RESEARCH/INVESTIGACIÓN

BIOLOGICAL CONTROL OF *ROTYLENCHULUS RENIFORMIS* ON SOYBEAN BY PLANT GROWTH-PROMOTING RHIZOBACTERIA

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ABSTRACT

Xiang, N., K. S. Lawrence, J. W. Kloepper, and P. A. Donald. 2018. Biological control of *Rotylenchulus reniformis* on soybean by plant growth-promoting rhizobacteria. Nematropica 48:116-125.

Rotylenchulus reniformis is the primary economic nematode pathogen of cotton and also causes significant economic losses on soybean production in the southern region of the United States. Two plant growth-promoting rhizobacteria (PGPR) strains, Bmo3 (*Bacillus mojavensis*) and Bve2 (*B. velezensis*), previously reported to reduce *Meloidogyne incognita* population density on cotton, were evaluated for potential biocontrol of *R. reniformis* on soybean in greenhouse and field trials. In greenhouse trials, strains Bmo3 and Bve2 significantly reduced total numbers of *R. reniformis* eggs at 45 days after planting (DAP) compared to the untreated control ($P \leq 0.05$). Strain Bmo3 significantly increased soybean plant biomass (shoot fresh weight and root fresh weight) at 45 DAP compared to the biological control product Poncho®/Votivo® (Clothianidin plus *B. firmus* I-1582) ($P \leq 0.05$). In soybean field trials, strain Bmo3 significantly reduced *R. reniformis* eggs/g root at 45 DAP compared to the untreated control ($P \leq 0.05$). Strain Bmo3 also had a statistically equivalent level of eggs/g root as the chemical standard abamectin ($P \leq 0.05$). Soybean yield was statistically similar among all the treatments over the two trials. PGPR strain Bmo3 showed biological control potential for *R. reniformis* on soybean.

Key words: *Bacillus*, PGPR, plant growth-promoting rhizobacteria, reniform nematode, *Rotylenchulus reniformis*, soybean

RESUMEN

Xiang, N., K. S. Lawrence, J. W. Kloepper, y P. A. Donald. 2018. Control biológico de *Rotylenchulus reniformis* en soja por rizobacterias promotoras del crecimiento de las plantas Nematropica 48:116-125.

Rotylenchulus reniformis es el patógeno principal del nematodo económico del algodón y también causa pérdidas económicas significativas en la producción de soja en la región sur de los Estados Unidos. Dos cepas de rizobacterias promotoras del crecimiento vegetal (PGPR), Bmo3 (*Bacillus mojavensis*) y Bve2 (*B. velezensis*), previamente reportadas para reducir la densidad de población de *Meloidogyne incognita* en algodón, fueron evaluadas para el posible control biológico de *R. reniformis* en la soja en invernadero y campo ensayos. En ensayos en invernadero, las cepas Bmo3 y Bve2 redujeron significativamente el número total de huevos de *R. reniformis* a los 45 días después de la siembra (DAP) en comparación con el control no tratado ($P \leq 0.05$). La cepa Bmo3 aumentó significativamente la biomasa de la planta de soja (peso fresco del tallo y peso fresco de la raíz) a 45 DAP en comparación con el producto de control biológico Poncho® / Votivo® (Clothianidin plus *B. firmus* I-1582) ($P \leq 0.05$). En ensayos de campo de soja, la cepa Bmo3 redujo significativamente los huevos de *R. reniformis* / g a 45 DAP en comparación con el control no tratado ($P \leq 0.05$). La cepa Bmo3 también tuvo un nivel estadísticamente

equivalente de huevos / g de raíz como el estándar químico abamectina ($P \leq 0.05$). El rendimiento de soja fue estadísticamente similar entre todos los tratamientos en los dos ensayos. La cepa BPR3 de PGPR mostró potencial de control biológico para *R. reniformis* en soja.

