# Suppressiveness of root-knot nematodes mediated by rhizobacteria

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## ARTICLE INFO

## ABSTRACT

Plant growth-promoting rhizobacteria (PGPR) are beneficial bacteria that colonize the rhizosphere and plant roots resulting in enhancement of plant growth or protection against certain plant pathogens. Studies were conducted to test the hypothesis that induction of soil suppressiveness against *Meloidogyne incognita* using rhizobacterial inoculants is related to soil microbial activity and rhizosphere bacterial populations. Commercially-available rhizobacterial inoculants (Equity®, BioYield®, and AgBlend®) and FZB42, strain in the product RhizoVital®, were selected based on elicitation of growth promotion in tomato and pepper in previous tests. The inoculants Equity (multiple strains), BioYield (two strains), and FZB42 induced significant reductions in nematode eggs per gram root, juvenile nematodes per ml of soil, and galls per plant on tomato. AgBlend, containing microbial metabolites, reduced number of galls. Treatment with each of the inoculants also increased root weight. Rhizosphere populations of total bacteria and aerobic endospore-forming bacteria (AEFB) were increased following treatment with AgBlend, BioYield and FZB42. Strain FZB42 had an unique colony morphology, allowing its detection in the rhizosphere where it became the dominant strain. Soil microbial activity, as assessed by fluorescein diacetate hydrolysis, was not affected by inoculants. These results indicate that the selected microbial inoculants increase rhizosphere bacterial populations, and in the case of FZB42, actively colonize the rhizosphere, thereby inducing suppressiveness to nematodes, without necessarily enhancing soil microbial activity. Further, induction of soil suppressiveness against *M. incognita* was related to bacterial population size in the rhizosphere, when inoculants that contained two PGPR strains and also microbial metabolites were used.

## 1. Introduction

Due to environmental concerns and increased regulations on use of chemical fumigants, more management strategies for control of root-knot (*Meloidogyne* spp.) nematodes are currently being investigated (Nico et al., 2004). Biological control using microbial antagonists is one potential alternative to chemical nematicides. Among the biological control agents that have been assessed are egg-parasitic fungi, nematode-trapping fungi, bacteria, and polyphagous predatory nematodes (Gray, 1988; Kerry, 1988; Kerry and Hidalgo-Diaz, 2004; Kiewnick and Sikora, 2005). These antagonists can limit nematode abundance. *Paecilomyces lilacinus* (Thom) Samson, for example, is a fungus that significantly reduces soil populations of *Meloidogyne incognita* (Kofoid and White) Chitwood and increases tomato yield (*Lycopersicon esculentum* Mill) (Lara et al., 1996). *Pseudomonas aeruginosa* (Schroeter) Migula and *Bacillus subtilis* (Ehrenberg) Conn are bacteria that suppress root-knot nematode infection and nematode population densities under greenhouse and field conditions in mungbean (*Vigna radiata* (L.) R. Wilczek) (Siddiqui et al., 2001). Antagonists are also involved in soil suppressiveness, whereby a specific group of soil microorganisms antagonizes plant pathogens leading to suppression of disease (Weller et al., 2002). Given that antagonists are the underlying bases of soil suppressiveness, studies have been done to identify and characterize root-knot nematode suppressive soils (Fernandez et al., 2001; Pyrowolakis et al., 2002).

A number of studies have reported on the role of ecosystem health in managing plant diseases (e.g., Kokalis-Burelle and Kloepper, 2004). Specific cultural practices, such as crop rotation, cover cropping, and addition of organic amendments, have resulted in enhanced plant health at certain environments (Schippers et al., 1987; Weller et al., 2002). These cultural practices result in changes to the soil microbial community which facilitate development of disease suppressiveness (Bending et al., 2002; Kloepper et al., 1991; Larkin et al., 1993; Mazzola, 2002). Consequently, understanding soil microbial community interactions is fundamental for developing practices to manage plant diseases. The knowledge that

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agricultural production depends on complex biological equilibria in soil will ultimately aid in modifying agro-ecosystems and obtaining more favorable conditions for plant growth and health. One practical challenge to implementing this approach is establishing beneficial microbial communities, such as plant growth-promoting rhizobacteria (PGPR), to promote soil ecosystem health that contribute to suppression of plant pathogens and other pests. PGPR-based inoculants include formulations containing a single-strain, a mixture of two strains, or complex mixtures of over 10 strains of *Bacillus* spp. (Kloepper and Ryu, 2006; Lucy et al., 2004). PGPR have shown positive effects in plants on such parameters as germination rate, tolerance to drought, weight of shoots and roots, yield, and plant growth under salt stress (Kloepper et al., 2004; Kokalis-Burelle et al., 2006; Yildirim et al., 2006). Another major benefit of PGPR is their use as biological control agents for plant disease-causing organisms (Ji et al., 2006; Zehnder et al., 2001).

