Biological Control of *Meloidogyne incognita* by Spore-forming Plant Growth-promoting Rhizobacteria on Cotton

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Abstract

In the past decade, increased attention has been placed on biological control of plant-parasitic nematodes using various fungi and bacteria. The objectives of this study were to evaluate the potential of 662 plant growth-promoting rhizobacteria (PGPR) strains for mortality to *Meloidogyne incognita* J2 in vitro and for nematode management in greenhouse, microplot, and field trials. Results indicated that the mortality of *M. incognita* J2 by the PGPR strains ranged from 0 to 100% with an average of 39%. Among the PGPR strains examined, 212 of 662 strains (or 33%) caused significantly greater mortality percent of *M. incognita* J2 than the untreated control. *Bacillus* was the major genus initiating a greater mortality percentage when compared with the other genera. In subsequent trials, *B. velezensis* strain Bve2 reduced

M. incognita eggs per gram of cotton root in the greenhouse trials at 45 days after planting (DAP) similarly to the commercial standards Abamectin and Clothianidin plus *B. firmus* I-1582. *Bacillus mojavensis* strain Bmo3, *B. velezensis* strain Bve2, *B. subtilis* subsp. *subtilis* strain Bssu3, and the Mixture 2 (Abamectin + Bve2 + *B. altitudinis* strain Bal13) suppressed *M. incognita* eggs per gram of root in the microplot at 45 DAP. *Bacillus velezensis* strains Bve2 and Bve12 also increased seed-cotton yield in the microplot and field trials. Overall, results indicate that *B. velezensis* strains Bve2 and Bve12, *B. mojavensis* strain Bmo3, and Mixture 2 have potential to reduce *M. incognita* population density and to enhance growth of cotton when applied as in-furrow sprays at planting.

Meloidogyne incognita (Kofoid & White) Chitwood, the southern root-knot nematode, is one of the most important plant-parasitic nematodes affecting cotton (*Gossypium hirsutum*) production in the United States (Creech et al. 1995; Robinson 2007). In 2015, cotton yield in the United States was estimated to be 7.9 million bales, but losses due to *M. incognita* were estimated at 215,500 bales, which was equivalent to 1.35% of total production (Lawrence et al. 2016). Due to environmental and health concerns with the use of chemical nematicides for nematode management, many alternative strategies such as biological agents for plant-parasitic nematode control have been investigated (Burkett-Cadena et al. 2008; Kiewnick and Sikora 2006). Plant growth-promoting rhizobacteria (PGPR) promote plant growth and elicit significant reductions in the incidence or severity of various diseases on a diversity of hosts (Kloepper et al. 2004). Strains of PGPR that also exhibit nematocidal activity and/or elicit induced systemic plant resistance to plant-parasitic nematodes could be potential alternatives to chemical nematicides.

Many studies have reported antagonistic activity of various bacterial strains of *Bacillus* spp. against plant-parasitic nematodes. Twelve species of *Bacillus* have been documented for *M. incognita* management, including *B. amyloliquefaciens* (syn. *B. velezensis*) (Burkett-Cadena et al. 2008), *B. cereus* (Siddiqui and Mahmood 1999), *B. circulans*, *B. coagulans* (Serfoji et al. 2010), *B. firmus* (Mendoza et al. 2008; Terefe et al. 2009), *B. licheniformis* (Siddiqui and Husain 1991; Siddiqui and Mahmood 1992), *B. megaterium* (Kloepper et al. 1992), *B. polymyxa* (Khan and Akram 2000), *B. sphaericus* (Krechel et al. 2002), *B. subtilis* (Kavitha et al. 2007; Siddiqui and Mahmood 1999), and *B. thuringiensis* (Mohammed

et al. 2008; Zuckerman et al. 1993). Among these *Bacillus* spp., some have been developed into commercial products for controlling plant disease and nematodes. BioNem-WP/BioSafe, a *B. firmus* product developed by AgroGreen, was reported effective against *M. incognita*, *M. hapla*, *Heterodera* spp., *Tylenchulus semipenetrans*, *Xiphinema index*, and *Ditylenchus dipsaci* (Keren-Zur et al. 2000). BioYield, a combination of *B. velezensis* strain IN937a and *B. subtilis* strain GB03, was developed by Gustafson in a flowable formulation for management of soil-borne pathogens and suppression of *M. incognita* population density on tomato (Burkett-Cadena et al. 2008; Kloepper et al. 2004). Nemix, a *Bacillus* spp. product developed by AgriLife/Chr. Hansen, was reported for control of root-knot nematodes on vegetables and fruit trees (Hallmann et al. 2009). VOTiVO, *Bacillus firmus* GB-126, is marketed by Bayer CropScience as a seed treatment for the control of plant-parasitic nematodes on corn, cotton, sorghum, soybean, and sugar beet (Wilson and Jackson 2013). Pathway Consortia, a product containing *B. subtilis*, *B. licheniformis*, *B. megaterium*, *B. coagulans*, *Pseudomonas fluorescens*, *Streptomyces* spp., and *Trichoderma* spp. developed by Pathway Holdings, was reported for the management of plant-parasitic nematodes (Askary 2015).

Modes of action for biocontrol of plant-parasitic nematodes of some *Bacillus* strains have been studied. Mendoza et al. (2008) studied in-vitro activity of *B. firmus* against burrowing nematode *Radopholus similis*, root-knot nematode *M. incognita*, and stem nematode *Ditylenchus dipsaci*, and detected rates of mortality of these nematodes and significant reduction of *M. incognita* hatching after incubation with a low concentration of pure culture filtrates. The mode of action for the observed nematode paralysis and mortality was attributed to secondary metabolites produced by the bacteria (Mendoza et al. 2008).

Induced systemic resistance (ISR) caused by some *Bacillus* strains has been documented. Sikora (1988) found that *B. subtilis* can induce protection against *M. incognita* in cotton. Kloepper et al. (2004) reported that specific strains of *B. velezensis*, *B. cereus*, *B. mycoides*, *B. pasteurii*, *B. pumilus*, *B. sphaericus*, and *B. subtilis* can elicit significant reductions in the incidence or severity of various diseases on a diversity of hosts through ISR. Schrimsher (2013) studied the ISR of *B. firmus* GB-126 against *Heterodera glycines* and *M. incognita* in split-root experiments in the greenhouse and found that ISR was evident in the *H. glycines* split-root assay but not in the *M. incognita*

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Table 1. Effect of plant growth-promoting rhizobacteria strains on *Meloidogyne incognita* J2 mortality percentage higher than untreated control^a in laboratory trials

