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Efficacy of a Novel Nematicidal Seed Treatment against *Meloidogyne incognita* on Cotton

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Abstract: The efficacy of abamectin as a seed treatment for control of *Meloidogyne incognita* on cotton was evaluated in greenhouse, microplot, and field trials in 2002 and 2003. Treatments ranging from 0 to 100 g abamectin/100 kg seed were evaluated. In greenhouse tests 35 d after planting (DAP), plants from seed treated with abamectin were taller than plants from nontreated seed, and root galling severity and nematode reproduction were lower where treated seed were used. The number of second stage juveniles that had entered the roots of plants from seed treated with 100 g abamectin/kg seed was lower during the first 14 DAP than with nontreated seed. In microplots tests, seed treatment with abamectin and soil application of aldicarb at 840 g/kg of soil reduced the number of juveniles penetrating seedling roots during the first 14 DAP compared to the nontreated seedlings. In field plots, population densities of *M. incognita* were lower 14 DAP in plots that received seed treated with abamectin at 100 g/kg seed than where aldicarb (5.6 kg/ha) was applied at planting. Population densities were comparable for all treatments, including the nontreated controls, at both 21 DAP and harvest. Root galling severity did not differ among treatments at harvest.

Key words: abamectin, Avermectin, G*ossypium hirsutum*, *Meloidogyne incognita*, nematicide.

Cotton (*Gossypium hirusutum* L.) is an important agricultural commodity worldwide, and the US is one of the largest cotton-producing countries with 4.9 million ha harvested in 2003 (National Agricultural Statistics Service, 2004). The root-knot nematode (*Meloidogyne incognita*) is a significant economic pathogen in all 17 of the states in the United States where cotton is produced (Blasingame and Patel, 2005). Root-knot nematodes damage cotton by disrupting normal uptake of water and nutrients (Kirkpatrick et al., 1991, 1995). This disruption in normal plant function can result in substantial yield suppression (Thomas and Kirkpatrick, 2001). Although crop rotation and in some areas the limited use of moderately resistant cultivars have been useful for root-knot control in cotton, the application of nematicides is the most widely used control measure in the US cotton belt (Koenning et al., 2004). The two most commonly used nematicides for controlling *M. incognita* in cotton are aldicarb, a nonfumigant insecticide/nematicide, and 1,3-dichloropropene, a soil fumigant. Dramatic increases in yield can be obtained with the use of nematicides to control *M. incognita* (Kinlock and Rich, 1998; Baird et al., 2000; Wrather et al., 2002), with greater nematode suppression generally expected with the soil fumigant (Kinloch and Rich, 1998). However, disadvantages of both aldicarb and 1,3-dichloropropene include its expense, toxicity to humans and animals, and significant environmental risk.

Avermectins are a class of macrocyclic lactones produced by *Streptomyces avermitilis* that are commonly used in treating gastrointestinal helminthic parasites in domestic animals (Fisher, 1997). Certain avermectins have been shown to suppress *M. incognita* on tobacco and tomato (Sasser and Kirkpatrick, 1982; Garabedian and Van Gundy, 1983) and *M. arenaria* on tobacco (Nordmeyer and Dickson, 1985) at levels comparable to those achieved by application of carbamate and organophosphate nematicides. Avermectins have not yet been utilized in commercial agriculture for nematode control, most likely because of their low water solubility and rapid decomposition in the soil (Putter et al., 1981; Bull et al., 1984). Recently, however, abamectin (avermectin B_1) has received renewed interest as a possible suppressant for plant-parasitic nematodes when delivered as a seed treatment. The objective of this study was to determine the efficacy of abamectin as a seed treatment for control of *M. incognita* in cotton.

Materials and Methods

Greenhouse: Meloidogyne incognita was collected from an infested field in Drew County, AR, and then maintained on tomato (*Lycopersicon esculentum*) cv. Rutgers in a greenhouse. Tests were initiated on 5 June 2002 in a greenhouse in 10.2-cm-diam. clay pots filled with a steam-pasteurized (0.5 hr at 70°C) mixture (v/v) of 50% fine quartz sand and 50% Smithdale sandy loam (fine loamy siliceous, thermic Typic Paleudult, 85% sand, 14% silt, and 1% clay). Abamectin (Syngenta Crop Protection, Greensboro, NC), applied as a seed treatment at 10, 50, 75, and 100 g a.i./100 kg of seed, was evaluated on the *M. incognita*-susceptible cotton cultivar Stoneville 4892 BR. Two seeds of each treatment were planted into each pot at an approximate seed spacing of 7.6 cm. Pots were all inoculated with 5×10^3 *M. incognita* eggs collected from tomato cultures by NaOCl extraction (Hussey and Barker, 1973). After planting, treatments were arranged in a randomized complete block design with 10 replications/treatment. Plant populations were thinned to one plant/pot after emergence.

