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GREENHOUSE EVALUATION OF EXPERIMENTAL HARPIN-BASED SEED AND FOLIAR TREATMENTS ON ROOT KNOT NEMATODES IN COTTON

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Abstract

Harpin proteins activate natural stress-defense responses in plants that increase plant vigor and result in better overall plant health. Root-knot nematode (RKN), *Meloidogyne incognita*, is an important nematode pest of many crop species including cotton. A study was initiated to examine the effects of harpin proteins on cotton infected with nematodes. Replicated tests were established in Alabama, Arkansas, Mississippi, and South Carolina. An independent University or Extension scientist conducted each test. The harpin proteins were applied to cottonseed (EBC-151ST and EBC-152) and to foliage (EBC-351A) at two weeks after the two-leaf stage of cotton variety PM1218. All plants were grown in a greenhouse or growth room environment. At planting, each pot was inoculated with 2,000 eggs of *M. incognita*. Trials were terminated at approximately 45 days after initiation. Plant measurements, root galling, numbers of nematode eggs and juveniles were recorded. EBC-151ST and EBC-152 seed treatments and EBC-351A foliar sprays of harpin protein onto cotton averaged less root galling and fewer numbers of *M. incognita* eggs and juveniles compared with untreated control plants. As with previously reported findings, these results suggest that harpin proteins may be useful tools in reducing numbers of nematodes infesting cotton.

Introduction

Harpins are naturally occurring proteins from a novel group of compounds first reported from *Erwinia amylovora* (Wei et al., 1992). Harpin proteins, including harpin$_{Ea}$ and harpin$_{\alpha\beta}$, elicit the expression of genes involved in the hypersensitive response and plant growth enhancement and activate an induced systemic defense response (Wei & Beer, 1996). This response has been associated with enhanced resistance in plants to pathogens and certain other pests. A consequence of these discoveries is the development of commercial Plant Health Regulator products containing harpin proteins, such as **Messenger® STS**, **N-HIBIT™**, **Mighty Plant™**, and **ProAct™**.

Harp-N-Tek™ is the brand name for technologies originating from harpin proteins. Harpin$_{Ea}$ and other harpin proteins activate the natural stress-defense and growth responses in plants that increase plant growth, stamina, and vigor, improve overall plant health, and can lead to improved output quality, increased marketable yields, and enhanced shelf-life. Other desirable attributes of Harp-N-Tek products include low use rates, rapid degradation in the environment, little or no dietary exposure, negligible toxicity, ease of application, and mass production using simple, environmentally friendly, and cost-effective water-based fermentation technology. Harp-N-Tek products are formulated with low risk inert ingredients.

In many parts of the world, plant-parasitic nematodes are a major production problem for cotton (*Gossypium hirsutum* L.). Root-knot nematode, *Meloidogyne incognita* (Kofoid & White) Chitwood, can be a major production constraint (Thomas & Kirkpatrick, 2002). Foliar application of harpin$_{Ea}$ to field-grown cotton where a significant

M. incognita population was present resulted in improved seed cotton yield in Arkansas (French, 2001). The primary objective of this study was to investigate if seed and foliar treatments with harpin protein reduce reproduction of *M. incognita* on cotton.

Materials & Methods

Design. In four greenhouse and growth chamber experiments, seed treatments with EBC-151ST and EBC-152 and foliar applications with EBC-351A were evaluated for effects on reproduction of *M. incognita* and growth of cotton. The experimental design of each trial was completely randomized, and all treatments were replicated 10 times in each experiment. Two to three cottonseeds were planted into 10.2 cm-d clay pots, and fungicide and nematicide treatments were not applied at planting. Nematodes (*M. incognita* host race 3) were obtained from tomato stock cultures by NaOCl extraction (Hussey & Barker, 1973). At planting, pots were infested by pipetting 2×10^3 eggs into three holes (0.5 cm-d × 2.5 cm deep) around the seedling. At five days after planting, emerged plants were thinned to one seedling per pot. Plants were watered daily and fertilized as needed for vigorous growth. Trial locations and cooperators are summarized in Table 1. Experiment 4 is ongoing; consequently, findings will be reported only from experiments 1-3.