Code	Scientific name	<i>Meloidogyne incognita</i> J2 mortality (%) ^b	Dunnett's <i>P</i> versus ^c (<i>P</i> ≤ 0.05)		
			Clothianidin + <i>B. firmus</i>	Aldicarb ^d	Water
Ad1	<i>Arthrobacter defluvii</i>	53.5	0.8886	0.0681	0.0150
Bal2	<i>Bacillus altitudinis</i>	71.1	0.0535	0.9365	<0.0001
Bal3	<i>Bacillus altitudinis</i>	96.4	<0.0001	1.0000	<0.0001
Bal4	<i>Bacillus altitudinis</i>	97.4	<0.0001	1.0000	<0.0001
Bal5	<i>Bacillus altitudinis</i>	100.0	<0.0001	1.0000	<0.0001
Bal11	<i>Bacillus altitudinis</i>	59.7	0.9047	0.6812	0.0483
Bal12	<i>Bacillus altitudinis</i>	52.5	0.9375	0.0531	0.0201
Bal13	<i>Bacillus altitudinis</i>	75.9	0.1480	1.0000	0.0009
Bal14	<i>Bacillus altitudinis</i>	87.7	0.0003	1.0000	<0.0001
Bal15	<i>Bacillus altitudinis</i>	94.5	<0.0001	1.0000	<0.0001
Bal16	<i>Bacillus altitudinis</i>	61.3	0.3699	0.3157	0.0013
Bal17	<i>Bacillus altitudinis</i>	84.4	0.0010	1.0000	<0.0001
Bar6	<i>Bacillus aryabhatai</i>	78.2	0.0077	1.0000	<0.0001
Bar7	<i>Bacillus aryabhatai</i>	75.1	0.0193	0.9992	<0.0001
Bar8	<i>Bacillus aryabhatai</i>	88.1	0.0003	1.0000	<0.0001
Bar9	<i>Bacillus aryabhatai</i>	79.4	0.0054	1.0000	<0.0001
Bar14	<i>Bacillus aryabhatai</i>	63.3	0.2694	0.4263	0.0006
Bar15	<i>Bacillus aryabhatai</i>	64.6	0.2131	0.5124	0.0004
Bar16	<i>Bacillus aryabhatai</i>	67.8	0.4568	0.9930	0.0078
Bar17	<i>Bacillus aryabhatai</i>	60.3	0.4294	0.2671	0.0018
Bar19	<i>Bacillus aryabhatai</i>	87.6	0.0003	1.0000	<0.0001
Bar20	<i>Bacillus aryabhatai</i>	86.8	0.0004	1.0000	<0.0001
Bar21	<i>Bacillus aryabhatai</i>	90.8	<0.0001	1.0000	<0.0001
Bar22	<i>Bacillus aryabhatai</i>	88.3	0.0002	1.0000	<0.0001
Bar24	<i>Bacillus aryabhatai</i>	55.8	0.7464	0.1116	0.0078
Bar25	<i>Bacillus aryabhatai</i>	54.1	0.8589	0.0768	0.0129
Bar27	<i>Bacillus aryabhatai</i>	100.0	<0.0001	1.0000	<0.0001
Bar28	<i>Bacillus aryabhatai</i>	95.4	<0.0001	1.0000	<0.0001
Bar29	<i>Bacillus aryabhatai</i>	96.7	<0.0001	1.0000	<0.0001
Bar31	<i>Bacillus aryabhatai</i>	68.5	0.0971	0.7917	<0.0001
Bar32	<i>Bacillus aryabhatai</i>	62.8	0.2907	0.3990	0.0007
Bar33	<i>Bacillus aryabhatai</i>	83.5	0.0014	1.0000	<0.0001
Bar41	<i>Bacillus aryabhatai</i>	97.6	0.0011	1.0000	<0.0001
Bar46	<i>Bacillus aryabhatai</i>	84.2	0.0315	1.0000	<0.0001
Bar47	<i>Bacillus aryabhatai</i>	57.8	0.5981	0.1692	0.0041
Bar49	<i>Bacillus aryabhatai</i>	66.7	0.1423	0.6615	0.0002
Bce4	<i>Bacillus cereus</i>	71.1	0.0538	0.9355	<0.0001
Bce6	<i>Bacillus cereus</i>	73.7	0.0275	0.9940	<0.0001
Bce7	<i>Bacillus cereus</i>	56.8	0.6688	0.1395	0.0056
Bce8	<i>Bacillus cereus</i>	94.3	<0.0001	1.0000	<0.0001
Bce14	<i>Bacillus cereus</i>	79.3	0.0056	1.0000	<0.0001
Bce15	<i>Bacillus cereus</i>	64.6	0.2121	0.5141	0.0004
Bce37	<i>Bacillus cereus</i>	51.0	0.9798	0.0369	0.0298
Bce38	<i>Bacillus cereus</i>	61.7	0.3489	0.3354	0.0011
Bce41	<i>Bacillus cereus</i>	73.3	0.0312	0.9895	<0.0001
Bce42	<i>Bacillus cereus</i>	94.2	<0.0001	1.0000	<0.0001
Bce44	<i>Bacillus cereus</i>	79.1	0.0059	1.0000	<0.0001
Bce45	<i>Bacillus cereus</i>	70.9	0.0564	0.9272	<0.0001
Bce46	<i>Bacillus cereus</i>	94.3	<0.0001	1.0000	<0.0001
Bce47	<i>Bacillus cereus</i>	50.3	0.9898	0.0310	0.0355
Bmt2	<i>Bacillus methylotrophicus</i>	76.7	0.0120	1.0000	<0.0001
Bmt5	<i>Bacillus methylotrophicus</i>	90.7	<0.0001	1.0000	<0.0001
Bmt7	<i>Bacillus methylotrophicus</i>	82.3	0.0021	1.0000	<0.0001
Bmt9	<i>Bacillus methylotrophicus</i>	68.1	0.1058	0.7640	<0.0001
Bmo2	<i>Bacillus mojavensis</i>	49.9	0.9940	0.0275	0.0398
Bmo3	<i>Bacillus mojavensis</i>	70.6	0.3255	0.9998	0.0039

(continued on next page)

^a In-vitro tests were performed in 96-well plates. Data of 212 PGPR strains indicating significantly higher mortality on *M. incognita* J2 than untreated control were presented in the table. All the PGPR strains had four replications and controls were based on 17 repeats. Data collected were analyzed in SAS 9.4 using PROC GLIMMIX procedure at significance level of $\alpha \leq 0.05$. *P* value less than 0.05 indicate a significant effect. LS-means and adjusted *P* values were presented in the table.

^b Mortality percentage was determined by the following equation: [(live J2 prior to exposure - live J2 at 48 hours) / live J2 prior to exposure] × 100.

^c Dunnett's option was used in the LS-means statement to assess the differences between bacterial strains and the Poncho/Votivo, Temik, and the untreated control.

^d Active ingredients for Poncho/Votivo are Clothianidin plus *B. firmus* I-1582, Temik is Aldicarb, and untreated control is sterile distilled water.

^e Indistinguishable species and unidentified strains.

Table 1. (continued from preceding page)

Code	Scientific name	<i>Meloidogyne incognita</i> J2 mortality (%) ^b	Dunnett's <i>P</i> versus ^c (<i>P</i> ≤ 0.05)		
			Clothianidin + <i>B. firmus</i>	Aldicarb ^d	Water
Bmo4	<i>Bacillus mojavensis</i>	66.8	0.1402	0.6670	0.0002
Bmy1	<i>Bacillus mycoides</i>	75.9	0.0154	0.9998	<0.0001
Bmy16	<i>Bacillus mycoides</i>	55.3	0.7762	0.1019	0.0089
Bmy17	<i>Bacillus mycoides</i>	71.1	0.0531	0.9375	<0.0001
Bmy18	<i>Bacillus mycoides</i>	85.4	0.0007	1.0000	<0.0001
Bmy20	<i>Bacillus mycoides</i>	67.8	0.1122	0.7446	0.0001
Bmy25	<i>Bacillus mycoides</i>	54.4	0.8406	0.0822	0.0118
Bmy26	<i>Bacillus mycoides</i>	70.0	0.0693	0.8845	<0.0001
Bmy30	<i>Bacillus mycoides</i>	94.9	<0.0001	1.0000	<0.0001
Bmy34	<i>Bacillus mycoides</i>	54.3	0.8453	0.0808	0.0121
Bmy36	<i>Bacillus mycoides</i>	58.9	0.5226	0.2073	0.0029
Bps4	<i>Bacillus psychrosaccharolyticus</i>	75.1	0.0193	0.9992	<0.0001
Bpu5	<i>Bacillus pumilus</i>	79.1	0.0059	1.0000	<0.0001
Bpu6	<i>Bacillus pumilus</i>	60.0	0.8922	0.6990	0.0454
Bsa1	<i>Bacillus safensis</i>	96.8	<0.0001	1.0000	<0.0001
Bsa4	<i>Bacillus safensis</i>	66.0	0.1643	0.6089	0.0002
Bsa6	<i>Bacillus safensis</i>	92.9	<0.0001	1.0000	<0.0001
Bsa7	<i>Bacillus safensis</i>	87.9	0.0003	1.0000	<0.0001
Bsa8	<i>Bacillus safensis</i>	100.0	<0.0001	1.0000	<0.0001
Bsa9	<i>Bacillus safensis</i>	53.7	0.8804	0.0705	0.0144
Bsa12	<i>Bacillus safensis</i>	90.2	0.0001	1.0000	<0.0001
Bsa26	<i>Bacillus safensis</i>	64.6	0.6376	0.9319	0.0167
Bsa28	<i>Bacillus safensis</i>	56.9	0.6651	0.1409	0.0055
Bsa31	<i>Bacillus safensis</i>	64.9	0.2007	0.5346	0.0003
Bsa34	<i>Bacillus safensis</i>	64.4	0.2220	0.4972	0.0004
Bsa35	<i>Bacillus safensis</i>	96.5	<0.0001	1.0000	<0.0001
Bsp2	<i>Bacillus simplex</i>	82.0	0.0023	1.0000	<0.0001
Bsp13	<i>Bacillus simplex</i>	65.1	0.1942	0.5468	0.0003
Bsp24	<i>Bacillus simplex</i>	76.2	0.0139	0.9999	<0.0001
Bsp32	<i>Bacillus simplex</i>	75.7	0.0161	0.9998	<0.0001
Bsp33	<i>Bacillus simplex</i>	65.5	0.1801	0.5749	0.0003
Bsp35	<i>Bacillus simplex</i>	81.6	0.0027	1.0000	<0.0001
Bsp36	<i>Bacillus simplex</i>	68.4	0.0986	0.7865	<0.0001
Bsp42	<i>Bacillus simplex</i>	79.0	0.0061	1.0000	<0.0001
Bsp44	<i>Bacillus simplex</i>	66.0	0.1643	0.6089	0.0002
Bsp45	<i>Bacillus simplex</i>	58.0	0.5856	0.1750	0.0039
Bsp46	<i>Bacillus simplex</i>	55.8	0.7429	0.1128	0.0077
Bsp47	<i>Bacillus simplex</i>	74.9	0.0199	0.9990	<0.0001
Bsp48	<i>Bacillus simplex</i>	71.0	0.0172	0.7796	<0.0001
Bsp50	<i>Bacillus simplex</i>	83.0	0.0017	1.0000	<0.0001
Bsp51	<i>Bacillus simplex</i>	75.5	0.0170	0.9996	<0.0001
Bsp52	<i>Bacillus simplex</i>	76.9	0.0116	1.0000	<0.0001
Bsp53	<i>Bacillus simplex</i>	82.7	0.0426	1.0000	0.0001
Bsp54	<i>Bacillus simplex</i>	83.1	0.0016	1.0000	<0.0001
Bsp55	<i>Bacillus simplex</i>	81.1	0.0031	1.0000	<0.0001
Bsp56	<i>Bacillus simplex</i>	75.3	0.0179	0.9995	<0.0001
Bsp57	<i>Bacillus simplex</i>	83.2	0.0016	1.0000	<0.0001
Bsp58	<i>Bacillus simplex</i>	88.3	0.0002	1.0000	<0.0001
Bsp59	<i>Bacillus simplex</i>	83.7	0.0013	1.0000	<0.0001
Bsp60	<i>Bacillus simplex</i>	64.3	0.2240	0.4939	0.0004
Bsp61	<i>Bacillus simplex</i>	84.8	0.0009	1.0000	<0.0001
Bsp62	<i>Bacillus simplex</i>	69.5	0.0773	0.8574	<0.0001
Bsp63	<i>Bacillus simplex</i>	76.4	0.0132	1.0000	<0.0001
Bsp64	<i>Bacillus simplex</i>	89.2	<0.0001	1.0000	<0.0001
Bsp66	<i>Bacillus simplex</i>	70.9	0.0568	0.9261	<0.0001
Bsp69	<i>Bacillus simplex</i>	51.2	0.9764	0.0385	0.0285
Bsp79	<i>Bacillus simplex</i>	76.8	0.0117	1.0000	<0.0001
Bsp81	<i>Bacillus simplex</i>	86.1	0.0006	1.0000	<0.0001
Bsp82	<i>Bacillus simplex</i>	73.5	0.0292	0.9921	<0.0001
Bsp84	<i>Bacillus simplex</i>	99.6	0.0006	1.0000	<0.0001
Bsp87	<i>Bacillus simplex</i>	81.3	0.0568	1.0000	0.0002
Bsp88	<i>Bacillus simplex</i>	91.5	<0.0001	1.0000	<0.0001
Bsp89	<i>Bacillus simplex</i>	84.3	0.0010	1.0000	<0.0001
Bsp91	<i>Bacillus simplex</i>	64.4	0.2220	0.4972	0.0004
Bsp92	<i>Bacillus simplex</i>	90.2	0.0001	1.0000	<0.0001

