Application for Rhizobacteria in Transplant Production and Yield Enhancement

J.W. Kloepper, M.S. Reddy and R. Rodríguez-Kabana
Department of Entomology and Plant Pathology
Auburn University
Auburn, AL 36849
USA

D.S. Kenney
Gustafson, LLC
Plano, TX 75075
USA

N. Kokalis-Burelle
USDA/ARS Horticultural Research Laboratory
Ft. Pierce, FL 34945
USA

N. Martinez-Ochoa
Plant Pathology Department
The University of Georgia
Tifton, GA 31793
USA

C.S. Vavrina
Southwest Florida Research and Education Center
The University of Florida
Immokalee, FL 34142
USA

Keywords: PGPR, biological control, induced systemic resistance, bacterization, vegetables

Abstract

PGPR (plant growth-promoting rhizobacteria) are root-colonizing bacteria that benefit plants by increasing plant growth or reducing disease. Current applications of PGPR as biocontrol agents rely on mixtures of PGPR as components in integrated management systems in which reduced rates of agrochemicals and cultural control practices are used. The finding that some strains of PGPR can elicit systemic disease protection has renewed interest in PGPR for practical application in agriculture and horticulture. We report here results of attempts to combine PGPR with different modes of action with organic amendments. Our hypothesis was that such an integrated system could be used for transplanted vegetables to produce more vigorous transplants that would be tolerant of nematodes and other diseases for at least a few weeks after transplanting to the field. The specific combination that we tested consisted of *Bacillus subtilis* strain GB03, *B. amyloliquefaciens* strain IN937a, and *B. subtilis* strain IN937b together with chitosan. Strain GB03 produces antibiotics while IN937a and IN937b elicit induced systemic resistance. Chitosan was added to stimulate a microflora antagonistic to nematodes. Results demonstrated that the combination of two bacilli strains with chitosan resulted in significant growth promotion that was correlated with induced resistance in tomato (*Lycopersicon esculentum)*, bell pepper (*Capsicum annuum*), cucumber (*Cucumis sativus*) and tobacco (*Nicotiana tabacum*). The preparation has been commercialized by Gustafson, LLC under the name "BioYield" and is discussed as a model for extending PGPR technologies to growers. BioYield is incorporated into the potting mix used to prepare transplants. Treated transplants demonstrate increased shoot and root growth, enhanced stem diameter, less transplant shock, and rapid development of new roots. Disease protection is sometimes observed, but the most reproducible effect is growth promotion resulting in yield increases with many tested transplant systems.

INTRODUCTION

Plant growth-promoting rhizobacteria (PGPR) are naturally occurring soil bacteria that aggressively colonize plant roots and benefit plants by providing growth promotion (Cleyet-Marcel et al., 2001; Kloepper, 1994; Glick, 1995). Inoculation of crop plants with certain strains of PGPR at an early stage of development improves biomass production

Proc. XXVI IHC – Transplant Production and Stand Establishment
Eds. S. Nicola, J. Nowak and C.S. Vavrina
Acta Hort. 631, ISHS 2004
Publication supported by Can. Int. Dev. Agency (CIDA)

through direct effects on root and shoot growth. Several reviews discuss specific aspects of growth promotion by PGPR (Cleyet-Marcel et al., 2001; Glick, 1995). Inoculation of ornamentals, forest trees, vegetables, and agricultural crops with PGPR may result in multiple effects on early-season plant growth, as seen in the enhancement of seedling germination, stand health, plant vigor, plant height, shoot weight, nutrient content of shoot tissues, early bloom, chlorophyll content, and increased nodulation in legumes.

Biological control using introduced PGPR against plant diseases has been extensively studied under greenhouse and field conditions. PGPR exhibit biological control via several mechanisms. Siderophores, antibiotics, hydrogen cyanide, and cell-wall degrading enzymes are among the metabolites produced by PGPR that reduce growth or activity of the pathogen. Biological control may also result from direct interactions between PGPR and the host plant. In this case, host disease defense reactions are stimulated, a process termed induced systemic resistance (ISR). Further, several studies shave shown that individual strains of PGPR elicit ISR against multiple pathogens - bacteria, fungi, and viruses - on one host plant, including tomato, bell pepper, and cucumber (Raupach et al., 1996; Raupach and Kloepper, 1998; 2000; Reddy et al., 1999; Reddy et al., 2000; Jetiyanon and Kloepper, 2002). To date, little research has been conducted to determine whether PGPR strains can elicit ISR in a range of host plants.

Improving the consistency of beneficial effects is a goal for PGPR research and development. Most approaches for biocontrol of plant diseases and plant growth promotion have used applications of single PGPR strains. Because one strain is not likely to be active in all soil environments or against all pathogens that attack the host plant, the use of a single strain may partially account for the reported inconsistent performance by PGPR. Supporting the use of a mixture of PGPR strains are two studies on ISR (Raupach and Kloepper, 2000; Jetiyanon and Kloepper, 2002) in which mixtures of PGPR provided greater activity against a broader range of plant pathogens than did single strains.