Palabras claves: *Bacillus*, nematodo reniforme, PGPR, Rhizobacteria promotora del crecimiento vegetal, *Rotylenchulus reniformis*, soja

INTRODUCTION

Rotylenchulus reniformis (Linford & Oliveira, 1940), the reniform nematode, is the primary nematode pathogen of economic importance in cotton production (Castillo *et al*., 2013) and also causes significant economic losses of soybean production individually or collectively with *Heterodera glycines* and *Meloidogyne incognita* in the southern region of the United States (Wrather and Koenning, 2009; Lee *et al*., 2015). *Rotylenchulus reniformis* became the most common plant-parasitic nematode on soybean in Alabama in 2008 and 2009 (Sikora *et al*., 2011). *Rotylenchulus reniformis* was first observed on cowpea in Hawaii in 1935 and Linford and Oliveira (1940) described the species. *Rotylenchulus reniformis* was first reported in the continental United States in Georgia in 1940 on turfgrass (Smith, 1940). In 1965, *R. reniformis* was observed on soybean in South Carolina (Fassuliotis and Rau, 1967), and since then, it has been reported in 11 southern states including Alabama, Arkansas, Florida, Georgia, Louisiana, Mississippi, Missouri, North Carolina, South Carolina, Tennessee, and Texas (National Cotton Council, 2017).

Nematicide application is an effective short-term method to manage *R. reniformis*. Although nematicides are registered for soybean, economics of soybean production often preclude their use. Biological control is one of the promising options for the management of *R. reniformis*. Three nematicides containing biologicals abamectin (Avicta®) (Syngenta, Greensboro, NC), Clothianidin plus *B. firmus* I-1582 (Poncho®/Votivo®) (Bayer CropScience, Raleigh, NC), and *Pasteuria nishizawae* - Pn1 (Clariva®) (Syngenta, Greensboro, NC) are now available on the market for management of plant-parasitic nematodes. More strategies need to be investigated to supplement the market.

Several fungi and bacteria have been reported with antagonistic activity against *R. reniformis*. Twelve fungal species associated with *R. reniformis* vermiform stages and eggs from cotton roots include *Arthrobotrys dactyloides*, *Aspergillus fumigatus*, *A. glaucus* group, *Cladosporium cladiosporioides*, *C. herbarum*, *Dactylaria brochophaga*, *Fusarium oxysporum*, *Purpureocillium lilacinus*, *Penicillium waksmanii*, *Phoma exigua*, *Torula herbarum*, and an unidentified basidiomycete (Castillo *et al*., 2010). Among these fungi, *P. lilacinus* has been studied most often. Reddy and Khan (1988) reported biological control activity of *P. lilacinus* on *R. reniformis* in tomato plants. Later, *P. lilacinus* was confirmed to have detrimental effects on *R. reniformis* population density under both greenhouse and field microplot conditions on tomato (Walters and Barker, 1994). A commercial product of *P. lilacinus* strain 251 (MeloCon® WG) was developed and found to be as effective as Vapam® (Amvac Chemical Corporation, Axis, AL) soil fumigant and significantly better than no treatment for management of root-knot and reniform nematodes in tomato (Schenck, 2004). The nematophagous fungus ARF (Arkansas fungi) was reported as an active parasite of *R. reniformis* with parasitism ranging from 48% to 79% after 10 d incubation in *in vitro* tests and reduced numbers of *R. reniformis* on cotton roots at 25 d after seedling emergence (Wang *et al*., 2004). *Pochonia chlamydosporia* was also reported to parasitize *R. reniformis* eggs and reduced numbers of *R. reniformis* on cotton root and in soil (Wang *et al*., 2005). In 2013, *Catenaria auxiliaxis* was reported to parasitize *R. reniformis* on cotton in Alabama (Castillo and Lawrence, 2013).

Bacterial strains within the genera *Pasteuria* and *Bacillus* have been reported to have antagonistic activity on *R. reniformis*. *Pasteuria* spp. were first reported to parasitize *R. reniformis* in cotton in the U.S. by Hewlett *et al*. (2009). Later, *Pasteuria* spp. were observed to infect and complete their life-cycle in juvenile, male, and female *R. reniformis* life stages (Schmidt *et al*., 2010). A strain of *B. thuringiensis* CR-371 reduced the population density of *R. reniformis* and root galls of *M. incognita* on tomato and pepper in field trials in Puerto Rico (Zuckerman *et al*., 1993). In

