MESSENGER® - HARPIN PROTEIN USE IN COTTON PRODUCTION

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Abstract

MESSENGER® is the first commercialized product from a new class of chemistry called harpin proteins. Harpin Ea, the active ingredient in MESSENGER, elicits an induced plant response resulting in pest management and growth promotion effects. Over 100 field trials evaluated MESSENGER on cotton during the 2000 season. Effects of MESSENGER application timing on cotton growth, yield, and nematodes are the focus of this report. Seven MESSENGER application regimes were tested from combinations of five foliar application timings: two-leaf (2-L), pinhead square (PHS), two weeks after pinhead square (PHS+2), first flower or first bloom (FB), and three weeks after first bloom (FB+3). In nematode trials, treatments of MESSENGER alone and aldicarb (Temik®) followed by MESSENGER were assessed for influence on yield and nematodes. All trials included an untreated control. Measurements included stand counts, plant mapping at three weeks after first bloom and at harvest, leaf tissue analysis for extractable nutrients at first bloom and six weeks after first bloom, nematode density and species, and lint yield and fiber quality.

Based on field trials conducted during the 2000 season and in previous years, MESSENGER appears to have significant beneficial effects on cotton yield and on cotton pests such as nematodes and potentially boll rot. MESSENGER timing field trials documented positive cotton yield effects. Across 20 timing trials, median cotton lint yield increases above the untreated control ranged from 11 to 96 lb lint/acre. Three MESSENGER timings (PHS, FB, FB+3; PHS, PH+2, FB, FB+3; and 2-L, PHS, PH+2, FB, FB+3) resulted in a median yield increase ranging from 83 to 96 lbs/acre of cotton lint above the untreated control. At some locations throughout the cotton belt, considerable yield increases were observed including a 230 lb/acre increase in cotton lint at one location. However, variation in yield response was observed. In many areas of the cotton belt, the 2000 production season was characterized as having severe environmental conditions. Influence of high temperature, low soil moisture, potassium availability, and time interval between MESSENGER applications on the timing trial results is discussed. In the nematode trials, treatments of MESSENGER alone and aldicarb followed by MESSENGER provided significant yield increases and positively influenced nematode densities (root-knot and reniform nematodes). MESSENGER applications provided benefits for cotton produced in soils infested with nematodes, and combinations of MESSENGER and aldicarb appeared to be complementary.

Introduction

Discovery of Harpin Protein

For over 30 years, scientists have investigated how plants recognize plant pathogens and what activates plant defense systems. Many plant defenses have been investigated and reviewed in great detail (Agrawal et al. 1999, Agrios 1997, Keen et al. 2001). In the early 1990s, Cornell scientists discovered that fire blight, *Erwinia amylovora*, releases a protein (harpin Ea) while attacking apple trees (Wei et al. 1992). Harpin protein was the first broad-spectrum elicitor of Hypersensitive Response (HR) discovered (Wei et al. 1992). Since then, agricultural scientists have documented that harpin proteins benefit plants by eliciting the expression of many plant genes including genes responsible for defending against diseases. Additionally, harpin proteins have been shown to activate plant growth systems that were previously inaccessible. Harpin Ea is an acidic, heat stable, glycine-rich, extracellular (cell-envelope-associated-protein) with a molecular weight of ~40 kilodaltons and consists of 403 amino acid residues with no cystiene.

EDEN Bioscience Corporation understood the significance of the harpin discovery and its potential use in crop protection and production. In early 1995, EDEN reached a licensing agreement with Cornell University for exclusive rights to harpins and harpin-related technologies. EDEN is a plant technology company focused on development, manufacturing, and marketing of innovative products for agriculture. Under the technical guidance of Zhongmin Wei, EDEN has expanded it's technological expertise within the novel class of harpin proteins and initiated discovery into new harpin proteins, harpin protein fragments, plant receptor systems, biochemical pathways, formulations, harpin alternatives, pre-mix with chemicals, and crop specific research. The first harpin protein to be commercialized by EDEN is MESSENGER®.

MESSENGER is an innovative technology for production agriculture. In April of 2000, MESSENGER was registered by the Environmental Protection Agency (EPA) as a biochemical pesticide. EDEN Bioscience has adapted standard pharmaceutical technologies to manufacture MESSENGER in substantial quantities. MESSENGER is formulated as a wettable dry granule with 3% harpin Ea. Numerous benefits to crop production and pest management have been demonstrated with MESSENGER. In a recent PESP Update from the EPA regarding MESSENGER, Horne (2000) stated, "For the first time in modern agriculture, growers will be able to harness the innate defense and growth systems of crops to substantially improve yields..."

Mode of Action of MESSENGER

After spraying MESSENGER onto the target plant, harpin Ea binds to plant receptor, triggering a series of complex signaling pathways that activates classical SAR and jasmonic acid/ethylene signaling pathways. EDEN has documented over 300 genes that expression is affected following an application of MESSENGER. Based on the current understanding of harpin protein, plant genes affected by MESSENGER can be broadly categorized into plant defense and growth promotion.