(continued on next page)

Table 1. (continued from preceding page)

Code	Scientific name	<i>Meloidogyne incognita</i> J2 mortality (%) ^b	Dunnett's <i>P</i> versus ^c (<i>P</i> ≤ 0.05)		
			Clothianidin + <i>B. firmus</i>	Aldicarb ^d	Water
Bsp93	<i>Bacillus simplex</i>	56.1	0.7196	0.1208	0.0070
Bsp94	<i>Bacillus simplex</i>	81.3	0.0029	1.0000	<0.0001
Bsp96	<i>Bacillus simplex</i>	95.5	<0.0001	1.0000	<0.0001
Bsp101	<i>Bacillus simplex</i>	98.0	0.0010	1.0000	<0.0001
Bsp114	<i>Bacillus simplex</i>	99.9	0.0006	1.0000	<0.0001
Bsp115	<i>Bacillus simplex</i>	99.9	0.0006	1.0000	<0.0001
Bsp116	<i>Bacillus simplex</i>	99.1	0.0007	1.0000	<0.0001
Bsp118	<i>Bacillus simplex</i>	69.3	0.0818	0.8422	<0.0001
Bsp123	<i>Bacillus simplex</i>	83.7	0.0350	1.0000	<0.0001
Bsp124	<i>Bacillus simplex</i>	67.5	0.1195	0.7232	0.0001
Bsp126	<i>Bacillus simplex</i>	76.2	0.0142	0.9999	<0.0001
Bsp131	<i>Bacillus simplex</i>	88.2	0.0003	1.0000	<0.0001
Bsp134	<i>Bacillus simplex</i>	62.9	0.2847	0.4064	0.0007
Bsp135	<i>Bacillus simplex</i>	71.4	0.0497	0.9477	<0.0001
Bsp143	<i>Bacillus simplex</i>	98.2	0.0009	1.0000	<0.0001
Bsp154	<i>Bacillus simplex</i>	99.9	0.0006	1.0000	<0.0001
Bsp187	<i>Bacillus simplex</i>	67.7	0.1146	0.7375	0.0001
Bsp195	<i>Bacillus simplex</i>	90.0	0.0001	1.0000	<0.0001
Bsp197	<i>Bacillus simplex</i>	64.3	0.2240	0.4939	0.0004
Bsp198	<i>Bacillus simplex</i>	80.6	0.0037	1.0000	<0.0001
Bsp199	<i>Bacillus simplex</i>	52.8	0.9251	0.0571	0.0185
Bsp200	<i>Bacillus simplex</i>	71.9	0.0437	0.9643	<0.0001
Bssin8	<i>Bacillus subtilis</i> subsp. <i>inaquosorum</i>	88.4	0.0002	1.0000	<0.0001
Bssin9	<i>Bacillus subtilis</i> subsp. <i>inaquosorum</i>	94.6	<0.0001	1.0000	<0.0001
Bssin10	<i>Bacillus subtilis</i> subsp. <i>inaquosorum</i>	94.6	<0.0001	1.0000	<0.0001
Bssin11	<i>Bacillus subtilis</i> subsp. <i>inaquosorum</i>	54.1	0.8544	0.0781	0.0126
Bssin12	<i>Bacillus subtilis</i> subsp. <i>inaquosorum</i>	94.3	<0.0001	1.0000	<0.0001
Bssin14	<i>Bacillus subtilis</i> subsp. <i>inaquosorum</i>	94.5	<0.0001	1.0000	<0.0001
Bssin15	<i>Bacillus subtilis</i> subsp. <i>inaquosorum</i>	90.6	<0.0001	1.0000	<0.0001
Bsssu2	<i>Bacillus subtilis</i> subsp. <i>subtilis</i>	84.4	0.0302	1.0000	<0.0001
Bsssu3	<i>Bacillus subtilis</i> subsp. <i>subtilis</i>	82.4	0.0457	1.0000	0.0001
Bte2	<i>Bacillus tequilensis</i>	93.5	<0.0001	1.0000	<0.0001
Bth2	<i>Bacillus thuringiensis</i>	58.8	0.5312	0.2026	0.0030
Bto18	<i>Bacillus toyonensis</i>	87.5	0.0003	1.0000	<0.0001
Bto21	<i>Bacillus toyonensis</i>	63.5	0.2603	0.4387	0.0006
Bto22	<i>Bacillus toyonensis</i>	82.9	0.0017	1.0000	<0.0001
Bto23	<i>Bacillus toyonensis</i>	73.3	0.0310	0.9898	<0.0001
Bto24	<i>Bacillus toyonensis</i>	76.8	0.0118	1.0000	<0.0001
Bto34	<i>Bacillus toyonensis</i>	74.0	0.0258	0.9957	<0.0001
Bto36	<i>Bacillus toyonensis</i>	93.1	<0.0001	1.0000	<0.0001
Bto40	<i>Bacillus toyonensis</i>	98.5	<0.0001	1.0000	<0.0001
Bto45	<i>Bacillus toyonensis</i>	82.1	0.0022	1.0000	<0.0001
Bto46	<i>Bacillus toyonensis</i>	64.2	0.2271	0.4889	0.0004
Bto49	<i>Bacillus toyonensis</i>	66.2	0.1572	0.6252	0.0002
Bto51	<i>Bacillus toyonensis</i>	87.2	0.0004	1.0000	<0.0001
Bto52	<i>Bacillus toyonensis</i>	89.4	0.0002	1.0000	<0.0001
Bto53	<i>Bacillus toyonensis</i>	75.6	0.0167	0.9997	<0.0001
Bto54	<i>Bacillus toyonensis</i>	81.3	0.0029	1.0000	<0.0001
Bto55	<i>Bacillus toyonensis</i>	91.3	<0.0001	1.0000	<0.0001
Bto57	<i>Bacillus toyonensis</i>	87.0	0.0004	1.0000	<0.0001
Bto58	<i>Bacillus toyonensis</i>	81.0	0.0032	1.0000	<0.0001
Bto59	<i>Bacillus toyonensis</i>	68.3	0.1019	0.7762	<0.0001
Bto61	<i>Bacillus toyonensis</i>	91.3	<0.0001	1.0000	<0.0001
Bto63	<i>Bacillus toyonensis</i>	84.5	0.0010	1.0000	<0.0001
Bto64	<i>Bacillus toyonensis</i>	66.6	0.1445	0.6560	0.0002
Bto65	<i>Bacillus toyonensis</i>	86.6	0.0004	1.0000	<0.0001
Bto66	<i>Bacillus toyonensis</i>	72.3	0.0398	0.9737	<0.0001
Bve2	<i>Bacillus velezensis</i>	72.8	0.2386	1.0000	0.0021
Bve4	<i>Bacillus velezensis</i>	54.1	0.8559	0.0777	0.0127
Bve5	<i>Bacillus velezensis</i>	54.7	0.8216	0.0879	0.0108
Bve12	<i>Bacillus velezensis</i>	81.1	0.0591	1.0000	0.0002
Bve13	<i>Bacillus velezensis</i>	61.9	0.7951	0.8106	0.0303
Bve14	<i>Bacillus velezensis</i>	89.3	0.0099	1.0000	<0.0001
Bve21	<i>Bacillus velezensis</i>	52.4	0.9423	0.0516	0.0208
Bve28	<i>Bacillus velezensis</i>	60.9	0.3960	0.2932	0.0015

