amendments on nematode viability and plant growth: Powdered chitin (25 g/kg mix), powdered fresh and composted pine bark (50 g/kg mix), and powdered hemicellulose (200 g/kg mix) were added to potting mix (Fafard's #2 peat medium). Either citral or benzaldehyde was combined with each of these amendments and with the non-amended treatment at 1.0 ml/kg mix. Two 'Rutgers' tomato seeds were planted after 24 hours. One cell from each group received alginate films containing root-knot nematode inoculum prepared as previously described. Films were placed in the potting mix 24 hours after treatment at the time of seeding and remained in place for 48 hours, after which they were evaluated for effects on nematode viability. Plants were thinned at 2 weeks, as previously described. One plant from each treatment and replication that did not receive alginate films was evaluated for growth by measuring shoot length, shoot weight, and root weight at transplanting. The 4-week-old seedlings were then transplanted into nematode-infested soil, and growth parameters, numbers of galls, root condition ratings, and gall ratings were determined as previously described 4 weeks after transplant. Treatments were randomized in complete blocks with six replications in the greenhouse.

Effects of combinations of phytochemicals, organic amendments, and PGPR on nematode viability and plant growth: Selection of organic amendment and phytochemical factors were based on previous experimental data. Selection of bacteria was based on their chitinolytic ability or their natural tolerance to the selected phytochemical. The experiment used a factorial design including two treatments of organic amendment (with and without chitin), two treatments of phytochemical (with or without benzaldehyde), and four treatments of bacteria (isolates 90–166, a chitinolytic isolate of *Serratia marcescens;* IN-844a, a phytochemical tolerant endophytic isolate of *Brevibacterium iodinum;* and 89B61, a phytochemical tolerant isolate of *Pseudomonas fluorescens*) (Table 1).

Bacterial inoculum was prepared by heavily streaking six plates of tryptic soy agar (TSA) (Difco/Becton Dickinson and Co., Sparks, MD) for each isolate and allowing cultures to grow for 24 hours at 28 °C. Cells were harvested by scraping plates and rinsing cells with a sterile 0.85% solution of NaCl. Bacterial concentrations were adjusted to between 10^9 and 10^{10} CFU/ml for application. Organic amendments and phytochemicals were applied to Fafard's #2 peat-based potting medium and mixed thoroughly in a cement mixer. Tomato seeds ('Rutgers') were placed in small depressions, and the bacterial suspensions were drenched over the seed in a volume of 1.0 ml/seed. Transplants were grown in flats for 4 weeks and then transplanted into sand inoculated with approximately 1,000 root-knot nematode eggs as previously described. Tomato plants were maintained for approximately 4 weeks in the greenhouse

TABLE 1. Treatments evaluated for effects on nematode viability and plant growth.

	Bacteria			
	Untreated	90-166	IN-844a	89B61
Untreated	1[a]	5	9	13
Chitin (20 g/kg)	2	6	10	14
Benzaldehyde (1 ml/kg)	3	7	11	15
Chitin (20 g/kg) + Benzaldehyde (1 ml/kg)	4	8	12	16

[a] Each number designates an individual treatment that consists of the combination of phytochemical or organic amendment in the row and the PGPR in the column.

and then evaluated for plant growth and root-knot nematode galling.

Statistical analysis: Data were statistically analyzed according to standard procedures including SAS general linear model (GLM), least-significant difference (LSD), and regression procedures (SAS Institute, Cary, NC). Unless otherwise stated, all differences referred to in the text were significant at the 5% level of probability.

Results

Effects of phytochemicals on nematode viability and plant growth: Increasing rates of both benzaldehyde and citral significantly reduced root-knot nematode egg and juvenile viability in vitro compared to the untreated control (Table 2). The 20% concentration rate of benzaldehyde reduced egg viability to less than 20%. Benzaldehyde was 100% efficacious as a nematicide against J2 at all rates tested. Citral became significantly ovicidal at the two highest concentrations (10% and 20%) (Table 2). Citral reduced juvenile viability to approximately one third of the untreated control at all rates tested (Table 2).

