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Control of Root-knot Nematodes on Tomato in Stone Wool Substrate with Biological Nematicides

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Abstract: The efficacy of four biological nematicides on root-galling, root-knot nematode (*Meloidogyne incognita*) reproduction, and shoot weight of tomato (*Solanum lycopersicum*) grown in stone wool substrate or in pots with sandy soil was compared to an oxamyl treatment and a non-treated control. In stone wool grown tomato, Avid® (a.i. abamectin) was highly effective when applied as a drench at time of nematode inoculation. It strongly reduced root-galling and nematode reproduction, and prevented a reduction in tomato shoot weight. However, applying the product one week before, or two weeks after nematode inoculation was largely ineffective. This shows that Avid® has short-lived, non-systemic activity. The effects of Avid® on nematode symptoms and reproduction on soil-grown tomato were only very minor, probably due to the known strong adsorption of the active ingredient abamectin to soil particles. The neem derived product Ornazin® strongly reduced tomato root-galling and nematode reproduction only in stone wool and only when applied as a drench one week prior to nematode inoculation, suggesting a local systemic activity or modification of the root system, rendering them less suitable host for the nematodes. This application however also had some phytotoxic effect, reducing tomato shoot weights. The other two products, Nema-Q™ and DiTera®, did not result in strong or consistent effects on nematode symptoms or reproduction.

Key words: control, *Meloidogyne incognita*, *Solanum lycopersicum*, stone wool, substrate.

Because of short rotations and fallow periods and relatively high soil temperatures, greenhouse vegetable and flower production has traditionally been susceptible to problems caused by soil-borne fungi and nematodes. To remediate problems, soil disinfection with steam was initially used, to be followed during the 1960's by cheaper and more effective soil fumigation with methyl bromide. Concerns about the toxicity and associated negative impact of methyl bromide on the environment led to a ban on its' use. As an alternative, cultivation of greenhouse vegetable and flower crops on artificial substrates was developed in the early 1970s (Amsing, 2004; Lehman, 1987). One type of substrate that is particularly popular in Europe is "rockwool" or "stone wool" made by Grodan®. The substrate is made by liquefying basalt under high temperatures, spinning it into threads, cooling it down, and compressing it into wool slabs, blocks or plugs (www.grodan.com). The major advantages of using substrate include that it allows growers to start a crop in a pathogen-free medium, to better control water, aeration, nutrition, and root distribution according to specific crop requirements (Ehret et al., 2001), and that poor or unsuitable soil types are no longer restrictive in choosing growing locations. According to Ehret et al. (2001) the majority of greenhouse crops are grown on artificial substrates. Specific data for the percentage or acreage of the different greenhouse crops that are grown on substrate, or the types of substrates used, are not available for California or the USA, but a large percentage of vegetable and flower propagation for greenhouse production in the USA is done on Grodan® stone wool plugs and blocks (R. Wyatt, Grodan Inc., pers. comm.).

An enquiry of a California grower of cut-flower roses on stone wool who had noticed a slow but steady decline in production, led us to sample the substrate for the presence of nematodes. Very high numbers of the second-stage juveniles (J2) of the root-knot nematode *Meloidogyne hapla* were found. A literature search revealed that in The Netherlands, where 90% of rose acreage is on substrate, approximately 10% of substrate-grown roses (80 ha) were infested with *M. hapla* (Amsing, 2004). The damage potential of *M. hapla* on roses remains unclear. In a pot study using a light clay soil, Amsing (1986) reported that plants grown in *M. hapla* infested soil (inoculum between 18-386 J2/100 ml soil) produced 19% fewer flowers per plant, and that flower weight was reduced by 12% compared to the no-nematode control over a 13 month period. Later however, Amsing et al. (2005) failed to observe significant effects of *M. hapla* on substrate-grown roses, in spite of high root infestations (1,800-2,800 *M. hapla* per g root) 12 months after nematode inoculation.

To manage nematode infestations in substrates, several studies have focused on identifying and eliminating the sources of nematode infestation, such as infested planting material or rainwater catch basins (Garcia Victoria and Amsing, 2007; Amsing, 2004). Once infestation has occurred however, there are few options to manage nematodes post-plant. In California, Vydate®, a systemic post-plant nematicide, is registered for use in several vegetable crops, but not in ornamentals. There are however several biological pesticides with potential nematicidal activity that could be used as a post-plant application to manage root-knot nematodes in substrate-grown crops. The goal of this study was to evaluate the efficacy of several of these products both when applied to stone wool substrate and when applied to soil. For the experiments in this study we used tomato as an assay

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plant, because of its' relative rapid growth and obvious root symptoms (i.e. galling) in response to nematode infestation, and because it is also grown on stone wool in greenhouse production, and the root-knot nematode species *M. incognita* because of its' relatively short life cycle and high reproductive rate on tomato. The biological products tested were DiTera®, and Nema-Q®, (registered nematicides), Ornazin® (insecticide/nematicide registered for greenhouse ornamentals and vegetables), and Avid® (insecticide registered for greenhouse ornamentals).