MESSENGER activates an induced systemic response of plant defenses associated with resisting attack by pathogens and herbivores (Wei and Beer 1996). After a topical application of MESSENGER, no symptoms of necrosis or phytotoxicity occur while a broad array of pathogenesis-related proteins, such as PR-1, PR-2 (B-1,3-glucanase), PR-5 (thaumatinlike protein), and others, are synthesized for plant resistance to some bacterial, fungal, viral pathogens and nematodes. Gossett et al. (2000) demonstrated this in cotton. The jasmonic acid/ethylene signaling pathway of SAR is independent of salicylic acid and is characterized by the production of defensin (Pdf1.2), protease inhibitors, and other defense proteins and enzymes that offer resistance to some species of pathogens as well as insects and mites.

Eliciting of resistance with harpin Ea has been demonstrated on a broad range of plant species (Wei and Beer 1996). Following a MESSENGER application, activation occurs within 15 minutes of harpin Ea binding to the plant receptor and is followed by full systemic response within 3 to 5 days. Due to the quick binding of harpin protein and rapid initiation of activation, MESSENGER is rainfast within 30 minutes of application.

Plant growth systems are also activated following an application of MESSENGER. In field trials, growth promotion benefits with MESSENGER have been shown to include increased root mass, leaf biomass, flowering, fruiting, and yield as well as improved quality and earliness. An additional benefit of MESSENGER has been increased storageability of harvested produce.

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Recent studies by the National Aeronautics and Space Administration (NASA) have confirmed the growth promotion effects of increased photosynthesis and nutrient uptake following treatment with MESSENGER. In a highly controlled environment, NASA found that wheat treated with MESSENGER had increased net photosynthesis up to 15%, a dramatically altered daily respiration pattern, and increased nutrient uptake compared with untreated wheat plants (Wei, unpublished data).

MESSENGER Safety Profile

Acute toxicity tests document that MESSENGER is virtually non-toxic to mammals (Table 1 and 2). Similarly, MESSENGER is classified as practically non-toxic to all species tested (bobwhite quail, honeybee, plants, rainbow trout, *Daphnia magna*, and algae). Harpin protein is not persistent and does not accumulate in the environment. MESSENGER is classified as a Toxicity Category IV pesticide (EPA signal word "Caution" on label). Minimal protective clothing is required to mix and apply MESSENGER safely, and the restricted entry interval (REI) is 4-hours which is the minimum required by the EPA. Harpin is exempt from the requirement of a tolerance for all food crops.

MESSENGER Use Benefits in Various Crops

Field-testing of MESSENGER on various crops has demonstrated numerous benefits to pest management and plant growth promotion. For example, averaged across three replicated, small plot rice trials conducted in Arkansas during the 2000 season, a single application of MESSENGER at 2.23 oz/acre at the 2 to 3 leaf stage increased rice yields 11.4 bushels per acre above the average yield of the untreated control (unpublished data).

In a summary of 23 small plot, replicated trials and large block demonstrations from 1999 and 2000, early season applications of MESSENGER to staked tomatoes have improved management of diseases such as bacterial leaf spot, *Xanthomonas campestris* pv. *vesicatoria*, and bacterial speck, *Pseudomonas syringae* pv. *tomato*, (Short, personal communication, 2000). Early season applications of MESSENGER delayed the onset of bacterial leaf spot by 3 to 4 weeks compared with standard disease management practices. MESSENGER applications to tomatoes were shown to increase tomato yields as measured by bins per acre, boxes per bin, and % 5 x 6 or better.

MESSENGER applications influenced disease management and yield in a broad series of field trials conducted during 1999 in orange, grapefruit, and tangerine groves located in Florida (Remmick, personal communication, 2000). Citrus trees treated with MESSENGER had reduced infections of Alternaria brown spot, *Alternaria citri*, and citrus scab, *Elsinoe fawcetti*, compared with trees treated with grower standards. In citrus groves infested with citrus nematodes, citrus trees treated with MESSENGER were found to exhibit a healthier and better flush of leaves compared with untreated citrus trees. Positive growth promotion effects following MESSENGER applications to citrus were observed including earlier and larger flush of leaves, more uniform fruit set, earlier maturity, and increased yield based on more boxes per acre, larger fruit diameter, and higher fruit weight compared with trees not treated with MESSENGER.

During 1997 to 1999, three consecutive years of replicated, small plot field trials and large block demonstrations were conducted at the North Florida Research and Education Center near Quincy, FL. At this facility located adjacent to southwest GA and southern AL, Wright and his research associates have consistently documented yield increases from MESSENGER applications to cotton. Findings from these trials were reported at the Beltwide Cotton Conference (Wright et al. 2000).

The phenotypical response affected by MESSENGER may very by crop. For example, no obvious visual growth effects such as plant height were observed in trials in rice conducted in 2000 (unpublished data). However, as previously mentioned, a substantial yield effect with MESSENGER was documented. Whereas, obvious visual effects have been reported in leaf flush, leaf size, fruiting earliness and uniformity, and fruit size for citrus trees treated with MESSENGER.

MESSENGER is the first commercialized product from the new class of chemistry (harpin proteins). MESSENGER elicits an induced plant response resulting in pest management and growth promotion effects and perhaps other effects yet to be defined. Due to the uniqueness and novelty of this class of chemistry, the features and benefits of MESSENGER and other harpin proteins will likely take several years to clearly define across a broad range of crops. In many crops, EDEN has established a solid knowledge base for MESSENGER; however, EDEN realizes that we have more to learn about harpin proteins and their fit into production agriculture. EDEN looks forward to teaming up with growers, consultants, distributors, and University and Extension to further our understanding of the fit of MESSENGER and other harpin proteins in cotton.

