Cotton Root Protection from Plant-Parasitic Nematodes by Abamectin-Treated Seed

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Abstract: Abamectin is nematicidal to *Meloidogyne incognita* and *Rotylenchulus reniformis*, but the duration and length of cotton taproot protection from nematode infection by abamectin-treated seed is unknown. Based on the position of initial root-gall formation along the developing taproot from 21 to 35 d after planting, infection by *M. incognita* was reduced by abamectin seed treatment. Penetration of developing taproots by both nematode species was suppressed at taproot length of 5 cm by abamectin-treated seed, but root penetration increased rapidly with taproot development. Based on an assay of nematode mobility to measure abamectin toxicity, the mortality of *M. incognita* associated with a 2-d-old emerging cotton radicle was lower than mortality associated with the seed coat, indicating that more abamectin was on the seed coat than on the radicle. Thus, the limited protection of early stage root development suggested that only a small portion of abamectin applied to the seed was transferred to the developing root system.

Key words: abamectin, avermectin, cotton, *Gossypium hirsutum*, *Meloidogyne incognita*, nematicide, reniform nematode, root-knot nematode, *Rotylenchulus reniformis*, seed treatment.

The root-knot nematode, *Meloidogyne incognita* race 3, and the reniform nematode, *Rotylenchulus reniformis*, are important plant-parasitic nematodes affecting cotton (*Gossypium hirsutum*) production (Koenning et al., 2004). Second-stage juveniles (J2) of *M. incognita* penetrate just behind the 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 coarse-textured soils in nearly all areas of cotton production (Koenning et al., 1996). Vermiform females of *R. reniformis* 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 fine-textured soils in the southeastern US, but commonly it is 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 for 1 hr to concentrations greater than 0.39 µg/ml for *M. incognita* and 8.22 µg/ml for *R. reniformis* inhibits tomato root infection (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). However, commercialization of abamectin as a nematicide has been delayed because of limited foliar translocation, high affinity to bind to soil particles, and rapid decomposition in soil and sun light (Putter et al., 1981; Wislocki et al., 1989). In 2005, an abamectin formulation (Avicta 500 FS) was marketed by Syngenta Crop Protection as a seed treatment for early season control of

nematode damage to cotton seedlings. However, abamectin seed treatment has been variable in suppressing *M. incognita* and *R. reniformis* and increasing cotton yields in nematode-infested fields (Lawrence et al., 2006a, 2006b; Monfort et al., 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 to determine (i) suppression of nematode penetration of young cotton roots in response to abamectin seed treatment, and (ii) mortality of *M. incognita* exposed to the excised radicle tip or seed coat of an abamectin-treated seed.

Materials and Methods

Nematode cultures: Meloidogyne incognita and *R. reniformis* were isolated from cotton and maintained in the greenhouse on *Solanum lycopersicum* L. cv. 'Rutgers'. Eggs were collected from 8- to 10-wk-old *M. incognita* cultures with NaOCl (Hussey and Barker, 1973), from which J2 were collected in hatching chambers with a 20-µm-pore screen (Vrain, 1977). Only 24-hr-old J2 were evaluated in this study. *Rotylenchulus reniformis* was 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.

Seed treatments: Cotton (*G. hirsutum*) cv. Deltapine DP 444 BG/RR was used throughout this study. All seed treatments were applied by the manufacturer. Treated seed received 150 µg abamectin/seed (Avicta 500 FS, Syngenta Crop Protection, Greensboro, NC), the insecticide thiamethoxam (Cruiser 5FS, Syngenta Crop Protection) applied at 340 µg/seed, and a blend of the fungicides azoxystrobin, fludioxonil, and mefenoxam (Dynasty CST, Syngenta Crop Protection) applied at 30 µg/seed. Control treatments were seeds treated with thiamethoxam and azoxystrobin + fludioxonil + mefenoxam at the same rates.