Seedling height and the number of main stem nodes

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were recorded for five of the replications 35 DAP on 10 July. The root systems from these plants were removed from the pots and weighed, and root galling was evaluated on a scale where 0 = no galling visible, 1 = 1% to 10% of roots galled, 2 = 11% to 25% of roots galled, 3 = 26% to 50% galled, 4 = 51% to 75% galled, and 5 > 75% of root system showing galls. *Meloidogyne incognita* eggs were extracted from each root system using NaOCl, and the number of eggs per gram fresh root weight was calculated. The remaining five replications were allowed to grow for another 10 d, and then they were measured as with the original five replications. For statistical analyses, egg and juvenile numbers were transformed using $\log_{10}$ (x +1) to normalize variability among treatments. Actual egg and juvenile numbers are reported. Data were analyzed by analysis of variance and mean comparison by Waller-Duncan K-ratio t-test ($P < 0.05$) using SAS statistical software (SAS Institute, Inc., Cary, NC).

Field trial (2002): The experiment was conducted in a cotton field near Gin City, AR. The soil was a Rilla silt loam (fine-silty, mixed, thermic Typic Hapludalfs [Laurent et al., 1984] containing 50% sand, 45% silt, and 5% clay) that was naturally infested with *M. incognita*. Stoneville 4892 BR cotton seed treated with 0, 10, 50, 75, and 100 g abamectin/100 kg of seed was evaluated in this study. All treatments were planted at a rate of 13 to 14 seed/m of row. The experiment was planted in a randomized complete block design with five replications on 22 April. Individual plots were four rows (0.97-m spacing) by 15.2-m long. Soil samples for determination of *M. incognita* population density were collected on 30 April, 2 July, and 1 October. Samples were collected using a 2.5-cm-diam. sampling tube, and 16 individual cores/plot were collected to a depth of 20 cm within root zone at each sampling time. Nematodes were extracted from the samples using the semiautomatic elutriator (Byrd et al., 1976) followed by centrifugal flotation (Jenkins, 1964), and nematodes were identified and quantified using a stereoscope. Nematode numbers were transformed using $\log_{10}$ (x + 1) for statistical analysis.

Crop production practices were performed by the grower. Fertilization was based on soil test results. Because thrips (*Frankliniella fusca*) were abundant in the test, insect populations on the plants and plant damage caused by their feeding were assessed to aid in explaining any damage to plants not related to nematodes. Whole-plot visual ratings for thrip damage were made on 22 May using the scale: 1 = no visible effect, 2 = slight cupping of leaves, 3 = slight plant stunting, 4 = severe plant stunting and leaf cup, and 5 = plants dead. In addition, 10 arbitrarily selected plants from the outside two rows of each plot were collected, carefully placed in plastic bags to prevent thrips from escaping, and transported to the laboratory where the insects were washed from the plants using the procedure of Micinski et al. (1995) and counted.

Plant height, the number of main stem nodes, and root gall ratings were assessed from the plants following the thrip extraction. Root-gall ratings were made using a scale where: 0 = no galling visible, 1 = 10% of roots galled, 2 = 20% of roots galled, 3 = 30% galled, 4 = 40% galled, 5 = 50% galled, 6 = 60% galled, 7 = 70% galled, 8 = 80 % galled, 9 = 90% galled, and 10 = 100% of root system showing galls. Gall ratings were also made on six plants arbitrarily collected from the outside two rows of each plot on 2 July and again at harvest on 1 October. Seed cotton was machine harvested from the two center rows of each plot, and lint yield was estimated using 35% turnout. Data were analyzed by analysis of variance and mean comparison by Waller-Duncan K-ratio t-test ($P < 0.5$) using SAS statistical software.

