2006 Beltwide Cotton Conferences, San Antonio, Texas - January 3 - 6, 2006

INFLUENCE N-HIBIT™ AND PROACT™ ON NEMATODES IN FIELD COTTON

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Abstract

A regional study was initiated to examine the effects of two harpin proteins on cotton infected with nematodes. Replicated tests were established in Alabama, Florida, Louisiana, Mississippi, South Carolina, and Texas. An independent, University or Extension scientist conducted each test. The harpin proteins were applied to the cottonseed (N-Hibit™ CST, harpin$_{Ea}$) and /or the foliage (ProAct™, harpin$_{\alpha\beta}$) at the eight-leaf stage of DP 444 BG/RR or DP 555 BG/RR cotton. All plants were grown in microplots for the entire production season. Microplots were inoculated with nematodes as follows: Reniform nematode, *Rotylenchulus reniformis*, Alabama, Florida, and Mississippi; Columbia lance nematodes, *Hoplolaimus Columbus*, South Carolina; Root-knot nematode, *Meloidogyne incognita*, Louisiana and Texas. Four weeks after planting, soil samples were collected and analyzed for nematodes. Sampling was repeated through harvest. After harvest, roots were dug and rated for galling and necrosis. Plant growth measurements and yield were recorded. Due to severe weather conditions resulting in plant loss and replanting, trial locations in Louisiana, Mississippi, and South Carolina were significantly impact, and results are not reported. N-Hibit CST and N-hibit CST followed by ProAct provided reductions in nematodes and increases in cotton yield. These results parallel previously reported findings with harpins evaluated in greenhouse trials, which demonstrated that harpins may be useful in reducing damage to cotton due to nematodes while improving plant growth.

Introduction

Harpins proteins are from a novel group of naturally occurring compounds first isolated 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, Mighty-Plant™, N-HIBIT™ CST, and ProAct™.

Harp-N-Tek™ is the title or brand for technologies originating from harpin proteins. Harp-N-Tek products activate internal defense and growth mechanisms within plants, termed an “inside-out plant response,” and these mechanisms improve vigor, stamina, nutrient uptake, and reproductive growth and initiate natural self-defense mechanism, which in turn improves overall plant health and yield potential. N-Hibit CST (CST = commercial seed treatment) contains 3% harpin$_{Ea}$ protein and is specifically formulated as a seed treatment. In February 2005, the Environmental Protection Agency granted registration for ProAct^{TM}, a new plant health regulator. Used as a foliar spray to cotton and other crops, ProAct is a second-generation harpin product that contains 1% harpin$_{\alpha\beta}$ as the active ingredient.

Throughout the world, plant-parasitic nematodes are a significant impediment for cotton (Gossypium hirsutum L.) Reniform nematode, *Rotylenchulus reniformis* (Linford & Olivera) and root-knot nematode, *Meloidogyne incognita* (Kofoid & White) Chitwood can be a limiting constraint in cotton production (Thomas & Kirkpatrick, 2002; Lawrence & McLean, 2002) (Figure 1). In 1999, nematodes reduced cotton yields by 4.24% or 727,215 bales (Blasingame & Patel 2000), and losses were primarily attributed to *M. incognita* and *R. reniformis* (Mueller 2000). In greenhouse trials, seed and foliar treatment of cotton with harpin resulted in fewer *R. reniformis* and *M. incognita* eggs per gram of root weight compared with untreated cotton (Kirkpatrick et. al 2003, Kirkpatrick et. al 2005). 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 (N-Hibit CST) and foliar (ProAct) treatments with harpin protein reduce reproduction of *R. reniformis* or *M. incognita* nematodes on cotton grown in field microplots and improve cotton yield. Findings from that research are reported.

Materials & Methods

Design. The experimental design of each trial was completely randomized. If blocking factors were identified, then the design was adjusted to randomized complete block. Treatments were replicated 10 times. Each site was planted with a locally adapted variety that was tolerant to glyphosate and expressed *Bacillus thuringiensis* protein. Cotton cultivars DP 555 BG/RR and DP 444 BG/RR were utilized. Trial locations, cooperators, and other parameters are summarized in **Table 1**. Cotton was planted at a density of 8 to 12 seeds per microplot and thinned to 4 plants per microplot after complete emergence. Plots were inoculated with nematodes before or at planting by

Table 1. Summary of microplot cotton trial locations investigating N-Hibit^{TM} Seed Treatment and ProAct^{TM} Plant Health Regulator, 2005.