Nematode penetration: Two experiments were con-

ducted to determine the suppression of penetration by *M. incognita* and *R. reniformis* on developing roots in response to abamectin seed treatment. In the first experiment, conducted in a greenhouse, abamectin-treated and control seeds were planted into 656 cm^3 Deepots (Stuewe and Sons, Inc, Corvallis, OR) containing pasteurized sand:sandy loam (2:1 v/v; <1% organic matter; pH 8.0) soil mix infested with 1,000 *M. incognita* J2/500 cm^3. The root systems from these plants were harvested at 7, 14, 21, 28, and 35 d after planting (DAP). Nematode penetration of roots was determined at 7 and 14 DAP by staining J2 with acid fuchsin (Byrd et al., 1983). Infection was estimated at 21, 28, and 35 DAP based on total number of galls per root system. Reproduction was determined at 28 and 35 DAP based on numbers of eggs per gram of root. The experiment was arranged in a randomized complete block with each treatment replicated five times, and the experiment was conducted twice.

In a second experiment, taproots of 5-cm, 10-cm and 20-cm length of plants grown from abamectin-treated seed were inoculated with *M. incognita* or *R. reniformis* to determine suppression of nematode penetration at different taproot lengths. Abamectin-treated and control seeds were germinated on germination paper (Anchor Paper Co., St. Paul, MN) at 26°C for 3, 5, and 7 d to obtain taproots lengths of 5 cm, 10 cm, and 20 cm, respectively. Cotton taproots were inserted inside a 7-cm-long plastic cylinder (5-mm diam.), then covered in pasteurized sand (<710 μm) through 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 prepared as described above. 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 26°C. Nematode penetration of taproots was determined by staining nematodes with acid fuchsin (Byrd et al., 1983). Treatments were replicated six times per taproot length, and each experiment was conducted twice.

Toxicity associated with developing radicle: In this study, *M. incognita* J2 were exposed to a radicle tip or seed coat for 48 hr, and nematode mortality was measured. Abamectin-treated and control seeds were germinated in sand:sandy loam (2:1 v/v) soil mix for 48 hr at 26°C, resulting in a radicle length of 3 cm. The excised radicle tip (2 cm) and remaining seed coat were placed in separate 3-cm diam. glass petri dishes containing 30 to 40 J2 in 2 ml distilled water. Nematodes exposed to distilled water served as controls. Nematode mortality was estimated following a 48-hr exposure 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 conducted three times.

Statistical analysis: Data from repeated experiments were combined and subjected to general linear model analysis of variance using SPSS 11.5 (SPSS Inc., Chicago, Ill). Mean separations were established by least significant difference.

RESULTS

In the greenhouse experiment, suppression of *M. incognita* root infection by abamectin seed treatment varied depending on observation date (Fig. 1). The number of *M. incognita* in the taproot 7 DAP was higher for abamectin-treated than non-treated seed, but the reverse was observed at 14 DAP. Seedling heights, total galls per taproot, secondary roots, and eggs per gram of root from 21 to 35 DAP were similar between seed treatments (data not shown). Taproot length between the
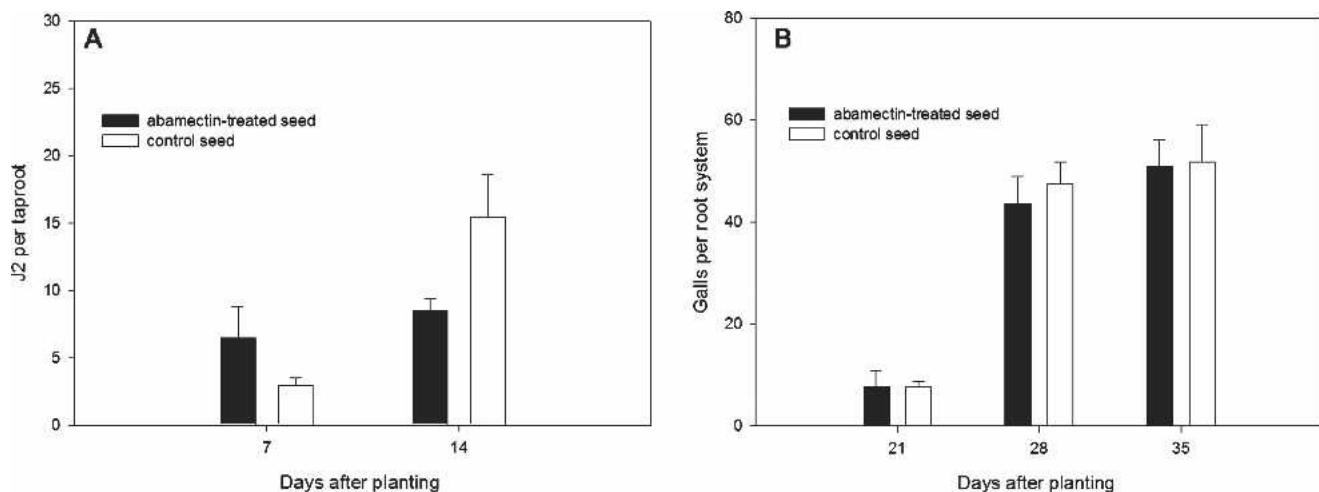