Field trial (2003): The test was conducted in a cotton field naturally infested with *M. incognita* near Portland, AR, in 2003. The soil in the research site was a Rilla silt loam soil (fine-silty, mixed, thermic Typic Hapludalfs [Gill et al., 1979] consisting of 47% sand, 49% silt, and 4% clay). All crop production practices were performed by the grower, and fertilization was based on soil nutrient analysis. Treatments consisted of either 0, 10, or 100 g abamectin/100 kg of seed or soil application of aldicarb (Temik 15 G, Bayer CropScience, Research Triangle Park, NC) at 840 and 1,176 g/ha in the planting furrow using seed that were not treated with abamectin. All treatments were planted at a rate of 13 to 14 seed/m of row. Treatments were arranged in a randomized complete block design with four replications. Individual plots were four rows (0.97-m spacing) by 15.2-m long. The trial was planted on 1 May using the cotton cultivar Stoneville 4892 BR. Soil samples for nematode assay were collected, processed and analyzed as described above for the 2002 trial. Soil samples were collected on 1 May, 14 May, 21 May, 28 May, and 10 October.

Plant height, number of main stem nodes, and root-galling severity were evaluated for 10 plants collected arbitrarily from the outside two rows of each plot on 5 June. A final gall rating was assessed on 10 plants from the center two rows of each plot on 10 November, immediately after harvest. Root-galling severity was estimated using the scale described for the 2002 trial. Seed cotton was machine harvested on 11 October, and lint yield was estimated using 35% turnout. Data were analyzed by analysis of variance and mean comparison by Waller-Duncan K-ratio t-test ($P < 0.05$) using SAS statistical software.

Microplots: The experiment was conducted three times (runs) during the 2003 growing season. The first test was initiated on 7 June and terminated on 21 June, and the second test was initiated on 2 July and terminated on 16 July. The final test began on 25 July and was terminated on 8 August. Experiments were con-

ducted in cylindrical concrete microplots (76-cm-diam. by 80-cm-depth) filled with a Smithdale sandy loam (fine loamy siliceous, thermic Typic Paleudult; 85% sand, 14% silt, and 1% clay) located at the Southwest Research and Extension Center. Plots were fumigated with methyl bromide (681 g/9.3 m^2) approximately three months prior to initiation of the first test. Soil from the microplots was assayed for soil fertility and amended with N, P, and K according to soil nutrient analyses. The treatments were evaluated using Stoneville 4892 BR cotton.

Inoculum of *M. incognita* for the microplots consisted of a mixture of finely chopped infected tomato roots and infested soil. Sufficient inoculum was incorporated into each microplot to give approximately 4×10^3 eggs and juveniles of *M. incognita*/500 cm^3 of soil in the upper 20 cm of each microplot. The inoculum was mixed into the soil using a rake immediately prior to planting. Inoculum was added to each plot for the second and third runs based on the population density at the end of the previous run to maintain a nematode population density at planting of 4×10^3 eggs and juveniles/500 cm^3 in the upper 20 cm.

Treatments consisted of seed treated with 100 g abamectin/100 kg of cottonseed, nontreated seed, and nontreated seed plus aldicarb at 840 g/ha broadcast on the soil surface and incorporated into the upper 5 cm of appropriate microplots using a rake immediately prior to planting. The experiment was arranged in a completely randomized design with four replications. Approximately 20 cotton seeds were planted into each microplot at an approximate seed spacing of 7.6 cm. Microplots were irrigated as needed and managed for insect and weed infestations in accordance with the University of Arkansas Cooperative Extension Service suggestions (Greene et al., 2003; Scott et al., 2003). Two seedlings were removed from each microplot at 5, 8, 11, and 14 DAP for all tests. The roots were washed free of soil, and then each root system was cleared and stained using the procedure described by Byrd et al. (1983) to determine the number of nematodes that had penetrated the root system. Root systems remained intact during the clearing and staining process, but each root system was arbitrarily divided into upper and lower portions (about 50% of the total root system length in each category) for easier observation under the dissecting microscope. Each root portion was examined using a stereomicroscope, and nematodes in the roots were counted. Plants that were not removed from the microplots for staining after the third test were rated for galling severity at 45 DAP using the same rating scale described for the greenhouse test. Data were analyzed by analysis of variance and mean comparison by Waller-Duncan K-ratio t-test ($P < 0.05$) using SAS statistical software.

Results

Greenhouse trial: Plants from seed treated with 100 g abamectin/100 kg seed were numerically taller than plants from nontreated seed 35 DAP (Table 1). Root galling was less severe ($P < 0.05$) on plants from all abamectin seed treatments, except 10 g/100 kg of seed, than from nontreated seed, and all abamectin seed treatments resulted in lower numbers of eggs than for the control. At 45 DAP, plants from seed receiving abamectin at rates of 50 or more g/100 kg of seed were taller ($P < 0.05$) than plants from the nontreated seed. There was no difference among treatments in the number of main stem nodes produced by plants at 35 DAP, but by 45 DAP, seed treated with 75 and 100 g/100 kg of seed produced plants with more nodes ($P < 0.05$) than the nontreated seed. Root-galling severity and nematode reproduction remained lower ($P < 0.05$) for all abamectin treatments than for the nontreated plants at 45 DAP.