Material and Methods

Nematode inoculum: A race 3 *M. incognita* population, originally isolated from cotton in the San Joaquin Valley, CA, USA, was maintained and multiplied in a greenhouse on tomato (*Solanum lycopersicum*) 'UC82', grown in sandy soil in 5 liter pots. Species and race identification were confirmed by iso-zyme electrophoresis and by reproduction on differential hosts (Eisenback and Triantaphyllou, 1991). Nematode inocula for the trials consisted of *M. incognita* eggs that were extracted from tomato roots with a 1% NaOCl solution (Radewald et al., 2003). Eggs released from the roots were collected on a 25 μm pore-size sieve and were counted in two 0.025 ml subsamples.

Establishing product rates: Tomato 'UC82' were seeded into 2.5 cm diameter stone wool plugs. Two weeks after seedling emergence, 80 plugs with seedlings were placed in the center of a stone wool block (l x w x h=10 x 10 x 6.35 cm), of which the sides and bottom had been covered with black plastic with five 3 mm holes in the bottom to allow for draining. Two weeks later, treatments were assigned to the blocks according to a completely randomized design, and 75 blocks were inoculated each with 10,000 *M. incognita* eggs by adding three times 1 ml of the egg suspension (containing 3,333 eggs/ml) in a triangular pattern on the top of each block at 1 cm from the center. Five blocks were not inoculated (no-nematode control). Products were prepared by mixing in distilled water, and were applied to each of five nematode-inoculated blocks within 1 hour after nematode inoculation, by drenching each block with 200 ml of the appropriate product solution (Table 1).

Three days after applying the treatments, 10 g of slow-release fertilizer (Osmocote®) was added to each block, a drip tube was placed above each block, and blocks were watered daily through an automated drip system. Plants were grown for six weeks. The plastic cover was removed from each block, the shoots were cut and weighed, and the roots visible at the bottom of each block were examined for root-galling. The severity of root-galling was indexed on a sale from 0 to 10 (0 = no galls seen, 10: roots completely covered in galls). Each block was then weighed, cut into 4 parts, and one quarter was weighed and placed in a mist chamber for extraction of nematodes. After 5 days, nematodes that had emerged were collected, and second-stage root-knot nematode juveniles were counted at 40x magnification under a dissecting microscope.

Timing of product application - stone wool: Tomato var. UC82 were seeded in to 2.5 cm diameter Grodan® stone wool plugs and then placed in the center of a stone wool block as described before. Products tested were Vydate® (150 ppm a.i.), Avid® (0.25 ppm a.i.), Ornazin® (18 ppm a.i.), Nema-Q™ (430 ppm a.i.), and DiTera® (900 ppm a.i.). Each of the products were applied according to the following schedule: 1) One-time drench of seedling plugs 2 weeks after seedling emergence, and 1 week before placing plugs on to stone wool blocks, 2) One-time drench of stone wool blocks at placement of plugs on blocks, 3) Drenching of blocks at plug-placement followed by additional drenches 2, and 4 weeks later,

Table 1. Products and rates applied to Grodan® stone wool blocks with tomato transplants. Application within one hour after inoculation of blocks with 10,000 *M. incognita* eggs (N = 5).

Product applied	Active ingredient	Distributor	Rate (active ingredient, ppm)
Water	n.a.		n.a.
Vydate® L	oxamyl	DuPont, Wilmington, DE	150
			300
Avid® 0.15 EC	abamectin	Syngenta, Greensboro, NC.	0.125
			0.25
			0.50
Ornazin® 3% EC	azadirachtin	SePro Corp., Carmel, IN.	18
			36
			72
Nema-Q™	*Quillaja saponaria* (soap tree) extracts	Monterey AgResources, Fresno, CA.	215
			430
			860
DiTera® DF	*Myrothecium verrucaria* fermentation solids and solubles	Valent, Walnut Creek, CA.	450
			900
			1,800

4) Drenching of blocks 2 weeks and 4 weeks after placement of plugs. Each treatment x product combination had 5 replicates. Blocks were inoculated with 10,000 *M. incognita* eggs at the time of placing the plugs on to the blocks as described before. Control consisted of five non-treated, no nematode blocks, and five non-treated nematode-inoculated blocks. Blocks were placed on a greenhouse bench in a completely randomized order, and plants were watered, fertilized, and harvested as described before. The entire experiment was repeated once.

