EFFICACY OF ABAMECTIN AS A SEED TREATMENT FOR CONTROL OF *Meloidogyne incognita* AND *Rotylenchulus reniformis* ON COTTON

A Dissertation

by

TRAVIS RYAN FASKE

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

August 2006

Major Subject: Plant Pathology

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Approved by:

Chair of Committee,	James L. Starr
Committee Members,	Thomas Isakeit
	Michael Kolomiets
	Leonard Pike
Head of Department,	Dennis Gross

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ABSTRACT

Efficacy of Abamectin as a Seed Treatment for Control of *Meloidogyne incognita* and *Rotylenchulus reniformis* on Cotton. (August 2006)

Travis Ryan Faske, B.S., Tarleton State University;

M.S., Oklahoma State University

Chair of Advisory Committee: Dr. James L. Starr

Abamectin is a blend of B_{1a} and B_{1b} avermectins that is being used as a seed treatment to control plant-parasitic nematodes on cotton. Data on the toxicity of abamectin and its effectiveness as a seed treatment to control *Meloidogyne incognita* or *Rotylenchulus reniformis* on cotton are lacking.

The toxicity of abamectin was based on an assay of nematode mobility, LD_{50} values of 1.56 µg/ml and 32.9 µg/ml were calculated based on 2 hr exposure for *M. incognita* and *R. reniformis*, respectively. There was no recovery of either nematode after exposure for 1 hr to its LD_{50} concentration. Sublethal concentrations greater than 0.39 µg/ml for *M. incognita* and 8.2 µg/ml for *R. reniformis* reduced (P = 0.05) infectivity on tomato.

In field trials, suppression (P = 0.05) of *M. incognita* was observed 32 DAP by abamectin seed treatment whereas no suppression of *R. reniformis* was observed. No suppression of *M. incognita* was perceived by abamectin seed treatment in microplots. Suppression of *M. incognita* was observed in

microplots by harpin$_{EA}$ and harping$_{\alpha\beta}$ as a seed treatment and foliar spray, respectively. Seed cotton yields were variable for abamectin-treated seed, but numerically positive for harpin-treated cotton.

Initial gall formation on developing taproots was suppressed (P = 0.001), and penetration of 5-cm long taproots by *M. incognita* and *R. reniformis* was numerically suppressed by abamectin-treated compared to non-treated seed, but infection increased with root development. Using an assay of nematode mobility, the proportion of dead second-stage juveniles (J2) was higher (P = 0.05) following exposure to an excised radicle from abamectin-treated seed than non-treated seed, but lower (P = 0.05) than J2 exposed to the abamectin-treated seed coat. Thus a higher concentration of abamectin remained on the seed coat than emerging radicle. The concentration of abamectin transferred from the seed coat to the developing roots was limited, which contributed to the variability in suppression of plant-parasitic nematodes on cotton.

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First I would like to give thanks to God for this opportunity and patience. Delight yourself in the LORD and he will give you the desires of your heart (Psalm 37:4).

Thanks to Dr. Jim Starr for his sound advice and support during my tenure as senior graduate student. I also appreciate the encouragement and counsel from my committee members: Drs. Tom Isakeit, Mike Kolomiets, and Leonard Pike.

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Finally, I would like to express a sincere thanks to my loving wife Jennifer who has always been supportive and encouraging. I would also like to acknowledge my family for their support and confidence.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Cotton production in the United States accounts for one quarter of the world's lint production. In 2004, 5.3 million hectares produced 2.2 x 10^{10} kg of cotton lint. Upland cotton (*Gossypium hirsutum* L.) makes up 95% of the cotton planted in the United States, with pima cotton (a hybrid of *G. hirsutum* and *G. barbadense*) making up the remaining acreage. An effective boll weevil eradication program and increased use of transgenic cotton cultivars resistant to herbicides and (or) insects has increased the overall profitability of cotton production. However, several biotic pathogens continue to be important factors in cotton production. These include plant-parasitic nematodes, which are found in every state where cotton is produced (Koenning et al., 2004).

Meloidogyne incognita (root-knot nematode) and *Rotylenchulus reniformis* (reniform nematode) are among the most important plant-parasitic nematodes affecting cotton production. *Meloidogyne incognita* is prominent throughout the cotton belt whereas *R. reniformis* can be found from the Carolina's to Texas. Other important nematodes include *Hoplolaimus columbus* (lance nematode) and *Belonalaimus longicaudatus* (sting nematode), which are concentrated in the southeastern United States. These four nematode species contribute to the estimated overall average yield loss of 2% with the highest

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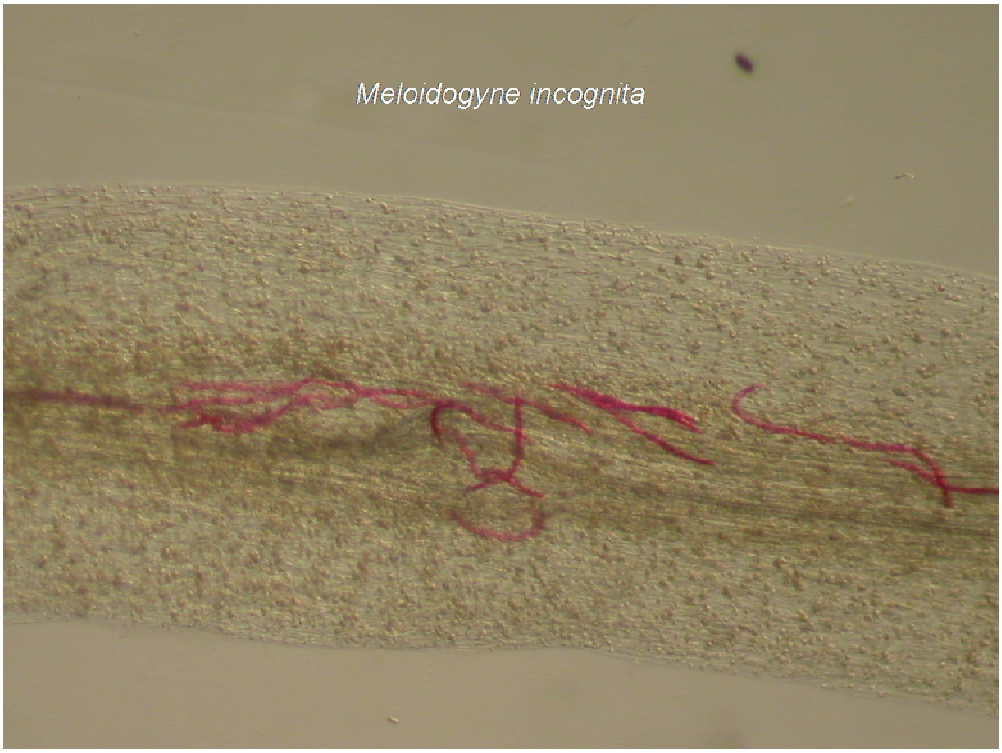
This dissertation follows the style of Journal of Nematology.

yield loss of 4.4% in 2000 according to The Cotton Foundation, resulting in a yield loss of $1.7x10^8$ kg (791,000 bales) equivalent to 196 million dollars (calculated based on USDA-NASS).

Meloidogyne incognita is subdivided into 4 races, with only race 3 and 4 parasitic to cotton. *Meloidogyne incognita* is a sedentary endoparasitic because the female remains stationary after feeding is initiated and second-stage juveniles (J2) are completely embedded inside the root. The life cycle is composed of four juvenile stages with the first-stage juvenile (J1) molting inside the egg and after hatch, the J2 infects at the root tips and migrates through the root (Fig. 1), becoming sedentary where the xylem and phloem differentiate, and establishes a permanent feeding site at that location. The feeding site consists of nurse cells (often called giant cells), which provide nutrients for the juvenile that swells in size as it feeds. After 10 to 14 d feeding, the J2 molts to a J3, followed by the J4 after 4 to 6 d. Neither the J3 or J4 stage feed. Females reinitiate feeding and begin egg production 5 to 7 d following the final molt and are capable of producing 750 eggs (Starr, 1998). Females reproduce by parthenogenesis, thus the vermiform males are not involved in reproduction and exit the root after the final molt. The eggs are deposited into a gelatinous matrix (the egg masses) that ruptures through the root-surface. Both eggs and J2 contribute to overwinter survival, with the J2 being the primary inoculum in the spring (Jeger et al., 1993).



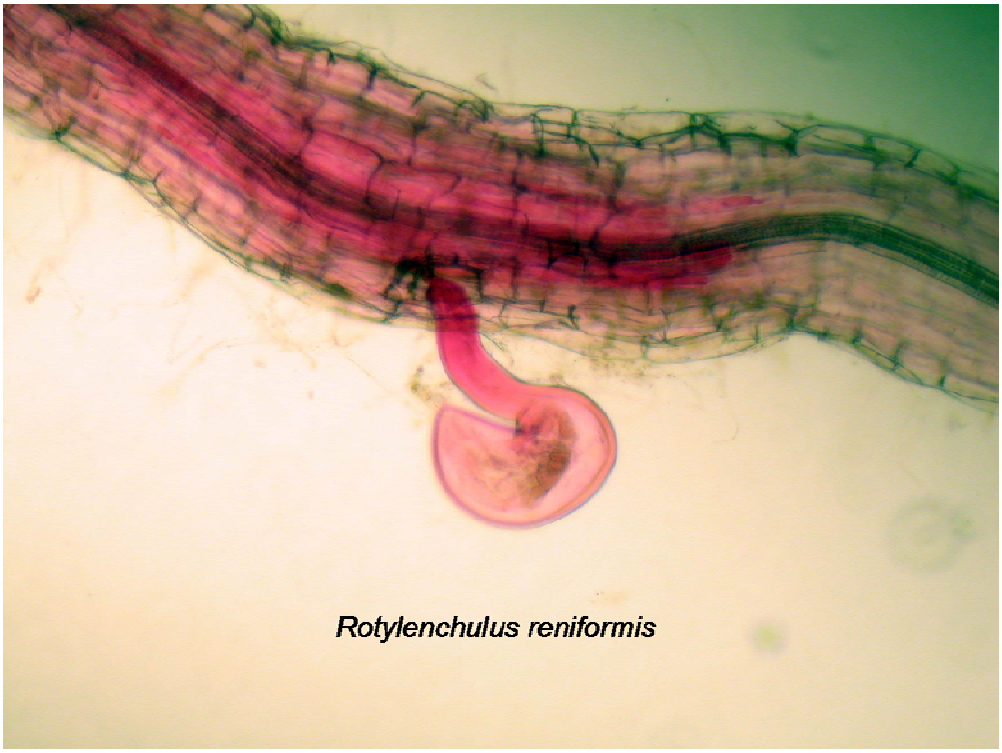


Fig. 1. Second-stage juveniles of *Meloidogyne incognita* and mature female of *Rotylenchulus reniformis* stained with acid fuchsin on cotton roots.

Foliar symptoms include slight to severe stunting, chlorosis, and nutritional deficiency. Galls that form on infected roots are a result of hypertrophy and hyperplasia of the root cortex cells. Galls are a good diagnostic indicator, but when initial inoculum densities are low (<100 J2/500 cm^3 soil) galls may be less conspicuous (Starr, 1998). Root galling causes a disruption in normal root cell function and limits nutrient and water transport, especially in young seedlings (Koenning et al., 2004). This nutrient disruption is related to foliar symptoms and yield loss.

Meloidogyne incognita is commonly found in coarsely textured soils with less than 40% clay. The large host range consists of mostly dicots and some monocots. *Meloidogyne incognita* prefers warmer soil temperatures with an optimum temperature for infection and reproduction at 28ºC. Its distribution is limited to the southern United States due to the cold winter soil temperature of the northern states. Distribution in a field is typically highly aggregated, with a higher population density of eggs than J2 during the summer when cotton is maturing, and with a greater portion of the population as J2 in the winter when the majority of the eggs have hatched. Both eggs and J2 are at their lowest levels just prior to planting (Starr, 1998) .

Rotylenchulus reniformis is a semi-endoparasite because only 1/3 of the female's anterior region is embedded in the root (Fig. 1). The life cycle consists of four juvenile stages with the J1 molting inside the egg and the J2 emerging at hatch. Unlike *M. incognita*, the juveniles remain in the soil during the J3 and J4 stages (Bird, 1984). Males and females develop at a 1:1 ratio. The vermiform

female is the only infective stage and penetrates the root at any location whereas the males are not parasitic. Reproduction is by amphimixis and occurs at a temperature of 16 to 36ºC, with the optimum temperature at 30ºC (Bird, 1984). Infection results in the establishment of a syncytium, a permanent feeding site of several multinucleated cells. Females will begin to produce eggs in 5 to 7 d after infection and each female can produce 100 eggs (Starr, 1998).

Because infection by *R. reniformis* does not result in galling of root tissue and foliar symptoms are similar to that of nutrient deficiencies, diagnosis of yield losses often go undetected. In addition, *R. reniformis* is commonly found in finely textured soils with higher percentage of silt and clay (Koenning et al., 1996). Vermiform stages can survive excessively dry soil conditions in an anhydrobiotic state for up to 2 years (Birchfield and Martin, 1967; Tsai and Apt, 1979), which impedes the effectiveness of crop rotation for management of this pathogen. Horizontal distribution of *R. reniformis* in the field is typically more uniform than *M. incognita*. Vertical distribution to depths >1 m is common (Lee et al., 2002; Robinson et al., 2000; Westphal and Smart, 2003). Highest populations of vermiform occur during the fall and are at a minimum in early spring. Populations as high as 49,000 nematodes/500 cm^3 soil have been reported (Jones et al., 1959) because all juvenile and mature adults can be found in the soil together.

Management practices for controlling *Meloidogyne incognita* and *Rotylenchulus reniformis* include cultural practices, resistance, and use of nematicides. Crop rotation is a common cultural practice in cotton, but a large host range of several crops and weeds by both nematode species limits the number of profitable crops that can be used in a rotation. Depending on soil texture, profitability, preference, and equipment; peanuts can be used in rotation to suppress populations of *M. incognita* and increase cotton yields (Kirkpatrick and Sasser, 1984). In areas infested with *R. reniformis*; peanuts, maize, sorghum, resistant soybean, and small grains can be used in a rotation with cotton to suppress nematode population densities (Davis et al., 2003; Westphal and Smart, 2003).

Because of recent efforts to develop cotton cultivars with improved fiber qualities, increased lint production, and with transgenic herbicide and bollworm resistance, commercial seed companies have put little effort into development into cotton cultivars that are resistant to *M. incognita*. Resistance has been identified in *G. hirsutum* (Robinson and Percival, 1997; Shepherd et al., 1988), and is being incorporated into breeding lines (Starr and Smith, 1999); however few lines with sufficient yield potential and lint/fiber qualities are available to producers. Acala NemX is commercially grown in the western states with competitive yield when grown in fields infested with *M. incognita* (Ogallo et al., 1997). In addition, Paymaster 1560 and Stoneville LA 887 also have high yield potentials in *M. incognita* infested soils, (Koenning et al., 2001; Robinson et al.,

1999; Starr et al., 2005; Zhou and Starr, 2003) in the southern portion of the cotton belt.

No useful level of resistance to *Rotylenchulus reniformis* in *G. hirsutum* has been identified, and only moderate levels in *G. barbadense* (Robinson and Percival, 1997; Robinson et al., 1999; Yik and Birchfield, 1984). Introgression of traits from *G. barbadense* into *G. hirsutum* is possible, but maintaining the desired agronomic traits is difficult in inter-specific hybrids. Tolerance to infection by *R. reniformis* has been reported in cotton germplasm lines (Cook et al., 1997; Koenning et al., 2000), but integration and screening of new pedigrees by cotton seed companies has been a secondary concern.

Nematode management continues to be highly dependent on nematicides, which are effective for nematode suppression and result in higher yields. The carbamate insecticide/nematicide, aldicarb (Temik), is commonly used on cotton to control thrips (*Frankliniella spp*.) and to suppress *R. reniformis* and *M. incognita*. Rates necessary to suppress nematode populations at plant maturity are 1.3 to 1.5 kg a.i./ha higher than rates used to control thrips (Starr, 1998). Even though effective and economically profitable at higher rates, producers are typically unwilling to invest in these higher rates due to a low profit margin. Another carbamate, oxamyl (Vydate), can improve management of *R. reniformis* and *M. incognita* in cotton when used as a foliar application, along with a pre-plant or at-plant application of aldicarb was also used, (Lawrence and McLean, 2000;2002). The fumigant nematicide, 1, 3-dichloropropene (Telone), is applied pre-plant and soil must remain undisturbed for at least 7 d to allow fumigant

dissipation from the soil prior to planting. Additional soil preparation prior to planting, a lengthy post-fumigation that delays planting, and need for specialized equipment has made this fumigant unattractive. Toxic nematicides are critically inspected by the public and governmental agencies due to toxicological, oncological, and environmental concerns (Ragsdale and Seiber, 1999). Chemicals with lower toxicity to handlers and the environment which provide nematode suppression are desirable. One such class of pesticides currently being evaluated are avermectins.

