DEVELOPMENT OF AN INTEGRATED BIOLOGICAL PREPARATION FOR GROWTH ENHANCEMENT OF VARIOUS VEGETABLE TRANSPLANT PLUGS SUPPRESSIVE TO PLANT DISEASES

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ABSTRACT

For the past 3 years, we have been developing an application of PGPR which combines several tactics with the goal of protecting tomato against root-knot nematode and fungal pathogens. We report here the initial development of the system, including evidence for synergy among the components of the application. The application, termed here, a "biological preparation" consisted of three components--an organic amendment (chitosan), designed for nematode control and selection of an antagonistic microflora, a PGPR strain previously shown to control seedling diseases by antifungal activity, and a PGPR strain previously shown to induce systemic protection against foliar pathogens. The formulated biological preparation could not be coated onto seeds due to the volume required after combining the three components, so we chose to use a seedling transplant system, in which the three components were incorporated into soil-less mix used to produce tomato transplants. An unexpected effect of the integrated system on tomato was a marked promotion of overall seedling growth. In initial field trials, growth promotion was retained for several weeks after transplanting and treated transplants exhibited some reductions in damage from nematodes, Fusarium crown rot, and bacterial spot. Further, a series of experiments were conducted to test the efficacy of the biological preparation (LS213) for growth promotion and ISR activity against foliar pathogens on the following crops: tomato against bacterial spot (*Xanthomonas axonopodis* pv. vesicatoria) and late blight (*Phytophthora infestans*), cucumber against angular leaf spot (*Pseudomonas syringae* pv. lachrymans), tobacco against blue mold (*Peronospora tabacina*) and pepper seedling growth. Additional treatments consisted of each bacterial strain with and without chitosan, chitosan alone, and a nontreated control. Results showed that the biological preparation significantly increased growth of all the crops across all the measurements when compared with the various individual components of the biological preparation. Also, the highest degree of disease protection against all the pathogens tested occurred with LS213. This indicates a clear synergy in plant growth promotion by the combination of chitosan and the two bacterial strains. Synergy in plant growth promotion translated into synergy in ISR activity.

INTRODUCTION

Induced systemic resistance (ISR) mediated by PGPR has been an area of intensive study in the last decade. Despite promising results in greenhouse and some field trials, practical use of this technology in agriculture has not yet been achieved. It has been suggested that PGPR and

ISR can be used as components in integrated approaches to managing plant health (Kloepper et al. 1999). This study was conceived several years ago in a group effort to accomplish such integration for vegetable transplants. The rationale was to combine PGPR approaches with organic amendments. We previously demonstrated that mixtures of PGPR used in the field enhanced overall performance in reducing incidence of naturally occurring diseases of cucumber (Raupach et al. 1998). In this new study, we planned to combine one strain of PGPR which produced antibiotics with strains that induced systemic resistance. These PGPR mixtures would then by added to an organic amendment. The organic amendment was envisioned to enhance indigenous antagonists to nematodes, and chitosan was selected because of the demonstrable role of chitin as an organic amendment to induce suppressiveness to plant parasitic nematodes (Hallmann et al., 1999). The specific goal of this project was to test the hypothesis that development of such an integrated biological preparation could enhance growth and health of vegetable transplants. We chose to incorporate the components of the biological system into the soil-less media used to grow transplants, rather than to use the traditional approach of seed treatment for applying PGPR.

METHODS

Field trials

Field trials were conducted at an experimental farm in Florida in 1997 with tomato cultivar Solar Set. Experimental treatments included chitosan as the organic amendment with one or more PGPR strains. PGPR used included *Bacillus subtilis* GB03 from Gustafson, LLC, *B. amyloliquefaciens* IN937b, and *B. subtilis* IN937a. The latter two strains were previously shown to induced systemic resistance on several crops, including tomato. The PGPR stains were used in the form of industrially prepared fermented spores, provided by Gustafson, LLC.. The specific treatment list is shown in Tables 1 a – d. The components were mixed into soil-less planting mix (peat-based media) at the time of sowing tomato seeds. Approximately 6 weeks after sowing, plants were transplanted into the field trials without further treatment with PGPR or chitosan.