(continued on next page)

Table 1. (continued from preceding page)

Code	Scientific name	<i>Meloidogyne incognita</i> J2 mortality (%) ^b	Dunnett's <i>P</i> versus ^c (<i>P</i> ≤ 0.05)		
			Clothianidin + <i>B. firmus</i>	Aldicarb ^d	Water
Bve34	<i>Bacillus velezensis</i>	58.9	0.7050	0.3464	0.0108
Bve37	<i>Bacillus velezensis</i>	76.5	0.1352	1.0000	0.0007
Bve40	<i>Bacillus velezensis</i>	76.5	0.1341	1.0000	0.0007
Bwe2	<i>Bacillus weihenstephanensis</i>	83.6	0.0013	1.0000	<0.0001
Bwe5	<i>Bacillus weihenstephanensis</i>	57.8	0.5999	0.1684	0.0042
Bwe10	<i>Bacillus weihenstephanensis</i>	94.3	<0.0001	1.0000	<0.0001
Bwe15	<i>Bacillus weihenstephanensis</i>	75.4	0.0174	0.9996	<0.0001
Bwe16	<i>Bacillus weihenstephanensis</i>	81.8	0.0025	1.0000	<0.0001
Brio1	<i>Brevibacterium iodinum</i>	87.8	0.0003	1.0000	<0.0001
Fso1	<i>Ficitibacillus solisalsi</i>	70.3	0.3385	0.9997	0.0042
Lma1	<i>Lysinibacillus macroides</i>	64.8	0.6287	0.9368	0.0161
Paam2	<i>Paenibacillus amylolyticus</i>	58.0	0.5838	0.1758	0.0039
Paam3	<i>Paenibacillus amylolyticus</i>	58.3	0.5608	0.1871	0.0035
Paam6	<i>Paenibacillus amylolyticus</i>	70.8	0.0574	0.9240	<0.0001
Paam7	<i>Paenibacillus amylolyticus</i>	82.8	0.0018	1.0000	<0.0001
Paam8	<i>Paenibacillus amylolyticus</i>	95.2	<0.0001	1.0000	<0.0001
Pala4	<i>Paenibacillus lautus</i>	70.4	0.0628	0.9065	<0.0001
Patu1	<i>Paenibacillus tundrae</i>	79.6	0.0050	1.0000	<0.0001
Paxy2	<i>Paenibacillus xylanexedens</i>	90.4	0.0001	1.0000	<0.0001
Spg8	<i>Sporosarcina globispora</i>	84.4	0.0010	1.0000	<0.0001
Uid4	<i>Bacillus aerophilus/stratosphericus</i> ^e	89.1	0.0002	1.0000	<0.0001
Uid6	<i>Bacillus aerophilus/stratosphericus</i> ^e	88.2	0.0002	1.0000	<0.0001
Uid7	<i>Bacillus aerophilus/stratosphericus</i> ^e	54.8	0.8134	0.0904	0.0104
Uid8	<i>Bacillus altitudinis/stratosphericus/aerophilus</i> ^e	98.9	<0.0001	1.0000	<0.0001
Uid9	<i>Bacillus altitudinis/stratosphericus/aerophilus</i> ^e	96.2	<0.0001	1.0000	<0.0001
Uid10	<i>Bacillus altitudinis/stratosphericus/aerophilus</i> ^e	96.8	<0.0001	1.0000	<0.0001
Control	Active ingredient^d				
Poncho/Votivo	Clothianidin and <i>B. firmus</i> I-1582	24.4	...	<0.0001	1.0000
Temik	Aldicarb	99.2	<0.0001	...	<0.0001
Untreated control	Sterile distilled water	2.0	1.0000	<0.0001	...

split-root assay. Collectively, these studies indicate that *Bacillus* spp. are promising candidates for nematode disease management through diverse modes of action.

The overall goal of this research was to investigate selected PGPR strains for their potential biological control of *M. incognita* on cotton. The specific objectives were to assess the potential of PGPR strains for mortality of *M. incognita* J2 in vitro and evaluate the efficacy of PGPR strains for reduction of *M. incognita* population density and plant growth promotion on cotton in greenhouse and microplot trials, and in field production systems.

Materials and Methods

PGPR strains. A total of 662 PGPR strains were included in in-vitro studies. PGPR strains were originally isolated, identified, and stored by J. W. Kloepper at Auburn University, Auburn, AL. Among these strains, 92.7% were *Bacillus* spp. including 208 strains of *B. simplex*, 70 strains of *B. toyonensis*, 53 strains of *B. aryabhattai*, 51 strains of *B. cereus*, 44 strains of *B. mycoides*, 41 strains of *B. velezensis*, 35 strains of *B. safensis*, 21 strains of *B. altitudinis*, 21 strains of *B. weihenstephanensis*, 15 strains of *B. subtilis* subsp. *inaquosorum*, 13 strains of *B. methylotrophicus*, six strains of *B. pumilus*, five strains of *B. psychrosaccharolyticus*, four strains of *B. mojavensis*, four strains of *B. subtilis* subsp. *subtilis*, four strains of *B. thuringiensis*, three strains of *B. siamensis*, three strains of *B. tequilensis*, and 13 strains of other *Bacillus* spp. Of the remaining 7.3% of the collection, 10 strains were *Sporosarcina globispora*, nine strains were *Paenibacillus amylolyticus*, four strains were *P. lautus*, and 25 strains were from multiple genera. The PGPR strains stored in 30% glycerol at -80°C were transferred to tryptic soy agar (VWR, Radnor, PA) plates and incubated at 35°C for 24 h. Vegetative cells of each strain were suspended in 5 ml of sterile distilled water in 25-ml glass tubes and the concentration was adjusted to 1 × 10⁷ CFU/ml.

Nematode inoculum. *Meloidogyne incognita*, originally isolated from an infested field at the Plant Breeding Unit (PBU) at E. V. Smith

Research Center of Auburn University and maintained on corn plants Mycogen 2H723 (Dow AgroScience, Indianapolis, IN) in 500-cm³ polystyrene pots in the greenhouse, was used as inoculum in the experiments. Eggs were extracted from corn roots by placing the root system in a 0.625% NaOCl solution and agitating the roots for 4 min using a rotary shaker at 120 rpm (Hussey and Barker 1973). Eggs were rinsed with tap water, collected on a 25-μm-pore sieve, then processed by sucrose centrifugation-flotation at 240 g for 1 min (Jenkins 1964). For in-vitro tests, *M. incognita* eggs were placed in a modified Baermann funnel (Castillo et al. 2013) on a slide warmer (Model 77) (Marshall Scientific, Brentwood, NH) and incubated at 31°C for 5 to 7 days to obtain second-stage juveniles (J2) (Xiang et al. 2014). The J2 were collected on a 25-μm-pore sieve, transferred to 1.5-ml microcentrifuge tubes, centrifuged at 5,000 g for 1 min, rinsed with sterile distilled water, and centrifuged at 5,000 g for 1 min. The J2 suspension was adjusted to 30 to 40 J2 per 10 μl of water (Xiang et al. 2014). For trials conducted in the greenhouse and microplot, eggs were enumerated at 40× magnification using an inverted TS100 Nikon microscope and standardized to 2,000 eggs per cone-tainer or 50,000 eggs per microplot.