	Alabama Location	**Florida Location**	**Louisiana Location**	**Mississippi Location**	**South Carolina Location**	**Texas Location**
Location	Auburn, AL	Quincy, FL	Bossier City, LA	Starkville, MS	Blackville, SC	Blackville, SC
Cooperator	K. M. Lawrence	J. R. Rich	T. L. Kirkpatrick and P. D. Colyer	G. W. Lawrence	J. D. Mueller	J. L. Starr
Nematode Species	*R. reniformis*	*R. reniformis*	*M. incognita.*	*R. reniformis*	*H. columbus.*	*M. incognita.*
Cultivar	DP 444 BG/RR	DP 555 BG/RR	DP 555 BG/RR	DP 444 BG/RR	DP 555 BG/RR	DP 555 BG/RR
Trial Status	Completed	Completed	Replanted and not included	Replanted and not included	Replanted and not included	Completed

mixing nematodes into 4-6 inches of soil before planting seed. Plant growth inputs, insects, mites, and weeds were managed according to locally accepted practices, and all plots within each trial were treated identically. Six trials were arranged with cooperators located in AL, FL, LA, MS, SC, and TX. The locations in LA, MS, and SC experienced severe weather and subsequent replanting during the season; consequently, these locations are not included in this report.

Treatments and Application. An independent agricultural scientist with G&H Associates acquired commercial samples of cottonseed, and treated seed with N-Hibit® CST seed treatment at 3 oz per cwt. After treatment, untreated and treated seed samples were shipped to each cooperator. Foliar treatment consisted of ProAct applied at the eight-leaf stage. Each trial included an untreated control and N-Hibit treatment that was not treated with ProAct. Cooperators were requested to apply ProAct treatments with calibrated ground equipment utilizing a shielded spray boom with two spray nozzles per row and to apply each plot as single pass. Water was used as the carrier, and treatments were applied at a finished spray volume of 10 gal/acre. Cooperators applied ProAct as stand alone applications without adjuvants or pesticides. Spray equipment was carefully rinsed prior to each application. Sprays were made on a day and at a time when the plants are actively growing.

Field Observations. Prior to or at planting a composite soil sample was collected and analyzed for OM, micronutrients, macronutrients, soil pH, calculated CEC, and percent cation saturation. As appropriate, phytotoxicity ratings were taken and expressed as percent damaged or stunted. Because no adverse effects were observed, results will not be further discussed. From each microplot, all plants were evaluated. If a plant was missing a terminal, the cooperator skipped the atypical plant and moved on to the next plant. At approximately nodes above white flower (NAWF) 5 or 6, NAWF was assessed on each plant by counting nodes above first position white flower to the unfurled leaf. The number of mainstem nodes between uppermost first-position cracked and last harvestable boll (nodes above cracked boll, NACB) on each plant were assessed at approximately NACB = 7. At harvest or within two weeks of harvest, plant height and number of nodes for each plant was measured. Numbers of plants and open, green, hard lock, and rotten bolls from each plot were recorded for each plant. At maturity, open cotton bolls were harvested from each microplot and converted to harvested weight per plant.

Nematode Measurements. Cooperators attempted to collect and analyze soil samples from each microplot at 4 weeks after planting, repeat at approximately four week intervals during the season, and finish with end of season samples. Nematode densities were expressed as per 500 cc of soil. After harvest, cotton plants were dug from the soil and roots were rated for galling, necrosis, and architecture. Galling Index: 0 = no galls or egg masses are present, 1 = 1 or 2 galls, 2 = 3-10 galls, 3 = 11-30 galls, 4 = 31-100, and 5 = >100 galls. Necrosis Rating: 0 = no necrosis, 1 = <10% necrotic, 2 = 11-15% necrotic, 3 = 25-50% necrotic, 4 = 51-75% necrotic, and 5 = 76-100% necrotic. Root Architecture: 1 = good taproot; 2 = j-rooted taproot, and 3 = taproot missing.

Data Analysis. Data were subjected to an analysis of variance (ANOVA) and Duncan's New MRT (p=0.05, protected) means separation test (Duncan 1955, Cochran & Cox 1957). Significance is reported at P=0.05 for analyses unless otherwise indicated.