Although the uses and benefits of PGPR-based inoculants are becoming better understood, little is known about the mechanisms by which PGPR induce suppressiveness to plant pathogens, specifically root-knot nematode. For example: Do PGPR suppress soil pathogens via root colonization or through increasing soil microbial activity? Do inoculants that contain multiple strains of PGPR increase soil microbial activity or affect nematode suppression more than inoculants containing one or two strains?

Sustainable agricultural practices typically function through the activity of soil microorganisms, and obtaining beneficial communities that promote soil health can contribute to reducing root-knot nematode damage. We conducted greenhouse experiments to determine if PGPR-based inoculants can be used to induce suppressiveness to soil-borne pathogens, i.e., nematodes, by enhancing soil microbial activity and by sustaining stable populations of PGPR in the rhizosphere. In addition, we tested whether or not soil microbial activity would be increased more by inoculants containing multiple strains of PGPR than by inoculants containing two strains.

## 2. Materials and methods

### 2.1. Plant material and nematode inoculum

Tomato transplants were produced in the greenhouse at the Plant Science Research Center, Auburn University, Alabama. Seeds of tomato hybrid 'Juliet' were planted into 32-cell polystyrene trays containing soil-less medium (Sunshine mix, Sun Gro Horticulture, Vancouver, British Columbia Canada) and grown for 3 weeks using overhead irrigation. Transplants were fertilized twice per week with 15–30–15 soluble fertilizer. Three-week-old tomato seedlings were used for all trials. Tomato seedlings were placed in 22 cm diameter (4500 ml) pots previously filled with autoclaved soil. The soil used in this study was a sandy loam, with a pH of 5.67, 0.9% organic matter content, 19.9 ppm of P, 86.7 ppm of K, 0.057% N, and 0.5% C (Auburn University Soil Testing Laboratory). Given the experimental aim of measuring changes in microbial activity due to application of rhizobacterial inoculants, soil used in this study was autoclaved twice to reduce any contribution from indigenous microorganisms. Inoculum of *M. incognita* race three was prepared by extracting nematode eggs from tomato roots following the method of Hussey and Barker (1973). A total of 25,000 eggs in 5 ml of water were applied to each pot as a drench after transplanting.

### 2.2. Rhizobacterial inoculants

Three commercially available PGPR-based inoculants (Equity®, BioYield®, and AgBlend®) and PGPR strain FZB42, which is formu-

lated in the product RhizoVital®, were tested. Equity (Naturize Biosciences LLC, Jacksonville, FL USA) contains 47 strains of bacilli in a liquid formulation. BioYield (Gustafson LLC, Plano TX, USA) contains *B. subtilis* strain GB03 and *Bacillus amyloliquefaciens* (ex Fukumoto) Priest et al. strain GB99 in a chitosan carrier. Ag-Blend (Advanced Microbial Solutions LLC, Pilot Point, TX USA) contains rhizobacteria and microbial metabolites produced during anaerobic fermentation of a microbial community. FZB42 (AbiTep GmbH, Berlin, Germany) is a strain of *B. amyloliquefaciens* that has been reported to enhance plant growth and suppress plant pathogenic organisms in the rhizosphere (Koumoutsi et al., 2004). Therefore, FZB42 and BioYield represented inoculants containing one and two strains, respectively; Equity represented inoculants containing multiple strains, and AgBlend represented inoculants containing primarily microbial metabolites. Stock suspensions of the inoculants were made by preparing a label-specified dose of each inoculant in water. Strain FZB42 was grown in flasks containing 50 ml of tryptic soy broth. After 48 h of incubation, flasks were centrifuged, and the supernatant was discarded. The pellet was re-suspended in distilled water to yield a concentration of $10^7$ colony forming units (cfu)/m. Each pot was inoculated with PGPR the day after transplanting of tomato seedlings by drenching soil with 100 ml of the stock suspension containing PGPR. This rate is equivalent to field application rate as labeled. Inoculants were selected based on results obtained in previous greenhouse experiments indicating positive effects on tomato growth (Cadena-Cepeda et al., 2006).