Cotton Production Challenges

Cotton growers face many difficulties in today's environment of production agriculture. Several challenges include nematodes, boll rot, and the plateau of cotton yields.

Nematodes are a devastating, unseen pest of cotton. Across the cotton belt, extension and research plant pathologists and agronomists estimated that nematode species caused a 4.24% reduction in cotton yields in 1999 or a loss of 727,215 bales (Blasingame & Patel 2000). In the southeastern states of AL, FL, GA, SC, and NC, nematodes resulted in an estimated loss of 261,037 bales of cotton. Similarly, 282,474 bales of cotton were lost to nematodes in the mid-south states of AR, LA, and MS. The primary species contributing to these losses are root-knot nematode, *Meliodogyne incognita*, and reniform nematode, *Rotylenchulus reniformis* (Mueller 2000). State specialists report that reniform nematodes continue to spread throughout cotton producing areas of Arkansas and Louisiana.

Boll rot or boll decay contributes to cotton yield reductions each year. In 1999, state specialists estimated that boll rot reduced yield by 348,882 bales in the US (Blasingame & Patel 2000). Southeastern states accounted for 191,337 bales lost to boll rot, and 109,430 bales were lost in the mid-south.

Another significant area of concern for cotton growers is the stifled growth in cotton yields (Helms 2000). Lewis & May (2000) reported that cotton yields improved from the early 1970s to the mid-1980s and reached an annual growth rate of more than 20 lb per acre per year by 1984. However, from the mid-1980s to the late 1990s, rate of yield growth shifted to a flat and then negative growth trend. By 1998, the annual rate of yield change had fallen to –18 lbs per acre per year. Clearly, cotton yields appear to be headed in the wrong direction.

Objectives

In 2000, EDEN Bioscience initiated a broad range of replicated small plot trials and large block demonstration trials on cotton. Several goals of these trials were to determine the optimal foliar timing of MESSENGER on cotton, to begin examining physiological effects of MESSENGER on cotton, and to initiate investigations into the responses of plant pests to MESSENGER treated cotton. The two primary target plant pests were nematodes and boll rot.

This report will review findings from the replicated, small plot cotton trials designed to investigate MESSENGER application timings and effects of MESSENGER on nematodes.

Materials and Methods

General Field Trial Design

The experimental design of each trial was a randomized complete block. Treatments were typically replicated six times, however, due to space limitations, some locations replicated each treatment four times. Plots contained four to six rows of cotton (generally 12.6 ft wide, 50 ft long) planted on a 30 to 40 inch row spacing. Each plot included a buffer of 10 ft between blocks and at least 2 rows between adjacent plots. Plant growth inputs, insects, mites, and weeds were managed according to locally accepted practices, and all plots treated identically. Most sites had irrigation available.

Treatment Application

Cooperators were requested to apply MESSENGER® treatments with ground equipment utilizing a four-row, shielded spray boom with two spray nozzles per row and to apply each plot as single pass. To eliminate concerns regarding variability in water quality, all MESSENGER applications used distilled water as the carrier applied at a total volume of 10 gal/acre [Note: Large acreage demonstrations utilized normal water]. Cooperators applied MESSENGER treatments as stand alone applications without adjuvants or pesticides. Spray equipment was carefully rinsed prior to MESSENGER applications. MESSENGER samples were used within 24 hours of opening, and treatments were applied within three hours of mixing MESSENGER with distilled water.

Field Trials

MESSENGER Application Timings. University agricultural scientists, state Extension specialists, and independent, private contractors conducted all trials. Twenty timing trials were initiated with cooperators located in AL, AR, FL, GA, LA, MS, and NC. Treatments consisted of seven MESSENGER application regimes that included combinations of five primary spray application timings (Table 3): two-leaf (2-L), pinhead square (PHS), two weeks after pinhead square (PHS+2), first flower or first bloom (FB), and three weeks after first bloom (FB+3). The use rate of MESSENGER in all timing trials was 2.23 oz/acre. Each trial included an untreated control that was not treated with MESSENGER (Table 3).

Nematodes. University plant pathologists and agronomists in AR, FL, LA, and MS initiated a series of trials focused on investigating effects on nematodes from foliar application of MESSENGER alone and in combination with an in-furrow application of aldicarb (Temik®, Aventis CropScience, Research Triangle Park, NC). The use rate of MESSENGER in all nematode timing trials was 2.23 oz/acre. Control treatments were an aldicarb check and an untreated check. Results are reported from four trials completed in AR, LA, and MS during 1999 and 2000; however, not all soil sample results have been compiled and analyzed.

Field Observations

Soil Samples. Prior to or at planting a composite soil sample was collected and analyzed for OM, micronutrients, macronutrients, soil pH, calculated CEC, percent cation saturation, and nematodes (by species).

Nematode Samples. In trials focused on nematodes, soil samples were collected from each plot prior to planting, several times during the production season, and at the end of season. Nematode samples were analyzed for nematode density and species. Data were transformed as log(x + 1) prior to ANOVA. Results from the Portland, AR trial were converted to percent reduction (or percent mortality) for each treatment using Henderson's Method, which corrects for natural fluctuations in the untreated check (Henderson & Tilton 1955).