Four separate field trials were conducted. The first trial was planted in a field with a known history of root-knot nematode, and methyl bromide fumigation was used as a positive control. The second trial was aimed at bacterial spot disease control, and the pathogen *Xanthomonas campestris* pv. vesicatoria was applied to the plants by foliar spray in mid-season. A passing hurricane created conditions favorable for spread of the pathogen and severe disease outbreak. The third trial evaluated control against crown and foot rot disease, using infestation at planting time with the pathogen *Fusarium oxysporum* f. sp. *lycopersici,* in a non-fumigated field; the fourth trial was identical to the third, except that methyl bromide fumigation was used. All field trials were randomized complete block designs with 6 replications of 10 plants per replication. The incidence and severity of disease was measured in each field trial.

Greenhouse tests

During the preparation of transplants for the field trial, plant growth promotion 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. In the first series, treatments included the complete biopreparation (chitosan plus PGPR strains GB03 and IN937a), and all combinations of the bacteria with and without chitin (Tables 2 and 3). As with the field trials, the PGPR and chitin were mixed into soil-less potting media prior to sowing seeds of tomato or cucumber. Two randomized complete block experiments were conducted, on for tomato and one for cucumber, each consisting of 8 treatments, with four replications of five seedlings per replication. Measurements were made at four weeks after planting on vigor, height, shoot fresh weight, number of leaflets per plant, and leaflet surface area.

Based on the results of the first series of greenhouse experiments, confirmatory trials were conducted with the complete biopreparation, also termed LS213, which contained chitosan plus PGPR strains GB03 and IN937a. In each experiment, the LS213 was compared to chitosan alone and to a non-treated control. One experiment evaluated induced systemic resistance on cucumber against angular leaf spot by challenge inoculation of seedlings with a foliar spray of *Pseudomonas syringae* pv. lachrymans. Separate experiments evaluated growth promotion on tomato, cucumber, pepper, and tobacco.

RESULTS

Field trials

Results of the field trials are presented in Tables 1 a – d. In the root-knot nematode trial (Table 1a), all combinations of PGPR strains with chitosan resulted in a significant reduction of the number of severe plants with symptoms, compared to the non-treated control. The root-knot index was significantly reduced, compared to the non-treated control only by treatments containing chitosan and two PGPR strains. In the trial for control of bacterial spot disease (Table 1b), all combinations of chitosan and PGPR significantly reduced both the number of fruits with lesions and the mean number of lesions per leaf, compared to the non-treated control. In this case, the magnitude of disease protection by the biological treatments was statistically equivalent to that of the standard chemical control, ManKocide, which is a combination of a fungicide and a copper compound. Protection was seen against crown and foot rot of tomato, both in fumigated and non-fumigated fields, with some of the combinations of chitosan and PGPR (Tables 1c and 1d). Although the level of disease, as indicated by the FORL index of the non-treated control, was moderately low, two biological treatments in the non-fumigated field and all four biological treatments in the fumigated field significantly reduced the FORL index.

Greenhouse tests

Based on the results of the field trial in which plant growth promotion was observed at the seedling transplant stage, experiments were designed to determine if there was synergy among the components of the biological system in regards to plant growth promotion. With tomato (Table 2), the maximum level of plant growth promotion occurred with the 3-way combination of chitosan + GB03 + IN937a. While chitosan alone significantly enhanced height, shoot fresh weight, number of leaflets per plant and leaflet surface area compared to the non-treated control, each of these parameters was significantly greater than the chitosan treatment with the 3-way combination (LS213). Results with cucumber (Table 3) were similar, with the

maximum plant growth promotion of each measured parameter occurring with the 3-way combination.