### 2.3. Induced suppressiveness to M. incognita in tomato rhizosphere soil by PGPR

Two experiments were conducted, each repeated twice, utilizing a completely randomized design (CRD) with four treatments and eight replicates per treatment per trial resulting in a total of 16 replicates per experiment. For the first experiment, four treatments were used: Equity, BioYield, AgBlend, and a water control. For the second experiment AgBlend, which contains mainly microbial metabolites, was replaced by FZB42, a single-strain of *B. amyloliquefaciens*. During the first study, collaboration with Humboldt University, (Berlin, Germany) was established to evaluate FZB42 as a PGPR inoculant. This gave us the opportunity to use a single-strain inoculum, which we deemed pertinent to the scope of the study.

Both experiments were maintained at 27 °C during the day and 24 °C during the night under natural light from May through August and under natural and artificial light (1000 W high pressure sodium lamps) from October through April. Studies were terminated 45 days after transplanting. At that time, root systems were rated for nematode-induced galling on a scale of 0–6 as follows: 0 = no galls, 1 = 1–10%, 2 = 11–25%, 3 = 26–50%, 4 = 51–75%, 5 = 69–90%, and 6 = 91–100% roots with galls. Eggs were recovered from excised roots by agitated extraction in a 10% sodium hypochlorite solution (Jenkins, 1964). The total number of eggs was counted under a dissecting microscope (4×) and expressed as number of eggs per gram of root. The extraction of juveniles from the soil was completed using Jenkins's method (Jenkins, 1964). Juveniles were then counted under the dissecting microscope, and data were expressed as juveniles per ml of soil.

In a second set of experiments, we investigated the effect of rhizobacterial inoculants on nematode population development in non-autoclaved field soil to test the hypothesis that similar reduction in *M. incognita* eggs and juveniles could be obtained in a more natural competition system. A completely randomized design with 2 × 3 factorial arrangement was used. Factors were rhizobial inoculants (BioYield and FZB4, plus a water control) and soil type (autoclaved and non-autoclaved). Each treatment × soil steriliza-

tion combination had eight replicates and the experiment was conducted once. Preparation of stock suspension of inoculants, application of inoculants, harvest, and nematode population assessment were done as previously described.

### 2.4. Soil microbial activity and rhizosphere bacterial population determinations

At the conclusion of each trial, tomato roots were shaken vigorously, and 10 g of rhizosphere soil in close association with roots were collected to determine bacterial population density and microbial activity. Total microbial activity was assessed by measuring fluorescein diacetate hydrolysis (FDA), using the procedure described by Schnurer and Rosswall (1982). Direct plate counts were used to quantify total culturable bacteria and aerobic endospore-forming bacteria (AEFB). For the counts, 1 g of rhizosphere soil was added to 50 ml sterile water in a 125 ml Erlenmeyer flask and shaken at 150 rpm for 20 min. Serial dilutions were made, and 50 μl of each concentration were plated onto 50% Tryptic Soy Agar (TSA, Difco, New York, NY, USA) for total bacteria ($10^{-1}$, $10^{-2}$, and $10^{-3}$ dilutions). The medium 50% TSA was prepared by dissolving 15 g of Tryptic Soy Broth (half of standard dose) and 18 g of agar in 1 l of sterile water. To quantify AEFB after serial dilution, tubes were placed into a water bath at 80 °C for 13 min, and the $10^{-1}$ and $10^{-2}$ dilutions were plated onto 50% TSA. All plates were incubated for 48 h at 28 °C. Numbers of colonies were counted, and population size was expressed as $\log_e$ (cfu)/g of soil for all treatments, a consequence of the log-normal modeling of response.

### 2.5. Statistical analysis

Response data from experiments using doubly autoclaved soils were analyzed jointly with treatments as the sole fixed effects; effects for experiment and repetition within experiment were subsumed in the residual error term. Response data from the experiment using soil (autoclaved vs. non-autoclaved) as a second factor were analyzed as a factorial. Since the experimental design was a CRD, the residual term is the pooled residual within treatment × soil combination variation. We first evaluated distributional assumption using the student panel graphs available in SAS® Proc GLIMMIX. The normality assumption was warranted only for gall rate and root mass. All other response variables followed a log-normal distribution. We then modeled the variance structure of the residual term (R-side modeling in SAS parlance) using the group option to create homogeneous variance groups. A good fit was indicated when the ratio of the Generalized Chi-Square divided by the residual degree of freedom approached unity while minimizing the number of variance groups. Finally, Dunnett's option was used to assess the differences between treatments and the untreated control. Actual adjusted P-values rather than significance classes are presented in the tables.