Fig. 1. Suppression of root penetration and infection by *Meloidogyne incognita* on developing seedling roots in response to abamectin-treated seed. Initial population density for *M. incognita* was 1,000 J2/500 cm^3 soil. A. Second-stage juveniles. B. Galls per root system. Bars indicate standard error.
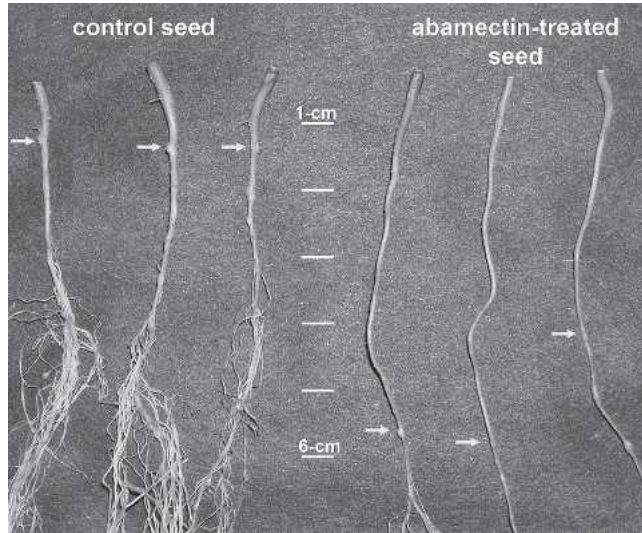
FIG. 2. Effect of abamectin seed treatment on the position of initial gall formation by *Meloidogyne incognita* on cotton taproots. To better visualize galling, secondary roots were removed 2 cm past the initial gall for both control and abamectin-treated seed. Arrows indicate position of first gall from crown.

crown and position of the initial gall was longer (5.6 cm, $P = 0.001$) for abamectin-treated than non-treated seed (2.4 cm, Fig. 2).

The length of taproot protection from *M. incognita* and *R. reniformis* infection by abamectin-treated seed varied at taproot lengths of 5 cm, 10 cm, and 20 cm (Fig. 3). Suppression of nematode penetration was greatest at taproot length of 5 cm for both nematode species. At taproot lengths of 10 cm and 20 cm, fewer *M. incognita* were observed on taproots from abamectin-treated seed whereas no suppression was observed for *R. reniformis.*

In preliminary experiments based on assays of nematode mobility, no mortality was observed in response to

pesticide-free cotton seed or fungicide seed treatment, and extremely low toxicities were observed for the insecticide seed treatment. 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 coats (0.87, Fig. 4).

DISCUSSION

Cotton yield response and suppression of *M. incognita* and *R. reniformis* by abamectin-treated seed has been variable in replicated field trials (Lawrence et al., 2006a, 2006b; Monfort et al., 2006; Phipps et al., 2006). In a greenhouse trial, Monfort et al. (2006) reported lower root gall rating and nematode reproduction on cotton roots grown in pasteurized soil 35 DAP by seed treated with only abamectin. However, in our greenhouse trial, root gall rating and nematode reproduction were similar between abamectin and non-abamectin treated seed, which were also treated with three fungicides and an insecticide. Interactions among these seed treatments may have contributed to the small portion of abamectin transferred along the developing cotton root system.

