EVALUATION OF *BACILLUS FIRMUS* (VOTIVO®) AND *PAECILOMYCES LILACINUS* STRAIN 251 (NEMOUT®) FOR THE BIOCONTROL OF RENIFORM NEMATODE *ROTYLENCHULUS RENIFORMIS*.

Juan David Castillo
Kathy S. Lawrence
Joseph W. Kloepper
Entomology and Plant Pathology Department
Auburn University
Auburn, AL

Abstract

Research in biological control alternatives for cotton nematodes is required due to the restriction in the use and rate of chemical nematicides. The objective of this study was to evaluate the commercial biocontrol products *Bacillus firmus* and *Paecilomyces lilacinus* 251 on cotton plants grown in autoclaved field soil under greenhouse conditions. *Bacillus firmus* was evaluated as a seed treatment at three different rates: 0.0071, 0.071, and 0.71 mg of spores per seed. *Paecilomyces lilacinus* was evaluated 0.1, 0.5, and 1 gr/100 ml. Data was recorded every five days until day thirty. Variables measured were plant height, shoot and root weight, females and eggs per gram of root, and vermiforms in 480 grams of soil. Results for *B. firmus* trial indicate no phytotoxicty was observed. Nematode numbers were affected. At the highest rate of *B. firmus,* 0.71 mg of spores per seed, numbers of reniform vermiforms ($P \leq 0.01$), females ($P \leq 0.01$), and eggs per gram ($P \leq 0.01$) of root were lower in compared to the control and the lower rates of *B. firmus* at 30 DAP. *Paecilomyces lilacinus* 251 did not affect plant growth when *R. reniformis* was present ($P \leq 0.88$). The highest rate of *P. lilacinus,* 1 gr/100 ml, resulted in a consistent reduction in reniform females and eggs per gram of root from 15 to 30 DAP ($P \leq 0.01$); a reduction in reniform vermiforms occurred at 30 DAP ($P \leq 0.01$). Results indicate these two biocontrol agents to do have potential in management options for *R. renifomis*.

Introduction

Rotylenchus reniformis is reducing the cotton production in the southeastern states of the U.S (Robinson, 2007). Current management is based on crop rotation and use of chemical nematicides. The Environmental protection agency (EPA) has restricted the use of Aldicarb that is the most common nematicide used in cotton production. Aldicarb application rate for cotton crop is restricted to 7 lb/acre, and will be retired from the market in August 31st, 2018 (Bayer, 2010). Therefore there is a need to explore nematode management alternatives such as biocontrol.

Currently there are two biocontrol commercial products that can be used on cotton crops for nematode control. *Bacillus firmus* is a bacterium originally isolated on Israel, and has been reported reducing *Radopholus similis*, *Meloidogyne incognita*, and *Ditylenchus dipsaci* under *in vitro* conditions (Mendoza *et al.*, 2008). This bacterium is commercialized as a seed treatment for cotton under the name of Votivo®.

Paecilomyces lilacinus is a widely studied nematode-egg-parasite fungus that has been reported reducing eggs of root-knot nematode (Freitas et al.,1995), and reniform nematode (Walters and Barker, 1994). The commercial formulation (NemOut®) contains *P. lilacinus* strain 251and of this fungus is a wettable powder. The objective of this study was to evaluate the commercial biocontrol products *Bacillus firmus* and *Paecilomyces lilacinus* 251 on cotton plants grown in autoclaved field soil under greenhouse conditions.

Materials and Methods

Two trials were conducted under greenhouse conditions. In the first trial, *B. firmus* was evaluated as a seed treatment at three different rates. The cotton plants (cv.ST5458B2RF) were grown in a silty clay loam (sand-silt-clay: 17.5-51.3-31.2%) autoclaved field soil (Nitrogen: 0.16%, organic matter: 2.2, pH 7.24). In the *B. firmus* trial seed treatments were: *1)* Untreated seed, *2)* Imidacloprid (500 g ai/100kg), *3) B. firmus* (0.0071 mg Al/seed), *4) B. firmus* (0.071 mg Al/seed), and *5) B. firmus* (0.71 mg Al/seed). In the second trial, *P. lilacinus* was evaluated in a

wettable powder formulation, were each gram of the product contains $1x10^{10}$ spores of the fungus. Treatments were: *1)* Control without nematodes, *2)* Control with nematodes, *3) P. lilacinus* (0.1 gr/100 ml), *4) P. lilacinus* (0.5 gr/100 ml), and *5) P. lilacinus* (1 gr/100 ml).