Avermectins are macrocyclic lactones produced as secondary metabolites by the soilborne actinomycete, *Streptomcyes avermentilis,* and were discovered by Merck Sharp and Dohme Research Laboratories (Merck & Co., Inc.) in 1975. Avermectins are made up of eight different components, denoted as A_{1a}, A_{1b}, A_{2a}, A_{2b}, B_{1a}, B_{1b}, B_{2a}, and B_{2b}. The A and B-components differ in a methoxy and hydroxyl group, respectively; the 1 and 2-components differ in a double and single bond, respectively; and the a and b-components differ by a secondary butyl side chain and an isopropyl substituent, respectively. The activity of a- and b-components is essentially identical and they are consistently produced in a 4:1 ratio during the fermentation process (Shoop et al., 1995). The B-components possess potent anthelmintic and insecticidal activity. The mode of action of avermectins is to block gamma-amino butyric acid stimulated chloride channels and open non-neurotransmitter-gated chloride channels (Jansson and Dybas, 1998; Schaeffer and Haines, 1989), causing an ion imbalance in the nervous system of treated nematodes. Ivermectin, a semi-synthetic hybrid of B_1

and B_2, was released for commercial use for controlling endo and ecto-parasites in livestock and domestic pets. Abamectin is avermectin B_1 and was initially released for ornamental and horticultural use as acaricide and insecticide. Abamectin is also incorporated into baits to control imported fire ants and cockroaches. Currently there are six pesticides from Syngenta Crop Protection with abamectin as the main active ingredient.

Chemical properties of abamectin include a short ½ life of <10 hr in sunlight and 20 to 47 d in the soil depending on the level of organic matter, with a great affinity to bind to soil particles. Based on a soil column leaching study, Wislocki et al. (1989) reported that abamectin molecules moved 6 cm in 28 d in a 38 cm high column with an equivalent of 56 cm of rainfall. Abamectin is essentially insoluble in water (7.8 ppb) and is not hydrolytic (Wislocki et al., 1989). In a few studies, low levels of abamectin are taken up by plant foliage via translaminar movement resulting in good control of mites (Dybas, 1989). Radiolabeled studies have shown that low levels of abamectin are transported within a plant root system from treated seeds (Long, 2005).

Avermectin (B_{2a}) at 0.3 ng/ml was reported to be sufficient to reduce the number of *M. incognita* juveniles infecting cucumber roots in vivo (Wright, 1983). Abamectin (B_1) has been reported to control several plant-parasitic nematodes in a variety of settings. Injecting abamectin into the pseudostem of banana was as effective as other commercial nematicide applications controlling *M. javanica* and *Radopholus similis*. A root dip assay provided moderate control of *M. incognita* on tomato (Jansson and Rabatin, 1997;1998). A soil drench

application of abamectin into the soil reduced penetration of *M. arenaria* on roots of tomato seedlings (Cayrol et al., 1993). Incorporating avermectins (B_1 or B_2) at low rates reduced root-gall ratings on tomato and tobacco in *M. incognita* infested fields relative to that of commercial nematicides (Garabedian and Van Gundy, 1983; Sasser et al., 1982). Nordmeyer and Dickson reported tobacco yield increase from abamectin treatments comparable to aldicarb and oxamyl in microplots infested with *M. incognita, M. javanica,* or *M. arenaria* (Nordmeyer and Dickson, 1985). Abamectin is toxic to plant-parasitic nematodes but not on non-target organisms and is effective at rates as low as 0.05 to 0.50 kg a.i./ha compared to 0.84 kg a.i./ha for commercially available nematicides. In all field trials, incorporating abamectin into the soil was the method of choice to increase the probability of contact with plant-parasitic nematodes. In 2002, Syngenta Crop Protection began testing seed treatments with various concentrations of abamectin for nematode control. Under controlled conditions, vegetable seed treated with 0.1 to 0.3 mg a.i./seed provided protection against *M. incognita* and *Pratylenchus penetrans* (Abawi et al., 2003; Becker and Hofer, 2004). In 2004, Syngenta Crop Protection began marketing Avicta Complete Pak – a cotton seed treatment that includes the nematicide abamectin (Avicta), insecticide thiamethoxam (Cruiser), and the fungicides azoxystrobin + fludioxonil + mefonoxam (Dynasty).

Objectives

The objectives of this study are (i) to determine the sensitivity of *M. incognita* and *R. reniformis* to abamectin using several in vitro assays, (ii) to evaluate abamectin as a cotton seed treatment for the management of *M. incognita* and *R. reniformis* in field and microplot trials, and (iii) to characterize abamectin seed treatment for suppression of root infection by *M. incognita* and *R. reniformis*.

CHAPTER II

SENSITIVITY OF *Meloidogyne incognita* AND *Rotylenchulus reniformis* TO ABAMECTIN

Introduction

The root-knot nematode *Meloidogyne incognita* is found in nearly all cotton (*Gossypium hirsutum*) production areas in the United States, especially in coarsely textured soil (Robinson et al., 1987; Starr et al., 1993). Root galling results in physiological changes in root tissue at the nematode feeding sites, which reduce nutrient and water flow, thus lowering yield (Koenning et al., 2004). The reniform nematode, *Rotylenchulus reniformis*, is the second most important nematode species on cotton. It is commonly found in the southeastern cotton belt in finely textured soil (Robinson et al., 1987; Starr et al., 1993). Because infection by *R. reniformis* does not result in galling of root tissue and foliar symptoms are similar to that of nutrient deficiencies, yield losses by this nematode often go undetected (Koenning et al., 2004).

Tactics for management of plant-parasitic nematodes continue to rely on nematicides for suppression of population densities (Koenning et al, 2004). The most effective non-fumigant nematicides are aldicarb and oxamyl, which are highly toxic. The use of highly toxic pesticides has been criticized by the public due to potential hazards to environmental and human health (Ragsdale and Seiber, 1999). Chemicals with lower toxicity to humans and the environment

that provide nematode suppression are desirable. One class of pesticide currently being re-evaluated for utility in management of plant-parasitic nematodes is avermectin.

Avermectins are 16-membered macrocyclic lactones produced by *Streptomyces avermitilis*. The anthelminthic, insecticidal, and acaricidal activities of avermectins are well known (Davies and Green, 1986; Dybas, 1989; Jansson and Dybas, 1998; Shoop et al., 1995), and several avermectin formulations are available to control insects and mites on plants. Avermectins block gamma-amino butyric acid-stimulated chloride channels and open non-neurotransmitter-gated chloride channels (Jansson and Dybas, 1998), causing an ion imbalance in the nervous system, resulting in paralysis. Abamectin (a blend of B_{1a} and B_{1b} avermectins) has been evaluated in soil applications, stem injections, root dips, bulb dips, and foliar sprays for potential control of plant-parasitic nematodes in several crops (Cayrol et al., 1993; Garabedian and Van Gundy, 1983; Jansson and Rabatin, 1997;1998; Nordmeyer and Dickson, 1985; Roberts and Matthews, 1995; Sasser et al., 1982). Successful treatments place the abamectin in contact with plant-parasitic nematodes. Commercialization of abamectin for controlling plant-parasitic nematodes has been delayed because abamectin has a short half-life in soil, 20 to 47 d depending upon the level of organic matter. Further, abamectin has a great affinity to bind to soil particles, is essentially insoluble in water, and is not hydrolytic (Wislocki et al., 1989). Abamectin-treated cotton seed has been evaluated for suppression of *M. incognita* and *R. reniformis*, and will be available to cotton producers in 2006

(Long, 2005). Few data on the sensitivity and behavioral effects of *M. incognita* and *R. reniformis* to abamectin are available. Given the renewed interest in this group of compounds for nematicide use, additional data on sensitivity of target species are needed.

The objectives of this study were (i) to characterize the lethal concentration of abamectin for *M. incognita* and *R. reniformis* in vitro, (ii) to determine if the effects of abamectin on each nematode species are reversible, and (iii) to determine the effect of sublethal concentrations of abamectin on infectivity of each nematode species.

Materials and Methods

Nematode cultures: *Meloidogyne incognita* and *R. reniformis* were originally isolated from cotton and maintained in the greenhouse on *Lycopersicon esculentum* cv. Rutgers. Eggs were collected from 8- to 10-wk-old *M. incognita* cultures with NaOCl (Hussey and Barker, 1973). Second-stage juveniles (J2) were collected in hatching chambers with a 20-µm pore screen that allows only hatched J2 to migrate into the collection dish (Vrain, 1977). Only 24-hr-old J2 were evaluated in this study. *Rotylenchulus reniformis* were collected from infested soil using Baermann funnels (Chapman, 1958). Mixed-life-stages of *R. reniformis* were collected with a 25-µm pore sieve after 48 hr and used immediately.

Lethal concentration response: Meloidogyne incognita J2 and *R. reniformis* mixed-life-stages were exposed to 21.5, 2.15, 0.22, 0.022, and 0 µg of abamectin/ml (Syngenta Crop Protection, Greensboro, NC), and mortality was determined visually at 2 hr and 24 hr post exposure. These tests were performed in BPI (Bureau of Plant Industries) watch dishes; each dish received 500 µl of 2X test concentration to which 30 to 40 nematodes in 500 µl of distilled water were added. Each treatment was replicated four times in two experiments for each nematode species. Nematodes were considered dead if they did not respond to being touched by a small probe. The abamectin carrier formulation (chemistry unknown) was also evaluated using the same procedure.

Estimating reversible effects of abamectin: Approximately 1000 of *M. incognita* or *R. reniformis* were exposed for 1 hr to its LD_{50} concentration (calculated based on a 2 hr exposure response). After the 1-hr exposure to abamectin, nematodes were carefully rinsed twice on a 25-µm pore sieve with distilled water, then transferred to a counting dish containing distilled water. Nematodes exposed to distilled water served as the control. Nematodes were examined using a dissecting microscope after 1-hr exposure, 1 hr after the rinse, and 2 hr after rinse. Nematodes were considered dead if they did not respond to being touched by a small probe. Each treatment was replicated four times and the proportion of dead nematodes was recorded for both nematode species.

A second experiment was conducted with an aldicarb (Bayer CropScience, Research Triangle Park, NC) treatment to compare nematicides. Preliminary experiments identified that 30 µg/ml of aldicarb resulted in

approximately 50% mortality for both *M. incognita* and *R. reniformis* after 2 hr of exposure. Nematode mortality was estimated at 2 hr and 24 hr after being removed from the abamectin or aldicarb treatments and rinsed with distilled water.

Effect of sublethal concentrations on infectivity: Approximately 120,000 of each nematode species was exposed to abamectin or aldicarb at its LD_{50} concentration (calculated based on a 2 hr exposure response) for 1 hr, then used to inoculate 2-wk-old tomato seedlings growing in sand-sandy loam (2:1 v/v) in 63 cm^3 planter flats. Each seedling received 2 ml of the abamectin solution containing 2000 nematodes. Inoculum was distributed among three cavities around the seedlings created by pushing a 1 ml pipette tip 3 cm into the root zone. Tomato seedlings with nematodes exposed to distilled water served as controls. Tomato plants were incubated at 28°C with 12 hr darkness in a 24 hr period. Seedlings inoculated with *M. incognita* were harvested 2 wk after inoculation, and those inoculated with *R. reniformis* were harvested 3 wk after inoculation.

The infectivity of *M. incognita* was evaluated using sublethal concentrations of 1.56, 1.17, 0.78, and 0.39 µg abamectin/ml. A root gall rating was used to estimate the effects of sublethal concentrations on infectivity of *M. incognita*, based on a six point scale with 0 = no galls and 5 = severe galling. The experiment was a randomized complete block design (RCBD) with each treatment replicated six times. Lower sublethal concentration treatments of 1.56, 0.75, 0.156, 0.016, and 0.002 µg abamectin/ml were evaluated to identify

the lowest sublethal concentration able to reduce infection in a second experiment.

Sublethal concentrations of 32.9, 24.7, 16.5, and 8.22 µg abamectin/ml were used to evaluate the infectivity of *R. reniformis* on tomato roots. Females were stained with acid fuchsin (Byrd et al., 1983) to aid in counting females per root system. The experiment was a RCBD with each treatment replicated six times and the experiment was repeated once.

Statistical analysis: Lethal concentration response data were subjected to probit analysis; whereas data collected from estimating reversible effects of abamectin and sublethal concentration on infectivity were analyzed using general linear model analysis of variance using SPSS 11.5 (SPSS Inc. Chicago, Ill).

Results

Meloidogyne incognita was more sensitive to abamectin than *R. reniformis* at all concentrations above 0.21 µg/ml (data not shown). Mortality of *M. incognita* after a 2 hr exposure was 99% at 21.5 µg abamectin/ml; whereas *R. reniformis* mortality at 2 hr was 28%. Mortality of *M. incognita* and *R. reniformis* reached 100% and 97%, respectively, after a 24 hr exposure to 21.5 µg abamectin/ml. The pesticide carrier had no detectible effect on mortality of either nematode species. No variation in mortality of *R. reniformis* was observed among male, female, or juvenile stages. The LD_{50} values of 1.56

µg/ml and 32.9 µg abamectin/ml were calculated based on 2 hr exposure for *M. incognita* and *R. reniformis*, respectively (Fig. 2). At 24 hr, the LD_{50} values for *M. incognita* and *R. reniformis* were 0.42 µg/ml and 3.49 µg/ml, respectively. The LD_{90} values at 24 hr exposure for *M. incognita* and *R. reniformis* were 0.82 µg/ml and 14.69 µg/ml, respectively.

Neither *M. incognita* nor *R. reniformis* exhibited any observable recovery from paralysis or mortality when removed from abamectin after 1 hr exposure to their respective 2 hr LD_{50} concentrations. Mortality continued to increase after *M. incognita* was rinsed and removed from the abamectin; whereas mortality for *R. reniformis* remained unchanged after removal from abamectin (Fig. 3). In contrast, a significant ($P < 0.05$) reversible effect was observed for *M. incognita* 2 hr after being rinsed and removed from aldicarb. Negligible recovery from aldicarb was observed for *R. reniformis* 24 hr after rinse and transfer to distilled water (Fig. 3). Nematode posture was rigid and straight for both nematode species when treated with abamectin, and neither responded to being touched by a small probe (Fig. 4). *Meloidogyne incognita* and *R. reniformis* were relaxed and undulated when exposed to aldicarb and responded to being touched by a small probe.

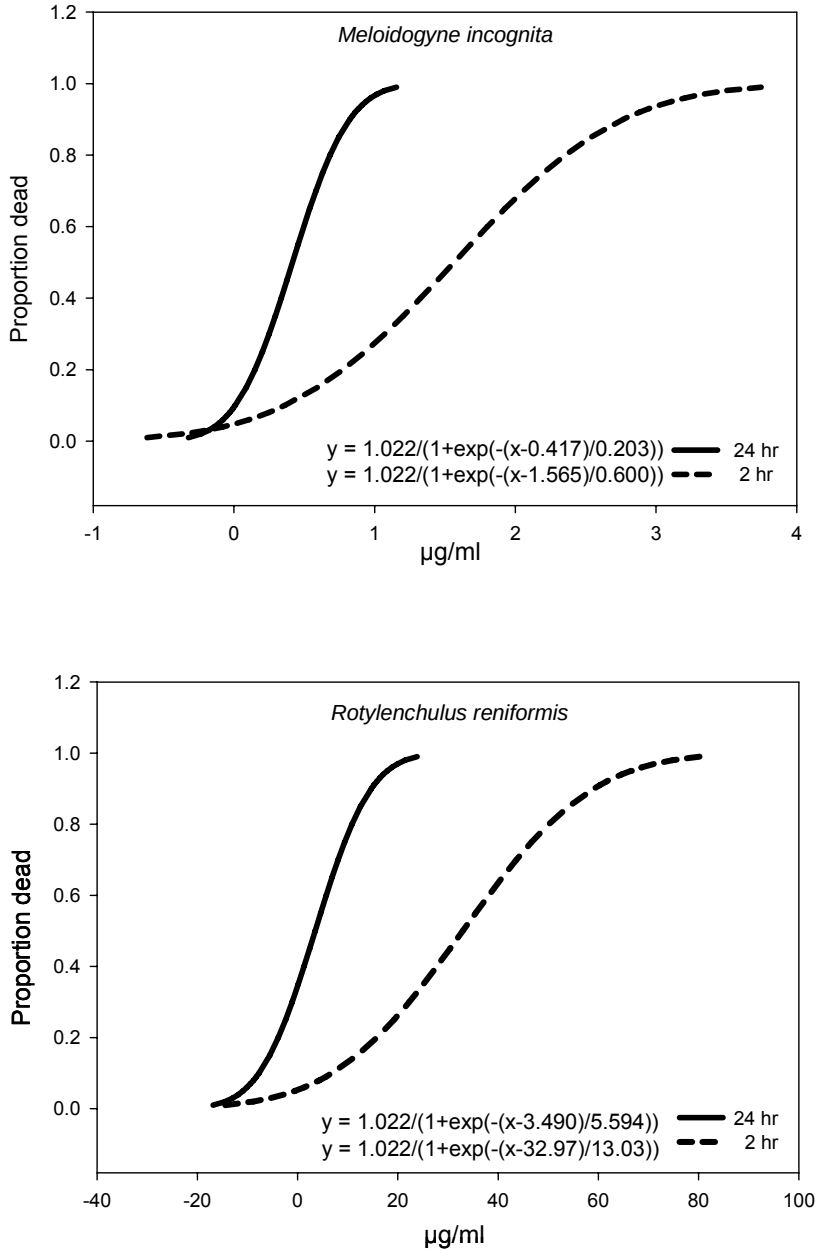


Fig. 2. Effect of abamectin on mortality of *Meloidogyne incognita* and *Rotylenchulus reniformis* after 2 hr and 24 hr exposure. Equations are derived by nonlinear regression of probit analysis.