The series of greenhouse tests designed to confirm benefits of LS213 (Tables 4 – 5d) confirm that the 3-way system was superior to chitosan alone for inducing systemic disease protection and promoting plant growth. Significant systemic protection against tomato bacterial spot disease and angular leaf spot on cucumber was achieved by treatment with LS213 but not by chitosan alone (Table 4). The plant growth promotion trials with LS213 on tomato, cucumber, pepper, and tobacco (Tables 5a – 5d) indicated that while chitosan alone significantly increased many of the tested parameters of plant growth, compared to the non-treated control, treatment with LS213 resulted in significant growth promotion over the chitosan effect.

DISCUSSION

The results presented here indicate that the plant growth promotion and disease control performance of individual PGPR strains can be enhanced via combination with other PGPR and with chitosan as an amendment to soil-less media used to prepare vegetable transplants. In the particular system developed, the biological preparation called LS213 consisted of chitosan together with spores of one PGPR strain with antibiotic production and one PGPR with ISR activity. Synergy of the components occurred such that the maximum level of plant growth promotion and the consistency of this and biological control was maximum with the full system. Individual components, such as chitosan alone, or PGPR alone, periodically promote significant effects on plant growth or disease control, but not as well as with the 3-way combination.

It is important to note that the benefits seen here are from the one-time application of the biological preparation. Hence, this approach to producing vegetable transplants with accelerated growth and some disease protection should be feasible to integrate into commercial production of transplants. Because the formulation contains fermented spores of the two bacilli PGPR strains, it has a long shelf-life. While the work presented here demonstrates that some disease protection can occur in the field with this one-time application, further work is needed to determine if the level of disease protection can be enhanced by booster treatments during the season and to determine effects on yield in the absence of disease.

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Table 1a. Potential of PGPR and chitosan on suppression of root-knot nematode severity in tomato cv. Solar Set in 1997 field trials

Treatment	Healthy stand[a]	% Dead plants[b]	No. of plants with severe symptoms[c]	Root-knot index[d]
Non-treated control	7.0	22	4.2	8.0
Chitosan + GBO3	6.8	25	2.4*	7.1
Chitosan + GBO3 + IN937b	7.4	18	2.6*	7.2
Chitosan + GBO3 + 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 stand was measured 45 days after planting and is a mean of 6 replicated plots. Each plot was originally planted with 10 plants.
[b]mean of 6 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.

Table 1b. Potential of PGPR and chitosan on suppression of bacterial spot in tomato cv. Solar Set caused by *Xanthomonas campestris* pv. vesicatoria in 1997 field trials

Treatment	No. of fruits with bacterial spot lesions[a]	No. of bacterial spot lesions per leaflet[b]
Non-treated control	11.3	58.5
Chitosan + GBO3	2.8*	19.7*
Chitosan + GBO3 + IN937b	4.0*	25.1*
Chitosan + GBO3 + 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 replications.
[b]Mean of 6 replications, 10 leaflets per replication.
*Significantly different from non-treated control at $P = 0.05$.

Table 1c. Potential of PGPR and chitosan on suppression of crown and foot rot of tomato cv. Solar Set caused by *Fusarium oxysporum* f. sp. *radicis lycopersici* in non-fumigated soil during 1997 field trials

Treatment	Healthy stand[a]	% Dead plants[b]	No. of plants with severe symptoms[c]	FORL index[d]
Non-treated control	10.0	3	8.0	1.3
Chitosan + GBO3	9.3	7	4.3*	0.5*
Chitosan + GBO3 + IN937b	9.7	3	5.0*	0.6*
Chitosan + GBO3 + 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

[a]Healthy stand was measured 45 days after planting and is a mean of 6 replicated plots. Each plot was originally planted with 10 plants.
[b]Mean of 6 replicated plots.
[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.

Table 1d. Potential of PGPR and chitosan on suppression of crown and foot rot of tomato cv. Solar Set caused by *Fusarium oxysporum* f. sp. *radicis lycopersici* in fumigated soil during 1997 field trials

Treatment	Healthy stand[a]	% Dead plants[b]	No. of plants with severe symptoms[c]	FORL index[d]
Non-treated control	7.8	22	7.2	1.5
Chitosan + GBO3	7.6	24	5.6	0.9*
Chitosan + GBO3 + IN937b	8.0	20	5.2*	0.9*
Chitosan + GBO3 + 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 stand was measured 45 days after planting and is a mean of 6 replicated plots. Each plot was originally planted with 10 plants.
[b]Mean of 6 replicated plots.
[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.
* Indicates significant difference from non-treated control at P = 0.05.