Cotton Stand Counts. Plant stand counts were recorded from the center two rows as plants per 10 row ft at 14 & 28 days after seedling emergence.

Cotton Leaf Tissue Samples. From the center two rows of each plot, five leaves from each of the six replicates were collected for a composite sample of 30 leaf samples for each treatment. Leaves were collected from separate plants at the fifth non-fruiting leaf from the terminal (uppermost portion of the plant). Leaves were sampled at first bloom and six weeks after first bloom. Care was taken to avoid exposing leaves to excessive heat prior to shipping. On the day of collection, all leaf samples were shipped via Priority Mail to Bradford AgriLab, Yazoo City, MS. Leaf samples were analyzed for nitrogen (%N), phosphorus (%P), potassium (%K), calcium (%Ca), magnesium (%Mg), copper (ppm Cu), iron (ppm Fe), manganese (ppm Mn), and zinc (ppm Zn), and Bradford AgriLab provided cooperators with quantities and ratings (high, sufficient, low, or deficient levels) for each element tested.

Cotton Plant Mapping. Plant maps were recorded for five typical plants per row from two center rows for a total of 10 plants per plot. At three weeks after first bloom, plant measurements included plant height, number of nodes, first position fruit (as square, flower, boll, no fruit), node number of white flower, and nodes above white flower. A single measurement, nodes above white flower, was recorded at two weeks after first open boll. A final plant map at or just prior to harvest recorded plant height, number of nodes, and first and second position fruit (as square, flower, green boll, open boll, rotten boll, or no fruit). Data analysis from the plant mapping observations is in progress, and results will not be presented.

Cotton Yield. At or just prior to harvest, numbers of open, green, and rotten (boll rot) bolls were recorded for two five-row ft samples from both center rows. The center two rows of each plot were harvested with a cotton picker, and harvested cotton from each plot was weighed and converted to a per acre yield estimate. A small sample from each plot (approximately the volume of one gallon) was labeled and shipped to a private laboratory for ginning. During the ginning process, pre-ginning weight, seed weight, and lint weight were recorded and percent turnout was calculated. A portion of each ginned sample was shipped to STARLAB (Knoxville, TN) for fiber analysis. Fiber characteristics measured in the analysis include length, uniformity of length, strength, percent elongation (E1), micronaire, reflectance or grayness (Rd), degree of yellowness (b), and Classer Grade. Fiber analysis is in progress, and results will not be presented.

Data Analysis. Data were subjected to an analysis of variance (ANOVA) and Student-Newman-Keuls means separation test (Newman 1939, Cochran & Cox 1957). Significance is reported at P=0.05 for analyses unless otherwise indicated. In many ANOVAs of the field trial data, results were not significant at the P=0.05 level. Consequently, most of the results and discussion will focus on trends and patterns.

Results and Discussion

MESSENGER® Cotton Field Trial Overview

In 2000, 114 field trials were planned (Table 4). Across the mid-south and southeastern states, 62 demonstration trials comparing one or two MESSENGER timing regimes with an untreated control were initiated. Fifty-two replicated trials including six laboratory or greenhouse trials were planned throughout the southeast, mid-south, and Texas. The vast majority of the replicated trials were completed. This report will focus on results from the replicated small plot field trials that investigated MESSENGER application timings and the influence of MESSENGER-treated cotton on nematodes.

MESSENGER Timing Trials

MESSENGER timing field trials documented positive yield effects. Three MESSENGER timings (PHS, FB, FB+3; PHS, PH+2, FB, FB+3; and 2-L, PHS, PH+2, FB, FB+3) resulted in a median yield increase ranging from 83 to 96 lbs/acre of cotton lint above the untreated control, and the other five timings ranged from 11 to 51 lbs/acre above the untreated control (Table 5).

However, all MESSENGER timings, even those with a large yield increase, generated variable yield results when assessed across all locations.

Substantial yield increases were observed in some trials across the testing area. Examples of positive results from trials completed in four states (FL, TX, MS, and NC) are provided (Tables 6, 7, 8, & 9). Compared with the untreated control, a MESSENGER timing resulted in maximum average lint yield increases of +192, +230, +184, +137 lb/acre across the four locations, and minimum yield differences were +18, -27, +23, and –24 lb/acre. When comparing results across these four locations, variations in yield effects for any given MESSENGER timing are apparent.

Large yield increases were not documented at all trial locations. For example, Snipes and Nichols observed considerable yield increases at the Tribbett, MS MESSENGER timing trial test site where three MESSENGER timings increasing lint yield by 122 to 184 lb/acre above the untreated control of 883 lb lint/acre (Table 8). However, in an identical MESSENGER timing trial conducted at nearby Stoneville, MS, Snipes and Nichols observed no yield increases.

Influence of Environmental Conditions

The 2000 cotton production season was unusual and severe due to very high day and night temperatures and limited rainfall, particularly in July and August. Cotton is a complex, perennial crop, and environmental conditions in 2000 reminded everyone of the complexity of producing a cotton crop. University of Arkansas plant pathologist T. Kirkpatrick and plant physiologist D. Oosterhuis have commented about what a difficult set of environmental conditions were encountered during the first season of extensively testing MESSENGER (Kirkpatrick & Oosterhuis, personal communication, 2000).