Treatments and Application. Samples of experimental harpin protein formulations, EBC-151ST, EBC-152, and EBC-351A were obtained from Eden Bioscience Corporation (Bothell, WA). At the research lab of G. Lawrence located on the North Farm, Mississippi State, MS, cotton cultivar Paymaster 1218 seed were treated with EBC-151ST and EBC-152 seed treatments at 3 oz per cwt. EBC-151ST was mixed into distilled water and then a food grade dye was added. Cottonseed were mixed with the EBC-151ST solution at a rate of 15 ml per kg of cottonseed. After seeds were allowed to dry, seed samples were packaged and shipped to each trial cooperator. At each trial location, cooperators treated cottonseed with EBC-152, which is used as a dry seed treatment. A measured amount (by weight) of cottonseed was thoroughly mixed with EBC-152. At two weeks after the two-leaf cotton stage, select pots were treated with EBC-351A. Foliar treatments with EBC-351A were prepared in distilled water, and applications were made in *M. incognita* experiments using a CO_2 pressurized backpack sprayer set to deliver 94.5 liters/ha total volume. Pots to be sprayed were moved and sprayed in a separate area, and cotton foliage was allowed to dry before returning pots to the growth chamber or greenhouse.

Measurements and Analysis. At the completion of each test, plants and root systems were removed carefully from the pots and separated from the soil. A root galling index was reported as 0 = 0, 1 =1 to 2, 2 = 3 to 10, 3 = 11 to 30, 4 = 31 to 100, 5 = >100 galls/root system. Plant measurements and root dry weights were recorded. Numbers of second stage juveniles were assayed from the soil in each pot by semi-automatic elutriation (Byrd et al., 1976) and centrifugal flotation (Jenkins, 1964). Twenty egg masses were selected arbitrarily, collected in small vials, and processed with NaOCl to determine the number of eggs per female. Each root system was then processed with NaOCl to determine total eggs per plant. Analysis of variance was conducted on all data, and means were compared by Duncan's Multiple Range Test at P=0.05). Differences between treatments and the untreated control were calculated as percent reduction.

Results & Discussion

Key Findings to Date:

- Differences in plant growth measurements among treatments were small (Table 1).
- Gall ratings were 11% to 16% lower for harpin treatments compared with the untreated control (Table 3). For example, in experiment 1 all treatments had a lower gall rating than the control, and three treatments were significantly lower than the control (Figure 1).
- Based on numbers eggs per 500 cc and eggs per root weight, reproduction of *M. incognita* was reduced 23% to 66% on treated plants compared with plants that did not receive harpin treatment (Table 3).
- Similarly, harpin-treated plants averaged 18% to 66% fewer juveniles per 500 cc in comparison with the untreated control. In experiment 3, numbers of juveniles per 500 cc were highly significant (P=0.0001),and all treatments averaged considerably fewer juveniles than the control (Figure 2). Reductions in juveniles per 500 cc were consistent for most treatments tested, except EBC-152 (Figure 3).

Conclusions

With three of four experiments completed, EBC-151ST and EBC-152 seed treatments and EBC-351A foliar sprays of harpin protein onto cotton adversely influenced *M. incognita* infesting cotton roots. Harpin treatments to seed and foliage reduced root galling and numbers of root knot nematode eggs and juveniles. A decrease in the fecundity of female root-knot nematodes in plants treated with harpin as well as a reduction in numbers of juveniles indicate that challenge of the plant by harpin proteins could result in a change in the suitability of cotton as a host for the nematode.

These results parallel previously reported findings with harpins evaluated in greenhouse trials (Kirkpatrick et. al 2003, Kirkpatrick et. al 2005), which suggested that harpins may be useful in reducing damage to cotton due to nematodes.

Acknowledgment

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Table 1. Summary of greenhouse trial locations investigating EBC-151ST and EBC-152 Seed Treatments and EBC-351A, 2004-2005.

	Experiment 1	Experiment 2	Experiment 3	Experiment 4
Location	Auburn, AL	Hope, AR	Starkville, MS	Blackville, SC
Cooperator	K. M. Lawrence	T. L. Kirkpatrick	G. W. Lawrence	J. D. Mueller

Table 2. Measurements of cotton plants growing in soil infested with *Meloidogyne incognita*, 2004 and 2005.

Treatment	Plant Height (in)[1/]	Nodes / Plant[1/]	Dry Root Weight (g)[1/]
EBC-151ST	23	11	0.83
EBC-151ST fb EBC-351A	23	10	0.75
EBC-152	22	11	0.61
EBC-152 fb EBC-351A	22	11	0.70
EBC-351A	24	11	0.68
CONTROL	22	11	0.70

[1/] Average across all trials.