Historically, PGPR have been applied as seed treatments to row crops. In our efforts to apply mixed PGPR inoculants to vegetables, we have been working on applying PGPR in the growing media used to prepare transplants. Our study was conceived several years ago from a group effort to integrate applications for vegetable transplants. The rationale was to combine PGPR with organic amendments, as some organic amendments, including chitin, can be mixed into agricultural soils with the effect of reducing nematode damage to plants (Rodríguez-Kabana, 1986; Hallmann et al., 1998; Suganda, 1999). We previously tested organic amendments of chitin, pine bark, and hemicellulose together with phytochemicals for effects on tomato transplant growth and root-knot nematode severity (Kokalis-Burelle et al., 2002a). Among the tested organic amendments, only treatments containing chitin increased plant root weight and reduced nematode galling compared to the control.

The specific goal of this project was to determine if an integrated biological preparation could protect vegetable transplants against diseases for several weeks after being transplanted into the field. The broader purpose was to accelerate development of vegetable transplant plugs and to increase plant health.

MATERIALS AND METHODS

PGPR Strains

Three spore-forming, bacilli PGPR strains were used: *Bacillus subtilis* strain GB03, *B. amyloliquefaciens* strain IN937a, and *B. subtilis* strain IN937b. Strain GB03 has shown biological control activity against *Rhizoctonia solani* and *Fusarium* spp. (Backman et al., 1997) and is part of a commercial product, Kodiak (Gustafson LLC, Dallas, Texas, USA) that is used as a seed treatment of cotton. Strains IN937a and IN937b elicit ISR in cucumber against cucurbit wilt caused by *Erwinia tracheiphila*, anthracnose caused by *Colletotrichum orbiculare*, and mosaic disease caused by cucumber mosaic virus (Raupach and Kloepper, 1998; 2000; Zehnder et al., 2000). These strains also elicit ISR in tomato against bacterial speck caused by *Pseudomonas syringae* pv. *tomato*, cucumber

mosaic virus, and tomato mottle virus (Ji et al., 1996; Murphy et al., 2000). PGPR strains were maintained for long-term storage at -80°C in tryptic soy broth (TSB) (Difco Laboratories, Detroit, Michigan, USA) supplemented with 20% glycerol. For experimental use, industrially formulated endospores by Gustafson were used.

Field Trials

A series of four field trials were conducted to test the biocontrol potential of PGPR preparations against nematodes, bacteria, and fungi with tomato cv. Solar Set in a sandy loam soil at the Uniroyal Chemical Company Research Farm located in Sanford, Florida. Each trial consisted of five main treatments: nontreated control, chitosan + GB03, chitosan + GB03 + IN937b, chitosan + GB03 + IN937a, and chitosan + IN937a + IN937b. In trial one, methyl bromide was included as a control for root-knot nematode. In trial two, ManKocide was used as a control for bacterial spot disease. Chitosan was used as a carrier for the biological preparation. In treatments containing PGPR strains, industrially formulated endospores were added to chitosan to reach 4.0×10^{10} colony forming units (CFU)/liter for each strain.

Tomato transplants were grown in Styrofoam Speedling trays (Speedling Inc., Sun City, Florida). Four to eight trays containing 128 cells were used for each treatment to produce enough seedlings for four trials. To produce the transplants, appropriate bacterial treatments were mixed thoroughly with Speedling soilless media at 1:40 (v/v) to reach a bacterial density of 10^9 CFU/liter media. The media were placed in the trays prior to seeding. One tomato seed was placed into each cell. Seedlings were grown for 4 weeks in the greenhouse at the Plant Science Research Facility, Auburn University, Auburn, Alabama Plants were fertilized weekly with 150 ppm N of Peter's professional solution (20-20-20) and watered regularly.

At the end of 4 weeks, seedlings were transplanted to the field into beds that were 20 cm high by 84 cm wide. There were 10 seedlings, 30 cm apart, per replicated bed. Prior to bedding, P_2O_5 fertilizer was broadcasted at 2.5 kg per 30.5 m of row. After bedding, 15-0-30 fertilizer was banded onto the bed shoulders at 9.0 kg per 30.5 m of row. In the trials designated for methyl bromide treatment, the beds were fumigated (335 kg/ha of 67% methyl bromide + 33% chloropicrin) before being covered with black polyethylene film. Single row treatment plots were replicated six times in a randomized complete block (RCB) design. At 30 days after transplanting, the number of healthy and dead plants per replication was counted and the percentage of dead plants per treatment was calculated.

Trial one evaluated biological control against the root-knot nematode *Meloidogyne incognita*. The trial was conducted in a field with a history of severe root-knot nematode damage. To rate root-knot severity, roots were harvested 60 days after planting and soil was removed by washing. Each plant was then rated on a scale of 1-10 according to the procedure described by Zeck (1971), where 1 = no galls and 10 = completely covered with galls.