another study (Niknam and Dhawan, 2003), an isolate of *B. subtilis* (Bst) significantly reduced the number of *R. reniformis eggs* on tomato and induced systemic resistance in tomato against *R. reniformis*. Two isolates of PGPR, *Pseudomonas fluorescens* (Pfbv22) and *B. subtilis* (Bbv57) significantly reduced *M. incognita* and *R. reniformis* infestation in soil and tomato roots when applied as seed treatment and soil application. (Jonathan *et al*., 2009). Castillo *et al*. (2013) reported that cotton seeds treated with *B. firmus* (1.4×10^7 CFU/seed), an application of *P. lilacinus* 251 (0.3% vol/vol of water), and the combination of *B. firmus* and *P. lilacinus* 251 reduced the population density of *R. reniformis* on cotton in the greenhouse, microplots, and field trials at 30 DAP. Collectively, these reports demonstrated that *Bacillus* spp. has potential on *R. reniformis* management on different hosts. More work is needed to find PGPR strains with biocontrol potential specifically for *R. reniformis* on soybean.

The overall objective of this study was to evaluate two PGPR strains (*B. mojavensis* Bmo3 and *B. velezensis* Bve2) for their biocontrol potential on *R. reniformis* on soybean. The specific objectives were to evaluate the efficacy of these two strains for reduction of *R. reniformis* population density and plant growth promotion on soybean in the greenhouse and under field production systems.

MATERIALS AND METHODS

PGPR strains

Two PGPR strains (*B. mojavensis* Bmo3 and *B. velezensis* Bve2) were included in this study. The strains were originally isolated, identified, and stored by the phytobacteriology lab at Auburn University, Auburn, AL, and were previously reported to reduce population density of *M. incognita* on cotton (Xiang *et al*., 2017a) and *H. glycines* on soybean (Xiang *et al*., 2017b). The strains stored in 30% glycerol at -80°C were transferred to tryptic soy agar (TSA) (VWR, Radnor, PA) plates, and incubated at 35°C for 24 hr. Vegetative cells of each strain were suspended in 5 ml of sterile distilled water in 25-ml glass tubes, and the cell concentration was adjusted to log 7.0 CFU/ml.

Nematode inoculum

Rotylenchulus reniformis, originally isolated from two naturally infested fields near Belle Mina in north Alabama and Huxford in south Alabama and maintained on cotton plants 'Stoneville 4946GLB2' (Bayer CropScience, Raleigh, NC) in 500 cm^3 polystyrene pots in the greenhouse, served as inoculum in the greenhouse experiments. Eggs were extracted from cotton 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, and further separated from soil by sucrose centrifugation-flotation method (Jenkins, 1964). For trials conducted in the greenhouse, *R. reniformis* eggs were enumerated at 40× magnification using an inverted TS100 Nikon microscope and standardized to 2,000 eggs per cone-tainer as described for *M. incognita* by Xiang and Lawrence (2016).

Plant materials

Soybean (*Glycine max*) variety 'Asgrow AG 5935', treated with metalaxyl, fluxapyroxad, imidacloprid, and pyraclostrobin by the manufacturer (Monsanto, St. Louis, MO), known to be susceptible to *R. reniformis* (Lawrence *et al*., 2016) was used for the greenhouse and field experiments.

Greenhouse trials

Two PGPR strains (Bmo3 and Bve2) were evaluated in the greenhouse for their capacity to reduce *R. reniformis* population density and promote soybean plant growth. All experiments were conducted at the Plant Science Research Center (PSRC) located at Auburn University, Auburn, AL. Experiments were performed in 150 cm^3 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 Plant Breeding Unit located at E.V. Smith Research Center of Auburn University, near Tallassee, AL. Soil was steam pasteurized at 180°C for 90 min, and cooled for 24 hr, then the steam pasteurizing process was repeated. Two soybean seeds were