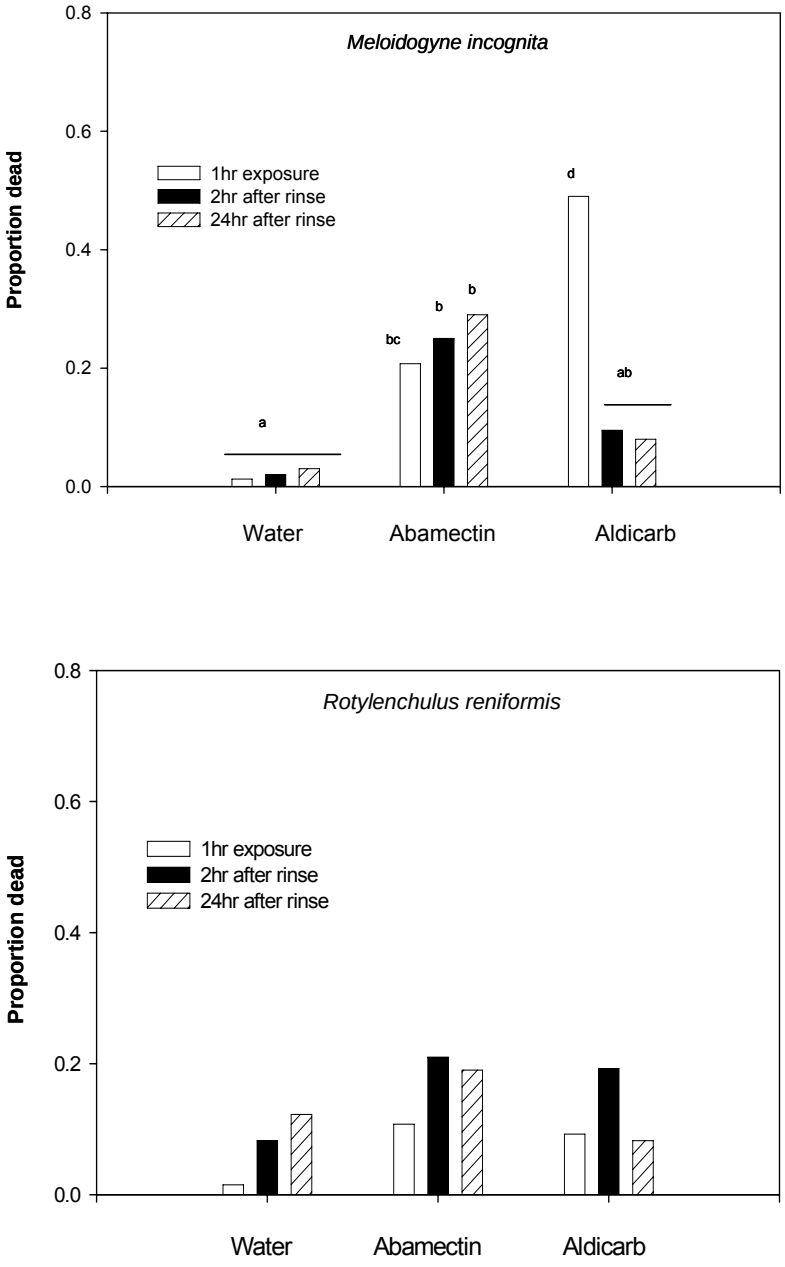


Fig. 3. Recovery of *Meloidogyne incognita* and *Rotylenchulus reniformis* post exposure to abamectin and aldicarb. Each species was exposed to its 2 hr LD_{50} for each nematicide for 1hr then rinsed and transferred to distilled water. Proportion of dead nematodes was recorded after the 1 hr exposure and at 2 hr and 24 hr after removal from the test solutions. Different letters over bars indicate significant differences at $\alpha = 0.05$ according to LSD for *M. incognita*.

Fig. 4. Posture of *Meloidogyne incognita* after 48 hr exposure to its respective LD_{50} concentration for abamectin and aldicarb. LD_{50} concentrations based on 2 hr exposure response were 1.56 µg abamectin/ml and 30.0 µg aldicarb/ml.

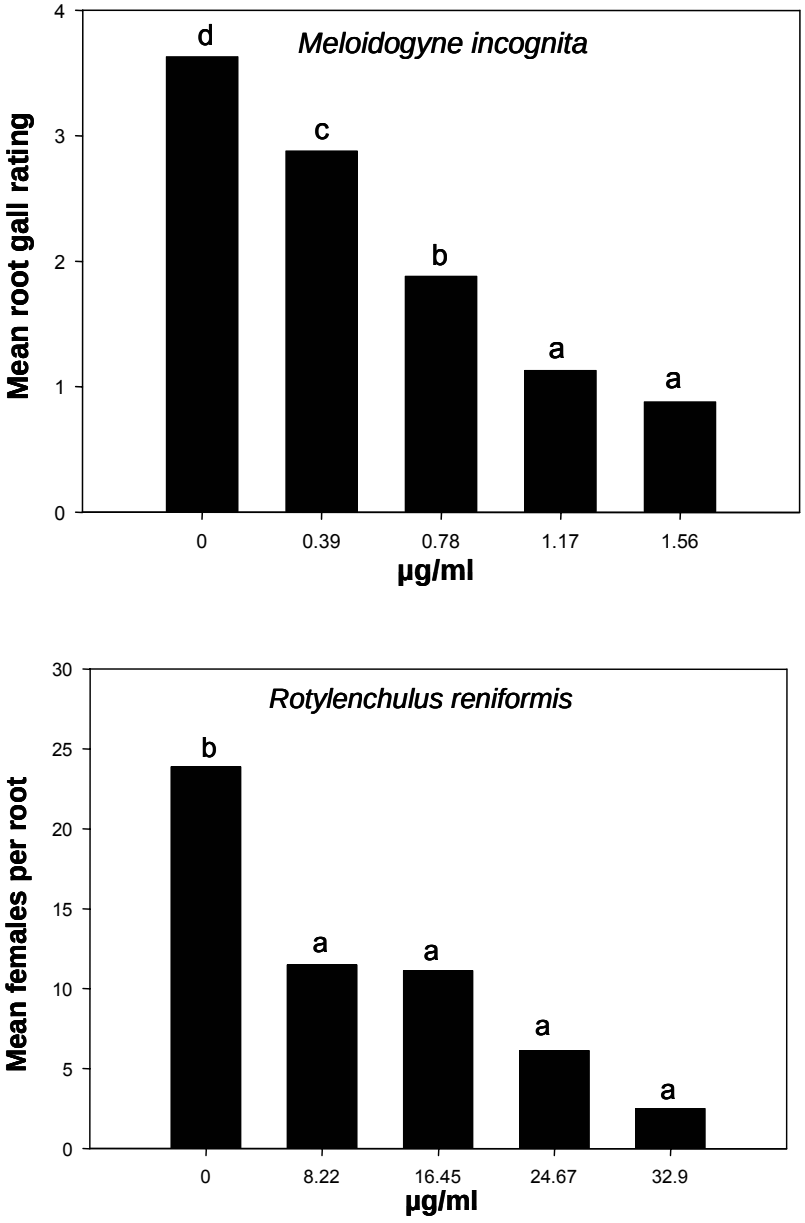


Fig. 5. Effect of treatment with sublethal concentrations of abamectin on infectivity of *Meloidogyne incognita* and *Rotylenchulus reniformis*. Root gall ratings are based on a six point scale where 0 = no galling and 5 = sever galling. Female *R. reniformis* were stained with acid fuchsin prior to counting. Different letters over bars indicate significant differences at α = 0.05 according to LSD.

All sublethal concentrations greater than 0.39 µg abamectin/ml inhibited ($P < 0.05$) infection of tomato roots by *M. incognita* (Fig. 5). No reduction of root galls occurred with abamectin concentration less than 0.15 µg/ml (data not shown). Sublethal concentrations greater than 8.2 µg abamectin/ml reduced ($P < 0.05$) the number of *R. reniformis* females observed per root (Fig. 5).

Discussion

Based on the lethal concentration response, *R. reniformis* was less sensitive to abamectin than was *M. incognita*. The effective LD_{90} based on 24 hr exposure of 0.82 µg/ml for *M. incognita* was similar to 0.2 µg/ml reported by Cayrol et al. (1993) for *Meloidogyne arenaria*. The LD_{90} based on 24 hr exposure for *R. reniformis* was 82% (14.7µg/ml) higher than *M. incognita*. These findings suggest that *Meloidogyne* species are generally more sensitive to abamectin than is *R. reniformis*.

Paralysis and mortality of *M. incognita* due to abamectin was irreversible and increased following removal of the pesticide. This response was also reported for *M. arenaria* to abamectin (Cayrol et al., 1993). There was no recovery by *R. reniformis* when treated with abamectin; however mortality of *R. reniformis* did not continue to increase. Reversible effects observed for *M. incognita* to aldicarb have also been reported in other plant-parasitic and free-living nematodes (Nelmes, 1970; Opperman and Chang, 1991). Thus, by this assay, *R. reniformis* was less sensitive to abamectin than *M. incognita.*

Plant infection was reduced when sublethal concentration rates were 25% of LD_{50} values for both *M. incognita* and *R. reniformis*. A sublethal concentration of 1.0 µg/ml of aldicarb has been reported to inhibit infection by *Meloidogyne javanica* and *Heterodera schachtii* to tomato roots and sugar beets respectively (Hough and Thomason, 1975). The concentration of abamectin necessary to cause paralysis and inhibit infection for both *M. incognita* and *R. reniformis* was very low and comparable to that of aldicarb.

Though the toxicity of abamectin is comparable to aldicarb; exposure to abamectin results in irreversible paralysis of *M. incognita* and *R. reniformis*. Abamectin is applied to cotton seed at a rate of 150 µg/seed, which far exceeds the LD_{50} values of either *M. incognita* or *R. reniformis*. The concentration of abamectin in the spermosphere and rhizosphere soil when seed is planted and germinates has not been adequately quantified, but even low concentrations can result in irreversible paralysis and inhibit infection.

CHAPTER III

EVALUATION OF ABAMECTIN SEED TREATMENT AND ALDICARB FOR MANAGEMENT OF *Meloidogyne incognita* AND *Rotylenchulus reniformis* ON COTTON

Introduction

The southern root-knot nematode, *Meloidogyne incognita* and the reniform nematode, *Rotylenchulus reniformis,* are among the most important plant-parasitic nematodes affecting cotton (*Gossypium hirsutum*) production (Koenning et al., 2004). Current management practices continue to depend on nematicides to suppress plant-parasitic nematode population densities. Aldicarb (Temik 15G), a non-fumigant nematicide, is commonly applied at 0.67 to 0.84 kg a.i./ha to suppress plant-parasitic nematodes. Aldicarb is absorbed into developing seedlings providing systemic protection from nematodes for ~60 d, depending on soil type, moisture, and microbial activity (McLean and Lawrence, 2003).

Abamectin is a blend of avermectin compounds that suppress *Meloidogyne* species when applied in direct contact with plant-parasitic nematodes (Cayrol et al., 1993; Jansson and Rabatin, 1997;1998). Development of an abamectin product to suppress plant-parasitic nematodes has been prolonged because abamectin is nearly insoluble in water, binds quickly to soil particles, and has limited local systemic movement in leaf tissue

(Wislocki et al., 1989). Radiolabeled studies have shown movement of abamectin applied as a seed treatment into the developing root system (Long, 2005). Abamectin as a seed treatment has been evaluated for suppression of some *Meloidogyne* species (Becker and Hofer, 2004; Becker et al., 2003; Kiewnick and Grimm, 2005; Long, 2005; Westphal and Egel, 2004). However, there is limited information on the suppression of plant-parasitic nematodes by abamectin-treated cotton seed compared to aldicarb. No information is available from commercial fields infested with both *M. incognita* and *R. reniformis*. Therefore, the objectives of this study were to evaluate abamectin as a seed treatment and aldicarb in field plots naturally infested with *M. incognita* and *R. reniformis* and microplots artificially infested with *M. incognita*.

Materials and Methods

Seed treatments: Cotton cv. DP 444 BG/RR was used for field and microplot trials. Seed treatments were applied by the manufacturer. These treatments included abamectin (Avicta, Syngenta Crop Protection, Greensboro, NC) applied at 150 µg/seed, thiamethoxam (Cruiser 5FS, Syngenta Crop Protection) at 340 µg/seed to control early season insects, and a blend of azoxystrobin + fludioxonil + mefonoxam (Dynasty CST, Syngenta Crop Protection) applied at 30 µg/seed to control seedling disease. The fungicides triadimenol + thiram (RTU-Baytan-Thiram, Bayer CropScience, Research Triangle Park, NC) were applied at 41 g a.i./100 kg seed for seedling disease

and pesticide STP15142 at 15 g a.i./100 kg seed. Aldicarb (Temik 15G, Bayer CropScience, Research Triangle Park, NC) was applied at 0.67 or 0.84 kg a.i./ha at planting in the seed row.

Field: The test site located in Robertson, Co., TX (N30.81341; W096.60181) was a Yahola fine sandy loam (77% sand, 21% silt, and 2% clay) naturally infested with *M. incognita* and *R. reniformis*. Abamectin-treated seed was evaluated based on five treatments, which included; i) Dynasty CST + Cruiser 5FS; ii) Dynasty CST + Cruiser 5FS +Avicta; iii) Dynasty CST + Temik 15G at 0.67 kg a.i./ha; iv) Dynasty CST + Temik 15G at 0.84 kg a.i./ha; and v) STP15142 + BTU-Bayton-Thiram + Temik 15G at 0.84 kg a.i./ha. Aldicarb was applied at planting (22 April 2005) in the seed row with chemical granular applicators. Treatments were arranged in a randomized complete block design (RCBD) and replicated four times. Plots consisted of four rows, 102-cm wide, and 12.2-m long. Blocks were separated by 3-m alley. All plots were maintained throughout the season as per commercial cotton production. Population densities of *M. incognita* and *R. reniformis* were measured three times during the growing season. Sixteen soil cores 2.5-cm dia. and 20-cm deep were taken from the inner rows of each plot and composited. Aliquots of 500 cm^3 soil were used for extraction of vermiform nematodes and cotton roots by elutriation. Vermiform nematodes were collected by a sucrose centrifugation technique (Jenkins, 1964) and eggs from cotton root tissue with NaOCl (Hussey and Barker, 1973). Second-stage juveniles of *M. incognita* and *R. reniformis* were collected in hatching chambers to identify reproduction per species (Vrain,

1977). Root-gall ratings were determined at harvest on 17 September 2005 from four plants collected from the outer two rows of each four row plot in a systematic sampling pattern. Root gall rating were based on a six point scale with 0 = no galling and 5 = severe galling. Seedling stand and vigor were evaluated 3 wk after planting and plant height was evaluated bimonthly.

Microplots: Wooden microplots (60-cm x 84-cm x 25-cm) were located at the *USDA/ARS,* Southern Crops Research Laboratory in College Station, Texas. Microplots were filled with a sandy soil (93% sand, 4% silt, and 3% clay) and were fumigated with 1, 3-dichloropropene (6 ml/m^2) to eliminate existing nematode populations. Microplots were infested 3 wk after fumigation with 2,500 eggs of *M. incognita*. Abamectin-treated seed was compared to aldicarb based on four treatment, which included; i) Dynasty CST + Cruiser 5FS; ii) Dynasty CST + Cruiser 5FS + Avicta; iii) Dynasty CST + Cruiser 5FS + Avicta + Temik 15G at 0.84 kg a.i./ha; and iv) Dynasty CST + Cruiser 5FS + Temik 15G at 0.84 kg a.i./ha. The 2 x 4 factorial arrangement of treatments was in a RCBD. The first treatment factor was infested and non-infested plots and the second treatment factors were nematicide treatments. Treatments were replicated six times. Plants were thinned 3 wk after planting to 5 plants per microplot. Population densities of *M. incognita* were determined twice during the season. Eight soil cores (2.5-cm dia. and 20-cm deep) were taken from each plot and composited. Extraction and collection of vermiform nematodes and cotton roots were processed as described above. Root-gall ratings were determined at harvest on 31 August 2005.

Statistical analysis: Data were analyzed using general linear model analysis of variance using SPSS 11.5 (SPSS Inc. Chicago, Ill) and mean separations were by least significant difference procedure.

Results

Field site pre-plant populations of *M. incognita* and *R. reniformis* were 68 and 513/500 cm^3 of soil, respectively. No differences due to treatments were observed for seedling stand, plant height, or vigor throughout the season. Dynasty CST + Cruiser 5FS + Temik 15G had a higher (P = 0.05) population density of *M. incognita* at 32 DAP, whereas no differences among treatment were observed for *R. reniformis*. Population densities of *M. incognita* or *R. reniformis* at 60 and 148 d after planting (DAP) were similar (P > 0.20) among treatments (Fig. 6). Mean egg counts of *M. incognita* and *R. reniformis* at 60 and 148 DAP were similar (P > 0.49) among treatments (Fig. 7). Percent vermiform nematodes from egg hatch at 60 DAP was 56% *R. reniformis* and 44% *M. incognita* across all treatments; whereas at 148 DAP 27% were *R. reniformis* and 73% were *M. incognita* across all treatments (Fig 8). Root-gall ratings were similar (P = 0.63) among treatments and averaged 1.75 across all treatments (Fig. 9).