The two trials had an experimental design in a randomized complete block design (RCBD) with five replications per treatment. Data were analyzed in SAS 9.1 (SAS Institute Inc.) using GLIMMIX procedure at the ($P \leq 0.05$) level of significance. Each trial was repeated twice for a total of 300 experimental units per trial.

Variables measured were plant height, shoot and root weight, females and eggs per gram of root, and vermiforms in 500 cc of soil. Data was recorded every five days until day thirty. Average temperatures were plants were grown was 29˚C. Moisture in soil was kept between 40-60% of the maximum water holding capacity, to warranty that all the experimental units receive the same amount of water. The presence of *B. firmus* on the cotton seed was confirmed culturing the treated seed on Triptic Soy Agar (TSA) with pH 8.0 and record the growth after 16 hours. The viability of spore germination of *P. lilacinus* was estimated culturing the fungus on Potato Dextrose Agar (PDA) and recorded the spores geminated after 12 hours.

The nematodes were extraction from the soil by combined gravity screening and centrifugation-flotation method. Eggs were extracted from the fresh root systems using the NaOCl method, and then were stained with fuschin acid to count the females invading the root. Vermiform life stages and eggs were counted under an inverted TS100 Nikon microscope, and females in the root under compound microscope Nikon SMZ800.

Results

In the *B. firmus* trial, the seed treatment with the highest rate of 0.71 mg of spores per seed supported more plant growth after 16 hours. The lower seed treatment rates produced no differences in plant height ($P \leq 0.49$), shoot weight ($P \leq 0.77$), or root weight ($P \leq 0.62$). Nematode numbers were affected. The highest rate of 0.71 mg of spores per seed of *B. firmus* reduced the number of females per gram of root at 20 and 30 DAP ($P \leq 0.01$) (Figure 1A). Additionally, the number of eggs per gram of root was reduced 15, 20, and 30 DAP by the 0.71 mg of spores per seed highest rate (Figure 1B). Numbers of vermiforms per 500 cc of soil was consistently reduced during the whole trial (Figure 1C). Seeds treated with the insecticide Imidacloprid show no reduction of females, eggs or vermiforms.

Paecilomyces lilacinus produced no phytotoxicity and no statistical differences of plant growth were observed ($P \leq 0.88$). The three rates, 0.1, 0.5, and 1 gm/100 ml had a consistent reduction in reniform females and eggs per gram of root from 15 to 30 DAP ($P \leq 0.01$); however 30 DAP, the highest rate of 1 gm/100 ml was the only one that reduced females and eggs per gram of root (Figure 2A). The eggs per gram of root were lower from 15 to 30 DAP with the highest rate (Figure 2B). Vermiforms per 500 cc of soil were only reduced at 25 and 30 DAP (Figure 2C).