## 3. Results

### 3.1. Induced suppressiveness to M. incognita in tomato rhizosphere soil by PGPR

In the combined analysis of experiments 1 and 2, the treatment effect was highly significant for all response variables ($P < 0.001$; data not shown). Treatment of tomato with AgBlend ($P = 0.004$), BioYield ($P < 0.001$), Equity ($P = 0.001$), and FZB42 ($P < 0.001$) suppressed galling compared to the untreated control. Eggs per gram of root were reduced by treatment with BioYield ($P = 0.039$) and FZB42 ($P = 0.052$), while numbers of juveniles extracted from roots were reduced by BioYield ($P = 0.027$), Equity ($P = 0.015$), and FZB42 ($P = 0.007$). Treatment with AgBlend, BioYield, and Equity resulted in increases in tomato root mass ($P \leqslant 0.077$; Table 1).

Results of the factorial experiment showed that autoclaving the field soil increased number of eggs/gram of root, number of juveniles, and gall rating compared with the non-autoclaved soil (data not shown). The analysis also indicated a main effect of treatments with rhizobial inoculants on eggs/gram of root and gall rating (Table 2). Analysis of the factorial design revealed a positive interaction among soil type and treatments for the variable gall rating. Gall rating was higher for treatments with FZB42, BioYield, and the untreated control in the autoclaved field soil compared to the non-autoclaved (data not shown). The effect of treatments on nematode population indicated that application of FZB42 resulted in a significant reduction in number of M. incognita eggs/g of root ($P = 0.009$) and application of BioYield resulted in suppression of galling ($P < 0.0001$). Additionally, a significant increase in root mass ($P \leqslant 0.08$) was observed for both rhizobial inoculants (Table 3).

### 3.2. Soil microbial activity and rhizosphere bacterial population

Applications of PGPR-based inoculants had significant effects on rhizosphere bacterial population density measured by direct plate counts. Populations of total bacteria and aerobic endospore-forming bacteria (AEFB) were greater in rhizosphere soil treated with AgBlend ($P = 0.015$) and BioYield ($P < 0.0001$) than in the untreated control or in other treatment (Table 4). Similarly, treatment with FZB42 ($P < 0.0001$) increased total bacteria plate counts in the tomato rhizosphere soil compared with the untreated rhizosphere

**Table 2**
P-values from the analysis of variance for individual treatments and interactions of treatments and soil types on gall formation, egg mass and juvenile counts of *Meloidogyne incognita* extracted from roots of tomatoes grown in autoclaved and non-autoclaved soils in the greenhouse

| Source | Gall rating | Eggs mass | Juvenile count | Root mass |
|---|---|---|---|---|
| PGPR treatment | <0.0001 | 0.01 | 0.88 | 0.01 |
| Soil type | <0.0001 | <0.0001 | 0.06 | 0.002 |
| PGPR treat * Soil type | 0.07 | 0.15 | 0.56 | 0.57 |

Treatments include PGPR-base inoculants.

**Table 1**
Least squares means, standard errors (SE), t-calculated (t-cal), and the probability of the difference to the untreated control (Dunnett's test) for PGPR-base inoculants on gall formation, egg mass and juveniles of *Meloidogyne incognita* extracted from roots of tomatoes grown in autoclaved soil in the greenhouse