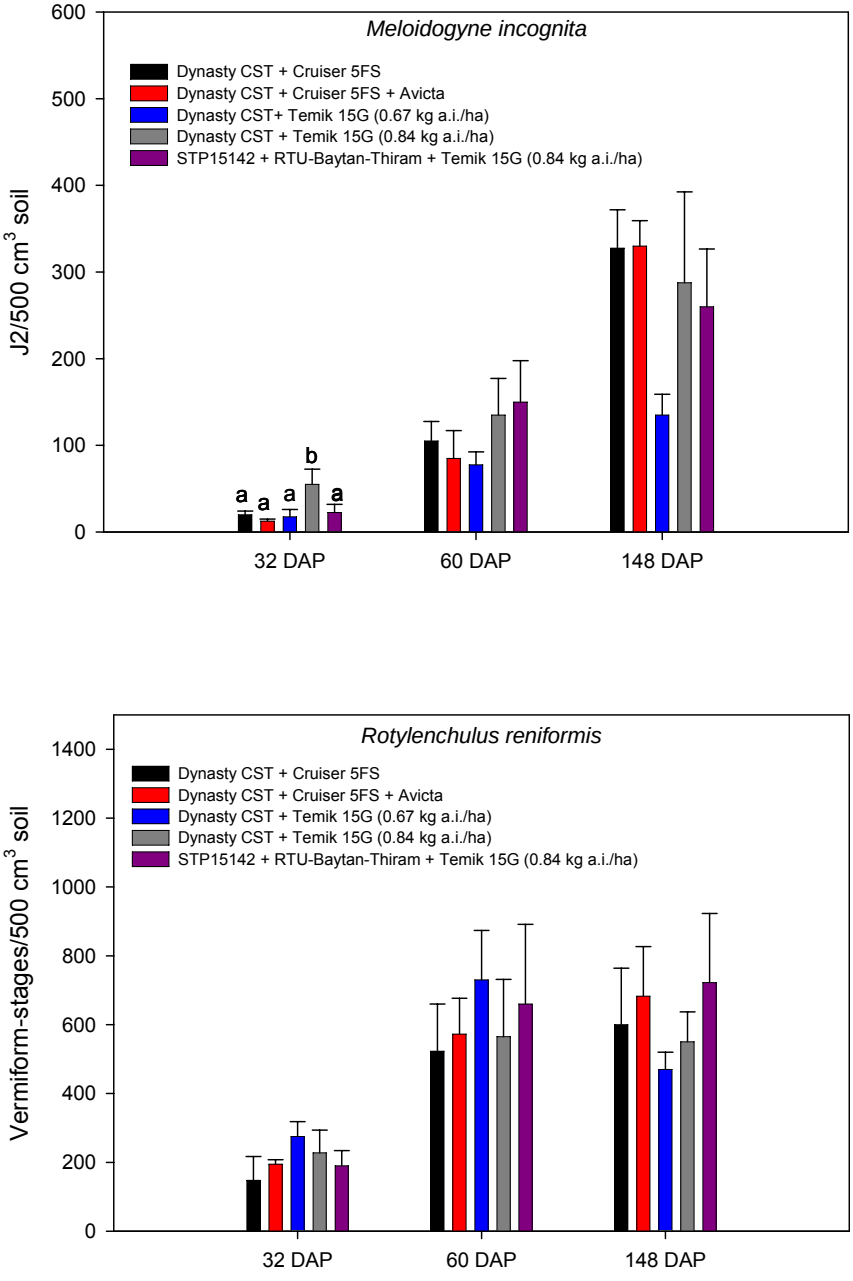


Fig. 6. Effect of Avicta and Temik 15G on *Meloidogyne incognita* and *Rotylenchulus reniformis* population densities on cotton. Dynasty CST, Cruiser 5FS, Avicta, STP15142, and RTU-Bayton-Thiram were applied as seed treatments whereas Temik 15G was applied at planting in seed row. Different letters over bars (*M. incognita*, 32 DAP) indicate significant difference according to LSD at $\alpha = 0.05$.

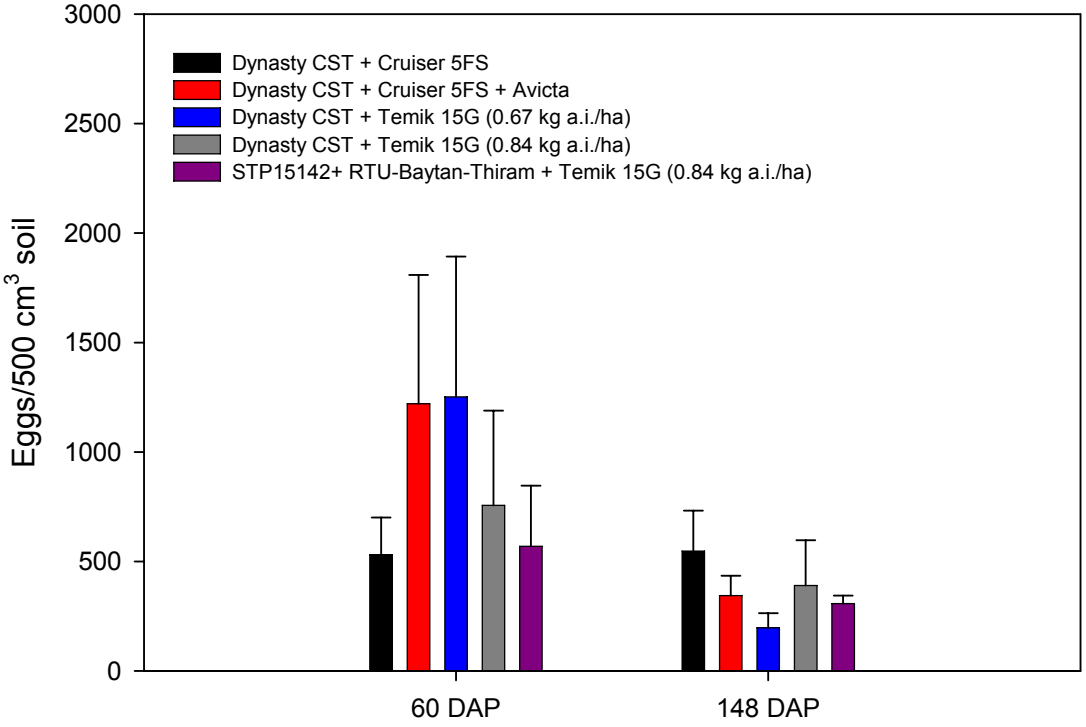


Fig. 7. Effect of Avicta and Temik 15G on egg production by *Meloidogyne incognita* and *Rotylenchulus reniformis* on cotton. Dynasty CST, Cruiser 5FS, Avicta, STP15142, and RTU-Bayton-Thiram were applied as seed treatments whereas Temik 15G was applied at planting in seed row.

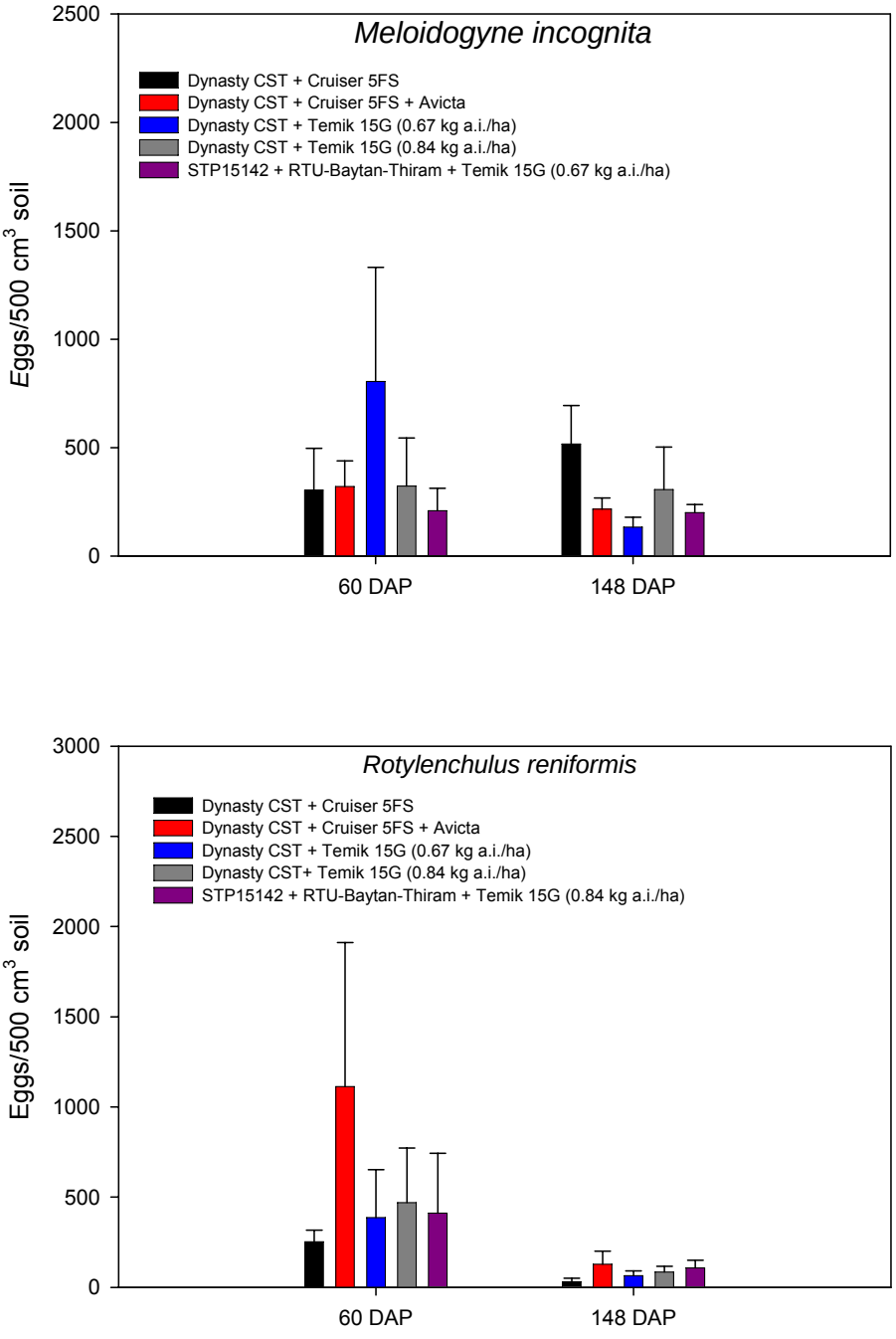


Fig. 8. Effect of Avicta and Temik 15G on reproduction by *Meloidogyne incognita* and *Rotylenchulus reniformis* on cotton. Vermiform were collected in a hatching chamber and identified. Dynasty CST, Cruiser 5FS, Avicta, STP15142, and RTU-Bayton-Thiram were applied as seed treatments whereas Temik 15G was applied at planting in seed row.

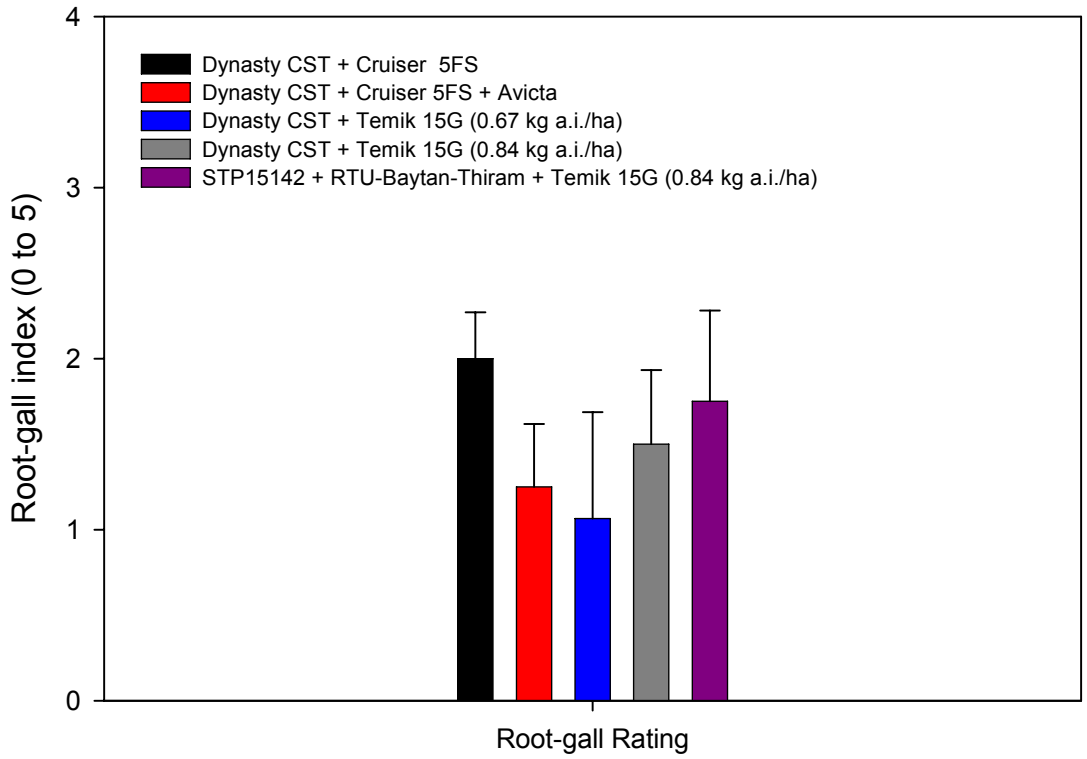


Fig. 9. Effect of Avicta and Temik 15G on cotton root-galling by *Meloidogyne incognita*. Four roots from outside two rows of the four row plats were used to determine root galling based on a six point scale with 0 = no galling and 5 = severe galling. Dynasty CST, Cruiser 5FS, Avicta, STP15142, and RTU-Bayton-Thiram were applied as seed treatments whereas Temik 15G was applied at planting in seed row.

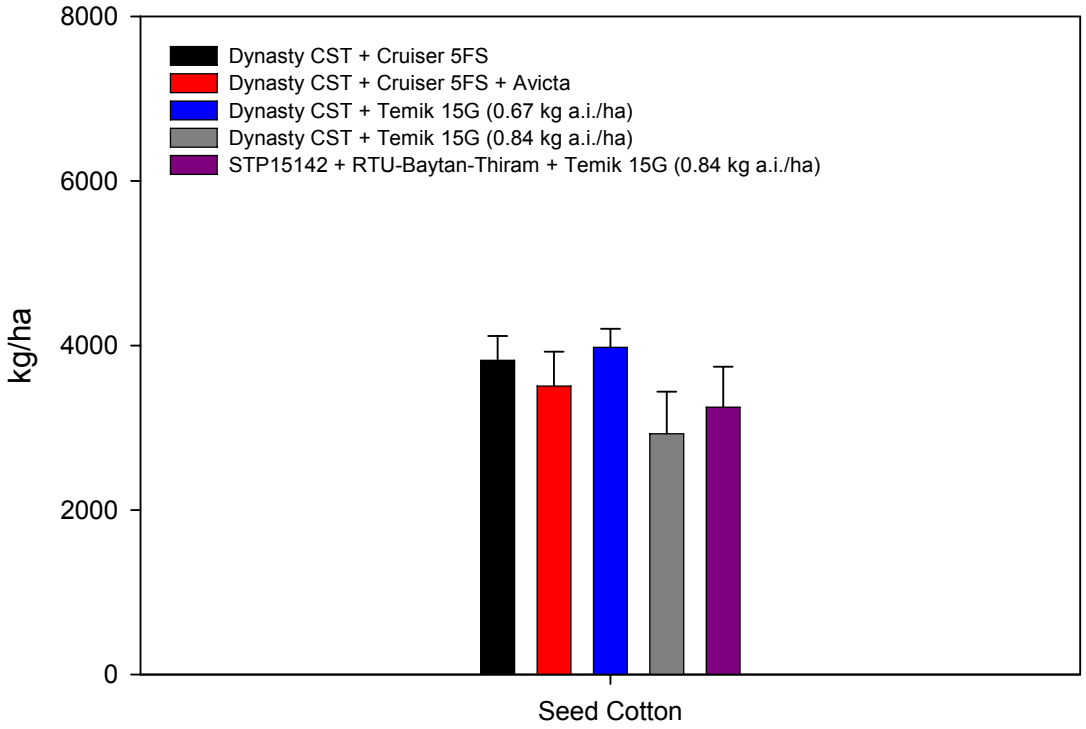


Fig. 10. Effect of Avicta and Temik 15G on seed cotton yield from field infested with *Meloidogyne incognita* and *Rotylenchulus reniformis*. Cotton was manually harvested from the center 3-m of the inner two rows of the four row plots. Dynasty CST, Cruiser 5FS, Avicta, STP15142, and RTU-Bayton-Thiram were applied as seed treatments whereas Temik 15G was applied at planting in seed row.

Mid-season weed control was neglected, thus plots were inundated at harvest with pigweed (*Amaranthus sp*.), red sprangletop (*Leptochloa filiformis*), and coloradograss (*Brachiaria texana*). Seed cotton yield was similar (P = 0.42) among treatments and averaged 3,495 kg seed cotton/ha across all treatments (Fig. 10).