Results & Discussion

Numbers of *R. reniformis* and *M. incognita* nematode were highest in all three experiments on control plants that did not receive treatment (Table 2, 3, and 4). At the Alabama location, numbers of *R. reniformis* in the N-Hibit CST and N-Hibit CST followed by ProAct plots were 36 to 42% lower than the control (Table 2). Differences in *R. reniformis* at the northern FL location were smaller than those observed in Alabama (Table 3); however, these smaller differences might be attributable to date of sample collection. Reductions in *R. reniformis* were similar for N-Hibit CST, N-Hibit CST followed by ProAct, Avicta, and Temik. Reductions of *M. incognita* ranged from 53 to 77% at the Texas location (Table 4). Treatment with N-Hibit CST or N-Hibit CST followed by ProAct resulted in an average reduction of nematodes of 40%to 46% (Table 5).

When compared with the untreated control, N-Hibit seed treatment increased seed cotton yields in the Texas (11%) and Florida (23%) experiments, but not at the Alabama experiment (-2%) (Table 2, 3, and 4). The combination treatment of N-Hibit CST followed by ProAct increased seed cotton yield by 6% to 25% above the untreated control (Table 2, 3, and 4). The average yield increase for N-Hibit CST or N-Hibit CST followed by ProAct was 11% and 16%, respectively (Table 5).

Table 2. Influence of N-Hibit and ProAct on reniform nematodes and cotton yield in replicated, microplot field trials, Alabama, 2005.

	Seed Cotton Yield				Root Knot Nematode (20-Jul-05)	
Treatment	**1st Pick (lb/plot)**	**2nd Pick (lb/plot)**	**Total (lb/plot)**	**% Difference**	**Per 500 cc**	**% Difference**
Untreated Control	49.4	39.4	88.8	---	21,672	---
N-Hibit CST (3 oz/cwt)	50.6	36.8	87.4	-1.8%	12,642	-35.7%
N-Hibit CST (3 oz/cwt) fb ProAct (1 oz/ac) at 8-leaf	52.3	41.8	94.1	5.7%	13,932	-41.7%

Differences among treatments not significant (P=0.05).

Table 3. Influence of N-Hibit and ProAct on reniform nematodes and cotton yield in replicated, microplot field trials, Quincy, Florida, 2005.
* Indicates value significantly difference from untreated control, Duncan's New MRT (p=0.05, protected).

	Seed Cotton Yield		Reniform Nematode (5-Dec-05)	
Treatment	**Total (lb/ac)**	**% Difference**	**Per 500 cc**	**% Difference**
Untreated Control	1,808	---	4.821	---
N-Hibit CST	2,219*	22.7%	3,631	-24.7%
N-Hibit CST (3 oz/cwt) fb ProAct (1 oz/ac) at 8-leaf	2,116*	17.1%	3,607	-25.2%
Avicta (0.15 mg/seed)	2,253*	24.6%	4,028	-16.5%
Temik (5 lbs/ac)	2,181*	20.6%	3,843	-20.3%

Table 4. Influence of N-Hibit and ProAct on root knot nematodes and cotton yield in replicated, microplot field trials, College Station, Texas, 2005.

	Seed Cotton Yield		Root Knot Nematode (90 DPI)	
Treatment	**Total (g / plant)**	**% Difference**	**Per 500 cc**	**% Difference**
Untreated Control	364.1	---	3,964	---
N-Hibit CST (3 oz/cwt)	403.8	10.9%	1,846	-53.4%
N-Hibit CST (3 oz/cwt) fb ProAct (1 oz/ac) at 8-leaf	454.1	24.7%	929	-76.6%

Differences among treatments not significant (P=0.05).

Table 5. Across average results with N-Hibit and ProAct on nematodes and cotton yield in replicated, microplot field trials, 2005.

	Average Difference (%) from Control	
Treatment	**Nematode Count**	**Seed Cotton Yield**
N-Hibit CST (3 oz/cwt)	-39.9%	10.6%
N-Hibit CST (3 oz/cwt) fb ProAct (1 oz/ac) at 8-leaf	-45.8%	15.8%

Conclusions

In three well-controlled field experiments, N-Hibit and N-Hibit followed by ProAct adversely influenced nematode population development and increased seed cotton yield. Results from these trials parallel previously reported findings with harpins evaluated in greenhouse trials, which demonstrated that harpins may be useful in reducing damage to cotton due to nematodes while improving plant growth. Harpin proteins, such as N-Hibit CST and ProAct, continue to demonstrate promise as useful tools that can be included within the arsenal of products available to manage nematodes in cotton.

Acknowledgment

We wish to gratefully acknowledge the efforts of Lisa Bednarski, Jessica Carothers, and other persons who assisted with trials presented in this manuscript.

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