Timing of product application – Pots: A similar experiment to the above was conducted, but with tomato plants seeded in seedling trays with potting mix (Sunshine Mix 5, Sungro, Vancouver, BC, Canada). Three weeks after emergence, the seedlings were carefully removed from the seedling tray, leaving the root plug intact, and were transplanted into 5 l pots filled with steam-sterilized sandy soil. Pots were inoculated at time of transplanting as described before but with 20,000 eggs per pot. Each treatment x product combination had six replicates. Pots were randomized over a greenhouse bench, and fertilized and watered as described before. Six weeks after transplanting, shoots were cut and weighed, and roots were carefully washed free of soil and examined for galling (0 = no galls seen, 10: roots completely covered in galls). Root-knot nematode eggs were extracted from each root system with a 1% NaOCl solution (Radewald et al., 2003), collected on a 25μm pore-size sieve and counted at 50x magnification using a dissecting microscope. The complete experiment was repeated once.

Statistical analysis: Data were analyzed using analysis of variance procedures (ANOVA), and means were separated using Fisher's Protected LSD test at the 95% confidence level. Prior to statistical analysis, nematode J2 and egg count data were log(x+1)-transformed, non-transformed data are shown. SAS (SAS Institute, Cary, NC, USA) statistical software was used for analysis.

Results

Experiment 1 - Establishing effective product rates: The nematode-inoculated control plants were heavily infested, exhibited severe root-galling, and their shoot weights were reduced by 32% relative to the non-inoculated control plants. All products at all rates, except the low rate of Nema-Q™, significantly reduced tomato root-galling compared to the nematode-inoculated controls. All three rates of Avid® nearly eliminated root-galling (galling index from 0 to 0.8), and tomatoes treated with Vydate® exhibited only minor galling. Avid® and Vydate® treatments also significantly reduced nematode reproduction compared to the nematode-inoculated controls. The only other treatment that significantly reduced nematode reproduction was the low rate of Ornazin®. Avid®, Vydate®, DiTera®, and the low and medium rate of Ornazin® prevented a significant reduction in tomato shoot weight relative to the non-inoculated control (Table 2). Based on these results, the medium rate of Avid®, Nema-Q™, and DiTera®, and the low rate of Ornazin®, were used in subsequent experiments. The low rate of Vydate® was used as the "standard" control.

Experiment 2 - Timing of product application, stone wool: Average root-galling in the nematode-inoculated control was moderately severe in both experiments (7.6 and 7.0 respectively). Strong and consistent reductions in root-galling (galling-index <2) resulted from the

Table 2. Effect of different rates of nematicides on root-knot nematode symptoms, infestation, and shoot weight of tomato grown on Grodan® stone wool blocks. Nematicides applied as a drench 1 hr. after nematode inoculation.

Product	Rate (ppm a.i.)	Root galling index	Second-stage *Meloidogyne* juveniles (J2) per root system	Fresh shoot weight (g)
No nematode control				77.2 (±8.9) abc
Plus nematode control		9.0 (±0.00) a[a]	174,135 (±42,784) a	52.3 (±4.4) ef
Vydate®	150	1.6 (±0.40) fgh	3,611 (±1,440) bcd	61.1 (±7.3) cdef
	300	1.4 (±0.51) gh	1,239 (±844) fg	64.7 (±2.3) bcdef
Avid®	0.125	0.8 (±0.37) gh	2,165 (±1,490) efg	92.8 (±11.2) a
	0.25	0.2 (±0.20) h	1,135 (±391) def	91.5 (±9.5) a
	0.5	0.0 (±0.00) h	267 (±178) g	66.3 (±8.1) bcdef
Nema-Q™	215	7.6 (±0.93) ab	40,075 (±10,1096) ab	54.8 (±6.2) def
	430	5.2 (±1.07) cde	86,579 (±38,487) a	47.0 (±6.9) f
	860	5.6 (±0.75) bcd	62,375 (±25,874) ab	46.7 (±6.7) f
DiTera®	450	6.2 (±1.50) bc	28,969 (±8,979) abc	72.9 (±6.7) abcde
	900	6.0 (±1.27) bcd	93,406 (±6,501) a	80.7 (±10.9) abc
	1,800	5.4 (±1.44) bcd	28,969 (±21,209) a	61.5 (±8.3) cdef
Ornazin®	18	2.6 (±0.40) fg	10,017 (±3,447) cde	73.8 (±4.3) abcd
	36	3.0 (±0.89) efg	18,348 (±8,258) abcd	83.1 (±7.3) ab
	72	3.8 (±0.37) def	25,545 (±7,363) abc	46.1 (±1.2) f

[a] Values shown are the mean of 5 replicates (n = 5) ± SE. Root galling index on a scale from 0-10 with 0 = no galls, 10 = 100% of roots on bottom surface of stone wool block galled. Values in a column followed by different letters are significantly different ($P \leq 0.05$) according to Fisher's LSD-test. Raw nematode data (J2 counts) were $\log_{10}(x+1)$-transformed prior to analysis; non-transformed data are presented.

at-plant/post-plant applications of Vydate® and Avid®, the at-plant application of Avid®, and the pre-plant drench with Ornazin® Significant reductions in galling in both experiments also resulted from the post-plant applications of Vydate®, the at-plant application of Nema-Q™, and the at-plant/post-plant applications of Ornazin®. However, all these treatments still resulted in moderate galling (galling index between 2 and 5). A few treatments: DiTera® (at-plant and at-plant/postplant), Avid® (post-plant) and Ornazin® (post-plant) only reduced galling compared to the nematode-inoculated control in one of the two replicated experiments (Tables 3 and 4).