In microplot trials no visible difference was observed for plant height, internode length, or boll count per plant among treatments. Microplots were sprayed weekly with endosulfan as per the Texas Department of Agriculture boll weevil eradication program, which controlled foliar insects. Infested microplots treated with Dynasty CST + Cruiser 5FS + Temik 15G at 90 DAP had fewer (P = 0.05) eggs/500 cm^3 soil than microplots treated with Dynasty CST + Cruiser 5FS + Avicta (Fig. 11). No difference (P = 0.82) in eggs/500 cm^3 soil was observed at 144 DAP among treatments. Root-gall rating for Dynasty CST + Cruiser 5FS + Temik 15G treated microplots was lower (P = 0.05) than Dynasty CST + Cruiser 5FS and Dynasty CST + Cruiser 5FS + Avicta treated microplots (Fig. 12). No yield difference (P = 0.81) was observed among treatments in infested (Fig. 13) or non-infested microplots. Across all treatments mean seed cotton/ha was of 6,498 for infested plots, and 7174 for non-infested plots.

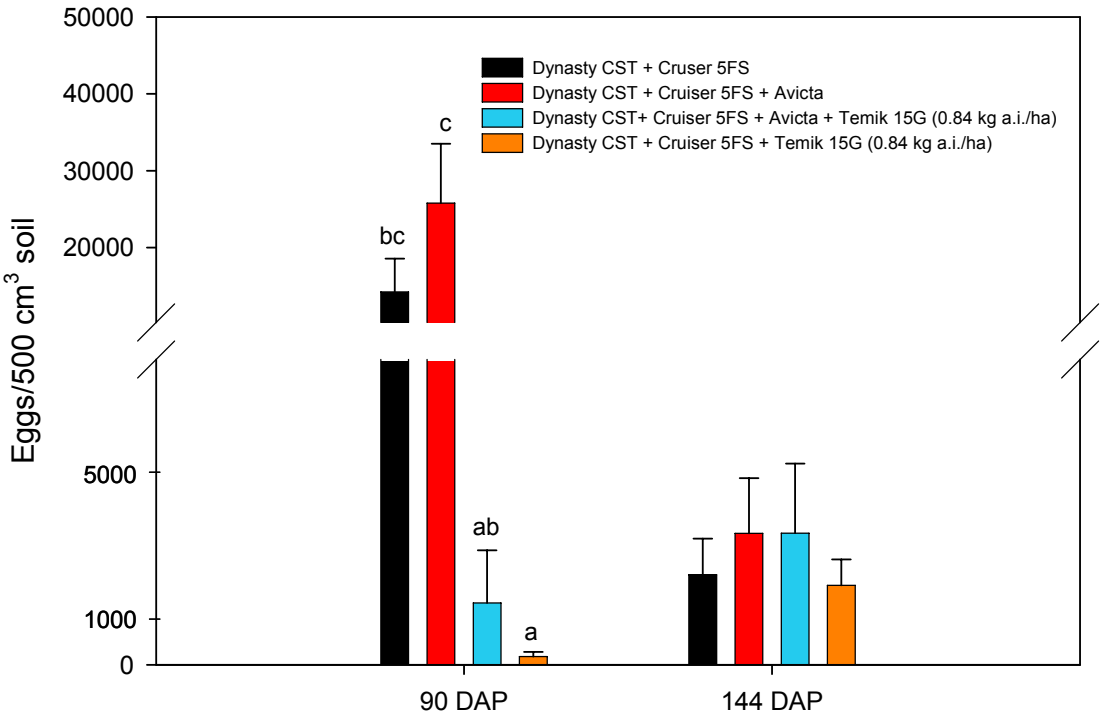


Fig. 11. Effect of Avicta and Temik 15G on reproduction by *Meloidogyne incognita* on cotton in microplots. Dynasty CST, Cruiser 5FS, Avicta, STP15142, and RTU-Bayton-Thiram were applied as seed treatments whereas Temik 15G was applied at planting in seed row. Bars (90 DAP) with different letter are significantly different according to LSD procedure at $\alpha = 0.05$.

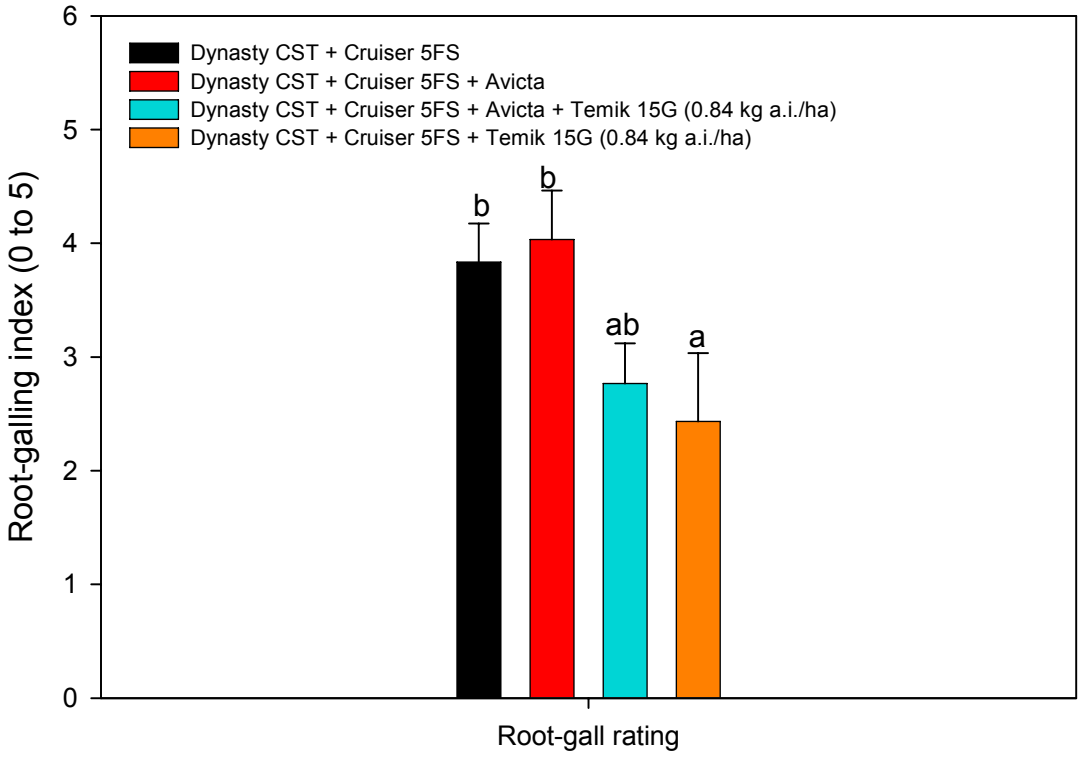


Fig. 12. Effect of Avicta and Temik 15G on cotton root-galling by *Meloidogyne incognita* in microplots. Root-gall rating is based on a six point scale with 0 = no galling and 5 = severe galling. Dynasty CST, Cruiser 5FS, Avicta, STP15142, and RTU-Bayton-Thiram were applied as seed treatments whereas Temik 15G was applied at planting in seed row. Bars with different letters are significantly different according to LSD procedure at $\alpha = 0.05$.

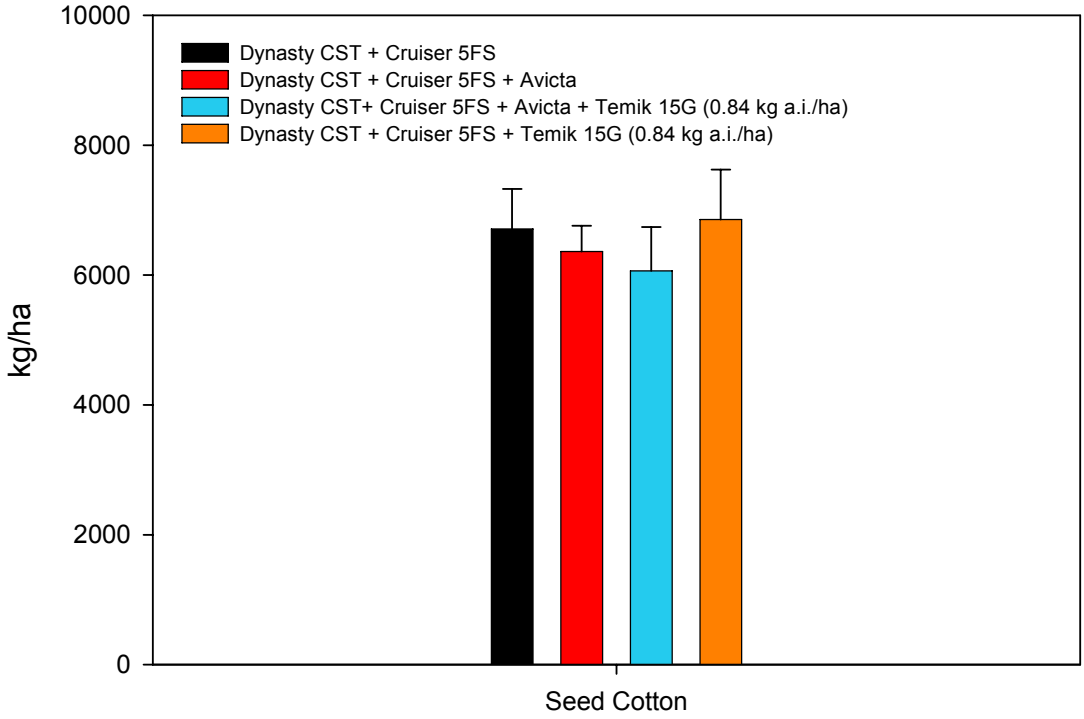


Fig. 13. Effect of Avicta and Temik 15G on seed cotton yield in microplots infested with *Meloidogyne incognita*. Dynasty CST, Cruiser 5FS, Avicta, STP15142, and RTU-Bayton-Thiram were applied as seed treatments whereas Temik 15G was applied at planting in seed row.

Discussion

At the field site, neither abamectin-treated seeds nor aldicarb treated soil were effective at consistently suppressing *M. incognita* or *R. reniformis* during the season. Only early season suppression of *M. incognita* by abamectin-treated seed was observed 32 DAP. Thus, protection of early season tap roots may be limited to one month because the concentration of abamectin may be significantly reduced on new root growth. This early season protection by abamectin seed treatment was not observed in microplots because neither eggs or juveniles were observed until 90 DAP in all treatments. A combination of abamectin-treated seed with aldicarb or a foliar application of oxamyl, a systemic insecticide with nematicidal activity, could be useful in extending the duration of nematode suppression. However, extended nematode suppression was not observed in microplots treated with abamectin and aldicarb. Thus, a lay-by treatment of aldicarb or oxamyl could be more effective at extending the duration of nematode suppression and provide a positive yield response. An infestation of mid-season weeds may have masked any treatment effect, but a similar yield response was observed by abamectin-treated seed and non-abamectin-treated control in microplots.

In similar field trials, suppression of *M. incognita* by abamectin-treated cucurbit seed was limited to an early season response (<30 DAP), with no positive yield or lower root-gall rating response at harvest (Becker and Hofer, 2004; Westphal and Egel, 2004). However, the concentration, duration, and

distance abamectin is translocated along developing roots is not known. Further, it is unknown how population densities, environmental conditions, or soil physical properties may affect plant-parasitic nematode suppression by abamectin seed treatment.

CHAPTER IV

EVALUATION OF HARPIN PROTEIN INDUCED RESISTANCE FOR MANAGEMENT OF *Meloidogyne incognita* ON COTTON

Introduction

Some plant-pathogenic bacteria produce harpins or pilinis, which are used to construct a type III secretion system used to deliver avr proteins across a plant cell wall and into the cytoplasm. The harpin protein $harpin_{EA}$ was first identified from *Erwinia amylovora*, and characterized as an acidic, heat stable protein with a molecular weight of ~40kD with no cystine amino acids (Wei et al., 1992). Harpins are general elicitors similar to glycoproteins, glucans, and chitin oligomers that can induce a plant defense response (i.e., hypersensitive response) to a broad spectrum of pathogens (Agrios, 2005; Wei and Beer, 1996; Wei et al., 1992). Commercialization of these elicitors as plant defense activators have been limited because some elicitors are too expensive to manufacture or do not control disease compared to commercial pesticides (Lyon and Newton, 1999). The three basic categories of plant defense activators commercially available for control of fungal and bacterial pathogens are composed of modified salicylic acid compounds that contain either benzothiadiazole (Actiguard, Bion, and WG50), utilize specific isolates of microbial species (Actinovate, Aspire, and YieldShield), or harpin proteins (Messenger) to manage various fungal and bacterial pathogens (Agrios, 2005).

Induced resistance to plant-parasitic nematodes has not been as thoroughly studied as fungi and bacteria. Systemic acquired resistance to *Meloidogyne hapla* on tomato was induced when pre-treated with non-pathogenic species of M*. incognita* or *M. javanica* (Ogallo and McClure, 1995). Chemical inducers of pathogenesis related proteins were moderately effective in suppressing *M. javanica* on tomato (Oka et al., 1999). Application of harpin$_{EA}$ as a foliar spray and seed treatment on cotton numerically reduced the number eggs produced by *M. incognita* (Kirkpatrick et al., 2005). Harpin$_{\alpha\beta}$, which is a blend of naturally occurring protein fragments, increased cotton yield when applied as a foliar spray (French, 2005). There is limited information on the effects of harpin induced resistance response to uniform infestations of *M. incognita*. Therefore, our objective was to evaluate commercial harpin proteins harpin$_{EA}$ as a seed treatment and harpin$_{\alpha\beta}$ as a foliar spray in microplots uniformly infested with *M. incognita*.

Materials and Methods

Microplots: Wooden microplots (60-cm x 84-cm x 25-cm), located at the *USDA/ARS,* Southern Crops Research Laboratory in College Station, Texas, were used for this study. The microplots contained a sandy soil (93% sand, 4% silt, and 3% clay, pH 7.3) and were fumigated with 1, 3-dichloropropene (6 ml/m^2) to eliminate existing nematode populations. Microplots were infested 3 wk after fumigation with 2,500 eggs of *M. incognita*. To evaluate harpin protein

induced resistance to plant-parasitic nematodes three treatments were evaluated, which included; i) $harpin_{EA}$ (N-hibit 3% $harpin_{EA}$ - Eden Bioscience Corporation, Bothell, WA), ii) $harpin_{EA}$ + $harpin_{\alpha\beta}$ (ProAct 1% $harpin_{\alpha\beta}$ - Eden Bioscience Corporation, Bothell, WA), and iii) non-treated control. Cotton cultivar DP 555 BG/RR was used in this study. $Harpin_{EA}$ was applied as a seed treatment (rate unknown) by Eden Bioscience Corporation. $Harpin_{\alpha\beta}$ was applied at 28 g/ha (1 oz/ac) with 81 L water/ha to cotton plants at the eight-leaf stage. Treatments were arranged in a randomized complete block design and replicated ten times. Microplots were thinned 3 wk after planting to 5 plants per plot. Population densities of *M. incognita* were determined twice during the season. Eight soil cores 2.5-cm dia. and 20-cm deep were taken from each plot and composited. Extraction of J2 and cotton roots was by elutriation. Collection of J2 was by sucrose centrifugation technique (Jenkins, 1964) and eggs from cotton roots by NaOCl (Vrain, 1977). Root-gall ratings were determined at harvest on 15 September 2005.

Statistical analysis: Data were analyzed using general linear model analysis of variance using SPSS (SPSS Inc. Chicago, Ill) and mean separation were by least significant difference procedure.

Results

There were no visual differences among treatments for plant height, internode length, or number of bolls per plant. Microplots were sprayed weekly with endosulfan (0.56 kg a.i./ha) as per the Texas Department of Agriculture boll weevil eradication program, which also controlled foliar insects. Harpin$_{\alpha\beta}$ foliar treatment was applied 42 days after plating (DAP). Harpin$_{EA}$ + harpin$_{\alpha\beta}$ treated plots had numerically lower egg count than harpin$_{EA}$ or non-treated seed at 95 DAP (Fig. 14). At 148 DAP harpin$_{EA}$ + harpin$_{\alpha\beta}$ treatment had a lower (P = 0.025) egg count than harpin$_{EA}$ treated seed. Second-stage juvenile population densities (P = 0.426) and root-gall ratings (P = 0.146) were similar among treatments at harvest (Figs. 15 and 16). No effect among treatments (P = 0.203) were observed for yield with an average of 4,827 kg seed cotton/ha across all treatments (Fig. 17).