planted 2.5 cm deep in each cone-tainer. One ml of bacterial cell suspension (1×10^7 CFU/ml) was added to each seed at planting. For the nematicide controls, soybean seeds were treated with each compound following agricultural industry recommendations: 0.13 mg a.i./seed of Poncho®/Votivo® (Clothianidin plus *B. firmus* I-1582), or 0.15 mg a.i./seed of Avicta® (abamectin) (Syngenta, Greensboro, NC) prior to planting. All seeds for nematicide treatments were treated using a Gustafson table-top seed treater (Bayer CropScience, Raleigh, NC), mixed for 3 min in a 3.78 L stainless-steel bucket and allowed to air-dry before packaging (Schrimsher *et al*., 2014). One ml of tap water was added to the untreated control seeds. One ml of water containing 2,000 *R. reniformis* eggs was pipetted into each cone-tainer at planting. Experiments were arranged in a randomized complete block design (RCBD) with five replications, and the experiment was repeated twice. Soybean seedlings were thinned to one per cone-tainer after emergence. Plants were watered as needed. Supplemental light of 1,000 watts halide bulbs producing 110,000 lumens was supplied to maintain a day length of 14 hr per day. Greenhouse temperatures ranged from 21°C to 35°C. Experiments were terminated at 45 DAP when plant and nematode measurements were recorded. Plant measurements included plant height (PH), biomass (Bio) with shoot and root fresh weights (SFW+RFW). Nematode measurements were number of *R. reniformis* eggs per cone-tainer (Tegg) and *R. reniformis* eggs per gram of root (eggs/g).

Field trials

The same PGPR strains assessed in the greenhouse were evaluated in field trials for their effect on early-season nematode population density, plant growth promotion, and yield enhancement in soybean. The experiments were established in Tennessee Valley Research and Extension Center (TVREC), Belle Mina, AL, in 2016. The artificially infested field had a *R. reniformis* population density of 5,000 vermiform life stages per 100 cm^3 of soil at planting. The experiments were arranged in a RCBD with 5 replications for each treatment. Experiment was repeated at the same research station in the same year in an adjoining field. The field trials were arranged in two-row plots that were 7.6 m long with 0.76-m row spacing. Blocks were separated by a 6.1-m alley. One hundred seventy-five soybean seeds were planted in each row with an Almaco plot planter (Almaco, IA). The PGPR treatments were applied as in-furrow sprays standardized to 1×107 CFU/seed and applied at 32.5 L/ha at planting. Seeds treated with Clothianidin plus *B. firmus* I-1582 and abamectin as previously described were included as industry standard controls. Tap water applied in-furrow was used as untreated control. All plots were maintained with standard herbicide, insecticide, and fertility production practices throughout the season as recommended by the Alabama Cooperative Extension System. At 45 DAP, four random soybean plants were carefully dug from each plot and transported to the laboratory. The same plant growth parameters evaluated in the greenhouse trials were evaluated in the field trials. *Rotylenchulus reniformis* population density was determined by extracting eggs from the roots as described previously. Soybeans were harvested mechanically with an Almaco plot harvester (Almaco, IA) at plant maturity approximately 160 DAP and yield recorded and adjusted to 13% moisture content.

Statistical analysis

Data collected from greenhouse and field trials were analyzed in SAS 9.4 (SAS Institute, Cary, NC) using the PROC GLIMMIX procedure. Dependent variables included plant height (PH), biomass (Bio), number of *R. reniformis* eggs per plot (Tegg), *R. reniformis* eggs per gram of root (egg/g) and yield. Fixed effects were PGPR strains or nematicides treatments and the random effects included replication and repeat in time. Student panels were generated to determine the normality of the residuals. A square root transformation was required for the Tegg and eggs/g data to satisfy the normal assumptions. LS-means were compared between the treatments, commercial standards Clothianidin plus *B. firmus* I-1582, abamectin and the untreated control by Tukey Kramer's method at a significance level of $\alpha = 0.05$. LS-means presented in the figures with different letters indicate a significant difference.

RESULTS

Greenhouse trials

In the greenhouse trials, both PGPR strains significantly reduced total numbers of *R. reniformis* eggs on soybean roots at 45 DAP as compared to the untreated control ($P \leq 0.05$) (Fig. 1). Strain Bmo3 significantly increased plant biomass (shoot fresh weight and root fresh weight) as compared to Clothianidin plus *B. firmus* I-1582 ($P \leq 0.05$) (Fig. 2).