Table 2. Potential of PGPR and chitosan on growth promotion of tomato cv. Solar Set under greenhouse conditions

Treatment	Vigor[a]	Height (cm)[b]	Shoot fresh weight (g)[c]	Number of leaflets/plant[d]	Leaflet surface area (cm2)[e]
Non-treated control	1.5	7.0	0.19	4.2	1.6
Chitosan	2.3	10.7*	0.71*	8.9*	3.9*
Chitosan + GBO3	2.8*	10.8*	0.72*	8.9*	4.1*
Chitosan + IN937a	2.8*	11.1*	0.74*	8.9*	4.8*
Chitosan + GBO3 + IN937a	4.5*	13.3*	0.91*	9.6*	5.8*
GBO3	1.8	7.4	0.23	4.6	1.8
IN937a	2.8*	8.8*	0.36*	5.8	2.7*
GBO3 + 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

[a]Seedling vigor was rated at three 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.
[b]Seedling height from the soil level to the tip. Mean of four replications, 5 seedlings per replication.
[c]Seedling shoot fresh weight is the mean of four replications, 5 seedlings per replication.
[d]Mean of 4 replications, 5 seedlings per replication.
[e]Largest leaflet surface area from the fourth or 5th true leaf. Mean of four replications, 5 plants per replication.
*Significantly different from nontreated control at $P = 0.05$.

Table 3. Potential of PGPR and chitosan on growth promotion of cucumber cv. SMR48 under greenhouse conditions

Treatment	Vigor[a]	Height (cm)[b]	Shoot fresh weight (g)[c]	Number of leafs/plant[d]	Leaflet surface area (cm2)[e]
Non-treated control	1.8	9.1	1.39	1.9	13.9
Chitosan	2.3	14.7*	2.23*	2.1	24.6*
Chitosan + GBO3	2.8*	12.3*	1.93*	2.1	20.8*
Chitosan + IN937a	2.8*	13.1*	2.12*	2.1	21.3*
Chitosan + GBO3 + IN937a [f]	4.5*	16.3*	2.97*	2.8*	27.7*
GBO3	1.5	8.8	1.40	1.9	13.6
IN937a	3.5*	12.9*	1.97*	2.0	18.5*
GBO3 + 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 three 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.
[b]Seedling height from the soil level to the tip. Mean of four replications, 5 seedlings per replication.
[c]Seedling shoot fresh weight is the mean of four replications, 5 seedlings per replication.
[d]Mean of 4 replications, 5 seedlings per replication.
[e]Largest leaf surface area from the fourth or 5th true leaf. Mean of four replications, 5 plants per replication.
[f]This treatment is the same as LS213.
*Significantly different from nontreated control at $P = 0.05$.

Table 4. PGPR and chitosan-mediated induced systemic resistance on tomato cv. Solar Set against bacterial spot caused by *Xanthomonas axonopodis* pv. vesicatoria and on cucumber cv. SMR48 against angular leaf spot caused by *Pseudomonas syringae* pv. lachrymans

Treatment	No. of bacterial spot lesions/leaflet[a]	No. of angular leaf spot lesions/leaf[b]
Non-treated control	18.9	18.5
Chitosan	17.6	17.9
Chitosan + GBO3 + IN937a (LS213)	7.9*	10.8*
LSD (P = 0.05)	3.1	3.2

[a]Values represent the mean number of lesions per leaflet of each seedling from four replications, 5 plants per replication.
[b]Values represent the mean number of lesions per leaf from four replications, 5 plants per replication.
*Significantly different from nontreated control at $P = 0.05$.