One consequence of the adverse environmental conditions may have been nutrient uptake. An analysis of average cotton lint yield partitioned by potassium level present in cotton leaf tissue revealed an interesting pattern (Table 10). Cotton yield from plots with 'sufficient' or 'high' levels potassium during early bloom averaged lint yields of 1,098 and 1,148 lbs/acre, respectively, compared an average of 929 lb/acre for plots with 'low' or 'deficient' levels of potassium. Cotton lint yields were 169 (+18%) to 219 (+24%) lbs/acre higher in plots with 'sufficient' to 'high' levels of potassium compared with 'deficient' or 'low' potassium plots. By late bloom, yields for plots with 'low' to 'sufficient' levels of potassium were 174 (+18%) to 138 (+15%) lbs/acre higher compared with potassium 'deficient' plots. No plots were 'high' in potassium at late bloom. Potassium levels in the cotton leaves at late bloom did not appear to be as closely associated with lint yield as early bloom levels of potassium. Deficiencies of one other element, zinc, appeared in a large portion of early and late bloom tissue samples.

Potassium serves many critical roles in cotton and other plants. Potassium is involved with plant processes, such as: osmotic regulation by controlling stomata and maintaining the water status of plants and turgor pressure in cells; cell growth primarily influencing cell elongation, cell thickness, and tissue stability; accumulation, translocation, and metabolism of newly formed carbohydrates; catalyst for over 60 enzymes; and synthesis of amino acids and proteins. (Bennett 1993, Jones 1998, Raven et al. 1981, Winegardner 1996). Reduced yield and quality coupled with increased lodging and sensitivity to plant pathogens are common problems in potassium deficient crops (Jones 1998, Winegardner 1996). Potassium moves to roots via diffusion in soil solution. Root contact (root density) and soil oxygen (O_2) strongly influence potassium uptake from soil (Jones 1998). Compared with other field crops, cotton has a low root length density and limited root development near the soil surface resulting in sensitivity to potassium deficiency (Cassman 1993). Soil potassium supply is generally sufficient until cotton reaches peak bloom (Cassman 1993). EDEN studies have shown that treating plants with MESSENGER increases nutrient uptake, especially potassium. For example, both greenhouse and field trials have documented that MESSENGER treated tomatoes exhibited a higher level of potassium uptake compared with untreated tomato plants (unpublished data).

A consideration for the 2000 MESSENGER timing trials is adequacy of irrigation. Drought stress can adversely influence cotton growth and lint yield (Earl 2000). In a replicated field trial conducted in Rohwer, AR, Coker and Oosterhuis (2000) documented a lint yield of 1546 kg/ha under well-watered conditions and 996 kg/ha under dryland conditions. Under severe drought stress and high temperatures of the 2000 production season in portions of the cotton belt, normal irrigation practices may not have been sufficient to meet the requirements of cotton plants. For example, in those trials that were furrow irrigated on every other row, irrigation water may not have been applied in sufficient quantities relative to the requirements of cotton plants.

Using the four timing trials presented in Tables 6, 7, 8, and 9 as an example, nitrogen, phosphorus, and potassium levels found in cotton leaf tissue at early bloom were reported 'sufficient' to 'high' at the Quincy, Raymondville, and Tribbett trial locations (Jamesville data not available). Whereas, at the previously mentioned Stoneville trial location that had no positive yield effects with MESSENGER, potassium levels were 'deficient' or 'low' for six treatments including the untreated control, and two other treatments were 'sufficient' and 'high.' Many other trial locations were found to have 'low' or 'deficient' levels of potassium in cotton leaves sampled during early bloom (Table 10). Based on soil fertility management practices by most trial cooperators, potassium deficiencies probably resulted from poor root development, low soil moisture, and other adverse environmental conditions instead of inadequate soil applications of fertilizer during spring or early season.

A combination of inadequate irrigation or rainfall and poor root development of cotton may have exacerbated potassium deficiencies in the MESSENGER timing trials. Coker and Oosterhuis (2000) reported that water deficit increased potassium deficiency in cotton. By irrigating every other cotton row or by maintaining insufficient soil moisture in the MESSENGER timing trials, adequate concentrations of potassium to meet the requirements of cotton may not have been present in the soil solution for uptake into the plant. If MESSENGER applications increased fruit load (earliness), severe heat stress, low or inadequate soil moisture, potassium deficiency, and a high demand for water and nutrients during boll filling could have masked some treatment effects with MESSENGER. To maintain adequate potassium in the soil solution and thus an adequate quantity within the plant, a more intensive irrigation schedule may be necessary to fully capture the benefits from treating cotton with MESSENGER. Although not tested in the 2000 timing trials, mid-season foliar applications of potassium may have significantly influenced the performance of MESSENGER treatments. Under dryland conditions with low or high levels of potassium in the soil, foliar applications of potassium increased the concentration of potassium in cotton leaves (Coker & Oosterhuis 2000).

Another consideration would be time intervals between MESSENGER applications. Time between MESSENGER applications with the tested timings of 2-L, PHS, PHS+2, FB, and FB+3 was anticipated to be approximately two to three weeks for each interval. An analysis of application dates across all timing trials revealed that actual intervals were 18.8 days between 2-L and PHS, 12.7 days between PHS and PHS+2, 9.8 days between PHS+2 and FB, and 20.3 days between FB and FB+3. The interval between PHS+2 and FB was less than 10 days instead of the projected 14 days. The reduction in the interval PHS+2 and FB, probably a consequence of high temperatures and associated heat unit accumulations, may have diminished the effectiveness of MESSENGER spray regimes that included applications at both PHS+2 and FB.