Field trials

In the field trails, both strains Bmo3 and Bve2 reduced total *R. reniformis* eggs on soybean roots at 45 DAP as compared to the untreated control (Fig. 3). Bmo3 significantly reduced *R. reniformis* eggs/g root at 45 DAP as compared to the untreated control and had an equivalent level of eggs/g root as abamectin ($P \leq 0.05$) (Fig. 3). Plant height and biomass at 45 DAP (data not shown) and soybean yield at harvest (Fig. 4) were similar among all the treatments.

DISCUSSION

The results demonstrated that PGPR Bmo3 consistently reduced *R. reniformis* population density on soybean in both greenhouse and field trials and promoted early soybean growth at 45 DAP in the greenhouse trials. Both PGPR strains tested in this study (Bmo3 and Bve2) were previously reported to reduce *M. incognita* eggs/g root on cotton, and Bve2 also enhanced cotton yield (Xiang, 2016). In a previous study (Liu *et al*., 2016) Bmo3 (previously called AP209) significantly reduced numbers of lesions of black rot caused by *Xanthomonas campestris* on Chinese cabbage and enhanced marketable yield. The current study added additional information to the finding that PGPR Bmo3 can promote early plant growth (Fig. 5) and reduce *R. reniformis* population density at an early plant growth stage on soybean.

Strains from several *Bacillus* spp. have been reported to reduce the population density of *R. reniformis* including *B. thuringiensis* on tomato and pepper (Zuckerman *et al*., 1993), *B. subtilis* on tomato (Niknam and Dhawan, 2003; Jonathan *et al*., 2009), and *B. firmus* on cotton (Castillo *et al*., 2013). Our study indicated that strains of two additional *Bacillus* species, *B. mojavensis and B. velezensis*, can reduce the population density of *R. reniformis* on soybean. Future studies should evaluate the effective strains on multiple crops with different nematode genera.

Several commercial products have been developed based on *Bacillus* spp. for management

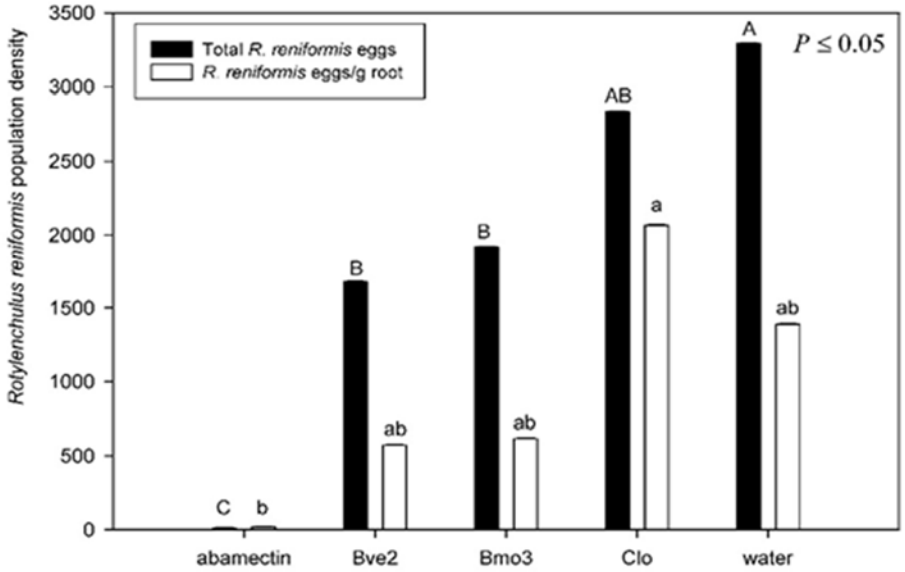

Fig. 1. Effect of abamectin (ai of Avicta®), Bve2 (*Bacillus velezensis*), Bmo3 (*B. mojavensis*), Clo (Clothiandin plus *B. firmus* I-1582, ai of Poncho®/Votivo®), and water (untreated control) on average total number of *Rotylenchulus reniformis* eggs from the roots and eggs/g of root on soybean at 45 DAP in greenhouse trials.