In potting mix, increasing rates of benzaldehyde improved seed germination whereas increasing rates of citral reduced seed germination (Table 3). The highest rate of citral tested reduced plant shoot weight compared to the lowest rate whereas the highest rate of benzaldehyde generally increased plant shoot and root weight compared to lower rates of benzaldehyde (Table 3).

All treatments including the untreated potting mix control reduced root-knot egg viability compared to the water control; no significant reduction in egg viability with increasing rates of citral was found when compared to the untreated mix control (Table 4). Benzaldehyde at rates 0.25 ml/kg and higher reduced egg viability compared to the untreated mix ($P \leq 0.05$) and reduced juvenile viability compared to the water control films ($P \leq 0.05$) (Table 4). Citral at 0.25 and 1.0 ml/kg reduced juvenile viability compared to the potting mix control, whereas benzaldehyde reduced juvenile viability compared to the mix control only at 1.0 ml/kg (Table 4).

Table 2. In vitro effects of phytochemicals on nematode eggs and juveniles from alginate films.

Treatment	Rate (v/v)	Viable eggs (%)	Viable juveniles (No.)
Control	—	76.0 a[a]	57.6 a
Citral	5%	64.0 ab	19.2 b
Citral	10%	44.8 cd	9.6 bc
Citral	20%	54.4 bc	21.2 b
Benzaldehyde	5%	32.0 de	0.0 c
Benzaldehyde	10%	33.6 d	0.0 c
Benzaldehyde	20%	19.2 e	0.0 c
LSD ($P = 0.05$)		12.8	13.4

[a] Means followed by the same letter are not different ($P > 0.05$).

Table 3. Effects of phytochemically treated potting mix on 'Rutgers' tomato germination and growth 4 weeks after seeding.

Treatment	Rate	Germination (%)	Shoot weight (g)	Root weight (g)
Control	—	41.5 bc[a]	0.58 abc	0.23 abc
Citral	0.10 ml/kg	33.2 bc	0.62 ab	0.22 abc
Citral	0.25 ml/kg	37.5 bc	0.55 abc	0.23 abc
Citral	0.50 ml/kg	12.5 c	0.51 bc	0.16 c
Citral	1.00 ml/kg	12.5 c	0.44 c	0.17 c
Benzaldehyde	0.10 ml/kg	50.0 ab	0.59 abc	0.19 bc
Benzaldehyde	0.25 ml/kg	45.7 abc	0.66 ab	0.25 ab
Benzaldehyde	0.50 ml/kg	62.5 ab	0.51 bc	0.21 bc
Benzaldehyde	1.00 ml/kg	79.0 a	0.68 a	0.30 a
LSD ($P = 0.05$)		37.2	0.16	0.07

[a] Means followed by the same letter are not different ($P > 0.05$).

Effects of organic amendments on plant growth and nematode viability: Pine bark at 100 and 200 g/kg mix reduced seed germination compared to the untreated control ($P \leq 0.05$) (data not shown). Hemicellulose and chitin did not reduced seed germination (data not shown). No reductions in root-knot nematode egg and juvenile viability were found among treatments with increasing rates of any organic amendment (data not shown).

Effects of combinations of phytochemicals and organic amendments on nematode viability and plant growth: Most combinations of organic amendments and phytochemicals did not affect seed germination (Table 5). However, composted pine bark, composted pine bark + benzaldehyde, and chitin + citral did reduce tomato seed germination compared to the untreated control (Table 5). All chitin treatments as well as hemicellulose alone and hemicellulose + benzaldehyde increased shoot weight compared to the untreated control (Table 5). Only treatments containing chitin increased plant root weight compared to the control ($P \leq 0.05$) (Table 5). Of these three treatments, chitin alone and chitin + benzaldehyde increased root weight more than the chi-

Table 4. Effects of potting mix treated with phytochemicals on nematode eggs in alginate films and on viability of juveniles hatched from films.