Nematode reproduction was high in the first, but much lower in the second experiment. In both experiments the at-plant/post-plant application of Vydate®, the at-plant and at-plant/post-plant applications of Avid®, and the pre-plant application of Ornazin® significantly reduced nematode reproduction relative to the nematode-inoculated control. The post-plant application of Vydate®, and the at-plant and at-plant/post-plant applications of DiTera® reduced nematode reproduction in the first experiment only. The post-plant application of Nema-Q™ was only effective in the second experiment (Tables 3 and 4).

Shoot weight was significantly reduced in the nematode-inoculated control compared to the non-inoculated control in the first experiment only. In the first experiment, shoot weights after the at-plant Vydate® application, the at-plant and at-plant/post-plant applications of Avid®, and the at-plant and at-plant/post-plant DiTera® applications were not significantly lower than in the non-inoculated control. The pre-plant drench and the post-plant applications of Ornazin® reduced shoot weights compared to the nematode-inoculated control (Table 3). In the second experiment, a number of treatments reduced shoot weights compared to the nematode-inoculated control. For example, all Ornazin® applications had lower shoot weights than the nematode-inoculated control (Table 4).

The timing of the application did not have a major effect on root-galling or nematode reproduction with Nema-Q™ or DiTera®. However, with the other products the timing of application did have significant effects on the reduction in root-galling and nematode reproduction. Vydate® was much more effective when it was applied repeatedly (at-plant/post-plant and post-plant), Avid® was much more effective when it was applied at-plant (at-plant and at-plant/postplant), and Ornazin® when applied as a pre-plant drench (Tables 3 and 4).

Experiment 3 - Timing of product application, Pots: Effects of the products on root-galling in pot-grown tomatoes were not as dramatic as in stone wool-grown tomatoes. Repeated applications (at-plant/post-plant and post-plant)

TABLE 3. Effect of application timing of nematicides on root-knot nematode symptoms, infestation, and shoot weight of tomato grown on Grodan® stone wool blocks. Tomato harvested 6 wk after inoculation with 10,000 *M. incognita* eggs. Nematicides applied as a drench. First replicated experiment.

Product (ppm, a.i.)	Application timing[a]	Root galling index	Second-stage *Meloidogyne* juveniles (J2) per root sytem	Fresh shoot weight (g)
No-nematode control				90.4 (±19.1) ab
Plus-nematode control		7.6 (±0.25) ab[b]	65,367 (±5,595) ab	66.8 (±4.4) cdefg
Vydate® (150)	pre-plant drench	7.2 (±0.58) ab	62,739 (±21,074) abcd	69.3 (±6.6) cdefg
	at-plant 1x	6.6 (±0.93) abcd	133,467 (±32,808) a	74.1 (±5.1) bcdef
	at-plant/post-plant	0.4 (±0.25) i	676 (±191) fg	63.9 (±1.6) efghi
	post-plant	3.2 (±1.32) gh	6,840 (±3,096) ef	62.1 (±6.1) efghi
Avid® (0.25)	pre-plant drench	7.8 (±0.20) a	89,188 (±50,420) abcd	55.1 (±3.9) fghi
	at-plant 1x	0.6 (±0.60) i	1,894 (±1,142) g	86.8 (±10.9) abc
	at-plant/post-plant	0.2 (±0.20) i	401 (±171) g	85.5 (±2.7) abcd
	post-plant	5.0 (±1.27) cdefg	107,988 (±39,113) abc	64.6 (±5.0) defghi
Nema-Q™ (430)	pre-plant drench	6.2 (±0.58) abcdef	56,107 (±12,552) abcd	67.5 (±6.7) cdefg
	at-plant 1x	3.2 (±0.92) gh	15,016 (±5,960) bcde	50.7 (±8.1) ghi
	at-plant/post-plant	5.6 (±0.68) bcdef	49,493 (±18,563) abcd	55.3 (±5.5) fghi
	post-plant	5.6 (±0.40) bcdef	57,922 (±34,279) abcd	66.2 (±9.1) cdefg
DiTera® (900)	pre-plant drench	6.8 (±0.37) abc	18,946 (±6,512) abcde	59.8 (±3.1) fghi
	at-plant 1x	4.2 (±0.97) fg	20,455 (±13,163) de	82.7 (±4.7) abcde
	at-plant/post-plant	4.4 (±0.93) fg	7,847 (±854) cde	98.8 (±9.6) a
	post-plant	7.6 (±0.25) ab	47,720 (±15,470) abcd	62.7 (±7.5) efghi
Ornazin® (18)	pre-plant drench	1.6 (±0.51) hi	995 (±690) fg	44.3 (±7.6) i
	at-plant 1x	6.4 (±0.81) abcde	38,035 (±13,897) abcd	65.6 (±2.8) defghi
	at-plant/post-plant	4.6 (±1.44) defg	28,638 (±8,674) bcde	66.0 (±10.7) cdefg
	post-plant	5.6 (±0.75) bcdef	52,764 (±14,797) abcd	44.6 (±2.7) hi