| Treatment | Gall rating | | | | $\log_e$ (Egg mass), g g$^{-1}$ roots | | | | $\log_e$ (Juvenile count), N ml$^{-1}$ | | | | Root mass, g | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean[a] | SE | t-cal | Dunnett's P | Mean[a] | SE | t-cal | Dunnett's P | Mean[a] | SE | t-cal | Dunnett's P | Mean[a] | SE | t-cal | Dunnett's P |
| Control | 4.2 | 0.16 | | | 8.11 | 0.18 | | | 7.08 | 0.15 | | | 15.08 | 1.29 | | |
| AgBlend | 3.3 | 0.22 | 3.2 | 0.004 | 8.15 | 0.53 | 0.1 | 1.000 | 7.39 | 0.23 | 1.1 | 0.646 | 27.18 | 1.90 | 5.3 | <0.001 |
| BioYield | 2.1 | 0.22 | 9.3 | <0.001 | 7.43 | 0.18 | 2.6 | 0.039 | 6.48 | 0.16 | 2.8 | 0.027 | 18.18 | 0.42 | 2.3 | 0.077 |
| Equity | 3.3 | 0.15 | 3.6 | 0.001 | 8.32 | 0.14 | 1.0 | 0.797 | 6.44 | 0.16 | 3.0 | 0.015 | 22.64 | 1.29 | 4.2 | <0.001 |
| FZB42 | 2.7 | 0.21 | 5.4 | <0.001 | 7.07 | 0.37 | 2.5 | 0.052 | 6.23 | 0.22 | 3.2 | 0.007 | 16.77 | 1.34 | 0.9 | 0.76 |

[a] Data from experiments 1 and 2 using doubly autoclaved soils were analyzed jointly. Degrees of freedom for error (df$_e$) = 91.

**Table 3**
Least squares means, standard errors (SE), degrees of freedom for error ($df_e$), *t*-calculated (*t*-cal), and the probability of the difference to the untreated control (Dunnett's test) for PGPR-base inoculants on gall formation, egg mass and juvenile counts of *Meloidogyne incognita* extracted from roots of tomatoes grown in autoclaved and non-autoclaved soils in the greenhouse

| Treatment | Gall rating | | | | | $\text{Log}_e$ (Egg mass), g g$^{-1}$ roots | | | | | $\text{Log}_e$ (Juvenile count), N ml$^{-1}$ | | | | | Root mass, g | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean[a] | SE | $df_e$ | *t*-cal | Dunnett's *P* | Mean | SE | $df_e$ | *t*-cal | Dunnett's *P* | Mean | SE | $df_e$ | *t*-cal | Dunnett's *P* | Mean | SE | $df_e$ | *t*-cal | Dunnett's *P* |
| Control | 4.2 | 0.19 | | | | 7.63 | 0.17 | | | | 7.27 | 0.44 | | | | 13.32 | 0.44 | | | |
| BioYield | 3.1 | 0.15 | 42 | 4.9 | <0.0001 | 7.07 | 0.25 | 21 | 1.8 | 0.138 | 7.05 | 0.44 | 21 | 0.8 | 0.412 | 14.68 | 0.44 | 36 | 2.2 | 0.079 |
| FZB42 | 3.7 | 0.15 | 42 | 1.9 | 0.128 | 6.95 | 0.14 | 33 | 3.1 | 0.009 | 7.35 | 0.44 | 33 | 0.8 | 0.351 | 16.60 | 0.95 | 13 | 3.1 | 0.010 |

[a] Response data from the experiment using soil (autoclaved vs. non-autoclaved) as a second factor analyzed as a factorial. Interaction effects were non-significant ($P > 0.07$).

soil. Despite these changes in populations, treatment with Ag-Blend, BioYield, or Equity showed no increases in microbial activity in soil as measured by FDA hydrolysis ($P = 0.69$; Table 5). Due to this lack of detection of changes in the microbial activity in the rhizosphere, FDA hydrolysis was not measured in subsequent experiments.

## 4. Discussion

Our results indicate that suppression of nematodes and nematode damage was induced by various PGPR-based formulations, including ones with a single PGPR strain (FZB42), two strains (Bio-Yield), complex bacterial mixtures (Equity), and microbial metabolites formed during batch fermentation (AgBlend). These inoculants appear to suppress root-knot nematode via different mechanisms. AgBlend, for example, reduced galling without significantly reducing numbers of juveniles/ml or eggs per gram of root (Table 1), indicating that the effects were predominantly exerted on the plant. In contrast, numbers of juveniles were reduced by treatment with Equity, BioYield, and FZB42, indicating that antagonistic interactions also occurred in the rhizosphere. Such antagonism might relate to antibiotic production. It has been reported that *B. amyloliquefaciens* strain FZB42 produces lipopeptides, surfactins, bacillomycin D, and fengycins, which are secondary metabolites with mainly antifungal activity (Chen et al., 2006). Antibiotic production by FZB42 might also explain both its colonization pattern and the finding that total bacterial carrying capacity of the rhizosphere was increased.