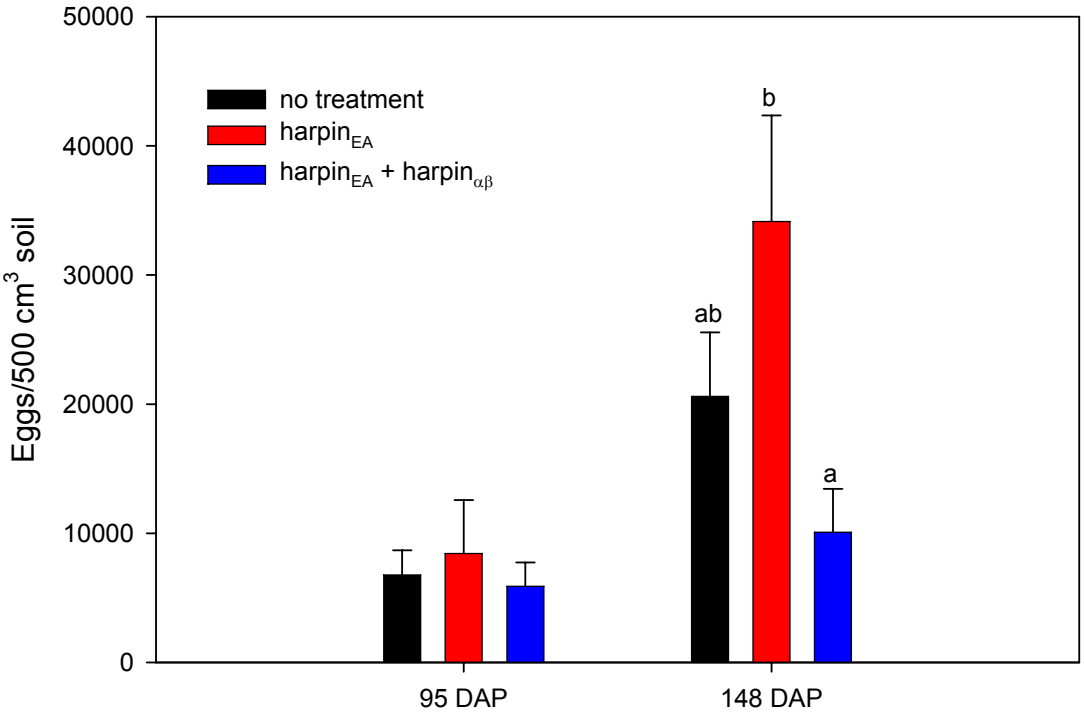


Fig. 14. Effect of $harpin_{EA}$ and $harpin_{\alpha\beta}$ on egg production by *Meloidogyne incognita* in microplots. $Harpin_{EA}$ (N-hibit 3%) was applied as a seed treatment and $Harpin_{\alpha\beta}$ (ProAct 1%) as a foliar spray 42 DAP. Different letters over bars (148 DAP) are significantly different according to LSD at $\alpha = 0.05$.

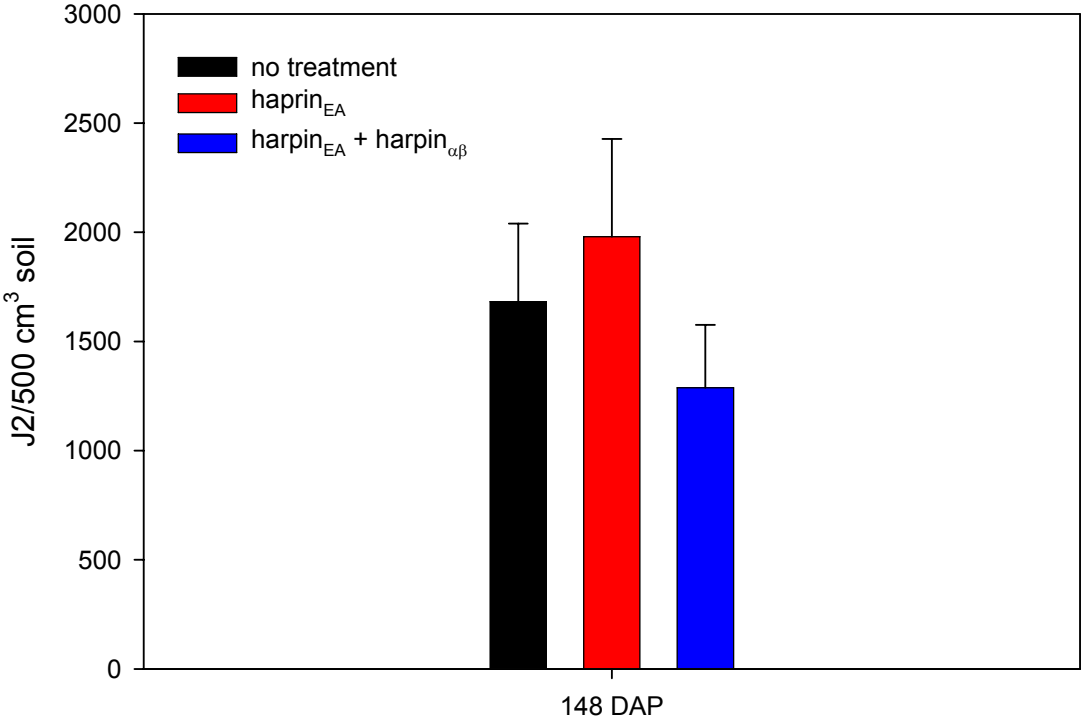


Fig. 15. Effect of harpin$_{EA}$ and harpin$_{\alpha\beta}$ on population densities of *Meloidogyne incognita* in microplots. Harpin$_{EA}$ (N-hibit 3%) was applied as a seed treatment and harpin$_{\alpha\beta}$ (ProAct 1%) as a foliar spray 42 DAP.

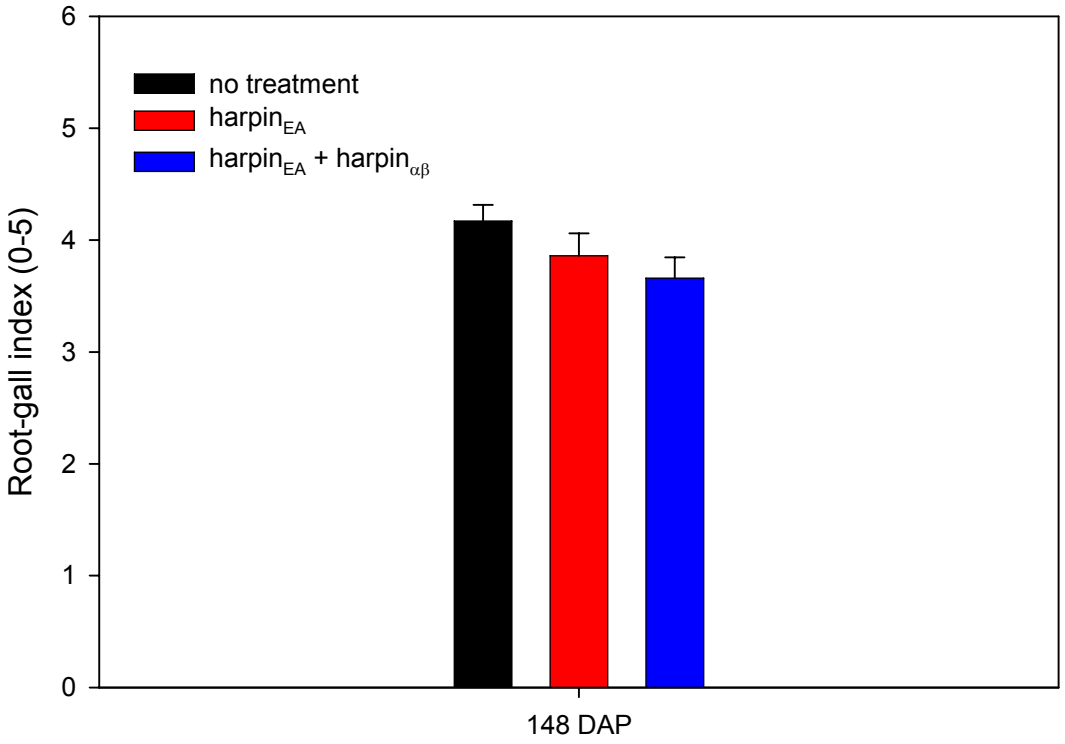


Fig. 16. Effect of harpin$_{EA}$ and harpin$_{\alpha\beta}$ on cotton root-galling by *Meloidogyne incognita* in microplots. Harpin$_{EA}$ (N-hibit 3%) was applied as a seed treatment and harpin$_{\alpha\beta}$ (ProAct 1%) as a foliar spray 42 DAP.

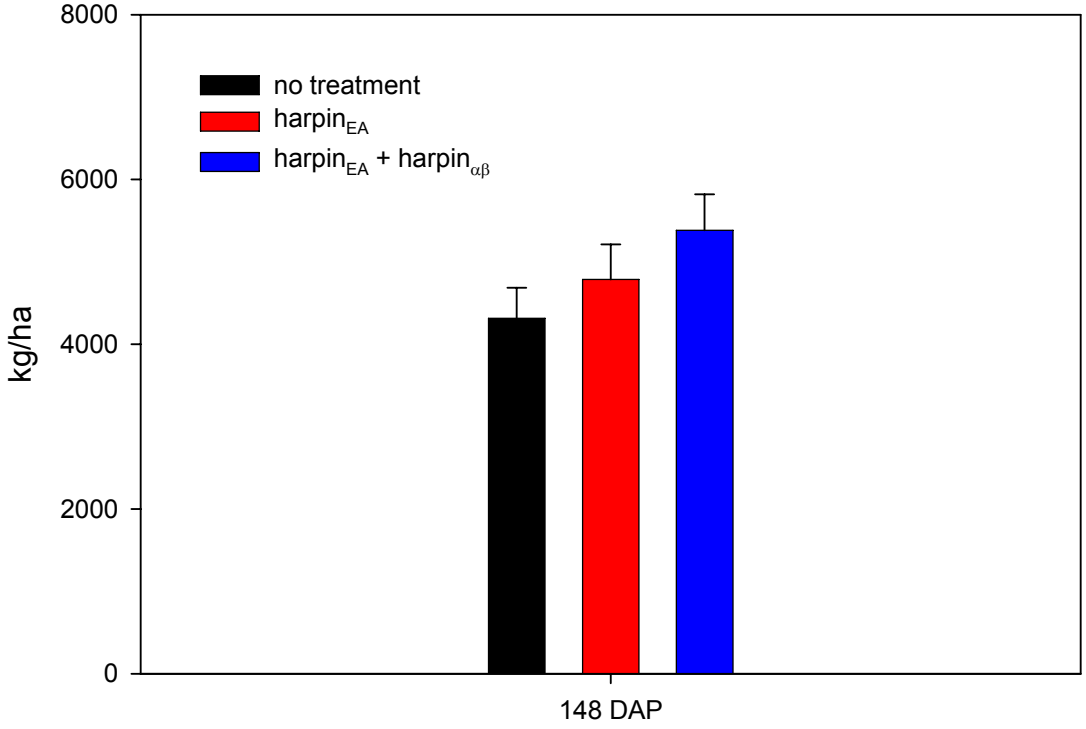


Fig. 17. Effect of harpin$_{EA}$ and harpin$_{\alpha\beta}$ on seed cotton yield in microplots infested with *Meloidogyne incognita*. Harpin$_{EA}$ (N-hibit 3%) was applied as a seed treatment and harpin$_{\alpha\beta}$ (ProAct 1%) as a foliar spray 42 DAP.

Discussion

The harpin$_{\alpha\beta}$ + harpin$_{EA}$ treatment was more effective at suppressing nematode population densities at harvest than harpin$_{EA}$ treatment only. In other studies, harpin$_{EA}$ applied to cotton as a foliar application with pre-plant applications of aldicarb, resulted in numerically lower population densities of *Rotylenchulus reniformis* at mid-season and at harvest than plots treated with a single application of aldicarb (McLean et al., 2002). Harpin$_{EA}$ + harpin$_{\alpha\beta}$ and harpin$_{EA}$ treatments produced 25% and 11% more seed cotton than non-treated cotton seed. Similar yield increases in field trials were also reported for harpin$_{\alpha\beta}$ treated cotton seed (French, 2005). This study suggests that harpin proteins harpin$_{EA}$ and harpin$_{\alpha\beta}$ are effective at suppressing population densities of *M. incognita* resulting in a positive yield response compared to non-treated seed.

Although, harpin$_{EA}$ was effective in suppressing root galling, applying harpin$_{\alpha\beta}$ as a foliar application to harpin$_{EA}$ treated seed plots was more effective at suppressing nematode population densities. Further, utilizing harpin$_{\alpha\beta}$ as a seed treatment or incorporating abamectin may also increase the effectiveness of harpin$_{EA}$ treated seed to suppress plant-parasitic nematodes. The pathogenesis-related proteins induced by harpin$_{\alpha\beta}$ compared to harpin$_{EA}$ could identify which metabolic processes are more effective at suppressing *M. incognita* as well as other plant-parasitic nematodes.

CHAPTER V

CHARACTERIZATION OF ABAMECTIN SEED TREATMENT FOR SUPPRESSION OF ROOT INFECTION BY *Meloidogyne incognita* AND *Rotylenchulus reniformis*

Introduction

The root-knot nematode, *Meloidogyne incognita*, and the reniform nematode, *Rotylenchulus reniformis*, are among the most important plant-parasitic nematodes affecting cotton (*Gossypium hirsutum*) production (Koenning et al., 2004). *Meloidogyne incognita* is a sedentary endoparasitic nematode. Second-stage juveniles (J2) penetrate root tips and migrate toward the developing vascular tissue where they establish a feeding site, resulting in the characteristic galling of root tissue. Root-knot nematodes are found in coarsely textured soils in nearly all areas of cotton production (Robinson et al., 1987; Starr et al., 1993). *Rotylenchulus reniformis* is a sedentary semi-endoparasitic nematode. Vermiform females penetrate the root perpendicular to the root axis and establish a feeding site in the endodermis and pericycle. The reniform nematode is found primarily in finely textured soil in the southeastern cotton belt, but commonly goes undetected because infection does not result in galling of root tissue (Koenning et al., 2004).

Management strategies for plant-parasitic nematodes continue to rely on nematicides because of limited availability of resistant cultivars with high yield potential (Koenning et al., 2004). Abamectin is nematicidal to both *M. incognita* and *R. reniformis* and exposure (1 hr) to concentrations greater than 0.39 µg/ml for *M. incognita* and 8.22 µg/ml for *R. reniformis* inhibit infection of tomato roots (Faske and Starr, 2006). Applying abamectin in close proximity to plant-parasitic nematodes has been effective in suppressing infection (Cayrol et al., 1993; Jansson and Rabatin, 1998). Commercialization of abamectin as a nematicide has been delayed because of limited foliar translocation, high affinity to bind to soil particles, and short ½ life in sunlight (Wislocki et al., 1989). Radiolabeled studies have shown movement of pesticides, including abamectin, applied as seed treatments into the developing root system (Long, 2005). However, abamectin seed treatment has been variable in suppressing *M. incognita* and *R. reniformis* and increasing cotton yields in nematode infested fields (Lawrence, Gazaway et al., 2006; Lawrence, Burmester et al., 2006;2006; Phipps et al., 2006). Further characterization of abamectin as a seed treatment is needed to better understand the variability in nematode suppression.

The objectives of this study were (i) to determine the effect of initial nematode population densities and different soil textures on nematode suppression by abamectin seed treatment, (ii) to determine suppression of nematode penetration of young cotton roots in response to abamectin seed-treatment, and (iii) to determine mortality of *M. incognita* exposed to the excised radicle or seed coat of an abamectin-treated seed.

Materials and Methods

Nematode cultures: *Meloidogyne incognita* and *R. reniformis* were originally isolated from cotton and maintained in the greenhouse on *Lycopersicon esculentum* cv. Rutgers. Eggs were collected from 8- to 10-wk-old *M. incognita* cultures with NaOCl (Hussey and Barker, 1973). Second-stage juveniles were collected in hatching chambers with a 20-µm-pore screen that allows only hatched J2 to migrate into the collection dish (Vrain, 1977). Only 24-hr-old J2 were evaluated in this study. *Rotylenchulus reniformis* was collected from infested soil using Baermann funnels (Chapman, 1958). Vermiform-stages of *R. reniformis* were collected with a 25-µm-pore sieve after 48 hr and used immediately.

Seed treatments: Cotton cv. DP 444 BG/RR that was used throughout this study. All treatments were applied by the manufacturer. Treated seed (Avicta, Syngenta Crop Protection, Greensboro, NC) received 150 µg abamectin/seed that were also treated with, the insecticide thiamethoxam (Cruiser 5FS, Syngenta Crop Protection) at 340 µg/seed and a blend of the fungicides azoxystrobin + fludioxonil + mefonoxam (Dynasty CST, Syngenta Crop Protection) applied at 30 µg/seed. Non-treated seeds were treated with thiamethoxam and azoxystrobin + fludioxonil + mefonoxam.

Effect of initial nematode population densities and soil texture: The effectiveness of abamectin seed treatment for suppressing plant-parasitic nematodes was determined at three initial population densities each for *M.*

incognita and *R. reniformis*. Abamectin-treated cotton seed was planted into 656 cm^3 Deepots (Stuewe & Sons, Inc. Corvallis, Oregon) containing soil infested with low, moderate, and high population densities of each nematode species. Infectivity of *M. incognita* was evaluated at 10, 100, and 1,000 J2/500 cm^3 soil in sand-peat soil mix (6:1 v/v, pH 7.7), and estimated 56 d after planting (DAP) based on root-gall rating and total eggs per root system. Root-gall rating was based on a six point scale with 0 = no galls and 5 = severe galling. This experiment was repeated in sand-sandy loam soil mix (2:1 v/v, pH 8.0). Infectivity of *R. reniformis* was evaluated at 50, 500, and 5,000 vermiform-stages/500 cm^3 soil in a sand-sandy loam soil mix (2:1 v/v, pH 8.0) and measured 56 DAP based on vermiform-stages per 500 cm^3 soil and eggs per root system. Non-abamectin-treated seed served as controls. A randomized complete block design (RCBD) was used with 10 replications of each treatment per initial population density and experiments were repeated once.