Table 3. Influence of harpin treatments on reduction of *Meloidogyne incognita* root gall rating, eggs, and juveniles, 2004 and 2005.

	Percent Reduction compared with Untreated Control[1/]			
Treatment	**Gall Rating**	**Eggs / 500 cc**	**Eggs / Root Wt**	**Juveniles / 500 cc**
EBC-151ST	15	23	30	51
EBC-151ST fb EBC-351A	16	66	39	55
EBC-152	12	32	23	18
EBC-152 fb EBC-351A	11	50	37	52
EBC-351A	15	61	52	66

[1/] Calculated averages using percent reduction from each experiment.

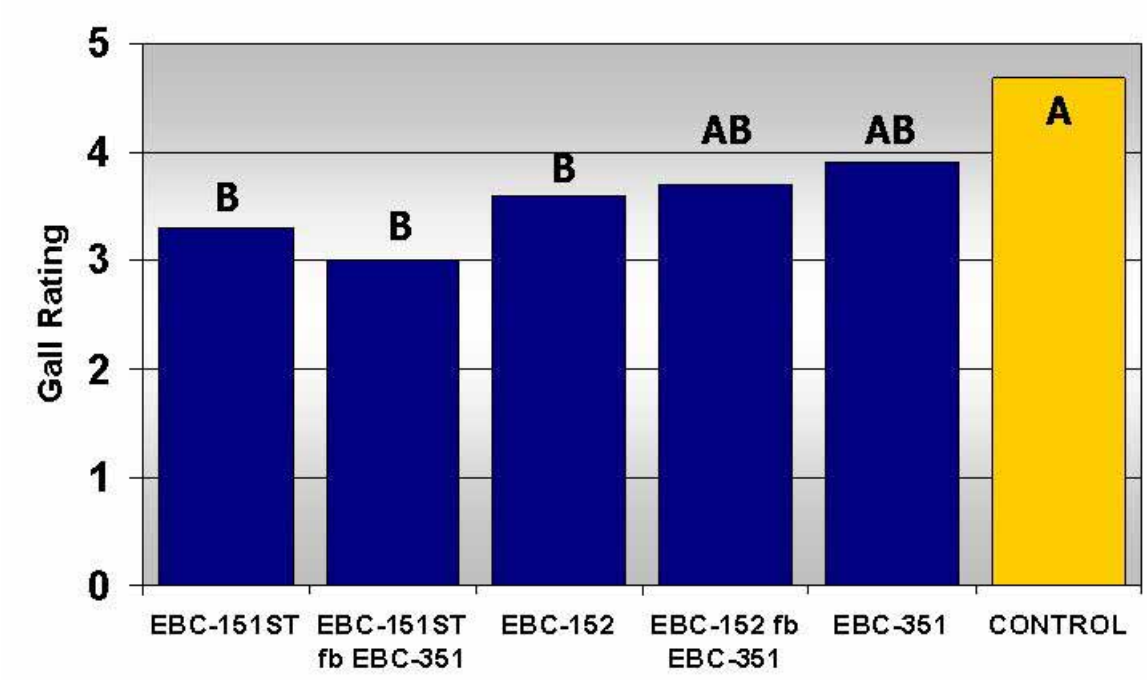

Figure 1. Average gall rating of cotton roots infested with *Meloidogyne incognita*, experiment 1, 2005. Bars with the same letter do not differ significantly, Duncan's New MRT (*P*=0.05, protected).

