AQUEOUS FORMULATIONS OF PLANT GROWTH-PROMOTING RHIZOBACTERIA FOR CONTROL OF FOLIAR PATHOGENS

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ABSTRACT

As part of our ongoing work aimed at using in commercial agriculture the concepts of PGPR-mediated plant growth promotion and induced systemic resistance, we have been assessing various methods for applying biological preparations to vegetable crops. Recently, this work has concentrated on applying powdered formulations into the soil-less potting mix used to prepare transplants. An alternative, or augmentative approach would be to use foliar sprays of the biological preparation in the field. We report here our initial attempts to use aqueous formulations as soil drenches or foliar sprays. Nine different biological aqueous preparations were made, each containing industrial formulated spores of *Bacillus subtilis* strain GB03 plus another PGPR strain and a formulation carrier. These were evaluated as drenches and foliar sprays on tomato against bacterial spot (*Xanthomonas axonopodis* pv. vesicatoria), cucumber against angular leaf spot (*Pseudomonas syringae* pv. lachrymans), tobacco against blue mold (*Peronospora tabacina*) and wild fire (*P. syringae* pv. tabaci). There were significant differences among the treatments in various crops and pathosystems tested. In general, foliar application was more effective than a drench application in reducing the incidence of the target pathogens. To date, our data suggest that drench or foliar applications of PGPR-based biological preparations can suppress foliar pathogens similarly to incorporation of the preparations into the mix used to grow transplants.

INTRODUCTION

Our group has tried for several years to apply PGPR-mediated induced systemic resistance to achieve practical control of foliar pathogens. Strains used in these adaptive studies were initially selected by screening for plant growth-promotion and for suppression of anthracnose, angular leaf spot, bacterial wilt on cucumber; early blight, late blight and bacterial spot on tomato; and blue mold on tobacco. These initial screens were designed for rapid testing of lab-produced PGPR strains under controlled growth chamber or greenhouse environments.

Initial field development of these strains was done in collaboration with Gustafson, LLC., which provided industrially fermented spore preparations of the bacilli PGPR. A series of "biological preparations" (called the LS series) was prepared in which each treatment consisted of two bacilli PGPR and chitosan as a formulation carrier. One of the two bacilli strains in each treatment was *Bacillus* subtilis strain GBO3, and the second strain was a different strain of bacilli which had previously shown induced systemic resistance activity. In our initial field studies with these LS treatments, the powdered formulation was mixed into soil-less media used to prepare vegetable

transplants, and the treatments were found to provide growth promotion and to induced systemic disease protection (Reddy et. al., 1999; Kenney et al., 1999; Kleopper et al., 1999; Martinez-Ochoa et al., 1999., Ryu et al., 1999; Yan et al., 1999; Zhang et al., 1999). Potential application of this system in the field may be enhanced by the use of mid-season "booster" applications of PGPR, but this requires development and assessment of aqueous formulations of PGPR. The current study was conducted to select specific PGPR mixtures efficacious in reducing foliar diseases when applied as aqueous treatments.

MATERIALS AND METHODS

Industrial formulated spores of *Bacillus subtilis* strain GBO3 with eight different strain-mixtures of bacilli in proprietary aqueous formulations produced by Gustafson LLC., were evaluated for effects on foliar pathogens. A series of greenhouse experiments was conducted to test the efficacy of these strains on tomato cv. Solar Set against bacterial spot (*Xanthomonas axonopodis* pv. vesicatoria), on cucumber cv. SMR48 against angular leaf spot (*Pseudomonas syringae* pv. lachrymans), and on tobacco cvs. KY14 and TN90 against blue mold (*Peronospora tabacina*) and wildfire (*P. syringae* pv. tabaci). There were 10 treatments, (eight different 2-strain aqueous mixtures, GBO3 alone and a formulation control) in each crop and pathosystem. Four to six-week-old seedlings produced in soil-less media were used for PGPR application. PGPR were applied either as drench or foliar spray 7 days before pathogen challenge. Pathogens were sprayed to run-off on all crops and pathosystems, except for wildfire on tobacco. The wildfire pathogen was applied as a drop inoculation on leaves. Individual treatments were arranged in a randomized complete block design with either six or 10 replications. Disease severity was assessed 5-10 days after challenge inoculation. Data were analyzed using ANOVA, and treatment means were separated by the LSD test at $P = 0.05$.

RESULTS

Treatments of PGPR applied either as a soil drench or a foliar spray significantly reduced disease severity compared with the formulation control to varying degrees on tomato against bacterial spot (Table 1), on cucumber against angular leaf spot (Table 2), and on tobacco against blue mold (Tables 3 and 4).

Table 1. Effect of aqueous formulations of PGPR on tomato cv. Solar Set against bacterial spot disease.