Tests in vitro. Tests in vitro were conducted to assess mortality of *M. incognita* J2 by PGPR strains. PGPR vegetative cell suspensions and *M. incognita* J2 inocula were prepared as mentioned previously. Ten-microliter nematode suspension containing 30 to 40 *M. incognita* J2 were added in each well of a 100-μl, 96-well plate. Ninety microliters of each PGPR bacterial vegetative cell suspension were transferred into each test well of the 96-well plate. Clothianidin plus *B. firmus* I-1582 (Poncho/VOTiVO) (Bayer CropScience, Raleigh, NC) at 0.7 μl/well (0.424 mg ai/seed) and 1 granule/well of Aldicarb (Temik 15G) (Bayer CropScience, Raleigh, NC) were used as chemical standards. Sterile distilled water was used as the untreated control. Each plate was sealed with parafilm and incubated at room temperature (22.2 to 25.5°C) for 48 h. Numbers of live *M. incognita* J2 were counted and recorded at experiment initiation and 48 h after

exposure to the bacterial strains. Viability of *M. incognita* J2 was determined using the sodium hydroxide technique developed by Xiang and Lawrence (2016). Mortality percentage of *M. incognita* J2 was calculated using the following equation: [(live J2 prior to exposure – live J2 at 48 h) / live J2 prior to exposure] × 100. Each treatment was replicated four times and the in-vitro screening experiment was repeated.

Plant materials. Cotton variety FM1944 GLB2 (Bayer CropScience, Raleigh, NC), known to be susceptible to *M. incognita* (Lawrence et al. 2015), was used for the greenhouse, microplot, and field experiments.

Trials in the greenhouse. Seventy-two PGPR strains were selected from the in-vitro screening for initial evaluation in the greenhouse for their efficacy to reduce nematode population density and promote cotton plant growth. Confidential agreements were signed during this research study and only nine *Bacillus* strains were available for further testing. These nine strains included one strain of *B. mojavensis* (Bmo3), two strains of *B. safensis* (Bsa25 and Bsa26), two strains of *B. subtilis* subsp. *subtilis* (Bssu2 and Bssu3), and four strains of *B. velezensis* (Bve2, Bve12, Bve37, and Bve40). All experiments were conducted at the Plant Science Research Center (PSRC) located at Auburn University, Auburn, AL. Experiments were performed in 150-cm³ plastic cone-tainers (Stuewe & Sons Inc., Tangent, OR) filled with a soil sand mix (60:40 v/v). The soil was a Kalmia loamy sand (80% sand, 10% silt, and 10% clay) collected from the PBU at E.V. Smith Research Center of Auburn University near Tallahassee, AL. Soil was steam pasteurized at 80°C for 90 min and cooled for 24 h, then the steam pasteurizing process was repeated prior to use. Two cotton seeds were planted 1.3 cm deep in each cone-tainer. One milliliter of bacterial cell suspension (1×10⁷ CFU/ml) was added to each seed at planting. For the nematicide controls, cotton seeds were treated with each compound following agricultural industry recommendations: 0.424 mg ai/seed of Clothianidin plus *B. firmus* I-1582, or 0.15 mg ai/seed of Abamectin (Syngenta, Greensboro, NC), or 1 granule of Aldicarb per seed was applied at planting. All seeds for Clothianidin plus *B. firmus* I-1582 treatment were treated with a Gustafson table-top seed treater (Bayer CropScience, Research Triangle Park, NC), mixed for 3 min in a 454-g stainless steel bucket and allowed to air-dry before packaging (Schrimsher et al. 2014). One milliliter of tap water was added to the untreated control seeds. One milliliter of water containing 2,000 *M. incognita* eggs was pipetted into a 2-cm depression in each cone-tainer at planting and covered with soil. Experiments were arranged in a randomized complete block design (RCBD). Each treatment had five replications and the experiment was repeated. Cotton seedlings were thinned to one per cone-tainer after emergence. Plants were watered as needed. Supplemental light of 1000 watt halide bulbs producing

110,000 lumens was supplied to maintain the day length of 14 h. Greenhouse temperatures ranged from 21°C to 35°C. Experiments were terminated at 45 days after planting (DAP). Plant and nematode measurements were recorded. Plant measurements included plant height (PH) and biomass (Bio) including shoot and root fresh weights (SFW+RWF). Nematode measurements were *M. incognita* eggs per gram of root (eggs/gr).

Trials in the microplots. Six PGPR strains and two mixtures of PGPR strains were evaluated for nematode population development, early plant growth promotion, and yield enhancement on cotton. The strains included *B. altitudinis* strain Bal13, *B. mojavensis* strain Bmo3, *B. subtilis* subsp. *subtilis* strains Bssu2 and Bssu3, and *B. velezensis* strains Bve2 and Bve12. Mixtures were formed from the best performing strains based on greenhouse studies. The two mixtures were Mixture 1 (Bve2 + Bal13) and Mixture 2 (seeds treated with Abamectin + Bve2 + Bal13). The experiments were conducted at the PSRC. Experiments were established in 26.5-liter pots filled with a Kalmia loamy sand collected from the PBU where *M. incognita* and *H. glycines* had not been detected. Experiments were arranged in a RCBD with 6 replications for each treatment and the experiment was repeated. Five cotton seeds were hand-planted at a 1.3-cm depth in a linear pattern to simulate a linear row-foot in the field (Schrimsher et al. 2014). One milliliter of bacterial suspension (1 × 10⁷ CFU/ml) was applied to each seed at planting. Five milliliters containing 50,000 *M. incognita* eggs as inoculum were pipetted into each pot at planting. Cotton seeds treated with Clothianidin plus *B. firmus* I-1582, and Abamectin as previously described, were used as standards. The untreated control included 1 ml of tap water per seed. Each microplot received water at 30 ml/min by an automated drip irrigation system adjusted throughout the season to run for 15 to 45 min twice a day, for a total of 450 to 1350 ml of water per microplot per day. At 48 DAP, one representative cotton plant from each microplot was removed for PH and Bio measurements. The *M. incognita* eggs were extracted from the root system as previously described and enumerated. At plant maturity, 142 DAP, seed cotton was handpicked, and yield was recorded as grams of seed cotton per microplot.

Trials in the field. The same strains and mixtures assessed in the microplot trials were also evaluated in field trials for their effect on early-season nematode population development, plant growth promotion, and yield enhancement in cotton. The experiments were established at PBU and at Prattville Agricultural Research Unit (PARU) in a sandy clay loam soil (64% sand, 10% silt, and 26% clay), Prattville, AL. Both fields were naturally infested with *M. incognita* and numbers of J2 were just at the detection level of 77 J2s per 100 cm³ of soil using the extraction technique as previously described. The experiment was arranged in a RCBD with 5 replications

Table 2. Efficacy of nine *Bacillus* plant growth-promoting rhizobacteria strains on plant height, biomass, and *Meloidogyne incognita* eggs/gr on cotton under greenhouse conditions at 45 DAP^{a,b}