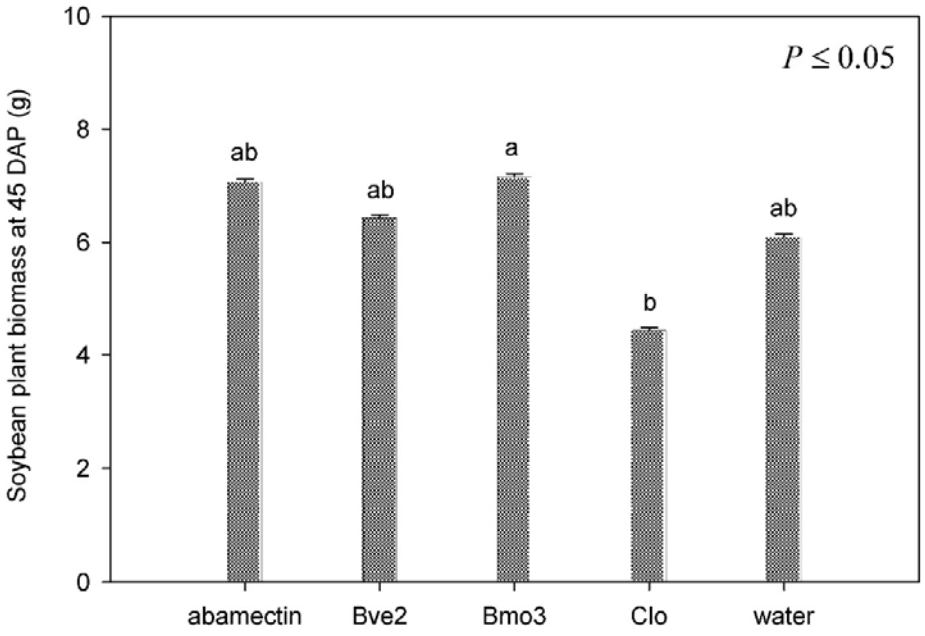

Fig. 2. Effect of abamectin (ai of Avicta®), Bve2 (*Bacillus velezensis*), Bmo3 (*B. mojavensis*), Clo (Clothiandin plus *B. firmus* I-1582, active ingredient of Poncho®/Votivo®), and water (untreated control) on soybean plant biomass (shoot fresh weight + root fresh weight) at 45 DAP in greenhouse trials.

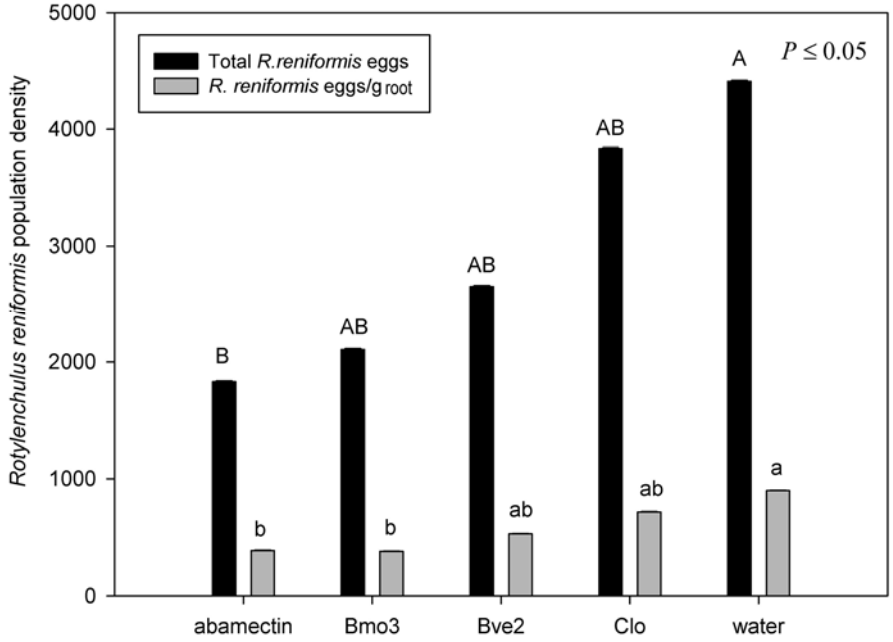

Fig. 3. Effect of different treatments on *R. Reniformis* population density in soybean at 45 DAP in the field trial. The treatments are abamectin (active ingredient Avicta), Bmo3 (*B. mojavensis*), Bve2 (*B. velezensis*), Clo (Clothianidin plus *B. firmus* I-1582, active ingredient Poncho-Votivo), and water (untreated control). LS-means were compared by Tukey's method at significance level of 0.05. Different letters mean significantly different.

