# EVALUATION OF MESSENGER® FOR ROOT-KNOT NEMATODE SUPPRESSION IN COTTON

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## RESEARCH PROBLEM

Root-knot nematodes are a significant problem for cotton producers throughout the state. Severe infestations can suppress lint yield by at least 150 lb/acre. Currently there are no root-knot- resistant cotton cultivars that are adapted for production in the mid-South, so nematode control is primarily through the application of either aldicarb (Temik) or Telone II (1,3-dichloropropene). Both of these materials are expensive and may be toxic to humans and animals. A biorational product, Messenger®, has been suggested as a means of mitigating nematode damage to cotton through a novel mode of action. These experiments were established to evaluate the potential of Messenger for root-knot suppression or control in cotton.

## BACKGROUND INFORMATION

Messenger is chemically identical to a protein that is produced by the plant pathogen *Erwinia amylovora*, which causes fireblight in pears and apples. This protein, named a harpin protein, was discovered by scientists at Cornell University about ten years ago. The protein is associated with a natural defense mechanism in plants known as a hypersensitive response, where host-plant cells die rapidly in localized areas in response to challenge by an incompatible pathogen. When harpin is exogenously applied to plants, the protein activates several different natural plant genes that are involved in plant growth and pest resistance, and this is the basis for interest in its potential for enhancing plant growth and pest resistance across a number of crop species.

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## RESEARCH DESCRIPTION

Messenger was evaluated in 2001 for its impact on cotton growth and the root-knot nematode in two field trials in Lafayette Coounty, and in one growth-chamber trial conducted at the Southwest Research and Extension Center. Experimental design for both field trials was a randomized complete block with six replications of each treatment. Individual plot size was four rows (38-inch spacing) by 30 ft. in length. Treatments used in trial 1 are listed in Table 1 and those used in trial 2 are listed in Table 2. Both tests were planted on 15 May 2001, and a seeding rate of four seed /row foot was used. The cultivar in trial 1 was Sure Grow 215 BR and the cultivar in trial 2 was Delta Pine 451 BR. All primary tillage, fertilization, weed, and insect control were provided by the grower. Temik 15 G and DiSyston 15 G were applied in-furrow in appropriate treatments at planting. For Messenger applications, the material was mixed with distilled water immediately prior to spraying and was delivered as a foliar spray (13 gpa total volume) to the two middle rows of each plot using a $CO_2$-pressurized backpack sprayer with a hand-held boom and two nozzles (8002) per row. All plots were sampled for nematodes at planting, in June and July, and at harvest. Root galling severity due to the nematode was rated from six plants in each plot immediately after harvest. In addition to root-knot nematode, Texas root rot (*Phymatotrichopsis omnivora*) was also found in the test site, so the percent of plants showing symptoms of this disease was also recorded. Seedcotton was harvested from the two middle rows of each plot with a two-row plot picker, and lint was calculated at 35%.

In the growth chamber trial, Stoneville 4892 BR was planted on 7 February 2001 into pots containing 500 $cm^3$ of sterilized fine sandy loam soil. Pots were placed into a growth chamber, and plants were maintained at a photoperiod of 16 hours of daylight and temperatures of 30°C daytime and 27°C at night. Messenger applications were made at either the second true leaf on 9 February (early) or two weeks after the two-leaf stage on 5 March (late). Plants were inoculated with ~5,000 root-knot nematode eggs, collected from stock tomato cultures, at either the time of planting or on 15 March, after Messenger treatment. A completely randomized experimental design was used, and treatments were replicated ten times (10 pots per treatment). Treatment and nematode inoculation timings and Messenger rates are listed in Table 1. Messenger applications were made with a backpack sprayer using $CO_2$ as the propellant. Distilled water was used for all applications and the material was delivered in a total volume of 10 gpa.

For treatments 1, 2, 4, 5, 7, 8, 13, and 14 (Table 3), the following physiological measurements were recorded on 27 March 2001: photosynthesis (µmol/$m^2$/sec); leaf temperature (°C); transpiration (as mol/$m^2$/sec/); stomatal conductance (µmol/$cm^2$/sec); and leaf intercellular $CO_2$ (ppm).

The experiment was terminated on 10 April 2001. Cotton plant heights, nodes per plant, and dry plant weights were recorded. Nematodes were extracted from the total volume of soil in each pot by semi-automatic elutriation and centrifugal flotation. Intact root systems were washed free of soil and 10 egg masses were arbitrarily removed and

placed in vials to determine the fecundity of individual females. The entire root mass was extracted using NaOCl to free eggs from egg masses that were attached to the roots. NaOCl was also used in the vials to free eggs from the handpicked egg masses. Nematodes (second-stage juveniles) and eggs per root mass were counted under a stereoscopic microscope, and juvenile and egg numbers were transformed by log (x+1) for statistical analysis.