Treatment	Scientific name	PH ^c	Dunnett's P					Dunnett's P					Dunnett's P				
			Clothianidin + <i>B. firmus</i>	Abamectin	Aldicarb	Water	Bio ^d	Clothianidin + <i>B. firmus</i>	Abamectin	Aldicarb	Water	Eggs/gr ^e	Clothianidin + <i>B. firmus</i>	Abamectin	Aldicarb	Water	
Bmo3	<i>B. mojavensis</i>	11.5	0.1391	0.0327	0.2625	0.9944	4.5	0.0854	0.9656	0.9929	0.9995	15.791	0.8793	0.0178	<0.0001	0.9316	
Bsa25	<i>B. safensis</i>	9.6	0.0055	0.0009	1.0000	0.9506	6.3	0.0212	0.4940	0.6013	0.7049	14.311	0.6254	0.0127	0.0001	1.0000	
Bsa26	<i>B. safensis</i>	10.6	0.0165	0.0024	0.8205	1.0000	4.4	0.1320	0.9945	0.9996	1.0000	14.789	0.6830	0.0087	<0.0001	0.9978	
Bssu2	<i>B. subtilis</i> subsp. <i>subtilis</i>	11.4	0.1168	0.0262	0.3030	0.9982	3.4	0.6943	1.0000	0.9998	0.9903	14.821	0.8849	0.0183	<0.0001	0.9268	
Bssu3	<i>B. subtilis</i> subsp. <i>subtilis</i>	12.4	0.5813	0.2230	0.0413	0.5017	4.5	0.0523	0.8865	0.9573	0.9905	18.163	0.8353	0.0145	<0.0001	0.9605	
Bve2	<i>B. velezensis</i>	11.5	0.1252	0.0286	0.2865	0.9971	4.5	0.0737	0.9470	0.9864	0.9986	7.825	1.0000	0.2167	0.0043	0.1225	
Bve12	<i>B. velezensis</i>	11.5	0.1404	0.0331	0.2603	0.9940	4.2	0.1542	0.9979	0.9999	1.0000	15.474	0.9701	0.0323	0.0002	0.7773	
Bve37	<i>B. velezensis</i>	9.0	0.0010	0.0001	1.0000	0.6093	3.9	0.4968	1.0000	1.0000	1.0000	21.514	0.9613	0.0545	0.0009	0.9733	
Bve40	<i>B. velezensis</i>	10.8	0.0731	0.0185	0.8411	1.0000	5.8	0.0190	0.4675	0.5729	0.6718	27.339	0.2840	0.0026	<0.0001	1.0000	
Control																	
Poncho/Votivo	Clothianidin + <i>B. firmus</i> I-1582	14.1	...	0.9996	0.0023	0.0405	2.3	...	0.6274	0.5187	0.3105	9.702	...	0.3217	0.0181	0.3400	
Avicta	Abamectin	14.7	0.9996	...	0.0004	0.0070	3.2	0.6274	...	1.0000	1.0000	1.815	0.3517	...	0.8740	0.0013	
Temik	Aldicarb	9.3	0.0023	0.0004	...	0.8122	4.0	0.5187	1.0000	...	1.0000	456	0.0181	0.8401	...	<0.0001	
Untreated control	Water	10.8	0.0341	0.0061	0.7270	...	3.8	0.2441	1.0000	1.0000	...	15.254	0.2687	0.0011	<0.0001	...	

^a Greenhouse trials were performed in plastic cone-tainers with mixed pasteurized soil and sand (60:40, v/v) for 45 days. The greenhouse trial was repeated twice and data were analyzed in SAS 9.4 using PROC GLIMMIX procedure at a significance level of $\alpha \leq 0.10$. Adjusted *P* values less than 0.10 indicated a significant effect. Adjusted *P* values were obtained by analyzing the data according to Dunnett's method. LS-means and adjusted *P* values were presented in the table.

^b DAP = days after planting.

^c PH = plant height (cm) at 45 DAP.

^d Bio = cotton plant biomass including shoot fresh weight (g) and root fresh weight (g) at 45 DAP.

^e Eggs/gr = *M. incognita* eggs per gram of root at 45 DAP.

for each treatment. The field plots were planted in two-row plots, 7 m long with 0.9-m row spacing. Blocks were separated by a 6-m alley. One hundred cotton seeds were planted in each row with an Almaco plot planter (Almaco, IA). The PGPR strains were standardized to 1×10^7 CFU/seed and applied as in-furrow sprays at 32.5 liter per hectare at planting. Two industry standards were used: seeds treated with Clothianidin plus *B. firmus* I-1582; or Abamectin as described previously. Tap water applied as an in-furrow spray was the untreated control at 32.5 liter/ha. At 40 DAP, four random representative cotton plants were removed from each plot. The same plant growth parameters evaluated in the microplots were also evaluated in the field. *Meloidogyne incognita* population density was determined by extracting eggs from four root systems per plot. Cotton was harvested mechanically with a cotton picker (Deere & Company, Moline, IL) at plant maturity which was near 150 DAP and seed cotton yield was recorded.

Statistical analysis. Data collected from in-vitro, greenhouse, microplot, and field trials were analyzed in SAS 9.4 (SAS Institute, Cary, NC) using the PROC GLIMMIX procedure. Dependent variables included J2 mortality, PH, Bio, *M. incognita* eggs/gr, and yield. Fixed effects were PGPR strains or nematicides treatments and the random effects included replication, test repeat, and location. Student panels were generated to determine the normality of the residuals. The PH, Bio, and eggs/gr data required a log-normal distribution transformation to satisfy the normal assumptions. LS-means were compared between the treatments, chemical standards Clothianidin plus *B. firmus* I-1582, Abamectin, Aldicarb, and the untreated control by Dunnett's method at significance levels of $P \leq 0.05$ or $P \leq 0.10$.

The LS-means are presented in the tables and adjusted *P* values are presented for statistical differences.

Results

Tests in vitro. The mortality percentage of *M. incognita* J2 ranged from 0.0 to 100% for the PGPR strains (662), with an average of 39%. Data presented are the results of 212 strains (33% of 662 strains) that had a greater percent mortality of *M. incognita* J2 than the untreated control (Table 1). Among all PGPR strains, 33% caused a significantly greater level of mortality of *M. incognita* J2 than the untreated control, 19% produced a significantly greater level of mortality percentage than the biological standard Clothianidin plus *B. firmus* I-1582, and 34% resulted in a statistically similar mortality percentage to Aldicarb ($P \leq 0.05$). Among all the strains, *Bacillus* spp. was the major genus initiating greater mortality percentage when compared with the other genera.

Trials in the greenhouse. In evaluations conducted in the greenhouse, plant height differences were observed (Table 2). Strain *B. subtilis* subsp. *subtilis* Bssu3 significantly increased plant height compared with Aldicarb ($P \leq 0.10$) (Fig. 1). Strain Bssu3 was the only strain not different from the Abamectin control; all other plants were shorter (Table 2). Strains *B. mojavensis* Bmo3, *B. subtilis* subsp. *subtilis* Bssu2, *B. subtilis* subsp. *subtilis* Bssu3, *B. velezensis* Bve2, and *B. velezensis* Bve12 were not different from the Clothianidin plus *B. firmus* I-1582 control with respect to plant height. Strains *B. mojavensis* Bmo3, *B. safensis* Bsa25, *B. subtilis* subsp. *subtilis* Bssu3, and *B. velezensis* Bve2 (Fig. 2) and Bve40 significantly increased plant

Fig. 1. Cotton plant height was increased from greenhouse trials at 45 DAP. Untreated control (left) and treatment with strain *Bacillus subtilis* subsp. *subtilis* Bssu3 (right).

Fig. 2. Cotton roots from greenhouse trials at 45 DAP. Untreated control (left) and treatment with strain *Bacillus velezensis* Bve2 (right).

biomass compared with the standard Clothianidin plus *B. firmus* I-1582 at 45 DAP ($P \leq 0.10$) (Table 2). All *Bacillus* strains supported similar numbers of *M. incognita* eggs/gr at 45 DAP as compared with the water control. None of the tested *Bacillus* strains reduced *M. incognita* eggs/gr similarly to the chemical standard Aldicarb. *Bacillus velezensis* strain Bve2 suppressed *M. incognita* eggs/gr at a level statistically equivalent to the Abamectin control (Table 2). Further, all nine *Bacillus* PGPR strains tested sustained levels of nematode eggs/gr statistically equivalent to the biological standard Clothianidin plus *B. firmus* I-1582 (Table 2).

Trials in microplots. None of the PGPR strains tested significantly affected either plant height or biomass in microplot tests 48 DAP as compared with the controls ($P \leq 0.10$) (Table 3). Plant heights ranged from 51.2 to 38.7 cm and biomass ranged from 108.0 to 58.5 g. In the microplot studies, *M. incognita* eggs/gr were reduced by *B. mojavensis* strain Bmo3, *B. subtilis* subsp. *subtilis* strain Bssu3, *B. velezensis* strain Bve2, and Mixture 2 (Abamectin + Bve2 (*B. velezensis*) + Bal13 (*B. altitudinis*)) at 48

DAP compared with the untreated control ($P \leq 0.10$) (Table 3). The *M. incognita* eggs/gr were statically similar to those recovered from Clothianidin plus *B. firmus* I-158. Strain Bve12 supported more *M. incognita* eggs/gr than the Abamectin standard ($P \leq 0.05$). At harvest, microplot yields ranged from 231 to 172 g per plot with no statistical differences between any of the biological or chemical treatments. The *B. velezensis* strain Bve12 resulted in the highest seed-cotton yield followed by the Mixture 2 and *B. velezensis* strain Bve2. These yields were statistically similar to the Clothianidin plus *B. firmus* I-158 and the Abamectin standards (Table 3).