Treatment	Rate	Viable eggs (%)	Viable juveniles (%)
Mix control	—	49.2 b[a]	43.8 b
Citral	0.10 ml/kg	50.0 b	45.7 b
Citral	0.25 ml/kg	39.2 bc	26.7 c
Citral	0.50 ml/kg	39.2 bc	44.0 b
Citral	1.00 ml/kg	46.0 bc	17.0 c
Benzaldehyde	0.10 ml/kg	35.2 bcd	48.3 ab
Benzaldehyde	0.25 ml/kg	31.2 cd	33.5 bc
Benzaldehyde	0.50 ml/kg	34.0 cd	43.5 b
Benzaldehyde	1.00 ml/kg	21.2 d	20.3 c
Water control	—	70.6 a	63.1 a
LSD ($P = 0.05$)		14.8	16.7

[a] Means followed by the same letter are not different ($P > 0.05$).

TABLE 5. Effects of organic amendments and phytochemicals on 'Rutgers' tomato seedling growth in the greenhouse at 28 days after treatment and galling by root-knot nematodes at 56 days after treatment.

Amendment	Rate	Phytochemical (1.0 ml/kg)	Germination (%)	Shoot weight (g)	Root weight (g)	Root condition[a]	Gall rate[b]	Galls/g
Control	—	—	83.37 a[c]	0.51 e	0.35 cd	1.53 a	5.18 e	88.36 abc
Control	—	Citral	79.12 a	0.66 bcde	0.47 bcd	1.06 c	5.20 e	80.47 bc
Control	—	Benzaldehyde	83.25 a	0.59 bcde	0.42 cd	1.03 c	5.43 cde	72.22 cd
Composted bark	50 g/kg	—	35.37 c	0.55 cde	0.30 d	1.07 c	5.95 ab	81.31 abc
Composted bark	50 g/kg	Citral	72.87 ab	0.53 de	0.35 cd	1.10 c	5.66 abcd	87.97 abc
Composted bark	50 g/kg	Benzaldehyde	45.75 bc	0.56 cde	0.28 d	1.00 c	6.08 a	102.36 ab
Fresh bark	50 g/kg	—	54.12 abc	0.51 e	0.34 cd	1.04 c	5.76 abc	87.67 abc
Fresh bark	50 g/kg	Citral	60.37 abc	0.61 bcde	0.37 cd	1.03 c	5.80 abc	104.33 a
Fresh bark	50 g/kg	Benzaldehyde	79.12 a	0.54 cde	0.44 bcd	1.06 c	5.53 bcde	89.92 abc
Chitin	25 g/kg	—	83.25 a	0.75 bcd	0.87 a	1.40 ab	4.50 f	56.49 de
Chitin	25 g/kg	Citral	47.87 bc	0.80 b	0.62 b	1.16 c	4.66 f	79.47 bcd
Chitin	25 g/kg	Benzaldehyde	60.37 abc	1.14 a	0.83 a	1.13 c	4.51 f	52.75 e
Hemicellulose	200 g/kg	—	72.87 ab	0.77 bc	0.53 bc	1.07 c	5.26 de	75.72 cde
Hemicellulose	200 g/kg	Citral	72.87 ab	0.73 bcde	0.47 bcd	1.10 c	5.46 cde	83.29 abc
Hemicellulose	200 g/kg	Benzaldehyde	75.00 ab	0.77 bc	0.52 bc	1.18 bc	5.53 bcde	72.15 cde
LSD ($P = 0.05$)			29.25	0.23	0.20	0.22	0.42	23.42