Trial two evaluated biological control against bacterial spot caused by *Xanthomonas axonopodis* pv. *vesicatoria*. The pathogen was inoculated 30 days after planting by spraying plants with a suspension of 10^8 CFU/ml. At 60 days after planting, foliar disease severity was assessed by sampling 20-50 leaflets from each replicated row in a treatment. Bacterial spot lesions on each leaflet were counted. Disease incidence on fruit was determined at the same time by counting the number of fruit with lesions in each plot. Symptoms of bacterial spot on fruit are typically not observed in the test area; however, hurricane condition two weeks prior to disease rating created optimum conditions of wind-blown rain and sand to inoculate fruit.

Trials three and four evaluated biological control against tomato crown and root rot caused by *Fusarium oxysporum* f. sp. *lycopersici* (FORL). FORL was grown on millet seed in preparation for both trials. At the time of transplanting, 25 cc of inoculum was placed onto soil near the stem of each plant. The percentage of dead plants was determined by counting the number of dead plants per replication at the end of the season.

Seedlings were harvested and rated for number of plants with severe symptoms caused by FORL and also for disease severity. Disease severity was rated on a scale of 0-3, where 0 = no symptoms and 3 = severe symptoms. Trial three was conducted in nonfumigated soil, and trial four was in soil fumigated with methyl bromide.

In the following year, two additional field trials, one in Sanford and the second in Cullman, Alabama, were conducted for further evaluation of the biological control potential of PGPR formulations against root-knot nematode on tomato. Treatments were nontreated control, chitosan control, chitosan + GB03, chitosan + GB03 + IN937b, chitosan + GB03 + IN937a, chitosan + IN937a + IN937b, GB03 alone, IN937a alone, IN937b alone, and IN937a + IN937b. Methyl bromide was included as a treatment at the Cullman trial. In the Sanford trial, indigenous nematode populations were used as inoculum. For the Cullman trial, an inoculum of 700 eggs of *M. incognita* was placed into each transplant hole at the time of field transplantation. The NaOCl procedure (Hussey and Barker, 1973) was used to extract *Meloidogyne* spp. eggs from galled roots of tomato seedlings maintained under greenhouse conditions. Experimental procedures and evaluation of root-knot severity were similar to those described above.

Greenhouse Trials

During the preparation of transplants for the field trials as described above, plant growth promotion of tomato was evident with some of the treatments, especially in the treatment with chitosan plus two PGPR strains. A series of greenhouse experiments were conducted to test the repeatability of the growth promotion of tomato and cucumber. Cucumber cv. SMR58 was used. In this trial, treatments included nontreated control, chitosan control, chitosan + GB03, chitosan + IN937a, chitosan + GB03 + IN937a, GB03, IN937a, and GB03 + IN937a. As with the field trials, the industrially prepared PGPR and chitosan were mixed into Speedling soilless potting media prior to being placed into Speedling trays. There were four Speedling trays with 128 cavities per replication per treatment. One-half of each replicated tray was seeded with tomato and the other half with cucumber, one seed per cavity. Replicated treatments were arranged in a randomized complete block design on a greenhouse bench.

Four weeks after seeding, plant vigor was rated on a scale of 1-5, where 1 = poor, 2 = average, 3 = good, 4 = very good, and 5 = excellent. At the same time, 10 seedlings per replication of each treatment were randomly selected. Shoot height, shoot fresh weight, number of true leaflets and/or leaves per plant, and surface area of leaflets and/or leaves were obtained for each selected seedling.

Using the results of the first series of greenhouse experiments, we conducted confirmatory trials with the following treatments: nontreated control, chitosan control, and chitosan + GB03 + IN937a. Tomato, cucumber, pepper, and tobacco were evaluated for growth promotion. The experimental procedure and measurements were as described above, except that each replicated Speedling tray was seeded with the four crops.

ISR Activity

Four weeks after seeding tomato and cucumber, 5 seedlings from each of the replicated treatments were transplanted into 10-cm^2 plastic pots containing 500 cm^3 of Speedling soilless growing medium. Seven days after transplanting, entire tomato seedlings were sprayed with the bacterial spot pathogen *Xanthomonas axonopodis* pv. *vesicatoria* (10^7 CFU/ml) until run-off. Inoculum of *X. axonopodis* pv. *vesicatoria* was produced on tryptic soy agar (TSA) at 28°C for 24 h and suspended in sterile distilled water (SDW). Pathogen-challenged plants were placed in a humidity chamber for 48 h. They were then moved to controlled growth rooms maintained at 28°C with 90% relative humidity (RH), photo period of 14-10 h day and night, respectively, with intermittent misting for 6-7 days until water soaked lesions were visible on the inoculated foliage. At this time, the cool air humidifiers were removed and misting was turned off. Ten leaflets per plant were randomly sampled 8 to 10 days after pathogen challenge, and lesion numbers were counted on each individual leaflet.

