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Sensitivity of *Meloidogyne incognita* and *Rotylenchulus reniformis* to Abamectin

T. R. Faske, J. L. Starr

Abstract: Avermectins are macrocyclic lactones produced by *Streptomyces avermitilis*. 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 and some vegetable crops. No LD_{50} values, data on nematode recovery following brief exposure, or effects of sublethal concentrations on infectivity of the plant-parasitic nematodes *Meloidogyne incognita* or *Rotylenchulus reniformis* are available. Using 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. Mortality of *M. incognita* continued to increase following a 1 hr exposure, whereas *R. reniformis* mortality remained unchanged at 24 hr after the nematodes were removed from the abamectin solution. Sublethal concentrations of 1.56 to 0.39 µg/ml for *M. incognita* and 32.9 to 8.2 µg/ml for *R. reniformis* reduced infectivity of each nematode on tomato roots. The toxicity of abamectin to these nematodes was comparable to that of aldicarb.

Key words: abamectin, avermectin, LD_{50}, *Lycopersicon esculentum*, *Meloidogyne incognita*, nematicide, *Rotylenchulus reniformis*, reniform nematode, root-knot nematode, seed treatment, tomato.

The root-knot nematode *Meloidogyne incognita* is found in nearly all cotton (*Gossypium hirsutum*) production areas in the US, 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 nonfumigant 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; Shoop et al., 1995; Jansson and Dybas, 1998), and several avermectin formulations are available to control insects and mites on plants. Avermectins block γ-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 (Sasser et al., 1982; Garabedian and Van Gundy, 1983; Nordmeyer and Dickson, 1985; Cayrol et al., 1993; Roberts and Matthews, 1995; Jansson and Rabatin, 1997, 1998). 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 to: (i) characterize the lethal concentration of abamectin for *M. incognita* and *R. reniformis* in vitro; (ii) determine if the effects of abamectin on each nematode species are reversible; and (iii) 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).

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Texas A&M University, Department of Microbiology and Plant Pathology, College Station, TX 77043–2132
Email address: j-starr@tamu.edu
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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 2X test concentration, to which 30 to 40 nematodes in 500 µl 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 1,000 *M. incognita* or *R. reniformis* were exposed for 1 hr to an LD_{50} concentration of abamectin (calculated based on a 2 hr exposure response). After the 1-hr exposure, 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 2,000 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. 1). 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.*

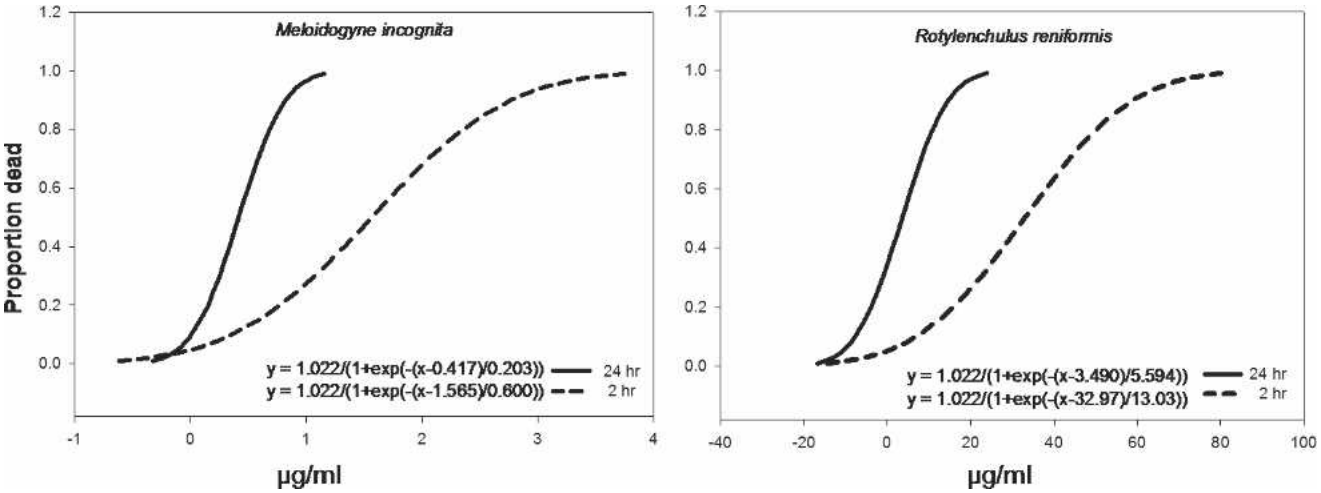

FIG. 1. Effect of abamectin on mortality of *Meloidogyne incognita* and *Rotylenchulus reniformis* after 2 hr and 24 hr exposure. Equations are derived by nonlinear regression of probit analysis.

reniformis remained unchanged after removal from abamectin (Fig. 2). 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. 2). Nematode posture was rigid and straight for both nematode species when treated with abamectin, and neither responded to being touched by a small probe. *Meloidogyne incognita* and *R. reniformis* were relaxed and undulated when exposed to aldicarb and responded to being touched by a small probe.

All sublethal concentrations greater than 0.39 µg abamectin/ml inhibited ($P < 0.05$) infection of tomato roots by *M. incognita* (Fig. 3). 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 lowered ($P < 0.05$) the number of *R. reniformis* females observed per root (Fig. 3).