Trials in the field. None of the PGPR strains tested significantly affected either plant height or biomass in field trials 40 DAP as compared with the controls ($P \leq 0.10$) (Table 4). Plant heights ranged from 25.4 to 19.9 cm and biomass ranged from 81.8 to 50.0 g. The *B. mojavensis* strain Bmo3 and Mixture 2 (Abamectin + Bve2 + Bal13) significantly reduced *M. incognita* eggs/gr on cotton at 40 DAP compared with untreated control ($P \leq 0.10$), which was similar

Table 3. Effect of six plant growth-promoting rhizobacteria strains and two mixtures on cotton plant height, biomass, *Meloidogyne incognita* eggs/gr at 48 DAP, and cotton yield in microplot trials at 142 DAP^a

Treatment	Scientific name	48 DAP ^b				48 DAP				48 DAP				142 DAP			
		Dunnett's <i>P</i> versus ($P \leq 0.05$)				Dunnett's <i>P</i> versus ($P \leq 0.05$)				Dunnett's <i>P</i> versus ($P \leq 0.05$)				Dunnett's <i>P</i> versus ($P \leq 0.05$)			
		PH ^c	Clothianidin + <i>B. firmus</i>			Water	Bio ^d	Eggs/gr ^e	Yield ^f	Water	Yield ^f	Water	Yield ^f	Clothianidin + <i>B. firmus</i>	Abamectin	Water	Yield ^f
			Abamectin	Water	Bio ^d												
Bal13	<i>B. altitudinis</i>	46.5	1.0000	0.9932	0.9874	95.1	1.0000	1.0000	0.9965	872	0.9933	0.1524	0.5332	185	0.9994	1.0000	1.0000
Bmo3	<i>B. mojavensis</i>	51.2	0.9988	0.6473	0.5630	108.0	0.8148	0.8738	0.6335	212	0.9983	0.8894	0.0459	199	1.0000	0.9980	0.9975
Bssu2	<i>B. subtilis</i> subsp. <i>subtilis</i>	38.7	0.6070	0.9974	1.0000	58.5	1.0000	0.9997	1.0000	409	1.0000	0.4690	0.1835	178	0.9949	1.0000	1.0000
Bssu3	<i>B. subtilis</i> subsp. <i>subtilis</i>	42.5	0.9646	1.0000	1.0000	65.3	0.9978	0.9928	1.0000	299	0.9730	0.9779	0.0231	190	0.9999	0.9999	0.9999
Bve2	<i>B. velezensis</i>	48.5	1.0000	0.9196	0.7918	85.4	0.9977	0.9995	0.9700	163	0.9928	0.9398	0.0340	215	1.0000	0.9494	0.9438
Bve12	<i>B. velezensis</i>	43.0	0.9810	1.0000	1.0000	67.4	1.0000	0.9999	1.0000	1,357	0.7459	0.0354	0.9340	231	0.9992	0.7555	0.7434
Mixture 1 ^g		43.8	0.9950	1.0000	0.9996	72.5	0.9996	0.9981	1.0000	913	1.0000	0.3256	0.2837	197	1.0000	0.9991	0.9988
Mixture 2 ^g		46.3	1.0000	0.9950	0.9687	65.9	1.0000	0.9999	1.0000	361	0.9884	0.9549	0.0301	229	0.9997	0.7915	0.7799
Control	Active ingredient																
Poncho/Votivo	Clothianidin + <i>B. firmus</i> I-1582	47.8	...	0.9575	0.9003	66.9	...	1.0000	1.0000	436	...	0.5022	0.1667	208	...	0.9829	0.9802
Avicta	Abamectin	42.3	0.9575	...	1.0000	81.3	1.0000	...	1.0000	69	0.5022	...	0.0022	173	0.9829	...	1.0000
Untreated control	Water	42.2	0.9494	1.0000	...	93.3	1.0000	...	1.551	1,551	0.1667	0.0022	...	172	0.9802	1.0000	...

^a Microplot trials were performed in 26.5 liter pots with a kalmia loamy sand soil. The microplot trial was repeated and analyzed in SAS 9.4 using PROC GLIMMIX procedure at a significance level of $\alpha \leq 0.05$. Adjusted *P* values less than 0.05 indicated a significant effect. Adjusted *P* values were obtained by analyzing data according to Dunnett's method. LS-means and adjusted *P* values were presented in the table.

^b DAP = days after planting.

^c PH = plant height (cm) at 48 DAP.

^d Bio = cotton plant biomass including shoot fresh weight (g) and root fresh weight (g) at 48 DAP.

^e Eggs/gr = *M. incognita* eggs per gram of root at 48 DAP.

^f Yield = grams of seed cotton yield handpicked at harvest.

^g Mixture 1 = strain Bve2 + strain Bal13; Mixture 2 = Abamectin + strain Bve2 + strain Bal13.

Table 4. Efficacy of six plant growth-promoting rhizobacteria strains and two mixtures on plant height, plant biomass, and *Meloidogyne incognita* eggs/gr at 40 DAP, and yield of cotton in a field production system at 150 DAP^a

Treatment	Scientific name	40 DAP ^b				40 DAP				40 DAP				150 DAP			
		Dunnett's <i>P</i> versus ($P \leq 0.05$)				Dunnett's <i>P</i> versus ($P \leq 0.05$)				Dunnett's <i>P</i> versus ($P \leq 0.05$)				Dunnett's <i>P</i> versus ($P \leq 0.10$)			
		PH ^c	Clothianidin + <i>B. firmus</i>			Water	Bio ^d	Eggs/gr ^e	Yield ^f	Water	Yield ^f	Water	Yield ^f	Clothianidin + <i>B. firmus</i>	Abamectin	Water	Yield ^f
			Abamectin	Water	Bio ^d												
Bal13	<i>B. altitudinis</i>	19.9	0.8818	0.9129	0.9793	58.9	0.9973	0.9999	1.0000	1,747	0.9961	0.9998	0.6523	3,902	1.0000	0.5795	0.4826
Bmo3	<i>B. mojavensis</i>	25.4	0.9997	0.9992	0.9879	81.8	0.9997	0.9952	0.9853	349	0.1023	0.6717	0.0126	4,235	0.9974	0.9823	0.1278
Bssu2	<i>B. subtilis</i> subsp. <i>subtilis</i>	22.6	1.0000	1.0000	1.0000	60.3	0.9989	1.0000	1.0000	1,358	0.8676	1.0000	0.3360	4,089	1.0000	0.8462	0.2211
Bssu3	<i>B. subtilis</i> subsp. <i>subtilis</i>	23.6	1.0000	1.0000	1.0000	67.0	1.0000	1.0000	1.0000	2,569	0.9998	0.5922	1.0000	4,204	0.9993	0.9622	0.1313
Bve2	<i>B. velezensis</i>	23.0	1.0000	1.0000	1.0000	65.5	1.0000	1.0000	1.0000	1,416	0.8906	1.0000	0.3486	4,415	0.9096	1.0000	0.0528
Bve12	<i>B. velezensis</i>	20.7	0.9681	0.9807	0.9983	50.0	0.9152	0.9749	0.9905	2,805	0.9867	0.3755	1.0000	4,396	0.9398	0.9999	0.0499
Mixture 1 ^g		20.5	0.9507	0.9681	0.9960	51.1	0.9351	0.9833	0.9944	2,191	1.0000	0.9172	0.9706	4,091	1.0000	0.8702	0.2403
Mixture 2 ^g		23.0	1.0000	1.0000	1.0000	77.9	1.0000	0.9997	0.9981	699	0.9364	0.9438	0.0454	3,582	0.9337	0.1532	0.9356
Control	Active ingredient																
Poncho/Votivo	Clothianidin + <i>B. firmus</i> I-1582	23.8	...	1.0000	1.0000	71.9	...	1.0000	1.0000	2,232	...	0.8926	0.9807	3,993	...	0.7291	0.3523
Avicta	Abamectin	23.5	1.0000	...	1.0000	67.8	1.0000	...	1.0000	1,419	0.8926	...	0.3509	4,563	0.7276	...	0.0199
Untreated control	Water	22.7	1.0000	1.0000	...	65.4	1.0000	...	...	2,889	0.9809	0.3521	...	3,186	0.3503	0.0198	...

^a Field trials were performed in two naturally infested fields in AL. Data were combined and analyzed in SAS 9.4 using PROC GLIMMIX procedure at significance level of $\alpha \leq 0.05$ for PH, Bio, and Eggs/gr and at a significance level of $\alpha \leq 0.10$ for cotton yield. Adjusted *P* values less than 0.05 or 0.10 indicated a significant effect. Adjusted *P* values were obtained by analyzing data according to Dunnett's method. LS-means and adjusted *P* values were presented in the table.

^b DAP = days after planting.