In addition to suppressing nematode damage, treatments with the tested inoculants increased tomato root weight, which could also account for some of the observed suppression. Plants with larger root systems have been reported to tolerate a given population of nematodes (Gierth et al., 2004; Kokalis-Burelle et al., 2006).

The suppression of root-knot nematode by PGPR inoculants, as found in our study, agrees with previous reports with BioYield in greenhouse and field trials (Kloepper et al., 1991; Kokalis-Burelle et al., 2002). BioYield, a product that contains spores of *B. subtilis* strain GB03, and *B. amyloliquefaciens* strain GB99 on a chitosan carrier, has been shown to induce growth promotion in tomato seedlings and reduce severity of diseases cause by

**Table 5**
Least squares means, standard errors (SE) of microbial activity measured by fluorescein diacetate hydrolysis (FDA) of tomatoes grown in autoclaved soil in the greenhouse

| Treatment | Fluorescein diacetate hydrolysis (µg fluorescein per gram of oven dry soil × h) | |
|---|---|---|
| | Mean | SE |
| Control | 1.62 | 0.248 |
| AgBlend | 1.71 | 0.088 |
| BioYield | 1.97 | 0.221 |
| Equity | 1.82 | 0.221 |

Die *F*-test for treatments had a $P = 0.69$.

several pathogens (Kloepper and Ryu, 2006; Kloepper et al., 2004). Reduction of soil-borne pathogens was related to antibiotic production by GB03, while promotion of indigenous soil predators and antagonists to root-knot nematodes was attributed to chitosan, and elicitation of induced systemic resistance was attributed to *B. amyloliquefaciens* (Kloepper et al., 2004). Additionally, application of BioYield significantly reduced galling by root-knot nematodes in tomato crops and resulted in increased yield (Kokalis-Burelle and Dickson, 2003). The current study extends the previous findings on BioYield by demonstrating that similar suppression of damage to nematodes may result by rhizobial inoculants formulated with different strategies.

The finding that treatment with BioYield and AgBlend resulted in higher AEFB populations in the rhizosphere was unexpected and likely resulted from either stimulation of indigenous AEFB or rhizosphere colonization by strains in the inoculants. However, additional colonization studies should be done to determine if the AEFB isolated from the rhizosphere match the PGPR strains applied. Nevertheless, the finding of increased AEFB populations in the rhizosphere agrees with a previous field study where BioYield was applied to the potting media at pepper seeding. The AEFB contained in this inoculant established stable populations in the rhizosphere of pepper that persisted throughout the growing season in the field (Kokalis-Burelle et al., 2006). The finding that AEFB also increased with AgBlend treatment is noteworthy because, although this product contains some *Bacillus* spp., the total mixed bacterial population is only 1000 cfu/ml. This population is consid-

**Table 4**
Least squares means, standard errors (SE), *t*-calculated (*t*-cal), and the probability of the difference to the untreated control (Dunnett's test) for PGPR-base inoculants on total bacterial and aerobic endospore-forming bacteria (AEFB) of tomatoes grown in autoclaved soil in the greenhouse

| Treatment | $\text{Log}_e$ (Total bacteria cfu), g$^{-1}$soil | | | | $\text{Log}_e$ (AEFB cfu), g$^{-1}$soil | | | |
|---|---|---|---|---|---|---|---|---|
| | Mean[a] | SE | *t*-cal | Dunnett's *P* | Mean[a] | SE | *t*-cal | Dunnett's *P* |
| Control | 16.29 | 0.059 | | | 14.08 | 0.13 | | |
| AgBlend | 16.95 | 0.281 | 2.3 | 0.091 | 14.62 | 0.12 | 3.0 | 0.015 |
| BioYield | 17.71 | 0.099 | 12.3 | <0.0001 | 15.88 | 0.08 | 11.7 | <0.0001 |
| Equity | 16.06 | 0.094 | 2.1 | 0.16 | 14.46 | 0.13 | 2.1 | 0.13 |
| FZB42 | 17.07 | 0.097 | 6.9 | <0.0001 | 14.55 | 0.23 | 1.8 | 0.22 |

[a] Data from experiments 1 and 2 using doubly autoclaved soils were analyzed jointly. Degrees of freedom for error ($df_e$) = 67.

erably lower than traditional microbial products. AgBlend, however, also contains microbial metabolites produced during anaerobic fermentation of a multi-trophic microbial community. Considering the relatively low population of microbes in the product, we surmise that the primary effect of AgBlend on rhizosphere bacteria occurs via activities of the microbial metabolites. Whether the metabolites are having a direct effect on rhizosphere bacteria or an indirect effect through stimulation of root growth needs to be evaluated in future studies.