Cucumber seedlings were challenge-inoculated with the angular leaf spot pathogen, *Pseudomonas syringae* pv. *lachrymans*. Inoculum of *P. syringae* pv. *lachrymans* was prepared by suspending cells from a 24 h culture grown on TSA in SDW to give a concentration of 10^8 cfu/ml. The pathogen was sprayed on the entire plant until run-off. After pathogen challenge, seedlings were placed into a humidity chamber for 24 h and then moved into controlled environmental growth rooms maintained at 25°C with 80% RH and photo period of 14-10 h day and night, respectively. Five days after pathogen challenge, the second and third leaves of each plant were assessed for angular leaf spot disease lesions. The experiments were conducted two times.

All data were subjected to analysis of variance (ANOVA). The treatment means were separated by Fisher's protected least significant difference (LSD) test at P = 0.05. All analyses were conducted with JMP software (SAS Institute Inc., Cary, North Carolina).

RESULTS

Field Trials

In trial one, there was no effect by any of the PGPR treatments on healthy stand of tomato seedlings and percentage of dead plants (Table 1a). However, methyl bromide increased healthy stand (seedlings with no disease symptoms) and reduced the percentage of dead plants compared to the nontreated control. All the treatments significantly reduced the number of plants with severe symptoms of root-knot nematode infestation compared to the nontreated control. In addition, two combinations of PGPR strains with chitosan reduced root-knot index compared to the nontreated control.

In trial two, all treatments reduced the number of fruit with bacterial spot lesions and number of bacterial spot lesions per leaflet compared to the nontreated control (Table 1b). With all PGPR treatments the level of protection against the incidence of fruit symptoms was statistically equivalent to protection afforded by ManKocide, the chemical control standard for bacterial spot. With disease incidence on foliage, two PGPR treatments resulted in protection that was significantly greater than the ManKocide control.

In trials three and four, there were no differences among treatments in healthy stand or percentage of dead plants grown in soil artificially infested with FORL in nonfumigated or fumigated soil (Tables 1c and 1d). However, in the nonfumigated field (Table 1c), all PGPR strains reduced the number of plants with severe FORL symptoms. Two treatments reduced the mean FORL index, compared to the nontreated control. In the fumigated field (Table 1d), one treatment reduced the number of plants with severe symptoms and all PGPR treatments reduced the mean FORL disease index.

Results of the second year field trials assessing protection against root-knot nematodes at two locations are shown in Table 2. In the field trial at Sanford, three treatments significantly reduced the mean root-knot index. At the Cullman field trial, the severity of root-knot was less than in Sanford, which can be seen in the relative root-knot index ratings for the controls in each location. In the Cullman trial, all treatments reduced the mean root-knot index compared to the nontreated control.

Greenhouse Trials

The initial greenhouse trial was designed to test the plant growth-promoting capacities of the individual PGPR strains, chitosan, and mixtures. The results obtained from both tomato (Table 3a) and cucumber (Table 3b) indicate a synergistic reaction when chitosan was combined with the two PGPR strains. With tomato, treatment with chitosan alone resulted in significant increases in height, number of leaflets, and leaflet surface area compared to the nontreated control; but it did not cause significant increases in vigor or weight. In contrast, treatment with the two PGPR strains in chitosan resulted in significant increases for all of these parameters compared to the nontreated control. The values of all five parameters resulting from the combination of the two PGPR strains plus

chitosan were significantly greater than the values for chitosan alone. Similar results occurred on cucumber. The combination of two PGPR strains and chitosan caused significant increases in all parameters, while treatment with chitosan alone increased three of the five parameters. The values of all five parameters resulting from the combination treatment were significantly greater than the values from treatment of chitosan alone.

Separate greenhouse experiments evaluated the plant growth-promoting effects of the combination treatment in comparison to chitosan and no treatment (Table 4). With tomato, cucumber, pepper, and tobacco, treatment with the combination of the PGPR strains with chitosan resulted in significant increases in vigor, height, shoot fresh weight, number of leaflets per plant, and leaf surface area compared to the treatment with chitosan alone.

ISR Activity

ISR activity was assessed on tomato and cucumber using the plants prepared in the previous experiment. Treatment with the combination of both PGPR strains with chitosan resulted in significant reductions in numbers of lesions of tomato spot and cucumber angular leaf spot compared to both the nontreated and chitosan-treated controls (Table 5).