The effectiveness of abamectin as a seed treatment for suppressing *M. incognita* and *R. reniformis* in different soil textures was determined experimentally. Abamectin-treated cotton seed was planted in Deepots (656 cm^3) containing soil that reflected a range of soil textures that each species commonly inhabits (Starr et al., 1993). Four parent soil types were mixed in different ratios to achieve the target concentration of silt (Table 1). Infectivity of *M. incognita* was evaluated in sandy soil with varying concentrations of silt (8, 15, 23, and 30% silt) infested with 500 J2/500 cm^3 soil, and assessed 56 DAP based on root-gall rating and eggs per root system (as described above).

Infectivity of *R. reniformis* was determined in loam soil with different concentration of silt (15, 30, 45, and 60% silt) infested with 2,000 *R. reniformis*/500 cm^3 soil, and estimated 56 DAP based on vermiform-stages per 500 cm^3 soil and total eggs per root system. Non-treated seed served as controls. A RCBD was used with 10 replications of each treatment per soil texture and experiments were repeated once.

Suppression of penetration and infection among early stage root development: Two studies were conducted to determine the suppression of penetration by *M. incognita* and *R. reniformis* by abamectin seed treatment at different lengths of taproot and DAP in nematode infested soil. In the first study, abamectin-treated seed was planted into Deepots (656 cm^3) containing sand-sandy loam soil mix (2:1 v/v) infested with 1,000 *M. incognita* J2/500 cm^3 soil and harvested at 7, 14, 21, 28, and 35 DAP. Non-treated cotton seed served as controls. Nematode penetration was determined at 7 and 14 DAP by staining *M. incognita* juveniles with acid fuchsin (Byrd et al., 1983). Infectivity was estimated at 21, 28, and 35 DAP based on total eggs and galls per root system. The experiment was a RCBD with each treatment replicated five times and the experiment was repeated once.

Table 1. Soil physical properties of parent and mixed soils.

Textural Class[a]	Sand	Silt	Clay	CEC[c]	pH
S*	96[b]	4	0	2.1	8.5
LS	88	7	5	N/A	N/A
LS*	84	11	7	6.3	8.5
LS	80	16	4	4.8	8.4
LS	74	23	3	N/A	N/A
SL*	67	28	5	6.8	8.3
SL	52	45	3	8.0	8.2
SIL*	37	61	2	9.4	8.2

[a] Soil textural classes with * indicate parent soils used in different proportion to achieve target concentrations of silt. Soil textural class abbreviations include; S = sand, LS = loamy sand, SL = sandy loam, and SIL = silt loam.
[b] Values indicate percent particle size based on hydrometer analysis
[c] Cation exchange capacity

In a second study, taproots at 5-cm, 10-cm and 20-cm lengths were inoculated with *M. incognita* or *R. reniformis* to determine suppression of nematode penetration as taproots elongate. Abamectin-treated seed was germinated on germination paper (Anchor Paper Co., St. Paul, MN) at 26°C for 3, 5, and 7 d for developing taproots lengths of 5-cm, 10-cm, and 20-cm long, respectively. Tapoot tips were sleeved inside a 7-cm long plastic cylinder (5-mm diam.) then covered in sand (< 710 µm) though a gap (2 x 40-mm) cut from the plastic cylinder. Approximately, 100 J2 in 100 µl distilled water were inoculated into the sand 1-cm below the root tips. Taproots were placed in a moisture chamber for 48 hr at 26°C. For *R. reniformis*, taproots were sleeved inside 7-cm long plastic cylinders, until target taproot lengths were centered in the plastic cylinder. Taproots were covered with fine sand (< 710 µm) and approximately 120 *R. reniformis* in 100 µl distilled water were inoculated into the sand at target lengths. Taproots were placed in a moisture chamber for 6 d at 26ºC. Non-treated seed served as controls. Treatments were replicated six times per taproot length and experiments were repeated once.

Toxicity associated with developing radicle from abamectin-treated cotton seed: In this experiment, *M. incognita* J2 were exposed to a radicle or seed coat from an abamectin-treated seed for 48 hr, and nematode mortality was measured. Abamectin-treated and non-treated seed were germinated in sand-sandy loam soil mix (2:1 v/v) for 48 hr at 26°C, resulting in a radicle length of 3-cm. Radicle tips (2-cm) were excised from the seed and placed in 3-cm diam. glass petri plates containing 30 to 40 J2 in 2 ml distilled water. The remaining

seed coats were placed in separate petri dishes with *M. incognita* J2. Nematodes exposed to distilled water served as control. Nematodes were incubated for 48 hr at 26°C. Nematodes were considered dead if they were not moving and did not respond to being touched by a small probe. Each treatment was replicated six times and the experiment was repeated twice. An activity response index was also used to qualitatively characterize nematode activity 48 hr after exposure to the radicle and seed coat. Activity response variables include: counts of active (nematode undulate without being touched), delayed response (slow undulation after being touched), and dead (no response after being touched) nematodes exposed to each treatment. The variables were utilized in the following equation to estimate the Activity Response = (active*3)(delayed response*2)(dead*1) /(active + delayed response + dead).

Statistical analysis: Data were subjected to general linear model analysis of variance using SPSS 11.5 (SPSS Inc., Chicago, Ill). Mean separation were by least significance difference.

Results

The effectiveness of abamectin seed treatment was variable for suppressing infection on cotton by *M. incognita* and *R. reniformis* across several initial population densities and across different soil textures (Figs. 18 and 19). Mean root-gall rating and egg production 56 DAP by *M. incognita* was numerically lower ($P \geq 0.14$) across all initial population densities and soil textures (≤ 23% silt) for abamectin-treated than non-treated seed. Abamectin seed treatment had no effect on infection of *R. reniformis* 56 DAP across initial nematode population densities or soil textures. Plant height, root weight, and foliage weight were similar between seed treatments for both nematode species (data not shown). No effect of cation exchange capacity was observed, which ranged from 4.8 to 9.4 and increased with silt concentration. Further, no effect of soil pH tested was observed because of overall similar results by *M. incognita* between soils that ranged in pH from 7.7 to 8.2 and results varied for *R. reniformis* even though soil pH (8.2 to 8.4) was very similar among soils tested.

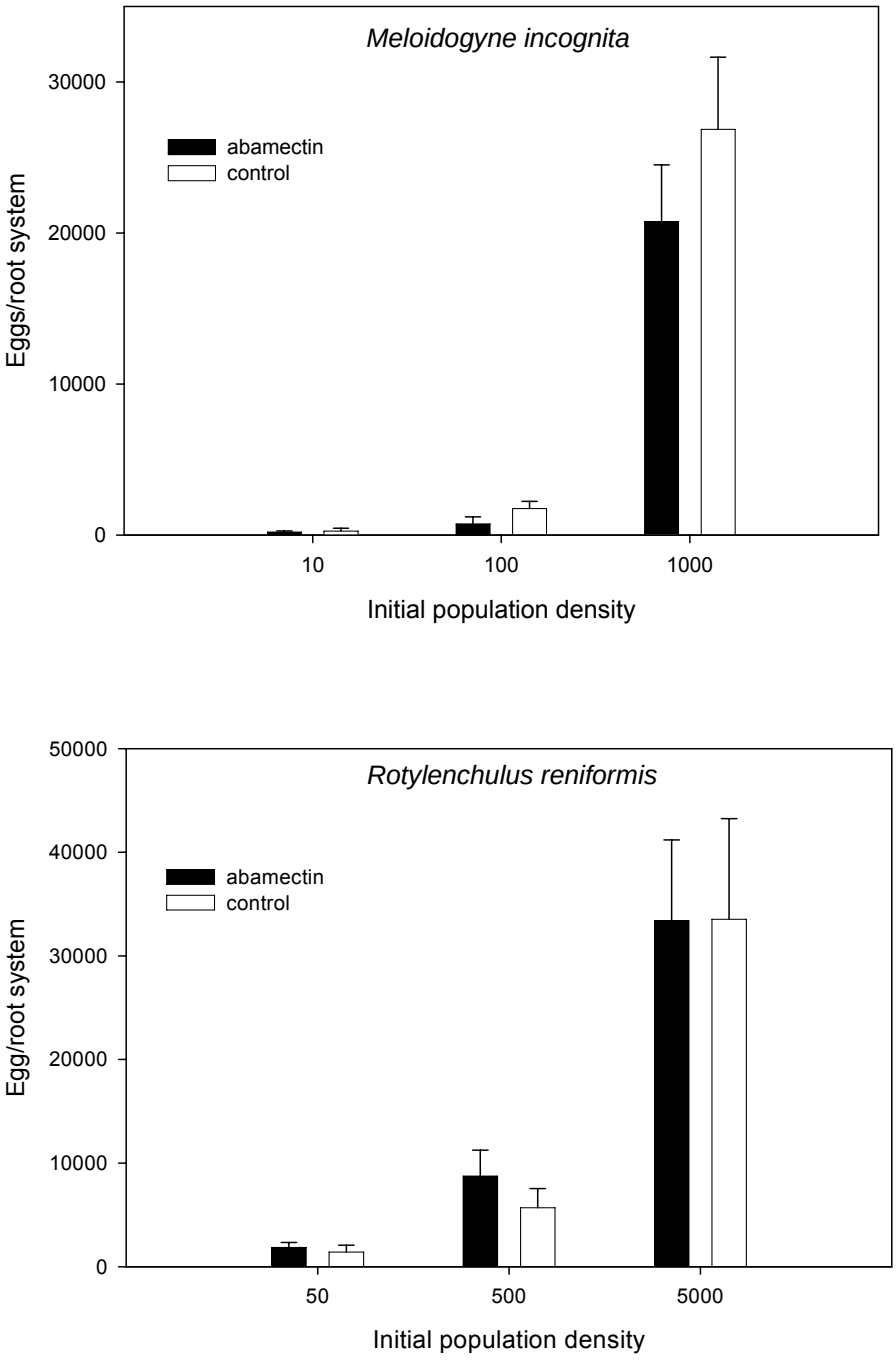


Fig. 18. Effect of different initial population densities on suppression of *Meloidogyne incognita* and *Rotylenchulus reniformis* by abamectin treated cotton seed. Egg counts were made 56 DAP.

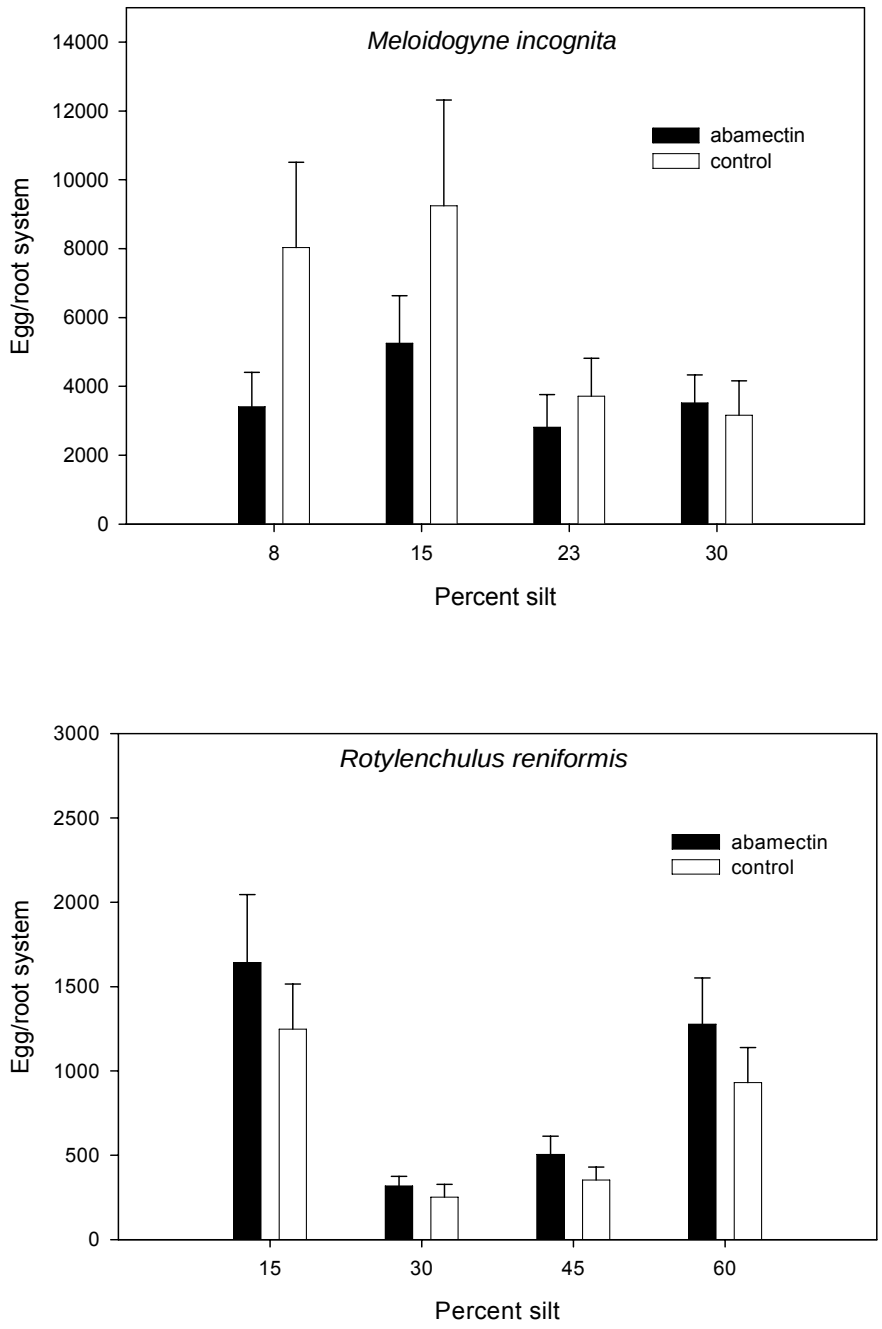


Fig. 19. Effect of different soil textures on suppression of egg production by *Meloidogyne incognita* and *Rotylenchulus reniformis* by abamectin treated cotton seed. Initial population density for *M. incognita* and *R. reniformis* was 500 J2/500 cm^3 soil and 2000 vermiform-stages/500 cm^3 soil, respectively. Egg counts were made 56 DAP.

Suppression of initial infection by *M. incognita* by abamectin seed treatment varied depending on observation date (Fig. 20). Taproot penetration by *M. incognita* 7 DAP (20-cm) was numerically higher for abamectin-treated than non-treated seed, but the reverse was observed at 14 DAP. Total galls per taproot and secondary roots from 21 to 35 DAP were very similar between seed treatments, but taproot length between the crown and first gall was longer (5.6 cm, $P = 0.001$) for abamectin-treated than non-treated seed (2.4 cm, Fig. 21).

The length of taproot protection by abamectin seed treatment varied in suppression of *M. incognita* and *R. reniformis* penetration at taproot lengths of 5-cm, 10-cm, and 20-cm (Fig. 22). Suppression decreased as taproot length increased from 5-cm to 10-cm for M*. incognita* and *R. reniformis*.

Based on the assay of nematode mobility, the proportion of dead J2 following 48 hr exposure to a radicle of an abamectin-treated seed was higher (0.39, $P = 0.05$) compared to non-treated seed (0.05) but, lower ($P = 0.05$) than J2 exposed to abamectin-treated seed coat (0.87, Fig. 23). The activity response (numbers close to 3 indicate active undulation) of J2 following exposure to an excised radicle from abamectin-treated and non-treated seed was 1.9 ($P = 0.05$) and 2.8, respectively.