[a] Root Condition Index Scale: 1 = white, firm, healthy; 5 = fully lesioned, discolored, deteriorated.
[b] Gall Rate: 1 = no galling; 10 = complete galling (Zeck, 1971).
[c] Means followed by the same letter are not different ($P > 0.05$).

tin + citral treatment ($P \leq 0.05$) (Table 5). All treatments except chitin alone improved root condition compared to the untreated control (Table 5). In addition, only treatments containing chitin reduced galling by root-knot nematodes and subsequently galls per gram (Table 5). Of these three treatments, chitin + benzaldehyde reduced the number of galls per gram of root tissue the most (Table 5). All of the pine bark treatments, except the highest rate of fresh bark combined with benzaldehyde, increased gall rating values ($P \leq 0.05$). However, these treatments did not increase the number of galls per gram of root tissue, nor did they decrease root weight compared to the untreated control. These data indicate that there was more compound-galling of older roots and less distinctly individual gall formation on younger roots.

There were no statistically significant interactions between organic amendments and phytochemicals in this study. Consequently, when organic amendments were analyzed disregarding phytochemicals as a factor, only chitin increased plant growth, improved root condition, and reduced galling by root-knot nematodes ($P \leq 0.05$) (Table 6). Chitin also improved root condition compared to the untreated control. However, chitin decreased seed germination compared to the control (Table 6). The combination of chitin and benzaldehyde, which exhibited the most potential for enhancing plant growth and reducing nematode viability, was therefore selected for use in further experiments.

Effects of combinations of phytochemicals, organic amendments, and PGPR on nematode viability and plant growth: When all three components were evaluated in combination and separately, treatments containing chitin alone increased shoot weight and significantly decreased galling. Combination of chitin and benzaldehyde increased shoot length, shoot weight, root weight, and root condition but did not reduce galling. The interaction between chitin and benzaldehyde was sta-

TABLE 6. Effects of organic amendments on tomato seedling growth in the greenhouse at 28 days after treatment and on galling by root-knot nematodes at 56 days after treatment.

	Germination (%)	Shoot length (cm)	Shoot weight (g)	Root weight (g)	Root condition[a]	Gall rate[b]	Galls/g
Unamended	81.87 a[c]	16.75 b	4.08 bc	2.34 c	2.75 bc	5.27 b	82.01 a
Composted pine bark[d]	51.37 c	14.65 d	3.67 c	2.19 c	3.36 a	5.90 a	90.54 ab
Fresh pine bark[e]	64.50 bc	15.07 cd	3.87 c	2.24 c	3.03 ab	5.68 a	93.71 a
Chitin[f]	63.87 b	21.61 a	6.86 a	4.11 a	1.41 d	4.55 c	61.92 c
Hemicellulose[g]	73.50 ab	16.48 bc	4.72 b	2.80 b	2.58 c	5.42 b	77.05 b
LSD ($P = 0.05$)	16.87	1.44	0.65	0.37	0.36	0.24	13.5

[a] Root Condition Index Scale: 1 = white, firm, healthy; 5 = fully lesioned, discolored, deteriorated.
[b] Gall rate: 1 = no galling; 10 = complete galling (Zeck, 1971).
[c] Means followed by the same letter are not different ($P > 0.05$).
[d] Composted pine bark was applied at 50 g/kg.
[e] Fresh pine bark was applied at 50 g/kg.
[f] Chitin was applied at 25 g/kg.
[g] Hemicellulose was applied at 200 g/kg.

tistically significant ($P \leq 0.05$) for shoot length, shoot weight, and root weight (Table 7). The combination of chitin + benzaldehyde + bacteria slightly improved some plant-growth parameters compared to the combination treatments without bacteria and the untreated control (Table 7). Chitin + benzaldehyde + 90-166, chitin + benzaldehyde + IN-844a, and chitin + benzaldehyde + 89B61 improved plant growth compared to the untreated control ($P \leq 0.05$) (Table 7). Chitin + benzaldehyde + 89B61 was the only three-way combination treatment that increased germination and improved shoot length, shoot weight, root weight, and root condition. However, this combination treatment did not reduce galling (Table 7). Only chitin alone exhibited a reduction in galling compared to the untreated control (Table 7). No treatment showed differences from the untreated control with regard to galls per gram (Table 7).