DISCUSSION

The ultimate objective in transplant production is to generate a strong, vigorous, compact plant that will establish and grow quickly in the field and produce an optimum yield. The results reported here indicate that combinations of two *Bacillus* PGPR strains and chitosan are useful components in production of tomato, cucumber, pepper, and tobacco transplants. Incorporating the selected combination treatment (chitosan and strains GB03 and IN937a) into planting media resulted in significant increases in vigor, height, leaf area, number of leaves, and shoot weight compared to nontreated control plants in all the crops tested. Plant growth promotion resulting from the combination treatment could be valuable in two ways. First, the combination could be used to produce a standard-sized transplant in less time than is needed without PGPR. Second, increases in the vigor and shoot weight of transplants typically result in less transplant shock, reduced vulnerability to drought, and greater resistance to attack by pathogens, nematodes, and insects early in the season (Vavrina, 1996).

Our results demonstrate that the combination treatment provided repeated growth promotion of tomato, cucumber, pepper, and tobacco under greenhouse conditions. The combination treatment also elicited ISR, as evidenced by reduced severity of bacterial spot diseases on tomato and cucumber under greenhouse conditions. With the same combination of PGPR and a chitosan carrier, we have also demonstrated reductions in root-knot nematode severity, crown and root rot, and bacterial spot disease of tomato under field conditions. Hence, certain individual and compatible mixtures of PGPR strains could provide a broad spectrum of PGPR-mediated ISR activity against multiple pathogens.

The beneficial effects of the chitosan controls on transplant growth and disease incidence may be due to the reported capacity of chitosan to induce low levels of disease resistance (Benhamou et al., 1998). Chitosan has also been reported to favor the development of nematode antagonistic microflora in soil (Rodríguez-Kábana et al., 1987) and to function as a slow release source of nitrogen. Although chitosan had some beneficial effects, the magnitude and repeatability of the benefits was greater with the combination treatment, which demonstrates that the components in the combination treatment interacted synergistically.

In the experimental field trials conducted in this study, several beneficial effects were noted from the PGPR treatments. Supporting our results is a recently published report by Kokalis-Burelle et al. (2002b) showing that similar preparations of PGPR and chitosan led to increased survival of tomato and pepper transplants, highly significant increases in plant growth, and significant yield increases with pepper. In our research, in addition to plant growth promotion, we found significant differences in the incidence or

severity of several diseases among treatments in both greenhouse and field environments. The benefits of PGPR treatments seen in our field trials included improvement in root ratings, reduction of galling by root-knot nematodes, and lower incidence of disease caused by common foliar pathogens such as bacterial spot and crown and root rot disease of tomato. Our results are also supported by recent work by Egel (2000), who found that root-knot nematode damage on watermelon was reduced with LS213, which is the same combination of two PGPR strains and chitosan that we used. Egel also demonstrated that when the combination treatment was used with Telone C35 as a soil fumigant, total yield was increased.

Yield responses to the combination treatment in tomato, pepper, and cucumber were significantly higher compared to the nontreated and chitosan controls in many repeated field trials across the United States (unpublished). In 2001, Gustafson, LLC commercialized this preparation under the name "BioYield". BioYield is incorporated into the potting mix used to prepare transplants. Treated transplants demonstrate increased shoot and root growth as well as enhanced stem caliper. After being transplanted to the field, treated seedlings typically show less transplant shock and develop new roots more quickly. Disease protection is sometimes observed; however, the most reproducible effect is growth promotion resulting in yield increases with many tested transplant systems.

ACKNOWLEDGEMENTS

We acknowledge the contributions over the years of collaborators on various aspects of this research, including Scott Enebak, John Murphy, Jane Polston, Edward Sikora, and Sadik Tuzun.

Literature Cited

Backman, P.A., Wilson, M. and Murphy, J.F. 1997. Bacteria for biological control of plant diseases. p.95-109. In: N.A. Rechcigl and J.E. Rechcigl (eds.), Environmentally Safe Approaches to Crop Disease Control, CRC Lewis Publishers, Boca Raton, Florida.

Benhamou, N., Kloepper, J.W. and Tuzun, S. 1998. Induction of resistance against Fusarium wilt of tomato by combination of chitosan with an endophytic bacterial strain: ultrastructure and cytochemistry of the host response. Planta 204:153-168.

Cleyet-Marcel, J.C., Larcher, M., Bertrand, H., Rapior, S. and Pinochet, X. 2001. Plant growth enhancement by rhizobacteria. p.185-197. In: J.-F. Morot-Gaudry (ed.), Nitrogen Assimilation by Plants: Physiological, Biochemical and Molecular Aspects, Science Publishers, Inc., Plymouth, U.K.

Egel, D.S. 2000. Evaluation of fumigants for the control of Fusarium wilt and root-knot nematode of watermelon. Fungicide and Nematicide Test Report 2001:N19.

Glick, B.R. 1995. The enhancement of plant growth by free-living bacteria. Can. J. Microbiol. 41:109-117.

Hallmann, J., Rodríguez-Kabana, R. and Kloepper, J.W. 1998. Chitin mediated changes in bacterial communities of the soil, rhizosphere and internal roots of cotton in relation to nematode control. Soil Biol. Biochem. 31:551-560.