[a]"pre-plant drench" applied to transplants 1 wk prior transplanting and nematode inoculation; "at-plant 1x" one time drench immediately after transplanting and nematode inoculation; "at-plant/post-plant" drench immediately after transplanting and nematode inoculation followed by additional drenches 2 and 4 wk later; "post-plant" drenches 2 and 4 wk after transplanting and nematode inoculation.

[b]Values shown are the mean of 5 replicates (n = 5) ± SE. Root galling index on a scale from 0-10 with 0 = no galls, 10 = 100% of roots on bottom surface of stone wool block galled. Values in a column followed by different letters are significantly different ($P \leq 0.05$) according to Fisher's LSD-test. Raw nematode data (J2 counts) were $\log_{10}(x+1)$-transformed prior to analysis; non-transformed data are presented.

TABLE 4. Effect of application timing of nematicides on root-knot nematode symptoms, infestation, and shoot weight of tomato grown on Grodan® stone wool blocks. Tomato harvested 6 wk after inoculation with 10,000 *M. incognita* eggs. Nematicides applied as a drench. Second replicated experiment.

Product (ppm, a.i.)	Application timing[a]	Root galling index	Second-stage *Meloidogyne* juveniles (J2) per root sytem	Fresh shoot weight (g)
No-nematode control				83.0 (±19.1) ab[b]
Plus-nematode control		7.0 (±0.32) abc	6,358 (±5,595) abc	82.3 (±4.4) ab
Vydate® (150)	pre-plant drench	7.8 (±0.20) a	6,428 (±21,074) abcd	61.2 (±6.6) def
	at-plant 1x	6.0 (±0.45) bcde	13,103 (±32,808) ab	60.9 (±5.1) def
	at-plant/post-plant	1.2 (±0.37) g	775 (±191) f	87.4 (±1.6) a
	post-plant	2.8 (±0.49) f	3,280 (±3,096) cde	62.6 (±6.1) def
Avid® (0.25)	pre-plant drench	6.2 (±0.80) bcd	3,753 (±50,420) abcd	75.6 (±3.9) abcd
	at-plant 1x	1.2 (±0.20) g	420 (±1,142) ef	84.0 (±10.9) ab
	at-plant/post-plant	0.6 (±0.40) g	128 (±171) g	83.3 (±2.7) ab
	post-plant	6.8 (±0.20) abc	8,091 (±39,113) abc	66.8 (±5.0) cdef
Nema-Q™ (430)	pre-plant drench	7.2 (±0.37) ab	8,565 (±12,552) ab	62.3 (±6.7) def
	at-plant 1x	4.6 (±0.40) e	12,996 (±5,960) ab	64.6 (±8.1) def
	at-plant/post-plant	7.0 (±0.32) abc	10,593 (±18,563) ab	69.3 (±5.5) bcdef
	post-plant	6.0 (±1.52) bcde	5,992 (±34,279) def	55.0 (±9.1) f
DiTera® (900)	pre-plant drench	6.2 (±0.71) bcd	3,174 (±6,512) bcd	73.1 (±3.1) abcde
	at-plant 1x	6.8 (±0.58) abc	9,570 (±13,163) ab	62.8 (±4.7) def
	at-plant/post-plant	7.8 (±0.20) a	16,398 (±854) ab	85.4 (±9.6) a
	post-plant	5.6 (±0.68) cde	3,555 (±15,470) abcd	80.3 (±7.5) abc
Ornazin® (18)	pre-plant drench	1.8 (±0.20) fg	618 (±690) ef	65.8 (±7.6) cdef
	at-plant 1x	6.4 (±0.40) abc	21,768 (±13,897) ab	58.4 (±2.8) ef
	at-plant/post-plant	4.8 (±0.37) de	13,503 (±8,674) ab	67.0 (±10.7) cdef
	post-plant	4.8 (±0.37) de	20,107 (±14,797) a	64.9 (±2.7) def

[a]"pre-plant drench" applied to transplants 1 wk prior transplanting and nematode inoculation; "at-plant 1x" one time drench immediately after transplanting and nematode inoculation; "at-plant/post-plant" drench immediately after transplanting and nematode inoculation followed by additional drenches 2 and 4 wk later; "post-plant" drenches 2 and 4 wk after transplanting and nematode inoculation.