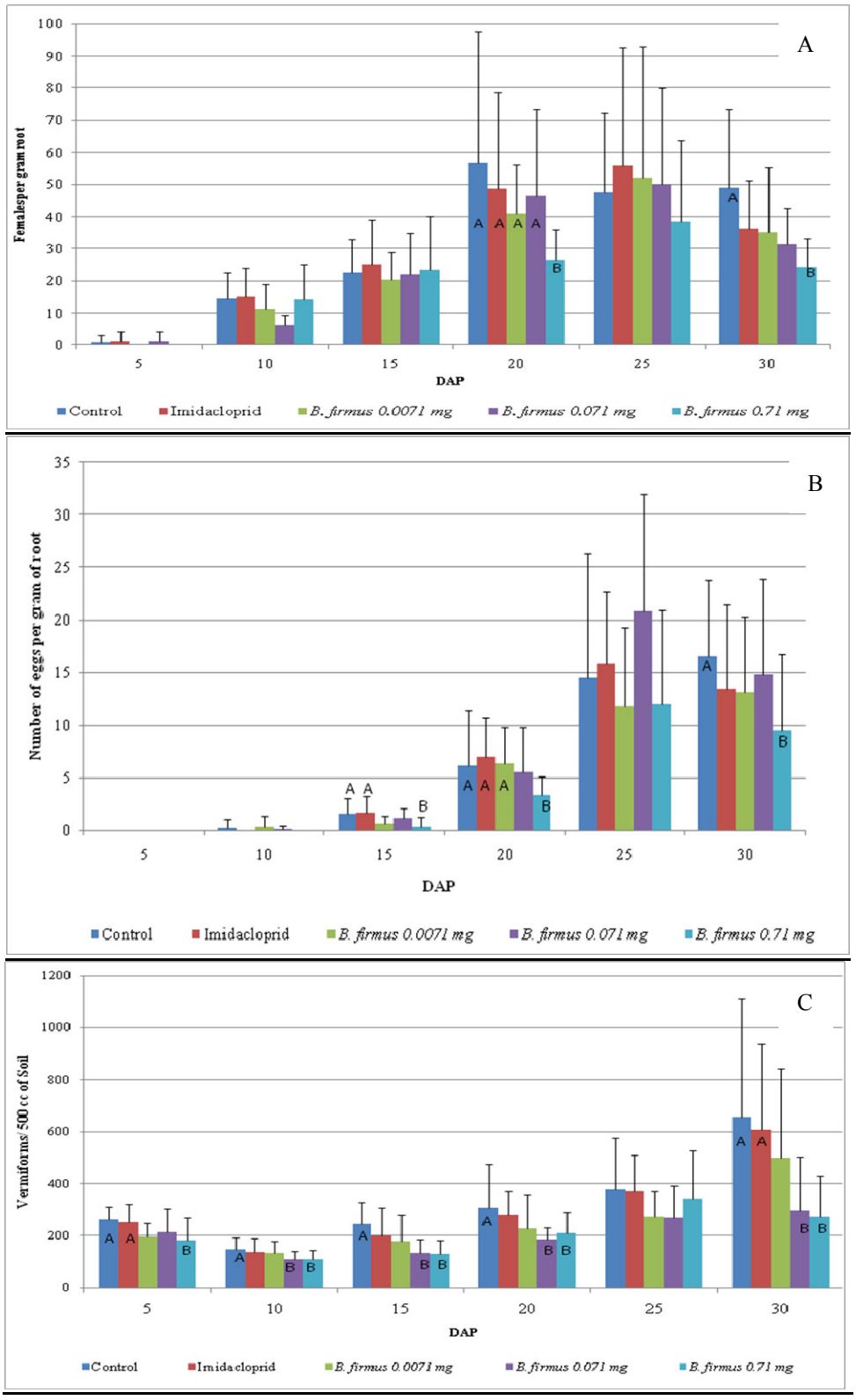

Figure 1. *Bacillus firmus* trial. (a) Number of females per gram of root; (b) Number of eggs per gram of root; and (c) Number of vermiforms per 500 cc of soil.