Hussey, R.S. and Barker, K.R. 1973. A comparison of methods of collecting inocula for *Meloidogyne* spp. including a new technique. Plant Disease Rep. 57:1025-1028.

Ji, P., Kloepper, J.W., Wilson, M. and Campbell, H.L. 1996. Rhizobacterial-induced systemic resistance in tomato against bacterial speck. Phytopathology 86:S50.

Jetiyanon, K. and Kloepper, J.W. 2002. Mixtures of plant growth-promoting rhizobacteria for induction of systemic resistance against multiple plant diseases. Biol. Control 24:285-292.

Kloepper, J.W. 1994. Plant Growth-Promoting Rhizobacteria. p.137-166. In: Y. Okon (ed.), *Azospirillum*/Plant Associations, CRC BOQ Raton, FL.

Kokalis-Burelle, N., Martinez-Ochoa, N., Rodriguez-Kabana, R. and Kloepper, J.W. 2002a. Development of multi-component transplant mixes for suppression of

Meloidogyne incognita on tomato (*Lycopersicon esculentum*). J. Nematol. (In Press).
Kokalis-Burelle, N., Vavrina, C.S., Rosskopf, E.N. and Shelby, R.A. 2002b. Field evaluation of plant growth-promoting rhizobacteria amended transplant mixes and soil solarization for tomato and pepper production in Florida. Plant Soil 238:257-266.
Murphy, J.F., Zehnder, G.W., Schuster, D.J., Sikora, E.J., Polstan, J.E. and Kloepper, J.W. 2000. Plant growth-promoting rhizobacteria mediated protection in tomato against tomato mottle virus. Plant Dis. 84:779-784.
Raupach, G.S. and Kloepper, J.W. 1998. Mixtures of plant growth-promoting rhizobacteria enhance biological control of multiple cucumber pathogens. Phytopathology 88:1158-1164.
Raupach, G.S. and Kloepper, J.W. 2000. Biocontrol of cucumber diseases in the field by plant growth-promoting rhizobacteria with and without methyl bromide fumigation. Plant Dis. 84:1073-1075.
Raupach, G.S., Liu, L., Murphy, J.F., Tuzun, S. and Kloepper, J.W. 1996. Induced systemic resistance in cucumber and tomato against cucumber mosaic virus using plant growth-promoting rhizobacteria (PGPR). Plant Dis. 80:891-894.
Reddy, M.S., Rodriguez-Kabana, R., Kenney, D.S., Ryu, C.M., Zhang, S., Yan, Z., Martinez-Ochoa, N. and Kloepper, J.W. 1999. Growth promotion and induced systemic resistance mediated by a biological preparation. Phytopathology 89:S39.
Reddy, M.S., Ryu, C.M., Zhang, S., Yan, Z., Kenney, D.S., Rodriguez-Kabana, R. and Kloepper, J.W. 2000. Approaches for enhancing PGPR-mediated ISR on various vegetable transplant plugs. Auburn University Website, Available: http://www.ag.auburn.edu/argentina/pdfmanuscripts/reddy.pdf (accessed 12/04/00).
Rodríguez-Kabana, R. 1986. Organic and inorganic nitrogen amendments to soil as nematode suppressants. J. Nematol. 18:129-135.
Rodríguez-Kábana, R., Morgan-Jones, G. and Chet, I. 1987. Biological control of nematodes: Soil amendments and microbial antagonists. Plant and Soil 100:237-247.
Suganda, T. 1999. Natural chitinous amendment for controlling root-knot nematode (*Meloidogyne* spp.) of tomato. J. Agrikultura. 10:17-19.
Vavrina, C.S. 1996. An introduction to the production of containerized vegetable transplants. Univ. FL., Cooperative Extension Service, Bulletin No. 302.
Zeck, W.M. 1971. A rating scheme for field evaluation of root-knot nematode infestations. Pflanzenschutz-Nachrichten 24:141-144.
Zehnder, G.W., Yao, C., Murphy, J.F., Sikora, E.J. and Kloepper, J.W. 2000. Induction of resistance in tomato against cucumber mosaic virus by plant growth-promoting rhizobacteria. Biological Control 45:127-137.

Tables

Table 1a. Suppression of root-knot nematode severity in tomato cv. Solar Set in a field trial in Sanford, FL.

Treatment	Number of healthy plants[a]	% Dead plants[b]	Number of plants with severe symptoms[c]	Root-knot index[d]
Nontreated control	7.0	22	4.2	8.0
Chitosan + GB03	6.8	25	2.4*	7.1
Chitosan + GB03 + IN937b	7.4	18	2.6*	7.2
Chitosan + GB03 + IN937a	7.8	13	0.8*	4.4*
Chitosan + IN937a + IN937b	7.0	22	1.0*	4.9*
Methyl bromide control	9.0*	0*	0*	0.6*
LSD (P = 0.05)	**2.0**	**15**	**1.7**	**1.4**

[a] Healthy plants were counted 30 days after planting. Values are means of six replications, each with10 plants.
[b] Mean of six replicated plots.
[c] Presence of large coalescent galls in the entire root system.
[d] Root-knot index was rated on a scale of 0-10, where 0 = no galls and 10 = completely galled.
* Significantly different from nontreated control at P = 0.05.