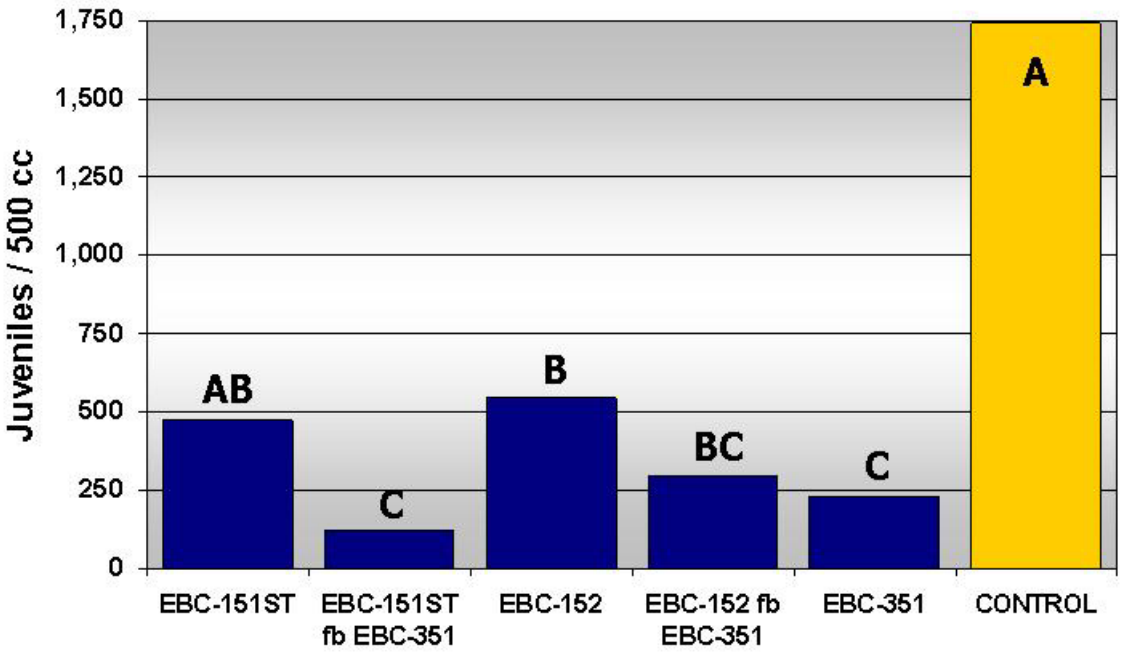

Figure 2. Average number of juvenile *Meloidogyne incognita*, per 500 cc of soil, experiment 3, 2005. Bars with the same letter do not differ significantly, Duncan's New MRT (*P*=0.05, protected).

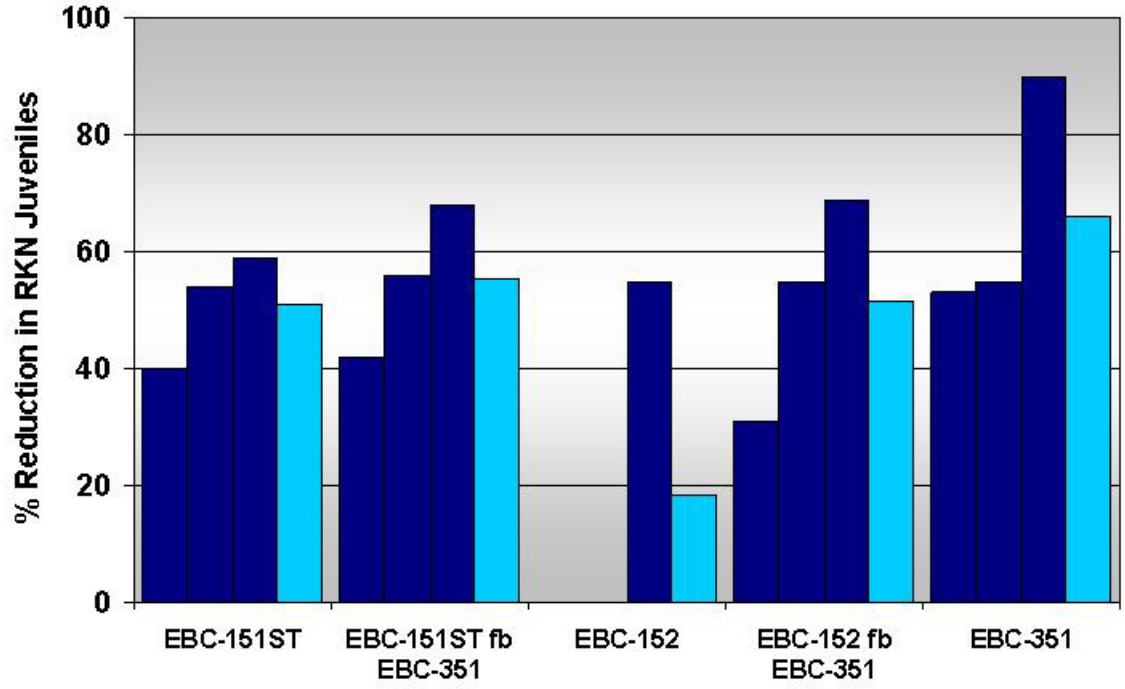

Figure 3. Average percent reduction of *Meloidogyne incognita* juveniles calculated from numbers of juveniles per 500 cc, 2004-2005. Dark blue bars depict results from each trial, and no bar is present if percentage reduction was not greater than zero. Each light blue bar represents the across trial average.