Discussion

These studies were undertaken to screen potential components of transplant mixes individually and, in combinations, for effects on plant growth and nematode viability. The most efficacious components were subsequently combined with selected PGPR and evaluated for growth promotion and root-knot nematode control on tomato. Initial studies on the direct effects of phytochemicals on root-knot nematode egg and juvenile viability illustrated the difference between citral and benzaldehyde with respect to nematicidal capabilities. Benzaldehyde was more toxic to root-knot nematode infective stage juveniles (J2) and eggs than citral in vitro. This direct toxicity to nematodes combined with the reduction in phytotoxicity observed with benzaldehyde led to its further investigation in a multicomponent system.

Phytotoxicity levels for the amendments tested were known for other crops, and this information provided a framework for initial studies with amendments for tomato (Cullbreath et al., 1985; Huebner et al., 1983; Kokalis-Burelle et al., 1994). The direct effects of chitin decomposition on nematodes, and the indirect effects on rhizosphere microorganisms antagonistic to nematodes (Culbreath et al., 1985; Rodríguez-Kábana et al., 1983), were previously established and instrumental in the inclusion of chitin in these experiments. It is likely that these mechanisms contributed to nematode suppression in our studies. Previous work by Benhamou and Thériault (1992) showed a reduction in Fusarium crown rot lesions on tomato with chitosan application to seed and indicates that chitosan may also be an elicitor of resistance responses in the host plant. To date, there is no direct evidence that lignin deposits negatively effect invasion by root-knot nematodes, but it is possible that such a mechanism is also operable in this host/pathogen system. The absence of nematode suppression with pine bark amendments is not consistent with previous work on soybean (Kokalis-Burelle et al., 1994) and indicates that it may be necessary to alter amendment composition with different crops. The suppressive effect of the peat-based potting mix alone on

Table 7. Effects of chitin, benzaldehyde, and bacteria on plant growth at 28 days after treatment and on galling by root-knot nematodes at 56 days after treatment.

Treatment			Germination (%)	Shoot length (cm)	Shoot weight (g)	Root weight (g)	Root condition[a]	Gall rate[b]	Galls/g
Chitin[c]	Benzaldehyde[d]	Bacteria							
No	No	Water	85.37 bcd[e]	13.58 e	7.61 c	2.96 f	2.91 a	5.28 ab	93.23 abc
Yes	No	Water	89.50 abcd	13.41 e	10.00 b	3.60 cdef	2.50 abc	4.73 c	72.14 c
No	Yes	Water	95.75 ab	14.16 de	7.65 c	2.80 f	2.66 ab	5.15 abc	94.43 abc
Yes	Yes	Water	95.75 ab	16.08 bc	12.45 a	4.44 ab	1.91 c	4.86 bc	75.34 bc
No	No	90-166[f]	83.25 cd	13.91 e	8.22 c	3.06 ef	2.66 ab	5.20 ab	95.28 ab
Yes	No	90-166	95.75 ab	14.83 cde	10.92 ab	3.96 abcd	2.41 abc	5.08 abc	90.82 abc
No	Yes	90-166	79.12 d	13.50 e	7.82 c	3.21 def	2.41 abc	4.98 abc	73.46 bc
Yes	Yes	90-166	93.75 abc	17.16 ab	12.50 a	4.46 ab	2.16 bc	4.95 abc	80.56 bc
No	No	IN-844a[g]	95.75 ab	14.25 de	8.10 c	3.17 def	2.58 abc	5.23 ab	86.34 bc
Yes	No	IN-844a	91.62 abc	15.95 bc	12.32 a	4.13 abc	2.16 bc	4.96 abc	83.17 bc
No	Yes	IN-844a	87.50 bcd	14.16 de	7.07 c	2.84 f	2.91 a	5.40 a	88.51 abc
Yes	Yes	IN-844a	93.75 abc	18.16 a	12.35 a	4.76 a	2.33 abc	4.96 abc	75.67 bc
No	No	89B61[h]	89.5 abcd	13.58 e	7.71 c	3.18 def	2.83 ab	5.18 abc	86.62 bc
Yes	No	89B61	95.75 ab	16.41 bc	11.09 ab	3.86 bcde	2.50 abc	4.88 bc	77.83 bc
No	Yes	89B61	93.75 abc	13.91 e	7.77 c	2.98 f	2.33 abc	5.28 ab	111.32 a
Yes	Yes	89B61	100.00 a	15.58 bcd	11.98 a	4.44 ab	1.91 c	4.83 bc	74.48 bc
LSD ($P = 0.05$)			11.62	1.63	1.64	0.83	0.72	0.45	22.84