Boll Rot Observation

Typically, boll rot is a significant problem in NC (Blasingame & Patel 2000). At the NC MESSENGER timing trial location, numerical differences in numbers of rotten bolls and percentage of rotten bolls were observed (Table 11; yield results, Table 9). All MESSENGER treatments averaged fewer rotten bolls and a lower percentage of rotten bolls compared with the untreated control. In studies conducted on cotton in China, EDEN has documented beneficial effects with MESSENGER against boll rot (unpublished data).

MESSENGER Nematode Trials

Results with MESSENGER on cotton in nematode trials were favorable. In two consecutive years, Lawrence has documented yield increases of 42 to 527 lbs seed cotton per acre in MESSENGER treated cotton compared with the untreated control (Table 12). The Starkville, MS and Inverness, MS trial locations were infested primarily with reniform nematode. At the Inverness trial, three different MESSENGER timings provided yield increases above the untreated control that were similar to Temik®. All four MESSENGER + Temik combinations provided yields higher than MESSENGER, Temik, and the untreated control. Large yield increases with MESSENGER and MESSENGER + Temik were observed at the Portland, AR trial which was infested with root-knot nematode (Table 13). At the Chase, LA trial., yield was very low for all treatments, but Padgett considered the irrigation of the trial to be inadequate (Table 13).

Effects on nematode populations were observed at the Portland and Chase locations (nematode estimates were not yet available for the Inverness location). Although densities of reniform nematode were very low at the Chase, LA location, MESSENGER and MESSENGER + Temik had significantly fewer nematodes present in 'Sample 2' (Table 14). As a proportion of the pretreatment count, seasonal root-knot nematode counts were lowest for MESSENGER and MESSENGER + Temik (Table 15). A similar result was observed after the data were corrected for changes in the untreated control (Table 16).

Conclusions

Based on field trials conducted during the 2000 season and in previous years, MESSENGER appears to have significant beneficial effects on cotton yield and on cotton pests such as nematodes and perhaps boll rot.

MESSENGER timing field trials documented positive cotton yield effects. However, unusually severe environmental conditions, insufficient soil moisture and low availability of potassium at some locations, a narrow reapplication interval between PHS+2 and FB, and other factors may have caused the variability in results across trial locations. Evaluations of MESSENGER timings on cotton will continue in the 2001 season. New considerations in trial design will include soil fertility, foliar applications of potassium, intensity of irrigation and resulting soil moisture, time intervals between MESSENGER applications, planting date, plant density, and other factors.

Based on current field trial results, MESSENGER applications appear to offer benefits for cotton plants grown in soils infested with nematodes. Treatments of MESSENGER alone and MESSENGER + Temik® provided significant positive effects on nematode management and cotton yield, and combinations of MESSENGER and Temik appear to be complementary. Future research will further investigate the influence of MESSENGER on nematodes infesting cotton.

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Table 1. Acute Toxicity of MESSENGER®

Toxicity Measurement	Result
Oral LD_{50} (rat)	>5,000 mg/kg
Dermal LD_{50} (rat)	>6,000 mg/kg
Inhalation LC_{50} (rat)	>2 mg/L
Skin and Eye Irritation (rabbit)	Nonirritating

Table 2. Acute Oral Toxicity Comparison

Product	Oral LD_{50}(mg/kg)	Toxicity level
Kerosene	50	High
Aspirin	1,240	Moderate
Table Salt	3,320	Moderate
MESSENGER®	>5,000	Low

Table 3. MESSENGER® application timing sequences tested in University and Extension small plot cotton trials,2000.

MESSENGER Treatments*	Time of Application* 2-L	PHS	PHS+2	FB	FB+3
Timing 1	X	X	X		
Timing 2	X	X		X	
Timing 3	X			X	X
Timing 4		X		X	X
Timing 5	X	X	X	X	
Timing 6		X	X	X	X
Timing 7	X	X	X	X	X
Untreated Control					

*MESSENGER treatment timings: **2-L**, two-leaf; **PHS**, pinhead square; **PHS+2**, two weeks after PHS; **FB**, first bloom; and **FB+3**, three weeks after FB.

Table 4. MESSENGER® field trials conducted in cotton with external cooperators, 2000.

State	Demonstration Trials	Replicated Trials*	Total Trials
AL	4	1	5
AR	12	16	28
FL	0	4	4
GA	23	2	25
LA	6	6	12
MS	16	5	21
NC	0	2	2
TN	1	2	3
TX	0	14	14
Totals	62	52	114

*Includes 6 trials classified as greenhouse, laboratory, or graduate student studies.

Table 5. Median cotton lint yield increase with MESSENGER® above the untreated control across all tested locations, 2000.

Treatments*	Median lint yield (lbs/acre)**	Difference in lint yield (as median lb/acre) between MESSENGER treatment* and untreated control
2-L, PHS, PHS+2	969	+50
2-L, PHS, FB	930	+11
2-L, FB, FB+3	970	+51
PHS, FB, FB+3	1,015	+96
2-L, PHS, PHS+2, FB	934	+15
PHS, PHS+2, FB, FB+3	1,002	+83
2-L, PHS, PHS+2, FB, FB+3	1,015	+96
Untreated Control	919	–

*MESSENGER treatment timings: 2-L, two-leaf; PHS, pinhead square; PHS+2, two weeks after PHS; FB, first bloom; and FB+3, three weeks after FB. MESSENGER tested at 2.23 oz/acre.
**Results based on 19 locations.