^c PH = plant height (cm) at 40 DAP.

^d Bio = cotton plant biomass including shoot fresh weight (g) + root fresh weight (g) at 40 DAP.

^e Eggs/gr = *M. incognita* eggs per gram of root at 40 DAP.

^f Cotton yield = seed cotton yield in kilogram/hectare at 150 DAP.

^g Mixture 1 = strain Bve2 + strain Bal13; Mixture 2 = Abamectin + strain Bve2 + strain Bal13.

to Clothianidin plus *B. firmus* I-1582 and Abamectin standards ($P \leq 0.10$) (Table 4). The *B. velezensis* strains Bve12 (Fig. 3) and Bve2 (Fig. 4) significantly increased seed-cotton yield compared with untreated control, and was similar to Clothianidin plus *B. firmus* I-1582 and Abamectin standards ($P \leq 0.10$) (Table 4).

Discussion

The results indicated that among all the PGPR strains, 33% caused a significantly greater level of mortality of *M. incognita* J2 than the untreated control and 34% caused statistically similar mortality to the level caused by Aldicarb ($P \leq 0.05$). *Bacillus* spp. was the primary genus causing greater mortality percentage of *M. incognita* J2 in the in-vitro tests. Further greenhouse, microplot, and field trials confirmed that specific strains of the *Bacillus* PGPR suppressed the population density of *M. incognita* in the greenhouse, microplot, and field evaluation systems, and increased seed cotton yield.

In vitro screening of the PGPR strains indicated that *Bacillus* spp. caused greater mortality of *M. incognita* J2 in vitro than other genera. Some strains of specific *Bacillus* spp. were previously reported to have nematocidal activity against plant-parasitic nematodes on different host

plants. Kloepper et al. (1992) reported that *B. megaterium* strain 1758 and *B. pumilus* strain 163 significantly reduced galls of *M. incognita* and cysts of *H. glycines* on soybean. Payne (1993) stated in a Bt patent that some strains of *B. thuringiensis* had nematocidal activity against nematodes including plant-parasitic nematodes *M. incognita* and *Aphelenchus avenae*. Siddiqui et al. (2001) reported that a *B. subtilis* strain isolated from the rhizosphere of *Helianthus annuus* had nematocidal activity on *M. javanica* in mungbean. Burkett-Cadena et al. (2008) found that *B. subtilis* strain GB03 and *B. velezensis* strain GB99 (BioYield, Gustafson LLC, Plano TX, USA) induced significant reductions in *M. incognita* eggs/gr, juvenile nematodes per cm³ of soil, and galls per plant on tomato. *Bacillus firmus*, the active ingredient of BioNem-WP (AgroGreen, Israel) was reported to control root-knot nematode on vegetables (Hallmann et al. 2009). In our study, we also found the specific strains of the species *B. pumilus*, *B. thuringiensis*, *B. subtilis*, *B. velezensis*, and *B. firmus* had nematocidal activity on *M. incognita* in our tests. In our trials, 17 different *Bacillus* species and subspecies, including *B. altitudinis*, *B. aryabhattai*, *B. cereus*, *B. galliciensis*, *B. lentus*, *B. methylotrophicus*, *B. mojavensis*, *B. mycoides*, *B. psychrosaccharolyticus*, *B. safensis*, *B. siamensis*, *B. simplex*, *B. subtilis* subsp. *inaquosorum*, *B. subtilis* subsp. *subtilis*, *B. tequilensis*,

Fig. 3. Cotton plants in Plant Breeding Unit (PBU) at 40 DAP. Untreated control (left) and treatment with strain *Bacillus velezensis* Bve12 (right).

Fig. 4. Cotton plants in PBU at 90 DAP. Untreated control (left) and treatment with strain *Bacillus velezensis* Bve2 (right).

B. toyonensis, *B. weihenstephanensis*, were found to have antagonistic activity against *M. incognita*. This is the first documentation of antagonistic activity by these *Bacillus* spp. to *M. incognita*.

The results from the greenhouse, microplot, and field experiments indicated that Bve2 (*B. velezensis*), Bmo3 (*B. mojavensis*), and Mixture 2 (Abamectin + Bve2 + Bal13) were relatively consistent in reduction of *M. incognita* eggs/gr, and *B. velezensis* strains Bve2 and Bve12 increased early plant growth and enhanced cotton yield. Many reports have shown that specific strains of PGPR or mixtures of PGPR strains can promote plant growth, reduce plant disease, and enhance yield with multiple hosts under greenhouse, microplot, or field conditions (Castillo et al. 2013; Jetiyanon and Kloepper 2002; Liu et al. 2016; Raupach and Kloepper 1998; Wei et al. 1996; Yan et al. 2002). Castillo et al. (2013) evaluated PGPR *B. firmus* GB-126 combined with *Paecilomyces lilacinus* 251 in commercial formulations in greenhouse, microplot, and field trials for the management of *Rotylenchulus reniformis* in cotton and reported that *R. reniformis* population density was decreased when exposed to *B. firmus* and *P. lilacinus* in the greenhouse, in the microplot at midseason, and in the field at harvest. Liu et al. (2016) found that specific PGPR strains Bve12 and Bve15 (*B. velezensis*), and Bmo3 (*B. mojavensis*), strain mixture-1 (Bve12 + Bmo3 + *Lysinibacillus macrolides* strain Lma1 + Bve15) and mixture-2 (mixture-1 + *B. safensis* strain Bsa27 + *B. pumilus* strain Bpu6 + *B. velezensis* strain Bve40) used in our studies also reduced black rot on Chinese cabbage caused by *Xanthomonas campestris* pv. *campestris* and increased marketable yield. Our studies added additional information to their findings that PGPR strains and mixtures of PGPR strains can promote early-season plant growth, increase yield, and reduce nematode numbers.

Mode of action of some PGPR strains has been studied. Mendoza et al. (2008) reported that mortality of sedentary and migratory endoparasitic nematodes *M. incognita*, *Radopholus similis*, and *Ditylenchus dipsaci* by *B. firmus* in in-vitro tests were closely associated with the production of bioactive secondary metabolites by the bacteria. Huang et al. (2010) demonstrated that PGPR strain *B. megaterium* YMF 3.25 significantly inhibited hatching of nematode eggs and reduced infection of *M. incognita* through production of nematocidal volatiles. They also confirmed that the nematocidal volatiles produced by the bacterium were mainly benzeneacetaldehyde, 2-nonanone, decanal, 2-undecanone, and dimethyl disulphide, which were active against juveniles and eggs at the concentration of 0.5 mmol, and that six other compounds also contributed to the nematocidal efficacy (Huang et al. 2010). Peng et al. (2011) tested three *B. thuringiensis* nematocidal crystal proteins Cry6Aa, Cry5Ba, and Cry55Aa against *M. incognita* and found that the combination of Cry6Aa and Cry55Aa caused significant synergistic toxicity against *M. incognita*. These reports indicated that the mode of action of the *Bacillus* PGPR strains with nematocidal activity is likely related to the production of bioactive secondary metabolites. Further research is needed to address the modes of action of the PGPR strains with nematocidal activity on *M. incognita*.

ISR elicited by *Bacillus* spp. against plant-parasitic nematodes is another important mode of action. Kloepper et al. (2004) summarized the published results and reported that specific strains of the species *B. velezensis*, *B. subtilis*, *B. pasteurii*, *B. cereus*, *B. pumilus*, *B. mycoides*, and *B. sphaericus* elicit significant reductions in the incidence or severity of various diseases, including root-knot nematode. The bacterial strains *B. sphaericus* B43 and *Rhizobium etli* G12 were reported to induce systemic resistance toward *M. incognita* on tomato as expressed in reduced juvenile penetration in the responder roots (Hauschild et al. 2000; Schäfer et al. 2006; Sikora et al. 2007). *Bacillus mojavensis* strain Bmo3 and *B. velezensis* strain Bve12, which were previously found to induce systemic resistance to black rot disease on Chinese cabbage and increase yield (Liu et al. 2016) were also found to reduce *M. incognita* population density and increase yield on cotton in our study. It is possible that the reduced *M. incognita* population density observed in our cotton trials could have resulted from induction of ISR by the PGPR strains Bmo3, Bve2, and Mixture 2 (Abamectin + Bve2 + Bal13), but further work is needed to test this.

In summary, *B. mojavensis* strain Bmo3, *B. velezensis* strains Bve2 and Bve12, and Mixture 2 (Abamectin + Bve2 + Bal13) are promising biological control agents which should be further evaluated for potential

use against plant-parasitic nematodes. These biological strains could potentially be alternatives to chemical nematicides or combined with chemical nematicides for the management of *M. incognita*.

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