[a] Root Condition Index Scale: 1 = white, firm, healthy; 5 = fully lesioned, discolored, deteriorated.
[b] Gall Rate: 1 = no galling; 10 = complete galling (Zeck, 1971).
[c] Chitin was applied at 20 g/kg.
[d] Benzaldehyde was applied at 1 ml/kg.
[e] Means followed by the same letter are not different ($P > 0.05$).
[f] 90-166 is a chitinolytic strain of *Serratia marcescens*.
[g] IN-844a is a phytochemical-tolerant endophytic strain of *Brevibacterium iodinum*.
[h] 89B61 is a phytochemical-tolerant strain of *Pseudomonas fluorescens*.

root-knot nematode egg and juvenile viability was apparent in studies conducted in potting mix using alginate films. This suppression by organic media components is not unexpected and serves to illustrate the potential of mix components for pathogen suppression.

Individually, chitin and benzaldehyde positively affected plant growth and negatively affected nematode viability and root galling. The combination of chitin and benzaldehyde consistently improved plant growth and decreased disease caused by *M. incognita* as compared to the organic amendments and phytochemicals applied to the potting mix alone. However, a significant interaction between chitin and benzaldehyde was observed only with respect to plant-growth parameters, not to a reduction in disease caused by root-knot nematodes. The addition of PGPR did not drastically improve the performance of these combinations in greenhouse trials. Further field evaluation is under way to determine if PGPR addition generates a different response under field environmental conditions. Recently completed studies to investigate the effects of PGPR/chitin transplant treatments on tomato and pepper growth and yield (Kokalis-Burelle et al., 2002) have shown an increase in seedling growth, transplant survival, and yield in Florida field trials. Studies are currently being conducted to evaluate the survival and colonization of transplant mix applied bacteria in the field throughout the growing season, and to assess the impact of applied bacteria on selected populations of indigenous rhizosphere colonizers.

The addition of microbial agents, organic amendments, and phytochemicals to the transplant mix is an ideal delivery mechanism for introducing these agents onto transplanted vegetable crops. Under greenhouse conditions in the pathogen-free transplant mix, the microbial agents have an advantage that allows them time and space to colonize the host root system. The use of transplants as a system for delivery of biocontrol agents is compatible with organic vegetable production and alternative chemical fumigants. With fumigation, the chemical leaves a pathogen-free soil environment in which the microbial product can survive, reproduce, and colonize the growing root system. The use of amendments such as chitin, that positively affect soil microbial communities and act as elicitors, in combination with microbial biocontrol agents, can improve plant performance and help to mitigate potential yield losses incurred by use of methyl bromide alternatives. Our studies show that chitin, benzaldehyde, and PGPR used as amendments to soil-less transplant media resulted in improved plant growth and reduced disease caused by root-knot nematodes on tomato.

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