Treatment	Number of bacterial spot lesions per leaflet[1]	
	Foliar spray	Drench
LS247 (GBO3 + SE34)	16.4	17.9*
LS265 (GBO3 + IN937a)	16.9	28.1*
LS266 (GBO3 + IN937b)	11.8	57.4
LS267 (GBO3 + INR-7)	22.1	44.6
LS268 (GBO3 + T-4)	17.5	32.5*
LS269 (GBO3 + 1PC-11)	27.1	19.5*
LS70 (GBO3 + 1PN-19)	6.4*	24.8*
LS271 (GBO3 + 3P-114)	10.1*	25.3*
GBO3 alone	22.9	22.5*
Formulation control	30.1	46.9
LSD (P = 0.05)	**8.4**	**14.3**

[1]Mean of five replications, 10 plants per replication and six leaflets per plant. Experiment was repeated two times. Disease severity is the number of lesions per leaflet.
*Significantly different from formulation control at P = 0.05.

Similar results were also obtained on wildfire of tobacco (data not shown here). The frequency with which various PGPR treatments provided significant control varied with the crop and pathosystem used. Overall, mixtures consisting of two strains showed significantly greater levels of disease suppression, compared to GBO3 alone. Foliar PGPR spray treatments led to higher disease suppression compared with the drench application, but there were significant differences in the magnitude of suppression among the PGPR treatments.

Table 2. Effect of aqueous formulations of PGPR on cucumber cv. SMR48 against angular leaf spot disease.

Treatment	Number of angular leaf spot lesions per leaf[1]	
	Foliar spray	Drench
LS247 (GBO3 + SE34)	17.3*	14.6*
LS265 (GBO3 + IN937a)	16.6*	19.6*
LS266 (GBO3 + IN937b)	34.5	31.8*
LS267 (GBO3 + INR-7)	30.3	49.9*
LS268 (GBO3 + T-4)	22.1*	69.4
LS269 (GBO3 + 1PC-11)	7.9*	9.1*
LS70 (GBO3 + 1PN-19)	16.3*	17.8*
LS271 (GBO3 + 3P-114)	34.9	42.9*
GBO3 alone	55.9	64.9
Formulation control	45.4	65.8
LSD (P = 0.05)	**15.8**	**13.4**

[1]Mean of five replications per treatment, 10 plants per replication and five leafs per plant. Experiment was repeated two times. Disease severity is the number of lesions per leaf.
*Significantly different from formulation control at P = 0.05.

Table 3. Effect of aqueous formulations of PGPR on tobacco cv. KY14 against blue mold disease.

Treatment	Number of blue mold lesions per plant[1]	
	Foliar spray	Drench
LS247 (GBO3 + SE34)	21.4*	40.4*
LS265 (GBO3 + IN937a)	20.6*	28.0*
LS266 (GBO3 + IN937b)	21.4*	30.4*
LS267 (GBO3 + INR-7)	22.0*	51.4*
LS268 (GBO3 + T-4)	24.6*	56.8
LS269 (GBO3 + 1PC-11)	19.4*	47.2*
LS70 (GBO3 + 1PN-19)	24.2*	51.0*
LS271 (GBO3 + 3P-114)	53.0	61.6
GBO3 alone	28.2*	43.8*
Formulation control	56.4*	67.8
LSD (P = 0.05)	**10.2**	**15.5**

[1]Mean of five replications per treatment, 10 plants per replication. Experiment was repeated two times. Disease severity is the number of blue mold lesions per plant
* Significantly different from formulation control at P = 0.05.

Table 4. Effect of aqueous formulations of PGPR on tobacco cv. TN90 against blue mold.

Treatment	Number of blue mold lesions per plant	
	Foliar spray	Drench
LS247 (GBO3 + SE34)	7.4*	8.6*
LS265 (GBO3 + IN937a)	6.2*	9.4*
LS266 (GBO3 + IN937b)	6.0*	12.2*
LS267 (GBO3 + INR-7)	7.6*	15.0*
LS268 (GBO3 + T-4)	10.6*	16.0*
LS269 (GBO3 + 1PC-11)	6.8*	11.2*
LS270 (GBO3 + 1PN-19)	8.2*	8.2*
LS271 (GBO3 + 3P-114)	8.8*	12.0*
GBO3 alone	12.4	9.6*
Formulation control	14.0	30.0
LSD (P = 0.05)	**3.2**	**5.9**

[1]Mean of five replications per treatment, 10 plants per replication. Experiment was repeated two times. Disease severity is the number of blue mold lesions per plant
*Significantly different from formulation control at P = 0.05.

DISCUSSION

Results presented here demonstrate that mixtures of two strains exhibited a more consistent and higher level of disease protection than did a single strain of GBO3 in aqueous formulation. Results also indicated that some aqueous PGPR treatments significantly reduced the incidence of all diseases, and that overall, foliar sprays were more effective than a drench application of the PGPR mixtures.

Our studies suggest that enhanced efficacy of plant growth promotion and biological control may be achieved when supplementary applications of PGPR are applied in an aqueous formulation either as drench or as foliar spray. A logical next step toward practical implementation of this technology is to evaluate the effects of aqueous formulations of PGPR on naturally occurring pathogens under field conditions.

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