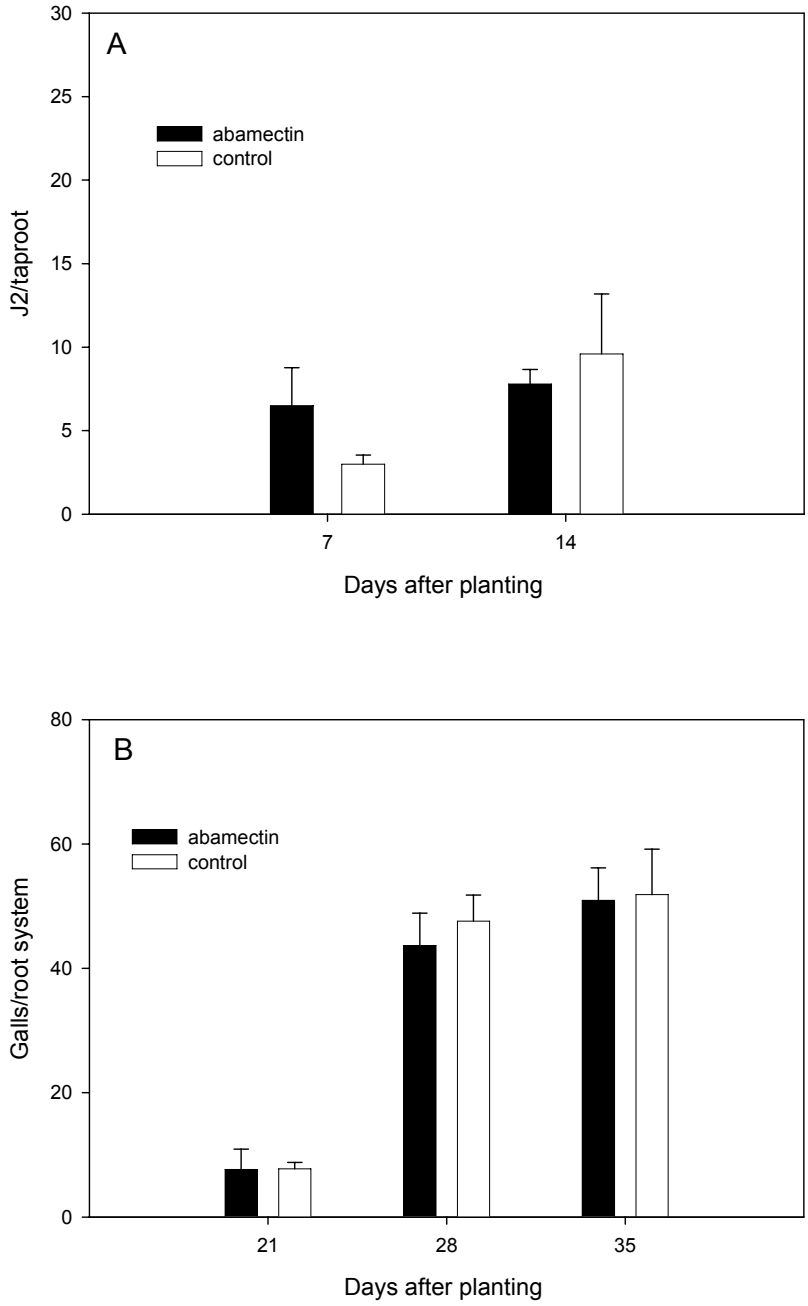


Fig. 20. Suppression of penetration and infection by *Meloidogyne incognita* by abamectin treated cotton seed among early stage root development. Initial population density for *M. incognita* was1,000 J2/500 cm^3 soil. A. Second-stage juveniles were stained with acid fuchsin prior to counting. B. Galls per root system were determined visually.

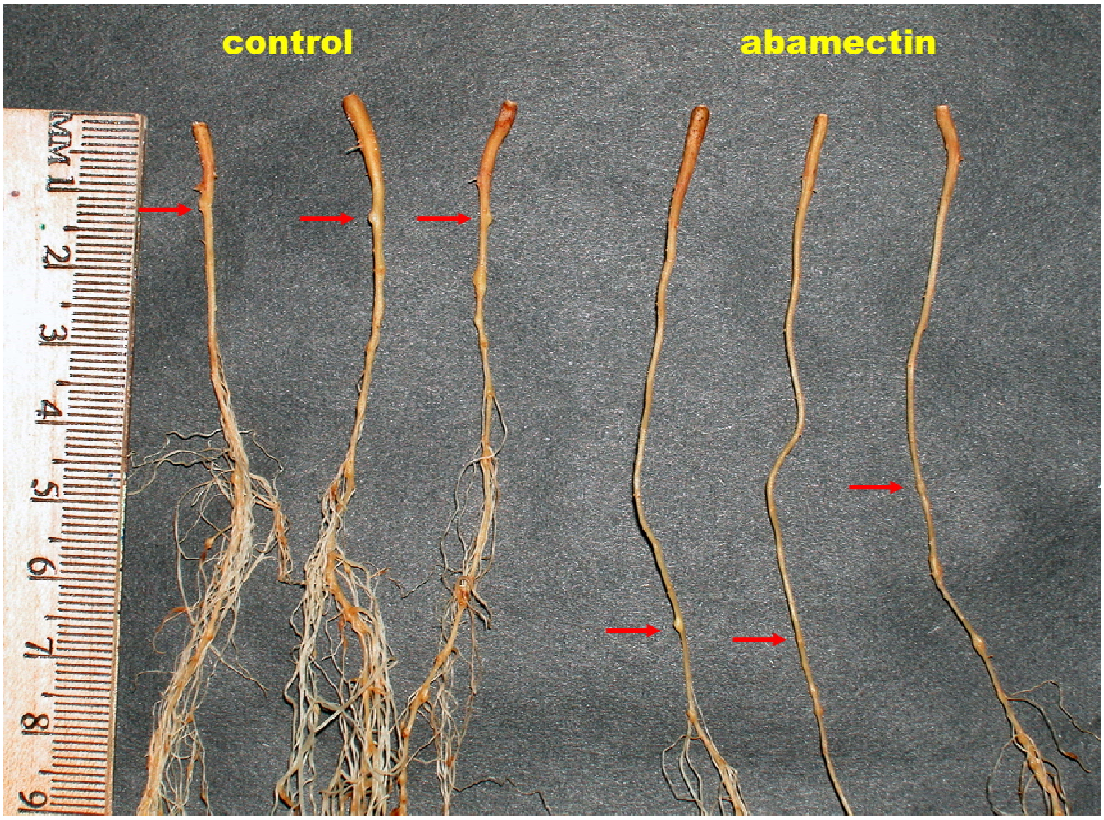


Fig. 21. Initial gall formation on cotton taproots. Secondary roots were removed 2-cm past the first gall for both control and abamectin treated seed to better visualize galling. Red arrows indicate first gall from crown.

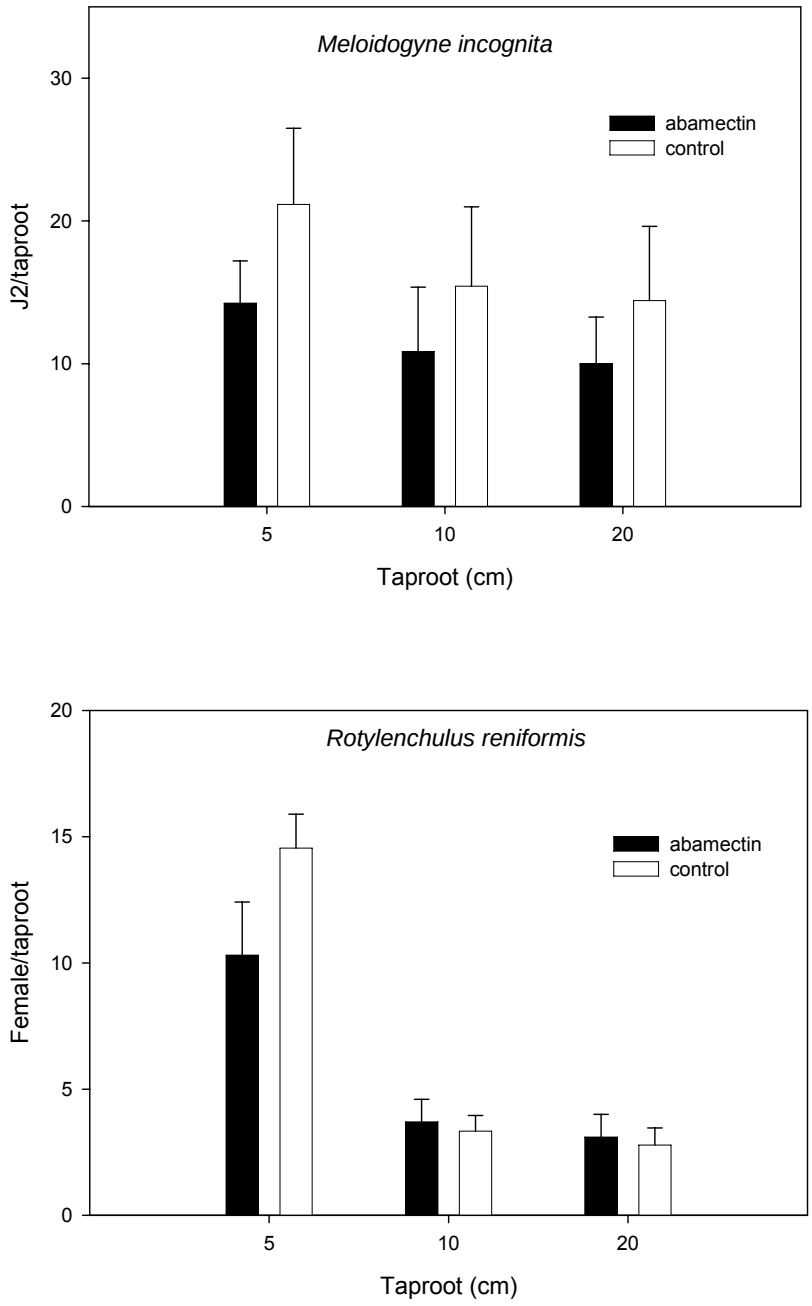


Fig. 22. Length of taproot protection by abamectin seed treatment for suppression of infection by *Meloidogyne incognita* and *Rotylenchulus reniformis*. Target taproot lengths were sleeved and centered inside a plastic cylinder then filled with sand. Taproot tips inoculated with 100 J2 *M. incognita* were harvested 48 hr after inoculation. Taproots inoculated with 180 mixed-life-stages of *R. reniformis* were harvested 6 d after inoculation.

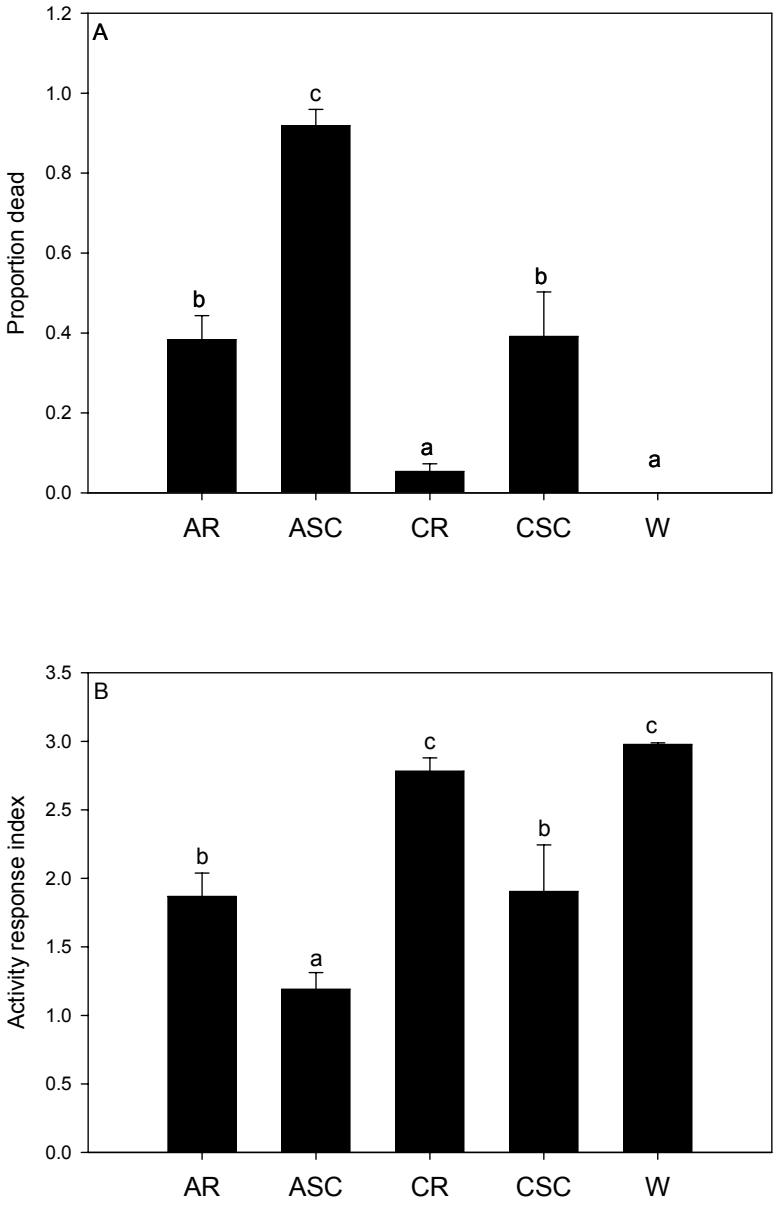


Fig. 23. Toxicity associated with abamectin treated cotton seed on mortality and activity response of *Meloidogyne incognita*. Treatment abbreviations include: abamectin radicle = AR, abamectin seed coat = ASC, control radicle = CR, control seed coat = CSC, and J2 in water = W. Seeds were germinated in soil for 3 d. Nematode J2 were exposed to seed coat or radicle for 48 hr. A. Proportion of dead J2. B. Activity Response formula = (undulating*3)(respond to touch*2)(no response*3)/(undulating + respond to touch + no response). Different letter over bars indicate significant difference according to LSD at $\alpha = 0.05$.

Discussion

Applying abamectin as a seed treatment efficiently distributes the nematicide in close proximity to the developing root system, and early season nematode suppression by abamectin-treated cucurbit seed has been reported (Becker and Hofer, 2004; Moreira and Barbosa, 2002; Westphal and Egel, 2004). In our studies, suppression of penetration and infection on cotton taproots by *M. incognita* and *R. reniformis* was greatest at taproot length of 5-cm, and decreased as taproots elongated, suggesting that abamectin concentration decreases rapidly as root length increases. The concentration of abamectin associated with developing roots was more effective against *M. incognita*, which is more sensitive than *R. reniformis* (Faske and Starr, 2006).

Based on the known concentration response ($y = 1.022/(1+\exp(-(x-0.417)/0.203))$ for *M. incognita* (Faske and Starr, 2006), and mortality of J2 after 24 hr exposure to the radicle or seed coat, the concentration of abamectin released into solution from the radicle and seed coat was estimated to be 0.13 µg and 0.68 µg abamectin/ml, respectively. Thus the majority of abamectin applied as a seed treatment remains on the seed coat. *Meloidogyne incognita* exposure to sublethal concentration less than 0.15 µg/ml for 1 hr did not reduce root-galling of tomato (Faske and Starr, 2006).

The proportion of J2 dead after 48 hr exposure to non-abamectin-treated seed coat was similar to the excised radicle from an abamectin-treated seed, suggesting nematicidal activity of the insecticide or fungicide applied seed

treatments. Although, no information on the sensitivity of *M. incognita* to thiamethoxam is available, exposure to thiamthoxan may explain the elevated mortality. Thiamethoxam is a neonicotinoid class of insecticide, which interferes with the neurotransmission of an insects nervous system (Tomizawa and Casida, 2003).

Cotton yield response and suppression of *M. incognita* and *R. reniformis* by abamectin-treated seed has been variable in replicated field trails (Lawrence, Gazaway et al., 2006; Lawrence, Burmester et al., 2006;2006; Phipps et al., 2006). Neither soil physical properties or initial population densities had a significant effect on the suppression of *M. incognita* or *R. reniformis* by abamectin seed treatment. Limited protection of early stage cotton root development was related to the small portion of abamectin transferred to the developing root system, which decreased rapidly as roots elongated. These observations contribute to the understanding of variability in suppression of plant-parasitic nematodes by abamectin seed treatment.

CHAPTER VI

CONCLUSIONS

Management practices of plant-parasitic nematodes continue to rely on nematicides for suppression of nematode population densities. In the past 25 years, no new nematicide has been developed because of the low market niche compared to other pesticides. Aldicarb is the most frequently applied nematicide in cotton that is potentially toxic to the handler and environment. Thus it is unique to evaluate a new nematicide that is also less harmful to humans and environment, and is applied as a seed treatment. The lethality of abamectin and its effectiveness as a seed treatment to control *Meloidogyne incognita* and *Rotylenchulus reniformis* are summarized below.

Abamectin is nematicidal to both *Meloidogyne incognita* and *Rotylenchulus reniformis. Meloidogyne incognita* was more sensitive to abamectin than *R. reniformis*. The concentration of abamectin necessary to cause irreversible paralysis and inhibit infection for both nematode species was very low and comparable to that of aldicarb.

Applying abamectin as a seed treatment efficiently distributes the nematicide in close proximity to the developing root system. Early season (32 DAP) suppression of *M. incognita* population density and reduced root-galling (148 DAP) were perceived in replicated field trials by abamectin seed treatment, but no suppression was observed for *R. reniformis*. Suppression of infection by

M. incognita was not observed in microplots. No positive yield response was observed in field or microplot trials by abamectin seed treatment.

Utilizing plants natural defense mechanisms to suppress nematode infection was perceived for $harpin_{EA}$ as a seed treatment and $harpin_{\alpha\beta}$ as a foliar spray. A positive yield response was observed for both $harpin_{EA}$ and $harpin_{EA}$ + $harpin_{\alpha\beta}$ treated plots. Although it has not been evaluated, combining harpins and abamectin in a seed treatment may be more effective in suppressing early seasons infection by plant-parasitic nematodes.

Neither soil physical properties or initial nematode population densities had a significant effect on suppression of *M. incognita* or *R. reniformis* by abamectin seed treatment. Limited protection of early stage cotton root development is related to the small proportion of abamectin transferred to the developing root system. These observations probably contribute to variability observed in suppression of plant-parasitic nematodes by abamectin seed treatment.

Although Syngenta promotes that abamectin seed treatment will result in higher or comparable yield to aldicarb 80% of the time, no significant response in yield was observed in our field or microplot trials. Controlling nematodes in a seed treatment is a unique delivery approach, and abamectin is lethal to plant-parasitic nematodes. However, a new abamectin variant that translocates a higher proportion of abamectin from the seed to developing root system would enhance the effectiveness of abamectin seed treatment in a pest management system.

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