[b]Values shown are the mean of 5 replicates (n = 5) ± SE. Root galling index on a scale from 0-10 with 0 = no galls, 10 = 100% of roots on bottom surface of stone wool block galled. Values in a column followed by different letters are significantly different ($P \leq 0.05$) according to Fisher's LSD-test. Raw nematode data (J2 counts) were $\log_{10}(x+1)$-transformed prior to analysis; non-transformed data are presented.

of Vydate® and Avid® significantly reduced root-galling in both experiments, but even in the best treatment the root-galling index was still relatively high (4.3), compared to the root-galling index after the best treatment in stone wool-grown tomatoes (0.2) (Table 5 and 6). In the second replicate of the pot experiment, the pre-plant and at-plant applications of Vydate®, and the at-plant/post-plant application of Nema-Q™ also significantly reduced galling (Table 6).

Nematode reproduction was high, with close to 1 million eggs recovered per root system in the nematode-inoculated control. Significant reductions in nematode reproduction occurred only after some of the Vydate® treatments, although not consistently between the two replicated experiments (Tables 5 and 6).

Compared to the non-inoculated control, tomato shoot weight was reduced in the nematode-inoculated control in the second replicated experiment only. In this second replicate, repeated Vydate® applications (at-plant/post-plant and post-plant) prevented a significant reduction in shoot weight relative to the non-inoculated control. Most treatments significantly increased shoot weights relative to the nematode-inoculated control (Tables 5 and 6).

Consistent effects of the different application timings occurred only with Avid®, where repeated applications (at-plant/post-plant, and post-plant) resulted in lower root-galling than the one-time applications (pre-plant drench, and at-plant) (Tables 5 and 6).

DISCUSSION

Our experiments confirm results by others (Lehman, 1987; Amsing 2004) that showed that root-knot nematodes can cause high levels of infestation and reproduce on roots of susceptible crops grown in stone wool substrate. Several studies have been done on the management of plant-parasitic nematodes in soil-less cultures, and these have mostly been focused on identifying and eliminating the sources of nematode infestation through proper preventative measures and hygiene (Amsing, 2004; Garcia Victoria and Amsing, 2007). Very few studies however have dealt with the potential to control plant-parasitic nematodes in the soil-less substrate in a growing crop. Amsing (1990) reported that Vydate® L, added at 0.02% to the nutrient tank solution effectively controlled *Pratylenchus vulnus* in rose for approximately 5 weeks. In this system, roots were flooded with the recirculating nematicide-containing solution five times a day.

In our initial experiment, a reduction in tomato root-galling was achieved by all tested products. However, Avid® in particular showed promising results as this product nearly eliminated root-galling, reduced nematode reproduction by over 98%, and also prevented a significant

TABLE 5. Effect of application timing of nematicides on root-knot nematode symptoms, infestation, and shoot weight of tomato grown in pots with steam-sterilized sand. Tomato harvested 6 wk after inoculation with 20,000 *M. incognita* eggs. Nematicides applied as a drench. First replicated experiment.

Product (ppm, a.i.)	Application timing[a]	Root galling index	*Meloidogyne* eggs per root system	fresh shoot weight (g)
No-nematode control				111.2 (±12.6) a[b]
Plus-nematode control		8.2 (±0.32) a	1,134,167 (±339,140) abcd	73.7 (±12.9) abcd
Vydate® (150)	pre-plant drench	8.3 (±0.20) a	581,333 (±179,284) de	44.5 (±12.0) d
	at-plant 1x	8.0 (±0.45) ab	1,021,667 (±141,814) abc	74.7 (±16.9) abcd
	at-plant/post-plant	6.3 (±0.37) cde	613,333 (±66,140) bcde	88.08 (±14.7) abc
	post-plant	6.5 (±0.49) bcde	387,500 (±56,121) e	107.6 (±15.2) ab
Avid® (0.25)	pre-plant drench	8.5 (±0.80) a	584,667 (±127,815) cde	63.0 (±4.3) cd
	at-plant 1x	7.7 (±0.20) abcd	1,431,667 (±223,810) a	93.9 (±15.6) abc
	at-plant/post-plant	6.0 (±0.40) e	1,619,167 (±308,741) a	97.5 (±15.4) abc
	post-plant	6.2 (±0.20) de	1,250,833 (±338,813) abcd	79.3 (±14.5) abcd
Nema-Q™ (430)	pre-plant drench	8.5 (±0.37) a	937,500 (±320,124) abcd	61.2 (±18.2) cd
	at-plant 1x	8.0 (±0.40) ab	1,408,333 (±298,436) a	65.2 (±15.6) cd
	at-plant/post-plant	8.3 (±0.32) a	891,667 (±140,592) abcd	60.5 (±8.7) cd
	post-plant	7.5 (±1.52) abcde	708,333 (±174,005) bcde	60.1 (±18.2) cd
DiTera® (900)	pre-plant drench	8.3 (±0.71) a	1,005,833 (±350,798) abcd	74.3 (±14.7) abcd
	at-plant 1x	8.2 (±0.58) a	1,090,833 (±207,514) abc	73.5 (±8.9) abcd
	at-plant/post-plant	7.7 (±0.20) abcd	1,742,500 (±388,432) a	85.0 (±19.9) abcd
	post-plant	7.3 (±0.68) abcde	1,694,167 (±442,501) a	78.5 (±14.2) abcd
Ornazin® (18)	pre-plant drench	8.3 (±0.20) a	1,061,667 (±286,289) abcd	64.7 (±19.9) cd
	at-plant 1x	8.0 (±0.40) ab	1,470,833 (±284,372) a	68.1 (±15.0) bcd
	at-plant/post-plant	7.8 (±0.37) abc	1,305,833 (±347,245) ab	70.3 (±13.9) abcd
	post-plant	7.3 (±0.37) abcde	609,583 (±63,759) bcde	64.5 (±11.5) cd