Table 6. Influence of MESSENGER® on cotton lint yield, D. Wright, University of Florida, NFREC, Quincy, FL, 2000.

Treatments*	Lint yield (lbs/acre)	Difference in lint yield between MESSENGER treatment* and untreated control
2-L, PHS, PHS+2	864	+85
2-L, PHS, FB	821	+42
2-L, FB, FB+3	797	+18
PHS, FB, FB+3	924	+145
2-L, PHS, PHS+2, FB	870	+91
PHS, PHS+2, FB, FB+3	971	+192
2-L, PHS, PHS+2, FB, FB+3	918	+139
Untreated Control	779	--

*MESSENGER treatment timings: 2-L, two-leaf; PHS, pinhead square; PHS+2, two weeks after PHS; FB, first bloom; and FB+3, three weeks after FB. MESSENGER tested at 2.23 oz/acre.

Table 7. Influence of MESSENGER® on cotton lint yield, Raymondville, TX, 2000.

Treatments*	Lint yield (lbs/acre)	Difference in lint yield between MESSENGER treatment* and untreated control
2-L, PHS, PHS+2	1,277	+70
2-L, PHS, FB	1,365	+158
2-L, FB, FB+3	1,286	+79
PHS, FB, FB+3	1,204	-4
2-L, PHS, PHS+2, FB	1,181	-27
PHS, PHS+2, FB, FB+3	1,437	+230
2-L, PHS, PHS+2, FB, FB+3	1,242	+35
Untreated Control	1,207	--

*MESSENGER treatment timings: 2-L, two-leaf; PHS, pinhead square; PHS+2, two weeks after PHS; FB, first bloom; and FB+3, three weeks after FB. MESSENGER tested at 2.23 oz/acre.

Table 8. Influence of MESSENGER® on cotton lint yield, C. Snipes and S. Nichols, Mississippi State University, Tribbett, MS.

Treatments*	Lint yield (lbs/acre)	Difference in lint yield between MESSENGER treatment* and untreated control
2-L, PHS, PHS+2	910	+27
2-L, PHS, FB	925	+43
2-L, FB, FB+3	961	+79
PHS, FB, FB+3	1,005	+122
2-L, PHS, PHS+2, FB	1,067	+184
PHS, PHS+2, FB, FB+3	905	+23
2-L, PHS, PHS+2, FB, FB+3	1,053	+170
Untreated Control	883	–

*MESSENGER treatment timings: 2-L, two-leaf; PHS, pinhead square; PHS+2, two weeks after PHS; FB, first bloom; and FB+3, three weeks after FB. MESSENGER tested at 2.23 oz/acre.

Table 9. Influence of MESSENGER® on cotton lint yield, Jamesville, NC, 2000.

Treatments*	Lint yield (lbs/acre)	Difference in lint yield between MESSENGER treatment* and untreated control
2-L, PHS, PHS+2	998	+112
2-L, PHS, FB	862	-24
2-L, FB, FB+3	968	+82
PHS, FB, FB+3	1,023	+137
2-L, PHS, PHS+2, FB	920	+34
PHS, PHS+2, FB, FB+3	895	+9
2-L, PHS, PHS+2, FB, FB+3	970	+85
Untreated Control	886	–

*MESSENGER treatment timings: 2-L, two-leaf; PHS, pinhead square; PHS+2, two weeks after PHS; FB, first bloom; and FB+3, three weeks after FB. MESSENGER tested at 2.23 oz/acre.

Table 10. Average cotton lint yield across all treatments, including untreated control, partitioned by level of potassium found in cotton leaf tissue at two sampling periods, 2000.

Potassium (%K) level in cotton leaf tissue samples*	Average lint yield (lbs lint/acre)			
	Early Bloom		Late Bloom	
	Lint (lb/acre)	N**	Lint (lb/acre)	N
Low and Deficient (pooled)	929	26	1,002	58
Deficient	955	11	945	39
Low	910	15	1,119	19
Sufficient	1,098	31	1,083	50
High	1,148	15	---	0

*Early bloom results were categorized as Deficient = <1.25 %K; Low= 1.25-1.45 %K, Sufficient = 1.45-2.5 %K; and High = >2.5 %K. Late bloom results were categorized as Deficient = <1 %K; Low = 1-1.2 %K, Sufficient = 1.2-2.25 %K; and High = >2.25 %K.

**N = Sample size indicates number of treatment x location combination used to calculate average yield. Differences in cumulative sample size by bloom period resulted from some locations not reporting results from the early bloom sample.

Table 11. Influence of MESSENGER® on boll rot in cotton, Jamesville, NC, 2000.

Treatments*	Open Bolls /10 row ft	Rotten Bolls /10 row ft	% Rotten of Total Bolls**
2-L, PHS, PHS+2	170	24	14%
2-L, PHS, FB	150	16	10%
2-L, FB, FB+3	154	35	18%
PHS, FB, FB+3	164	24	13%
2-L, PHS, PHS+2, FB	169	12	6%
PHS, PHS+2, FB, FB+3	155	28	15%
2-L, PHS, PHS+2, FB, FB+3	154	16	10%
Untreated Control	143	38	21%

*MESSENGER treatment timings: 2-L, two-leaf; PHS, pinhead square; PHS+2, two weeks after PHS; FB, first bloom; and FB+3, three weeks after FB. MESSENGER tested at 2.23 oz/acre.