Field 2002: Plant height and the number of nodes on the main stem were not affected by any of the treatments (Table 2), and no differences were detected in nematode population density in the soil 8 DAP (data

Table 1. Plant growth, root-galling severity, and *Meloidogyne incognita* reproduction on cotton in the greenhouse after seed treatment with abamectin.

	35 DAP[a]				45 DAP[a]			
Abamectin rate[b]	Plant height (cm)	No. nodes[c]	Gall rating[d]	Reproduction[e] (eggs/g)	Plant height (cm)	No. nodes[c]	Gall rating[d]	Reproduction[e] (eggs/g)
0	18.8 ab[f]	5.6 a	4.2 a	32,357 a	21.5 b[f]	5.4 b	5.0 a	109,445 a
10	18.4 ab	5.0 a	4.2 a	15,157 b	33.8 ab	6.0 ab	3.4 bc	59,011 b
50	17.5 b	5.4 a	2.0 b	2,784 c	35.1 a	6.0 ab	3.8 b	37,154 bc
75	20.5 ab	5.8 a	2.3 b	6,639 bc	38.0 a	6.8 a	3.2 bc	15,415 bc
100	23.1 a	6.0 a	2.0 b	8,472 bc	39.7 a	6.4 a	2.6 c	7,111 c

[a] Days after planting.
[b] Grams abamectin per 100 kg cotton seed.
[c] Number of main stem nodes.
[d] Rating scale: 0 = no galls visible, 1 = 1% to 10% of roots galled, 2 = 11% to 25% of roots galled, 3 = 26% to 50% of roots galled, 4 = 51 to 75% of roots galled, and 5 >75% of roots galled.
[e] Number of *Meloidogyne incognita* eggs per gram fresh root.
[f] Means within columns followed by the same letter do not differ ($P < 0.05$) by Waller-Duncan K-ratio t-test.

TABLE 2. Cotton growth, root-knot nematode populations and galling, and thrip populations and damage 30 DAP in field plots after seed treatment with abamectin, 2002.

Abamectin (g/100 kg cotton seed)	Plant height (cm)	No. nodes[a]	*Meloidogyne incognita* J2/500 cm^3 [b]	*Meloidogyne incognita* Gall rating[c]	Thrips[d] No./plant	Thrips[d] Damage rating[e]
0	11.1 a[f]	3.0 a	227 a	0.15 a	10.5 ab	3.2 a
10	9.8 a	3.1 a	284 a	0.00 a	17.0 ab	3.0 ab
50	10.3 a	3.2 a	738 a	0.07 a	19.8 a	3.0 ab
75	10.3 a	3.1 a	341 a	0.10 a	6.3 b	2.5 b
100	10.1 a	3.1 a	625 a	0.10 a	11.3 ab	2.8 ab

[a] Number of main stem nodes.
[b] J2/500 cm^3 = number of second stage juveniles per 500 cm^3 of soil.
[c] Rating scale of 0 to10 where 0 = no galling and 10 = 100% of the root system galled 30 DAP.
[d] Primarily *Frankliniella fusca.*
[e] Visual damage rating using the scale: 1 = no visible effect, 2 = slight cupping of leaves, 3 = plant stunting, 4 = severe stunting and leaf cup, 5 = severe stunting and defoliation.
[f] Means within columns followed by the same letter do not differ ($P < 0.05$) by Waller-Duncan K-ratio t-test.

not shown). The number of thrips recovered from plants one month after planting were lower ($P < 0.05$) where seed was treated with 0.75 g abamectin/100 kg, and visual damage ratings were also lower with this treatment. Root-gall ratings were similar among treatments at 30 DAP. There were no differences among treatments in the population density of *M. incognita* either at mid-season or at harvest, and root galling and cotton yield were similar among treatments (data not shown).

Field 2003: The initial population density of *M. incognita* second stage juveniles was similar at planting among all treatments, except where the 100 g abamectin/100 kg seed was located (Table 3). Population density was lower ($P < 0.05$) 2 wk after planting in plots receiving seed treated with 100 g abamectin/100 kg seed than with either aldicarb treatment or in the nontreated control. No differences among treatments were found in plant height, number of main stem nodes, or root-galling severity at mid-season, and nematode population density, root-galling severity, and lint yield were similar among all treatments at harvest (data not shown).