## RESULTS AND DISCUSSION

### Lafayette County, Messenger Trial 1

Root-knot nematodes were detected in all plots at planting, and numbers were similar among treatments (Table 4). There were no differences in nematode numbers among treatments in June, although the numbers declined following treatment 1 which had received Messenger two weeks prior to sampling. There were no differences in the number of eggs found at mid-season (July) among treatments. Nematode numbers were highest at mid-season in some treatments that received DiSyston (treatments 9 and 11) and in treatment 3 that received Temik. Both nematodes and eggs increased substantially in all plots by harvest. There were no differences among treatments in the number of nematodes that were found at harvest. Root galling was moderately severe in all treatments (Table 5). Galling was greatest after treatment 9 (DiSyston + 4 Messenger applications) and least severe after treatment 7 (Temik + 3 early Messenger applications). Texas root rot was most severe in treatment 1 (Temik + 4 Messenger applications), but this was most likely due to variability of this disease in the test site. There were no differences among treatments in lint yield. Messenger application has no significant effect for galling or yields with any treatment.

### Lafayette County, Messenger Trial 2

There were no clear trends of Messenger effects on nematode population densities throughout the season (Table 6), and populations increased in all treatments. Multiple applications of Messenger resulted in slightly lower nematode numbers at harvest (October) than single applications, and nematode reproduction, as measured by the number of eggs in harvest samples, was lower where multiple applications were made. Three applications of Messenger, with the first initiated at the 3$^{rd}$-leaf stage, resulted in the lowest number of eggs at harvest. There were no differences among treatments in root-galling severity or the percent of plants showing Texas root rot and lint yield was not significantly affected by treatments (data not shown).

### Growth Chamber Study Results

No significant differences in photosynthesis, transpiration, leaf temperature, stomatal resistance, and intercellular $CO_2$ was found among Messenger treatments or nematode inoculation treatments although photosynthesis and stomatal resistance were slightly lower and intercellular $CO_2$ was slightly higher in Messenger-treated plants (data not shown).

When nematodes were added at planting, a single application of Messenger at 2.23 oz/acre lowered nematode reproduction (Table 7). Total eggs that were extracted from the cotton roots following this treatment were lower than all other treatments. In addition, the number of eggs that were produced by individual adult females was lower, indicating that Messenger adversely affected the reproductive capability of the females. The number of juveniles that were recovered from the soil following this treatment was also numerically lower than in other treatments. Two applications of Messenger or a single early application at 4.46 oz/acre tended to follow this same trend. Juveniles, total eggs, and eggs per female were slightly, although not significantly, lower than the control. When nematode inoculation was delayed until after Messenger was applied to the plants, only the 4.46 (early) application resulted in lower numbers of juveniles than the control (Table 8). None of the treatments resulted in lower numbers of eggs or eggs per female when inoculation with nematodes was delayed.

Control plants that did not receive either Messenger or nematodes were tallest (Table 9). When nematodes were applied at planting, Messenger resulted in a numerical, although not statistically significant, increase in plant height compared with nematode-infested plants that did not receive the material. A single application of Messenger either early or late, and two applications of the material to nematode-infested plants provided a numerical increase in the number of stem nodes. There were no differences among treatments in plant weight. When nematodes were added later in the experiment, no trends in plant height, nodes, or weight were apparent (data not shown).

## PRACTICAL APPLICATION

Although the novel mode of action of Messenger and its biorational nature make this material extremely interesting, significant efficacy for suppression of nematode damage will be needed before Messenger can be recommended to growers. Also, there was no significant effect on yield. The lower nematode reproduction in Messenger-treated plants in the growth chamber study is encouraging. Further study will be needed to determine if rates and timing of Messenger can be found that are efficacious in the field for root-knot management in cotton.