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 *M. arenaria*. The LD_{90} based on 24 hr exposure for *R. reniformis* was 82% (14.7 µg/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 re-

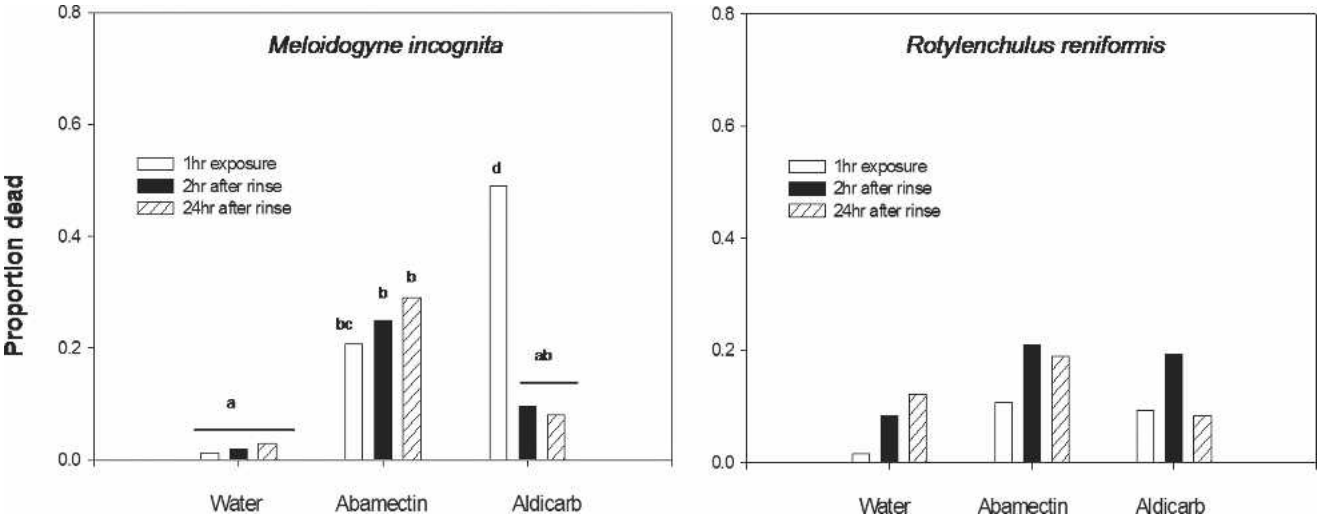

FIG. 2. Recovery of *Meloidogyne incognita* and *Rotylenchulus reniformis* post exposure to abamectin and aldicarb. Each species was exposed to its 2 hr LD_{50} for each nematicide for 1hr, then rinsed and transferred to distilled water. Proportion of dead nematodes was recorded after the 1 hr exposure 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*.

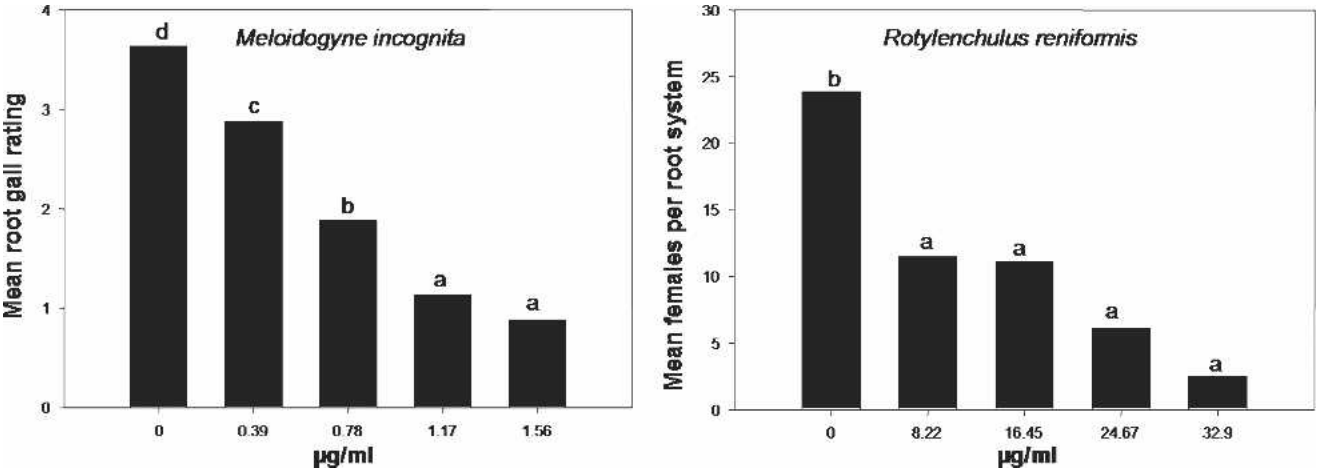

FIG. 3. Effect of treatment with sublethal concentrations of abamectin on infectivity of *Meloidogyne incognita* and *Rotylenchulus reniformis*. Root gall ratings are based on a six point scale where 0 = no galling and 5 = severe galling. Female *R. reniformis* were stained with acid fuchsin prior to counting. Different letters over bars indicate significant differences at $\alpha = 0.05$ according to LSD.

moval 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 *M. javanica* and *Heterodera schachtii* of 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 cottonseed 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.

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