Although the observed nematode suppression was generally related to bacterial populations in the rhizosphere, suppression was not related to microbial activity. Surprisingly, the inoculants did not cause measurable increases in microbial activity as estimated by FDA hydrolysis. While it is possible that the FDA was not sensitive enough to detect enhanced microbial activity, we think this is unlikely because this method is widely used in soil microbial ecology, and it is recommended by the USDA for use in commercial soil testing laboratories to determine soil microbial activity (Green et al., 2006).

In summary, treatment with several representative formulations of microbial inoculants induced soil suppressiveness against *M. incognita* in both autoclaved and non-autoclaved field soil and enhanced tomato growth. Additionally, increases in bacterial population density in the rhizosphere were detected by direct plate count for inoculants containing one and two bacterial strains and containing microbial metabolites, and there was no correlation between microbial activity and population density in the rhizosphere. Hence, microbial inoculants can be used as components in integrated approaches to managing diseases and changing microbial population dynamics in the rhizosphere.

## References

Bending, G.D., Turner, M.K., Jones, J.E., 2002. Interactions between crop residue and soil organic matter quality and the functional diversity of soil microbial communities. Soil Biology and Biochemistry 34, 1073–1082.

Cadena-Cepeda, M., Kokalis-Burelle, N., Runion, G.B., Kloepper, J.W., 2006. Assessing soil microbial populations and enzyme activity following the use of microbial inoculants. In: Seventh International Workshop of Plant Growth Promoting Rhizobacteria. Noordwijkerhott, The Netherlands, p. 28. 28 May–2 June.

Chen, X.-H., Vater, J., Piel, J., Franke, P., Scholz, R., Schneider, K., Koumoutsi, A., Hitzeroth, G., Grammel, N., Strittmatter, A.W., Gottschalk, G., Sussmuth, R.D., Borriss, R., 2006. Structural and functional characterization of three polyketide synthase gene clusters in *Bacillus amyloliquefaciens* FZB 42. Journal of Bacteriology 188, 4024–4036.

Fernandez, C., Rodriguez-Kabana, R., Warrior, P., Kloepper, J.W., 2001. Induced soil suppressiveness to a root-knot nematode species by a nematicide. Biological Control 22, 103–114.

Gray, N.F., 1988. Ecology of nematophagous fungi: effect of the soil nutrients N, P and K, and seven major metals on distribution. Plant and Soil 108, 286–290.

Green, V.S., Stott, D.E., Diack, M., 2006. Assay for fluorescein diacetate hydrolytic activity: optimization for soil samples. Soil Biology and Biochemistry 38, 693–701.

Gierth, K., Hallmann, J., Schlang, J., Müller, J., Sikora, R.A., 2004. Plant tolerance for managing plant parasitic nematodes. OILB/SROP Bulletin 27, 67–73.

Hussey, R., Barker, K., 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. Plant Disease Report 57, 1025–1028.

Jenkins, W., 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Disease Report 48, 692.

Ji, P., Campbell, H., Kloepper, J., Jones, J., Suslow, T., Wilson, M., 2006. Integrated biological control of bacterial speck and spot of tomato under field conditions using foliar biological control agents and plant growth-promoting rhizobacteria. Biological Control 36, 358–367.

Kerry, B.R., 1988. Fungal parasites of cyst nematodes. Agriculture, Ecosystem and Environment 24, 293–305.

Kerry, B.R., Hidalgo-Diaz, L., 2004. Application of *Pochonia chlamydosporia* in the integrated control of root-knot nematodes on organically grown vegetable crops in Cuba. IOBC WPRS Bulletin 27, 123–126.

Kiewnick, S., Sikora, R., 2005. Biological control of the root-knot nematode *Meloidogyne incognita* by *Paecilomyces lilacinus* strain 251. Biological Control 38, 179–187.

Kloepper, J.W., Rodríguez-Kábana, R., McInroy, J.A., Collins, D.J., 1991. Analysis of populations and physiological characterization of microorganisms in rhizospheres of plants with antagonistic properties to phytopathogenic nematodes. Plant and Soil 136, 95–102.