**Total Bolls = Open Bolls + Rotten Bolls.

Table 12. Average cotton yield and yield increase with MESSENGER® above the untreated control in two trials conducted by G. Lawrence, Mississippi State University, at Mississippi State, MS in 1999 and Inverness, MS in 2000.

	Average seed cotton yield (lb/acre) and increase above untreated control			
Treatments*	**Starkville, MS**		**Inverness, MS**	
Untreated Control	889 b	--	2,023	–
Temik	--	--	2,221	+198
2-L, PHS, PHS+2	--	--	2,236	+214
2-L, PHS, PHS+2 + Temik	--	--	2,326	+303
2-L, PHS, PHS+2, FB	--	--	2,065	+42
2-L, PHS, PHS+2, FB + Temik	--	--	2,304	+281
2-L, PHS, PHS+2, FB, FB+3	--	--	2,217	+195
2-L, PHS, PHS+2, FB, FB+3 + Temik	--	--	2,545	+552
2-L, FB, FB+3, FB+6	1,427 a	+538	--	–
2-L, FB, FB+3, FB+6 + Temik	--	--	--	–
2-L, FB, FB+3	1,331 ab	+442	2,293	+271
2-L, FB, FB+3 + Temik	--	--	2,376	+354

*MESSENGER treatment timings: 2-L, two-leaf, pinhead square (PHS), PHS+2 weeks, first bloom (FB), FB+3 weeks, and FB+6 weeks. MESSENGER tested at 2.23 oz/acre. Temik applied in-furrow as nematicidal treatment. Means followed by the same letter do not significantly differ.

Table 13. Average cotton yield and yield increase with MESSENGER® above the untreated control in trials conducted by T. Kirkpatrick, University of Arkansas, Portland, AR, 2000 and by B. Padgett, Louisiana State University, Chase, LA, 2000.

	Average seed cotton yield (lb/acre)			
Treatments*	**Arkansas**		**Louisiana**	
Untreated Control	2,905 a	--	625	–
Temik	3,285 ab	+380	714	+89
MESSENGER	4,215 ab	+1,310	664	+39
MESSENGER + Temik	3,693 b	+788	706	+81

*MESSENGER treatment timing: 2-L, two-leaf, pinhead square (PHS), two weeks after PHS; first bloom (FB), and three weeks after FB. MESSENGER tested at 2.23 oz/acre. Temik applied in-furrow as nematicidal treatment. Means followed by the same letter do not significantly differ (P=0.05, Student-Newman-Keuls).

Table 14. Average numbers of reniform nematode, B. Padgett, Louisiana State University, Chase, LA, 2000.

	Average numbers of reniform nematode per soil sample**	
Treatments*	**'Sample 2'**	**'Sample 3'**
Untreated Control	219 a	1,498
Temik	384 a	83
MESSENGER	2 b	2,627
MESSENGER + Temik	11 b	587

*MESSENGER treatment timing: 2-L, two-leaf, pinhead square (PHS), two weeks after PHS; first bloom (FB), and three weeks after FB. MESSENGER tested at 2.23 oz/acre. Temik applied in-furrow as nematicidal treatment.

**Average number converted from log(x+1) transformation of raw data. Means followed by the same letter do not significantly differ (P=0.05, Student-Newman-Keuls).

Table 15. Seasonal root knot nematode counts, T. Kirkpatrick, University of Arkansas, Portland, AR, 2000.

	May**	**Root knot nematode counts as proportion of May sample**			
	Avg.	**June**	**July**	**August**	**October**
Untreated Control	406	0.3	0.4	2.1	10.2
Temik	236	0.4	2.18	2.8	23.8
MESSENGER	979	0.4	0.4	1.7	6.4
MESSENGER + Temik	2,559	0.1	0.1	0.4	2.5

*MESSENGER treatment timing: 2-L, two-leaf, pinhead square (PHS), two weeks after PHS; first bloom (FB), and three weeks after FB. MESSENGER tested at 2.23 oz/acre. Temik applied in-furrow as nematicidal treatment.

**Average numbers of root knot nematode juveniles and eggs per 500 cc of soil converted from log(x+1) transformation of raw data.

Table 16. Percent reduction in root knot nematode counts, T. Kirkpatrick, University of Arkansas, Portland, AR, 2000.

	May**	**Percent Reduction*****			
	Avg.	**June**	**July**	**August**	**October**
Untreated Control	406	--	--	--	–
Temik	236	0%	0%	0%	0%
MESSENGER	979	85%	0%	17%	38%
MESSENGER + Temik	2,559	54%	67%	82%	75%

*MESSENGER treatment timing: 2-L, two-leaf, pinhead square (PHS), two weeks after PHS; first bloom (FB), and three weeks after FB. MESSENGER tested at 2.23 oz/acre. Temik applied in-furrow as nematicidal treatment.

**Average numbers of root knot nematode juveniles and eggs per 500 cc of soil converted from log(x+1) transformation of raw data.

***Percent reduction calculated using Henderson's Method (Henderson & Tilton 1955).