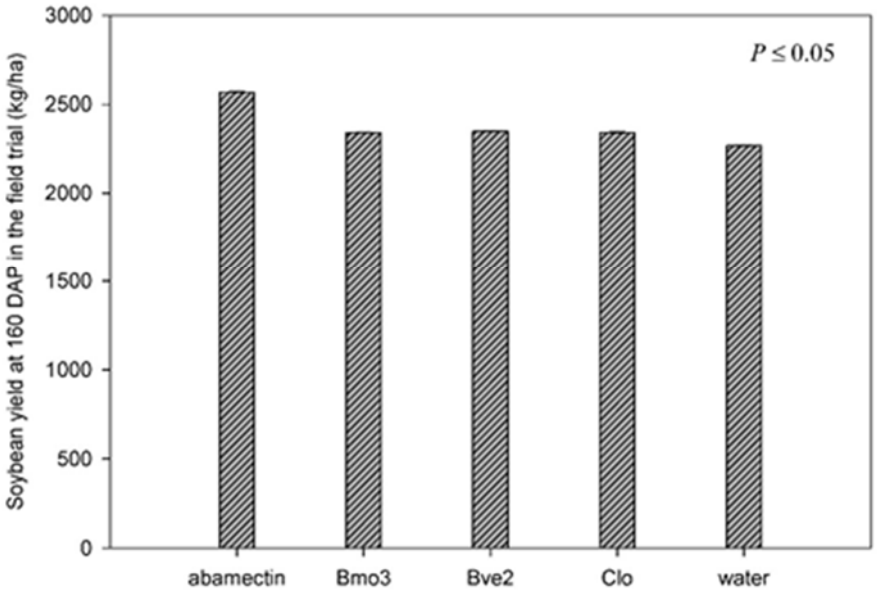

Fig. 4. Field trial effects of abamectin (ai of Avicta®), Bve2 (*Bacillus velezensis*), Bmo3 (*B. mojavensis*), Clo (Clothiandin plus *B. firmus* I-1582, ai of Poncho®/Votivo®), and water (untreated control) on soybean yield at plant maturity.

Fig. 5. Visual differences of soybean plants (Asgrow 5935) in the untreated control (CK left), abamectin treated (middle), and biological Bmo3 (*Bacillus mojavensis*) treated (right) TVREC at 37 days after planting (above) and 80 days after planting (below).

of plant-parasitic nematodes, including the reniform nematode. VOTiVO®, *B. firmus* GB-126, by Bayer CropScience was developed as a seed treatment for the control of multiple plant-parasitic nematodes attacking 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. was developed for management of plant-parasitic nematodes in vegetable and fruit production systems (Askary, 2015). BioSafe-WP, BioNem-WP, and Chancellor, products containing *B. firmus*, were developed by Agro Green (Israel) for management of plant-parasitic nematodes (Askary, 2015). Nemix, *Bacillus* spp. was developed by Chr. Hansen (Brazil) for management of plant-parasitic nematodes (Askary, 2015). At present, VOTiVO® is the key biological product containing *Bacillus* spp. for the management of plant-parasitic nematodes in row crops in the United States.

Our study indicated that Bmo3 has displayed promising biological control potential on *M. incognita* (Xiang, 2016) and *R. reniformis*. Future studies are needed to determine the mode of action for Bmo3. Studies with other *Bacillus* spp. strains for management of plant-parasitic nematodes have identified two main modes of action, including production of various bioactive secondary metabolites to kill or paralyze the plant-parasitic nematodes (Mendoza *et al*., 2008; Huang *et al*., 2010; Peng *et al*., 2011) and elicitation of induced systemic resistance (ISR) (Kloepper *et al*., 2004; Schäfer *et al*. 2006; Sikora *et al*. 2007; Schrimsher, 2013). No reports were found on the mechanism of *Bacillus* spp. on *R. reniformis* in soybean plants.

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