[a]"pre-plant drench" applied to transplants 1 wk prior transplanting and nematode inoculation; "at-plant 1x" one time drench immediately after transplanting and nematode inoculation; "at-plant/post-plant" drench immediately after transplanting and nematode inoculation followed by additional drenches 2 and 4 wk later; "post-plant" drenches 2 and 4 wk after transplanting and nematode inoculation.

[b]Values shown are the mean of 6 replicates (n = 6) ± SE. Root galling index on a scale from 0-10 with 0 = no galls, 10 = 100% of roots galled. Values in a column followed by different letters are significantly different ($P \leq 0.05$) according to Fisher's LSD-test. Raw nematode data (egg counts) were $\log_{10}(x+1)$-transformed prior to analysis; non-transformed data are presented.

reduction in tomato shoot weight in nematode-inoculated plants. Only Vydate® performed in a similar manner. In the next series of experiments with stone wool-grown tomato, the highest levels of control were again obtained with Avid® and Vydate®. However, there were significant effects of the timing of product application on the levels of control that were achieved. The Avid® treatments were only effective when an at-plant application was included. In these applications, Avid® was applied at the same time as plants were inoculated with the root-knot nematode eggs. Wright et al. (1983) reported an almost complete, but reversible inhibition of *M. incognita* egg hatching by abamectin. Stretton et al. (1987) suggested that this resulted from the immobilization of the juveniles, rather than from effects on egg development. Still, our results clearly show that abamectin applied to stone wool blocks at time of nematode inoculation provided very high levels of control. The fact that a pre-plant drench with Avid® one week prior to nematode inoculation, and post-plant applications starting two weeks after nematode inoculation were largely ineffective, indicate respectively that the activity and/or presence of the product in the stone wool was fairly short-lived (< 1 wk), and that the product is largely ineffective once host plant roots have been infected. It is known that abamectin degrades rapidly by photo-oxidation (Mrozik, 1994). In a soil environment it's efficacy is compromised because it binds tightly to soil particles, and has a low water solubility resulting in poor movement of the product through the soil profile (Bull, 1985; Bull et al., 1984; Chukwudebe et al., 1996; Mrozik, 1994). The fate of abamectin in stone wool is unknown, but our results show that in stone wool the efficacy of the product is also short-lived. The failure of abamectin to affect the development of *M. incognita* once inside the host roots agrees with findings by Stretton et al. (1987), and further confirms findings by others that show that root uptake of abamectin is minimal (Chukwudebe et al., 1996; Wislocki et al., 1989).

Ntalli et al. (2009) recently reported that a neem-based product or its' active ingredient azadirachtin used at recommended rates, did not affect the motility of second-stage *M. incognita* juveniles in *in vitro* tests, and also failed to reduce nematode reproduction on tomato in greenhouse pot tests. This corresponds with our results, as we also did not observe an effect of Ornazin® on tomato root-galling or nematode reproduction in the pot tests. Ornazin® did reduce tomato root-galling and nematode reproduction in stone wool blocks when applied to tomato transplants as a pre-plant drench one week prior to nematode inoculation. However, this application also had phytotoxic effects as tomato shoot weights were reduced compared to the nematode-inoculated controls. This suggests Ornazin® either had some local systemic activity, or directly affected the tomato roots making them less suitable for nematode

TABLE 6. Effect of application timing of nematicides on root-knot nematode symptoms, infestation, and shoot weight of tomato grown in pots with steam-sterilized sand. Tomato harvested 6 wk after inoculation with 20,000 *M. incognita* eggs. Nematicides applied as a drench. Second replicated experiment.