Table 1b. Suppression of bacterial spot in tomato cv. Solar Set caused by *Xanthomonas axonopodis* pv. *vesicatoria* in a field trial in Sanford, FL.

Treatment	Number of fruit with bacterial spot lesions[a]	Number of bacterial spot lesions per leaflet[b]
Nontreated control	11.3	58.5
Chitosan + GB03	2.8*	19.7*
Chitosan + GB03 + IN937b	4.0*	25.1*
Chitosan + GB03 + IN937a	5.7*	20.2*
Chitosan + IN937a + IN937b	3.7*	22.3*
ManKocide control	4.5*	30.2*
LSD (P = 0.05)	**2.6**	**9.4**

[a] Mean of six replications; 20 fruits per replication.
[b] Mean of six replications; 10 leaflets per replication.
* Significantly different from nontreated control at P = 0.05.

Table 1c and d. Suppression of crown and root rot of tomato cv. Solar Set caused by *Fusarium oxysporum* f. sp. *radicis lycopersici* in field trials in Sanford, FL.

c. Nonfumigated Soil

Treatment	Number of healthy plants[a]	% Dead plants[b]	Number of plants with severe symptoms[c]	FORL index[d]
Nontreated control	10.0	3	8.0	1.3
Chitosan + GB03	9.3	7	4.3*	0.5*
Chitosan + GB03 + IN937b	9.7	3	5.0*	0.6*
Chitosan + GB03 + IN937a	9.0	10	5.3*	0.8
Chitosan + IN937a + IN937b	8.3	17	4.7*	0.9
LSD (P = 0.05)	**1.2**	**12**	**2.3**	**0.7**

d. Fumigated Soil

Treatment	Number of healthy plants[a]	% Dead plants[b]	Number of plants with severe symptoms[c]	FORL index[d]
Nontreated control	7.8	22	7.2	1.5
Chitosan + GB03	7.6	24	5.6	0.9*
Chitosan + GB03 + IN937b	8.0	20	5.2*	0.9*
Chitosan + GB03 + IN937a	7.8	22	6.2	1.0*
Chitosan + IN937a + IN937b	7.4	26	5.6	1.0*
LSD (P = 0.05)	**2.9**	**29**	**2.0**	**0.4**

[a] Healthy plants were counted 30 days after planting. Values are means of six replications, each with10 plants.

[b] Mean of six replications, each with 10 plants.

[c] Symptoms were determined by the presence of internal discoloration of the cortex and vascular tissue at the crown level.

[d] FORL index was rated on a scale of 0-3, where 0 = no symptoms and 3 = severe and extended discoloration.

* Significantly different from nontreated control at P = 0.05.

Table 2. Suppression of root-knot nematode severity on tomato in field trials.

	Root-Knot Index[a]	
Treatment	**Sanford, Florida**	**Cullman, Alabama**
Nontreated control	6.9	5.3
Chitosan control	7.1	4.2*
Chitosan + GB03	6.1*	4.6*
Chitosan + GB03 + IN937b	7.9	3.8*
Chitosan + GB03 + IN937a	6.1*	4.9*
Chitosan + IN937a + IN937b	7.2	4.5*
GB03	6.9	4.6*
IN937a	7.2	3.2*
IN937b	6.4*	4.4*
IN937a + IN937b	7.1	4.3*
Methyl bromide	NI[b]	1.1*
LSD (P = 0.05)	**0.3**	**0.2**

[a] Mean of 60 plants (6 replications, each with 10 plants). Root-knot index was rated on a scale of 1-10, where 1 = no galls and 10 = completely galled.
[b] Not included.
* Significantly less than nontreated control at P = 0.05.

Table 3a and b. Plant growth promotion under greenhouse conditions.

a. Tomato cv. Solar Set

Treatment	**Vigor[a]**	**Height (cm)[b]**	**Shoot fresh weight (g)[c]**	**Number of leaflets/plant[d]**	**Leaf surface area (cm^2)[e]**
Nontreated control	1.5	7.0	0.19	4.2	1.6
Chitosan control	2.3	10.7*	0.25	8.9*	3.9*
Chitosan + GB03	2.8*	10.8*	0.72*	8.9*	4.1*
Chitosan + IN937a	2.8*	11.1*	0.74*	8.9*	4.8*
Chitosan + GB03 + IN937a	4.5*	13.3*	0.91*	9.6*	5.8*
GB03	1.8	7.4	0.23	4.6	1.8
IN937a	2.8*	8.8*	0.36*	5.8	2.7*
GB03 + IN937a	1.5	7.3	0.25	4.7	1.8
LSD (P = 0.05)	**0.9**	**0.7**	**0.07**	**0.7**	**0.6**