In our studies, suppression of penetration and infection on cotton taproots by *M. incognita* and *R. reniformis* was greatest at a taproot length of 5 cm and decreased as taproots elongated, suggesting that abamectin concentration decreased rapidly as root length increased. The concentration of abamectin associated with developing cotton roots was more effective against *M. incognita,* which was reported to be more sensitive than *R. reniformis* to abamectin (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 the mortality of J2 after a
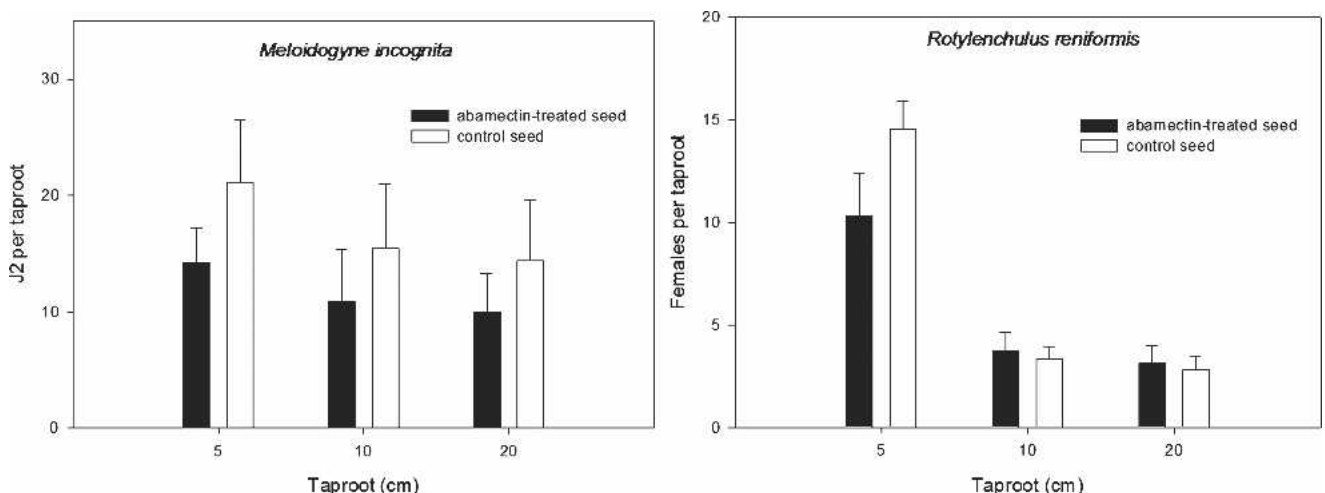

FIG. 3. Suppression of root penetration by plant-parasitic nematodes at different taproot lengths in response to abamectin seed treatment. Target taproot lengths were sleeved and centered inside a plastic cylinder then filled with sand. Taproot tips inoculated with 100 J2 of *Meloidogyne incognita* were harvested 48 hr after inoculation. Taproots inoculated with 120 vermiform-stages of *Rotylenchulus reniformis* were harvested 6 d after inoculation. Bars indicate standard error.
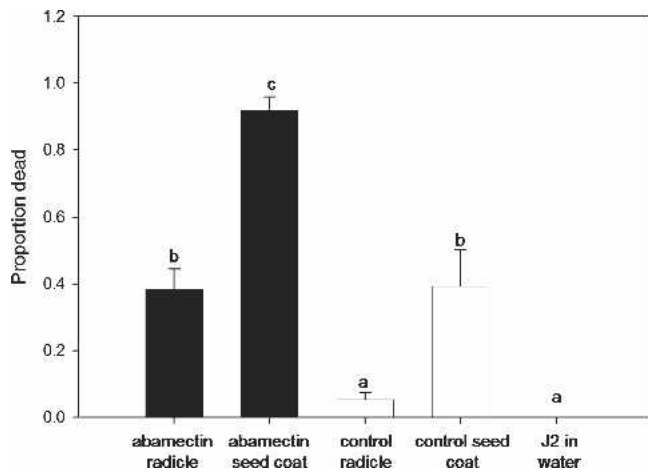
Fig. 4. Mortality of *Meloidogyne incognita* associated with the emerging radicle and residual seed coat from abamectin-treated cotton seed. Bars indicate standard error. Different letters over bars indicate significant difference according to LSD at $\alpha = 0.05$.

24 hr exposure, the concentrations of abamectin released into solution from the radicle and seed coat were 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. Exposing *M. incognita* to a concentration less than 0.15 µg abamectin/ml for 1 hr did not reduce root-galling of tomato (Faske and Starr, 2006).

Applying abamectin as a seed treatment delivers the nematicide in close proximity to the developing root system. 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.

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