Table 5a. Confirmation of growth promotion with LS213 on tomato cv. Solar Set under greenhouse conditions

Treatment	Vigor[a]	Height (cm)[b]	Shoot fresh weight (g)[c]	Number of leaflets/plant[d]	Leaflet surface area (cm2)[e]
Non-treated control	1.0	4.7	0.08	2.3	0.7
Chitosan	2.8*	9.1*	0.72*	10.4*	3.3*
LS213	4.8*	11.7*	1.04*	12.3*	4.8*
LSD (P = 0.05)	0.5	0.7	0.11	0.8	0.5

[a]Seedling vigor was rated at three 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.
[b]Seedling height from the soil level to the tip. Mean of four replications, 5 seedlings per replication.
[c]Seedling shoot fresh weight is the mean of four replications, 5 seedlings per replication.
[d]Mean of 4 replications, 5 seedlings per replication.
[e]Largest leaflet surface area from the fourth or 5th true leaf. Mean of four replications, 5 plants per replication.
*Significantly different from nontreated control at $P = 0.05$.

Table 5b. Confirmation of growth promotion by LS213 on cucumber cv. SMR48 under greenhouse conditions

Treatment	Vigor[a]	Height (cm)[b]	Shoot fresh weight (g)[c]	Number of leafs/plant[d]	Leaflet surface area (cm2)[e]
Non-treated control	1.0	4.4	0.79	2.0	5.7
Chitosan	2.5*	7.5*	1.39*	2.6*	15.3*
LS213	4.8*	11.7*	2.56*	3.4*	30.4*
LSD (P = 0.05)	0.5	0.7	0.31	0.3	2.9

[a]Seedling vigor was rated at three 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.
[b]Seedling height from the soil level to the tip. Mean of four replications, 5 seedlings per replication.
[c]Seedling shoot fresh weight is the mean of four replications, 5 seedlings per replication.
[d]Mean of 4 replications, 5 seedlings per replication.
[e]Largest leaf surface area from the fourth or 5th true leaf. Mean of four replications, 5 plants per replication.
*Significantly different from nontreated control at $P = 0.05$.

Table 5c. Confirmation of growth promotion by LS213 on pepper cv. California Wonder under greenhouse conditions

Treatment	Vigor[a]	Height (cm)[b]	Shoot fresh weight (g)[c]	Number of leafs/plant[d]	Leaflet surface area (cm2)[e]
Non-treated control	1.0	3.3	0.15	2.0	0.2
Chitosan	2.3*	6.2*	0.54*	5.7*	4.4*
LS213	3.3*	7.5*	0.59*	5.9*	5.1*
LSD (P = 0.05)	0.7	0.4	0.06	0.4	0.4

[a]Seedling vigor was rated at three 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.
[b]Seedling height from the soil level to the tip. Mean of four replications, 5 seedlings per replication.
[c]Seedling shoot fresh weight is the mean of four replications, 5 seedlings per replication.
[d]Mean of 4 replications, 5 seedlings per replication.
[e]Largest leaf surface area from the fourth or 5th true leaf. Mean of four replications, 5 plants per replication.
*Significantly different from nontreated control at $P = 0.05$.

Table 5d. Confirmation of growth promotion by LS213 on tobacco cv. TN90 under greenhouse conditions

Treatment	Vigor[a]	Height (cm)[b]	Shoot fresh weight (g)[c]	Number of leafs/plant[d]	Leaflet surface area (cm2)[e]
Non-treated control	2.5	1.1	0.15	2.1.	3.2
Chitosan	3.8*	2.7*	0.46*	4.5*	7.7*
Chitosan + GBO3 + 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]Seedling vigor was rated at three 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.
[b]Seedling height from the soil level to the tip. Mean of four replications, 5 seedlings per replication.
[c]Seedling shoot fresh weight is the mean of four replications, 5 seedlings per replication.
[d]Mean of 4 replications, 5 seedlings per replication.
[e]Largest leaf surface area from the fourth or 5th true leaf. Mean of four replications, 5 plants per replication.
*Significantly different from nontreated control at $P = 0.05$.