Microplots: As no treatment x time (DAP) or treatment x test interactions were detected, the data were pooled across DAP and tests. The number of nematodes that had entered the upper half of the root systems was similar for the aldicarb and the abamectin seed treatments (Table 4). The abamectin and the aldicarb treatments suppressed ($P < 0.05$) nematode penetration in both the lower and upper portion of the root systems (Table 4). Root galling on the plants that remained after the third test was less severe ($P < 0.05$) following either aldicarb or abamectin seed treatment than in the nontreated control (Table 4).

TABLE 3. Early season *Meloidogyne incognita* population densities and root galling after seed treatment with abamectin or soil application of aldicarb in field plots, 2003.

Treatment	DAP 0	DAP 14	DAP 21	DAP 28	Gall rating[a]
Nontreated	966 a[b]	171 ab	114 a	114 a	2.6 a
Abamectin (10 g/100 kg seed)	739 a	171 ab	114 a	0 a	2.8 a
Abamectin (100 g/100 kg seed)	170 b	56 b	114 a	56 a	2.6 a
Aldicarb (840 g/ha)	568 ab	511 a	57 a	0 a	1.6 a
Aldicarb (1,176 g/ha)	568 ab	284 ab	57 a	0 a	1.0 a

[a] 28 DAP. Ratings scale of 0–10 where 0 = no galling and 100 = 100% of root system galled.
[b] Means within columns followed by the same letter do not differ ($P < 0.05$) by Waller-Duncan K-ratio t-test.

DISCUSSION

The potential for loss of yield in fields due to *M. incognita* in cotton is relatively high in Arkansas and many other cotton-producing states due to its wide distribution. In the absence of acceptable cultivars with resistance to the nematode, the most effective method for root-knot management has been through annual applications of the nematicides 1,3-dichloropropene (Telone II) or aldicarb (Temik). These materials are toxic, expensive, and pose considerable environmental risk. Nematicide applied as a seed treatment is an attractive approach to nematode management in cotton due to its convenience and relatively low risk.

Under greenhouse and microplot conditions, abamectin applied as a seed treatment suppressed infection by *M. incognita* for 14 DAP and resulted in less severe root-galling severity early in the life of the plant. Nematode reproduction was also suppressed in greenhouse tests. Protection of the roots of cotton seedlings from infection by *M. incognita* during the first 2 wk after planting may improve the development and yield of the plant (Penteado et al., 2005). However, the effects of abamectin on nematode infection and reproduction were not as evident in the field evaluations. Greenhouse and the microplot trials both were conducted in relatively controlled environments where the soil was

TABLE 4. Cumulative number of *Meloidogyne incognita* in cotton roots 14 DAP and gall ratings in microplots[a].

Treatment	Root fraction[b] Upper	Root fraction[b] Lower	Root gall rating[d]
Nontreated	58 a[c]	128 a	2.7 a
Abamectin (100 g/100 kg seed)	8 b	24 b	2.2 b
Aldicarb (840 g/kg)	9 b	33 b	1.8 b

[a] Table 4 represents results of three microplot tests conducted in 2003.
[b] Intact root systems were arbitrarily divided into an upper 50% and a lower 50% fraction.
[c] Means within columns followed by the same letter do not differ at $P < 0.05$ by Waller-Duncan K-ratio t-test,
[d] Rating scale where 1 = 1% to 10% of root system galled, 2 = 11% to 25%, 3 = 26% to 50%, 4 = 51% to 75%, and 5 = 76% to 100%.

either steam pasteurized (greenhouse) or fumigated (microplots) prior to planting, which could have influenced the effect that was observed. In both of our field tests, the soil application of aldicarb, a material that has a long history of efficacy for nematode control in cotton, also had no effect on nematode population densities or cotton yield. It is possible that environmental effects on either the nematodes or the nematicides affected the efficacy of both materials in these sites.

Abamectin has recently received attention as a seed treatment against nematodes in certain vegetable crops (Becker et al., 2003) and appears to have considerable potential as a nematicide in this context. The relative consistency of its effects on nematode penetration and reproduction in our greenhouse and microplot trials is compelling and merits further study. More detailed studies of the potential for this novel approach to nematode control in cotton are needed across a range of field environments.

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