Kloepper, J.W., Ryu, C.-M., 2006. Bacterial endophytes as elicitors of induced systemic resistance. In: Schulz, B., Boyle, C., Siebern, T. (Eds.), Microbial Root Endophytes. Springer-Verlag, Heildelberg, pp. 33–51.

Kloepper, J.W., Ryu, C.-M., Zhang, S., 2004. Induced systemic resistance and promotion of plant growth by *Bacillus* spp. Phytopathology 94, 1259–1266.

Kokalis-Burelle, N., Kloepper, J.W., Reddy, M.S., 2006. Plant growth-promoting rhizobacteria as transplant amendments and their effects on indigenous rhizosphere microorganisms. Applied Soil Ecology 31, 91–100.

Kokalis-Burelle, N., Dickson, D.W., 2003. Effects of soil fumigants and bioyield[tm] on root knot nematode incidence and yield of tomato. Proceedings of International Research Conference on Methyl Bromide Alternatives and Emissions Reductions 50.1–50.3.

Kokalis-Burelle, N., Vavrina, C.S., Rosskopf, E.N., Shelby, R.A., 2002. Field evaluation of plant growth-promoting rhizobacteria amended transplant mixes and soil solarization for tomato and pepper production in Florida. Plant and Soil 238, 257–266.

Kokalis-Burelle, N., Kloepper, J.W., 2004. Soil ecosystem health and its role in plant disease suppression. In: Emerging concepts in plant health management, Research Signpost, Kerala, India, pp. 123–140.

Koumoutsi, A., Chen, X.-H., Henne, A., Liesegang, H., Hitzeroth, G., Franke, P., Vater, J., Borriss, R., 2004. Structural and functional characterization of gene clusters directing nonribosomal synthesis of bioactive cyclic lipopeptides in *Bacillus amyloliquefaciens* Strain FZB42. Journal of Bacteriology 186, 1084–1096.

Lara, J., Acosta, N., Betancour, C., Vincente, N., Rodriguez, R., 1996. Biological control of *Meloidogyne incognita* in Puerto Rico. Nematropica 26, 143–152.

Larkin, R., Hopkins, D., Martin, F., 1993. Effect of successive watermelon plantings on *Fusarium oxysporum* and other microorganisms in soils suppressive and conducive to fusarium wilt of watermelon. Phytopathology 83, 1097–1105.

Lucy, M., Reed, E., Glick, B.R., 2004. Applications of free living plant growth-promoting rhizobacteria. Antonie van Leeuwenhoek 86, 1–25.

Mazzola, M., 2002. Mechanisms of natural soil suppressiveness to soil-borne diseases. Antonie van Leeuwenhoek 81, 557–564.

Nico, A.I., Rafael, R.M., Jiménez-Díaza, M., Castillo, P., 2004. Control of root-knot nematodes by composted agro-industrial wastes in potting mixtures. Crop Protection 23, 581–587.

Pyrowolakis, A., Westphal, A., Sikora, R.A., Becker, J.O., 2002. Identification of root-knot nematode suppressive soils. Applied Soil Ecology 19, 51–56.

Schippers, B., Bakker, A.W., Bakker, P.A.H.M., 1987. Interactions of deleterious and beneficial rhizosphere microorganisms and the effect of cropping practices. Annual Review of Phytopathology 25, 339–358.

Schnurer, J., Rosswall, T., 1982. Fluorescein diacetate hydrolysis as a measure of total microbial activity in soil and litter. Applied Environmental Microbiology 43, 1256–1261.

Siddiqui, I.A., Ehetshamul-Haque, S., Shahid Shaukat, S., 2001. Use of rhizobacteria in the control of root rot-root knot disease complex of mungbean. Journal of Phytopathology 149, 337–346.

Weller, D.M., Raaijmakers, J.M., McSpadden, B.B., Thomashow, L.S., 2002. Microbial populations responsible for specific soil suppressiveness to plant pathogens. Annual Review of Phytopathology 40, 309–348.

Yildirim, E., Taylor, A.G., Spittler, T.D., 2006. Ameliorative effects of biological treatments on growth of squash plants under salt stress. Scientia Horticulturae 111, 1–6.

Zehnder, G.W., Murphy, J.F., Sikora, E.J., Kloepper, J.W., 2001. Application of rhizobacteria for induced resistance. European Journal of Plant Pathology 107, 39–50.