Product (ppm, a.i.)	Application timing[a]	Root galling index	*Meloidogyne* eggs per root system	fresh shoot weight (g)
No-nematode control				112.5 (±12.6) a[b]
Plus-nematode control		8.8 (±0.17) ab	936,667 (±193,536) abc	35.5 (±7.5) j
Vydate® (150)	pre-plant drench	5.0 (±1.03) g	390,833 (±181,261) e	57.4 (±12.1) fghij
	at-plant 1x	6.3 (±0.56) f	1,022,500 (±145,532) abc	87.9 (±6.5) bcd
	at-plant/post-plant	4.3 (±0.49) g	352,500 (±58,903) de	103.8 (±7.0) ab
	post-plant	6.7 (±0.42) f	712,500 (±124,624) bc	92.3 (±11.1) abc
Avid® (0.25)	pre-plant drench	8.5 (±0.34) ab	696,667(±108,241) bc	64.5 (±10.0) efghi
	at-plant 1x	8.0 (±0.26) abcd	724,167 (±75,735) abc	73.0 (±7.9) cdefg
	at-plant/post-plant	6.8 (±0.31) ef	1,443,333 (±318,896) a	83.9 (±6.4) bcde
	post-plant	7.2 (±0.31) def	1,233,333 (±164,533) ab	83.0 (±3.8) bcde
Nema-Q™ (430)	pre-plant drench	8.2 (±0.48) abcd	715,000 (±148,896) bc	48.6 (±7.4) hij
	at-plant 1x	8.0 (±0.26) abcd	1,294,167 (±96,431) a	58.7 (±4.4) fghi
	at-plant/post-plant	7.3 (±0.42) cdef	914,167(±195,522) abc	65.9 (±5.0) defghi
	post-plant	8.3 (±0.21) abc	583,333 (±102,304) cd	47.2 (±8.0) ij
DiTera® (900)	pre-plant drench	9.0 (±0.0) a	802.500 (±157,150) abc	53.4 (±7.9) hij
	at-plant 1x	7.3 (±0.40) bcde	845,833 (±113,089) abc	62.9 (±5.5) efghi
	at-plant/post-plant	8.5 (±0.22) ab	1,443,333 (±172,828) abc	72.5 (±13.1) cdefg
	post-plant	8.7 (±0.21) ab	724,167 (±147,447) bc	65.5 (±6.1) defghi
Ornazin® (18)	pre-plant drench	8.8 (±0.17) ab	1,080,833 (±212,894) abc	69.8 (±6.2) defgh
	at-plant 1x	7.8 (±0.31) bcde	1,050,000 (±160,603) abc	63.0 (±3.9) efghi
	at-plant/post-plant	7.8 (±0.17) bcde	1,112,500 (±226,096) abc	76.6 (±10.6) cdef
	post-plant	8.0 (±0.26) abcd	792,500 (±160,087) abc	54.0 (±5.1) ghij

[a]"pre-plant drench" applied to transplants 1 wk prior transplanting and nematode inoculation; "at-plant 1x" one time drench immediately after transplanting and nematode inoculation; "at-plant/post-plant" drench immediately after transplanting and nematode inoculation followed by additional drenches 2 and 4 wk later; "post-plant" drenches 2 and 4 wk after transplanting and nematode inoculation.

[b]Values shown are the mean of 6 replicates (n = 6) ± SE. Root galling index on a scale from 0-10 with 0 = no galls, 10 = 100% of roots galled. Values in a column followed by different letters are significantly different ($P \leq 0.05$) according to Fisher's LSD-test. Raw nematode data (egg counts) were $\log_{10}(x+1)$-transformed prior to analysis; non-transformed data are presented.

invasion and/or reproduction. Uptake of neem by plant roots – including tomato – and subsequent systemic activity has been reported by others studying the effect of neem applied as a soil drench on insect control (Prabhat Kumar and Poehling, 2006; Premachandra et al., 2005; Thoeming et al., 2003; Kumar et al., 2005), but there are no reports on systemic activity of neem against nematodes. Still, the apparent delayed effect of a soil-drench with neem on root-knot nematode infestation of tomato warrants further investigation. The other two products tested, Nema-Q™ and Diterra, did not result in consistent reductions in root-galling or nematode reproduction neither in soil-grown nor in stone wool-grown tomato. In conclusion, our research shows that Avid® (a.i. abamectin) and possibly Ornazin® (a.i. azadirachtin) may be useful to control root-knot nematode infestations in stone wool-grown crops. However, further research aimed at optimizing product rates and the methods of application (e.g. the feasibility of mixing the products with the nutrient tank solution) is still necessary.

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