**Table 1. Rates and timing of applications of nematicide and Messenger treatments. Gin City, AR, 2001. (Trial 1).**

| | | | Messenger application timing | | | |
|---|---|---|---|---|---|---|
| Treatment | Messenger rate | At-plant treatment | 2-leaf | Pin-head | First bloom | 3 wk after first bloom |
| | (oz/acre) | (lb/acre) | | | | |
| 1 | 2.25 | Temik 3.5 | X | X | X | X |
| 2 | 2.25 | Temik 3.5 | X | X | X | |
| 3 | 2.25 | Temik 3.5 | | X | X | X |
| 4 | none | Temik 3.5 | | | | |
| 5 | 2.25 | Temik 7 | X | X | X | X |
| 6 | 2.25 | Temik 7 | X | X | X | |
| 7 | 2.25 | Temik 7 | | X | X | X |
| 8 | none | Temik 7 | | | | |
| 9 | 2.25 | DiSyst.6.5 | X | X | X | X |
| 10 | 2.25 | DiSyst.6.5 | X | X | X | |
| 11 | 2.25 | DiSyst.6.5 | | X | X | X |
| 12 | none | DiSyst.6.5 | | | | |

**Table 2. Rates and timing of applications of nematicide and Messenger treatments. Gin City, AR, 2001. (Trial 2).**

| | | | Messenger application timing | | | |
|---|---|---|---|---|---|---|
| Treatment | Messenger rate | At-plant treatment | 2-leaf | Pin-head | First bloom | 3 wk after first bloom |
| | (oz/acre) | (lb/acre) | | | | |
| 1 | untreated | Disyston 5 | | | | |
| 2 | untreated | Temik 5 | | | | |
| 3 | 2.25 | Temik 5 | X | X | X | |
| 4 | 2.25 | Temik 5 | | X | X | X |
| 5 | 2.25 | Temik 5 | | X | X | |
| 6 | 2.25 | Temik 5 | | | X | X |
| 7 | 2.25 | Temik 5 | | X | | |
| 8 | 2.25 | Temik 5 | | | X | |
| 9 | 2.25 | Temik 5 | | | | X |

**Table 3. Timing and rate of Messenger and timing of nematode inoculation of cotton in growth chamber tests.**

| Timing and Messenger rate | | | Nematode inoculation timing | | |
|---|---|---|---|---|---|
| None | 2 leaf | 2 leaf + 2 weeks | None | At planting | 5 March |
| ---------- (oz/acre) ---------- | | | | | |
| X | | | X | | |
| X | | | | X | |
| X | | | | | X |
| | 2.23 | | X | | |
| | 2.23 | | | X | |
| | 2.23 | | | | X |
| | 2.23 | 2.23 | X | | |
| | 2.23 | 2.23 | | X | |
| | 2.23 | 2.23 | | | X |
| | | 2.23 | X | | |
| | | 2.23 | | X | |
| | | 2.23 | | | X |
| | 4.46 | | X | | |
| | 4.46 | | | X | |
| | 4.46 | | | | X |

**Table 4. Population density of *Meloidogyne incognita* juveniles and eggs at various sampling times. Gin City, AR. 2001. (Trial 1).**

| Treatment | *M. incognita* juveniles | | | | *M. incognita* eggs | |
|---|---|---|---|---|---|---|
| no. | May | June | July | October | July | October |
| | ---------- (#/500 cm³ soil) ---------- | | | | | |
| 1 | 189 | 38 | 341 | 1,200 | 1,036 | 1,164 |
| 2 | 114 | 265 | 190 | 1,000 | 1,358 | 1,088 |
| 3 | 38 | 76 | 720 | 1,300 | 370 | 824 |
| 4 | 455 | 341 | 493 | 1,500 | 1,428 | 1,036 |
| 5 | 720 | 303 | 303 | 1,150 | 600 | 2,161 |
| 6 | 455 | 303 | 114 | 1,300 | 1,302 | 1,652 |
| 7 | 379 | 151 | 152 | 1,400 | 2,212 | 918 |
| 8 | 455 | 190 | 341 | 1,300 | 609 | 2,006 |
| 9 | 493 | 303 | 720 | 2,250 | 900 | 1,377 |
| 10 | 493 | 76 | 190 | 1,000 | 321 | 1,324 |
| 11 | 76 | 227 | 758 | 1,450 | 997 | 1,636 |
| 12 | 644 | 152 | 379 | 1,950 | 646 | 851 |
| LSD(0.05) | 480 | 283 | NS[z] | NS | NS | NS |
| CV (%) | 110 | 121 | 119 | 81 | 175 | 78 |