b. Cucumber cv. SMR58

Treatment	Vigor[a]	Height (cm)[b]	Shoot fresh weight (g)[c]	Number of leaves/plant[d]	Leaf surface area (cm^2)[e]
Nontreated control	1.8	9.1	1.39	1.9	13.9
Chitosan control	2.3	14.7*	2.23*	2.1	24.6*
Chitosan + GB03	2.8*	12.3*	1.93*	2.1	20.8*
Chitosan + IN937a	2.8*	13.1*	2.12*	2.1	21.3*
Chitosan + GB03 + IN937a	4.5*	16.3*	2.97*	2.8*	27.7*
GB03	1.5	8.8	1.40	1.9	13.6
IN937a	3.5*	12.9*	1.97*	2.0	18.5*
GB03 + IN937a	1.8	9.2	1.42	1.8	13.8
LSD (P = 0.05)	**0.9**	**1.2**	**0.25**	**0.2**	**1.7**

[a] Seedling vigor was rated at 3 weeks after seeding on a scale of 1-5; 1 = poor, 2 = average, 3 = good, 4 = very good, and 5 = excellent. Mean of four replications, 10 plants each.
[b] Seedling height from the soil level to the tip. Mean of four replications, 5 seedlings each.
[c] Seedling shoot fresh weight is the mean of four replications, 5 seedlings per replication.
[d] Mean of four replications, 5 seedlings each.
[e] Largest leaf surface area from the 4th or 5th true leaf. Mean of four replications, 5 plants each.
* Significantly different from nontreated control at P = 0.05.

Table 4. Confirmation of PGPR efficacy of the selected biological system in the greenhouse.

Tomato cv. Solar Set

Treatment	Vigor[a]	Height (cm)[b]	Shoot fresh weight (g)[c]	Number of leaflets/plant[d]	Leaf surface area (cm^2)[e]
Nontreated control	1.0	4.7	0.08	2.3	0.7
Chitosan control	2.8	8.1	0.72	10.4	3.3
Chitosan + GB03 + IN937a	4.8*	10.7*	1.04*	12.3*	4.8*
LSD (P = 0.05)	**0.5**	**0.7**	**0.11**	**0.8**	**0.5**

Cucumber cv. SMR58

Treatment	Vigor[a]	Height (cm)[b]	Shoot fresh weight (g)[c]	Number of leaves/plant[d]	Leaf surface area (cm^2)[e]
Nontreated control	1.0	4.4	0.79	2.0	5.7
Chitosan control	2.5	7.5	1.39	2.6	15.3
Chitosan + GB03 + IN937a	4.8*	11.7*	2.56*	3.4*	30.4*
LSD (P = 0.05)	**0.5**	**0.7**	**0.31**	**0.3**	**2.9**

Pepper cv. California Wonder

Treatment	Vigor[a]	Height (cm)[b]	Shoot fresh weight (g)[c]	Number of leaves/plant[d]	Leaf surface area (cm^2)[e]
Nontreated control	1.0	3.3	0.15	2.0	0.2
Chitosan control	2.3	6.2	0.54	5.5	4.4
Chitosan + GB03 + IN937a	3.3*	7.5*	0.59*	5.9*	5.1*
LSD (P = 0.05)	**0.7**	**0.4**	**0.06**	**0.4**	**0.4**

Tobacco cv. TN90

Treatment	Vigor[a]	Height (cm)[b]	Shoot fresh weight (g)[c]	Number of leaves/plant[d]	Leaf surface area (cm^2)[e]
Nontreated control	2.5	1.1	0.15	2.1.	3.2
Chitosan control	3.8	2.7	0.46	4.5	7.7
Chitosan + GB03 + IN937a	4.8*	3.2*	0.55*	5.1*	9.1*
LSD (P = 0.05)	**0.8**	**0.3**	**0.07**	**0.5**	**0.7**

[a] Rated at 3 weeks after seeding on a scale of 1-5; 1 = poor, 2 = average, 3 = good, 4 = very good, and 5 = excellent. Mean of four replications, 10 plants each.

[b, c, d] Mean of four replications, 5 seedlings per replication.

[e] Largest leaflet surface area from the 4th or 5th true leaf. Mean of four replications, 5 plants each.

* Significantly different from the chitosan control at P = 0.05.

Table 5. Induced systemic resistance against bacterial spot on tomato and angular leaf spot on cucumber cv. SMR48.

Treatment	Number of bacterial spot lesions/leaflet[a]	Number of angular leaf spot lesions/leaf[a]
Nontreated control	18.9	18.5
Chitosan control	17.6	17.9
Chitosan + GB03 + IN937a	7.9*	10.8*
LSD (P = 0.05)	**3.1**	**3.2**

[a] Mean number of lesions per leaflet with four replications, each with 5 plants per replication.

* Significantly different from nontreated control at P = 0.05.