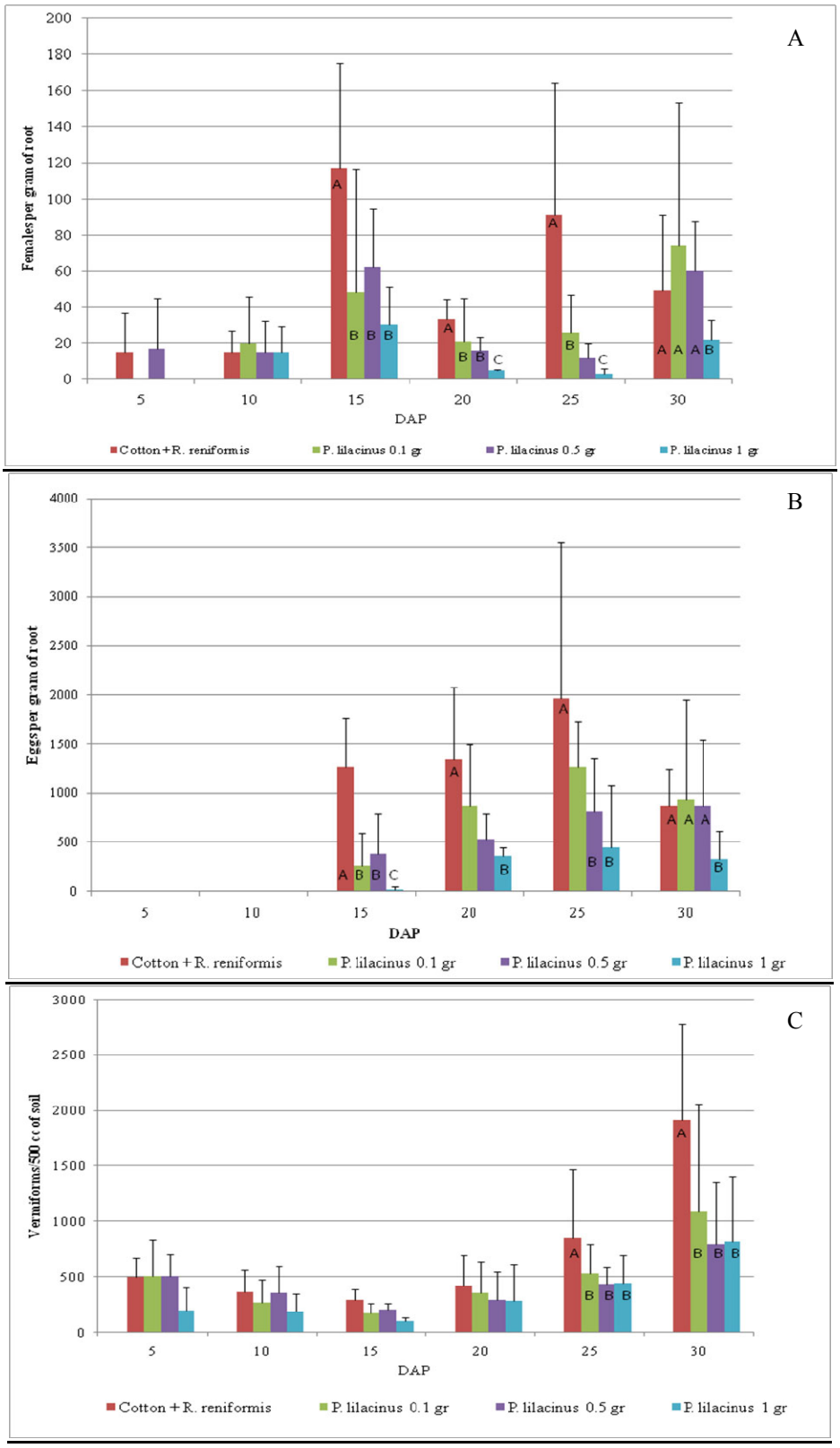

Figure 2. *Paecilomyces lilacinus* trial. (a) Number of females per gram of root; (b) Number of eggs per gram of root; and (c) Number of vermiforms per 500 cc of soil.

Conclusions

Commercial formulations of the biocontrol agents *B. firmus* and *P. lilacinus* reduced *R. reniformis* population 30 DAP. *Bacillus firmus* reduced *R. reniformis* numbers of eggs and vermiforms, therefore the reduction 20 and 30 DAP of females per gram of root is a consequence of the reduction in egg produced at 15 DAP and the vermiforms hatched from those eggs at 20 DAP. *Paecilomyces lilacinus* reduced numbers of eggs and females at 30 DAP. Vermiform stage of the nematode population was only reduced on 25 and 30 DAP what suggests that the reduction of this life stage is a consequence of the reduction of eggs during the initial days of the study. Both biological commercial indicated the potential to protect the cotton root in the first 30 days in autoclaved field soil. However is necessary now to evaluate them on non-autoclaved cotton field soil to test if they still provide control under the same conditions.

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