[z] NS = not significant

**Table 5. Root-galling severity, percent of plants showing Texas root rot symptoms, and cotton yield. Gin City, AR. 2001. (Trial 1).**

| Treatment no. | Root gall severity | Texas root rot | Lint |
|---|---|---|---|
| | | (%) | (lb/acre) |
| 1 | 2.6 | 56 | 864.3 |
| 2 | 2.3 | 22 | 898.7 |
| 3 | 2.5 | 17 | 873.0 |
| 4 | 2.4 | 36 | 930.3 |
| 5 | 2.9 | 36 | 900.3 |
| 6 | 2.3 | 36 | 862.9 |
| 7 | 2.0 | 31 | 909.1 |
| 8 | 2.5 | 28 | 846.4 |
| 9 | 3.1 | 33 | 838.6 |
| 10 | 2.9 | 35 | 831.0 |
| 11 | 2.6 | 17 | 942.2 |
| 12 | 2.5 | 36 | 828.5 |
| LSD (0.05) | 0.8 | 28 | NS[z] |
| CV (%) | 27 | 75 | 13 |

[z] NS = not significant

**Table 6. Population density of root-knot nematode juveniles and eggs at various sampling times. Gin City, AR. 2001. (Trial 2).**

| Treatment | *M. incognita* juveniles | | | | *M. incognita* eggs | |
|---|---|---|---|---|---|---|
| no. | May | June | July | October | July | October |
| | (#/500 cm$^3$ soil) | | | | | |
| 1 | 265 | 303 | 265 | 1,350 | 1,267 | 1,273 |
| 2 | 114 | 76 | 114 | 850 | 421 | 1,555 |
| 3 | 189 | 227 | 341 | 800 | 976 | 317 |
| 4 | 151 | 265 | 455 | 950 | 967 | 800 |
| 5 | 303 | 0 | 341 | 950 | 609 | 1,759 |
| 6 | 76 | 227 | 606 | 1,000 | 661 | 411 |
| 7 | 227 | 38 | 379 | 1,350 | 873 | 1,079 |
| 8 | 720 | 76 | 152 | 1,100 | 1,191 | 1,053 |
| 9 | 189 | 76 | 303 | 750 | 1,903 | 1,064 |
| LSD (0.05) | 458 | 266 | 419 | 797 | 1,539 | 1,105 |
| CV (%) | 158 | 159 | 109 | 68 | 134 | 92 |

**Table 7. Number of root-knot nematode juveniles per pot, eggs per plant, and eggs per adult female when nematodes were added at planting (before Messenger treatment).**

| Messenger timing and rate | Root-knot juveniles | Root-knot eggs | Nematode eggs |
|---|---|---|---|
| (oz/acre) | [#/pot (500 $cm^3$ soil) | (#/root system) | (#/adult female) |
| None applied (control) | 2,989 a[z] | 22,152 a | 318 a |
| Early (2.23) | 881 a | 1,690 b | 149 b |
| Early (2.23)+late (2.23) | 1,205 a | 6,988 ab | 261 ab |
| Late (2.23) | 2,975 a | 18,461 a | 382 a |
| Early (4.46) | 1,668 a | 4,007 ab | 304 ab |

[z] Numbers within a column followed by the same letter are not significantly different (P=0.05).

**Table 8. Number of root-knot nematode juveniles per pot, eggs per plant, and eggs per adult female when nematodes were added after Messenger treatment (March 15).**

| Messenger timing and rate | Root-knot juveniles | Root-knot eggs | Nematode eggs |
|---|---|---|---|
| (oz/acre) | [#/pot (500 $cm^3$ soil) | (#/root system) | (#/adult female) |
| None applied (control) | 807 a[z] | 15,976 a | 229 a |
| Early (2.23) | 366 a | 16,919 a | 204 a |
| Early (2.23)+late (2.23) | 549 a | 18,252 a | 222 a |
| Late (2.23) | 2,099 a | 13,714 a | 238 a |
| Early (4.46) | 130 b | 2,626 a | 230 a |

[z] Numbers within a column followed by the same letter are not significantly different (P=0.05).

**Table 9. Cotton plant height, weight, and number of stem nodes when nematodes were added at planting (before Messenger treatment).**

| Messenger timing and rate | Plant height | Stem nodes | Plant top weight |
|---|---|---|---|
| (oz/acre) | (cm) | (#) | (g) |
| No Messenger; no nematodes (control) | 35.6 a[z] | 10.0 a | 3.49 a |
| No Messenger (control) | 29.5 b | 8.4 b | 2.37 a |
| Early (2.23) | 31.4 ab | 9.2 ab | 3.02 a |
| Early (2.23)+late (2.23) | 30.3 b | 9.4 ab | 2.99 a |
| Late (2.23) | 31.6 ab | 9.3 ab | 2.96 a |
| Early (4.46) | 32.3 ab | 8.9 b | 2.71 a |

[z] Numbers within a column followed by the same letter are not significantly different (P=0.05).