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Drip Irrigation as a Delivery System for Infestation of Field Plots with Nematodes[1]

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Abstract: A drip irrigation delivery system was used to infest field sites with the plant-parasitic root-knot nematodes, *Meloidogyne incognita.* Juvenile or egg inocula passed through the system without blockage of emitters or harm to the nematodes. Field sites so infested were available for experimentation. Delivery of approximately 5×10^4 to 10^5 juveniles or 10^5 to 3×10^5 eggs per emitter through the drip system resulted in heavy root galling of tomatoes planted next to the drip emitters. Nematodes feeding on bacteria (*Acrobeloides* sp.) and on fungi (*Deladenus durus*) also were successfully applied through the drip system. This method has potential for uniformly infesting experimental sites with plant-parasitic or entomogenous nematodes and for manipulation of nematode community structure for soil ecological studies.

Key words: *Acrobeloides* sp., biogation, *Deladenus durus,* drip irrigation, field infestation, *Lycopersion esculentum, Meloidogyne incognita,* root-knot nematode.

Field studies with plant-parasitic nematodes often are not possible or not reliable because of lack of suitable test sites. In addition to standard requirements for a good experimental site, such as fairly uniform physical, chemical, and biological soil properties, a moderate to high plant-parasitic nematode population with little spatial variation throughout the test area is needed. The population also should be in a relatively stable condition (6). These conditions seldom occur. Naturally infested field sites usually have spatial aggregation of plant-parasitic nematodes (9,14) and this must be considered in the experimental design and conclusions. Artificial establishment and maintenance of a uniform nematode population in the field would be advantageous for nematode damage assessment, but this is difficult to achieve. One method frequently used to artificially infest a test site with sedentary plant-parasitic nematodes is to incorporate nematode-infested root material into the soil (6). A nematode-susceptible crop is then planted. Alternatively, plants in the greenhouse are infested with nematodes and planted in the test field, followed by one or more seasons of susceptible crops to increase the nematode population (6). Methods of introducing nematodes of the same life stage (i.e., eggs) have been used less frequently. Satisfactory infestation also has been obtained by dipping seedlings into a slurry of eggs of *Meloidogyne* spp. and a water absorbent polymer and transplanting into field plots (8). In another study, eggs of *Meloidogyne* spp. were suspended in weak agar suspension and injected under pressure ca. 10 cm deep under the seed row (2). The method is suitable for infesting tomato roots with *M. incognita* in most plots.

In this study, a drip irrigation system was used to introduce plant-parasitic, bacterivorous, and fungivorous nematodes into field sites. The primary focus of the study was to develop a method to uniformly infest field sites with root-knot nematode eggs or juveniles; however, the delivery of nematodes with different behavior or morphological characteristics through the drip system also was tested.

Materials and Methods

Nematode inoculum: Root-knot nematode inoculum was obtained from 2–3-month-old tomato plants (*Lycopersicon esculentum* Mill. cv. UC 82) that had been inoculated with race 1 juveniles of *M. incognita* 6 weeks after seeding. Nematode eggs were collected by a modified extraction technique (13). Galled roots containing egg masses

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were washed free of soil with tap water and cut into 2-cm-long pieces. These were triturated for two 30-second intervals at maximum speed with a two-speed blender (Waring, New Hartford, CT) in a 0.26% sodium hypochlorite solution. Nematode eggs were collected on a 30-μm-pore sieve, washed carefully, and adjusted to the desired concentration with tap water.

Root-knot nematode second-stage juveniles (J2) were obtained by incubating egg suspensions on a modified Baermann funnel at 26 C (15). Hatched J2 were collected daily and stored in shallow water at 15 C. The nematodes were used within 1 week of hatching. *Acrobeloides* sp. and *Deladenus durus* were from the collection of D. W. Freckman, Department of Nematology, University of California, Riverside. The *Acrobeloides* sp. was cultured on *Escherichia coli* on 10% tryptic soy agar (TSA) (Difco Laboratories, Detroit, MI) and *D. durus* on *Coleophoma* sp. on potato dextrose agar (Difco Laboratories) at 26 C. After 3 weeks, the agar was cut into small pieces and incubated on a modified Baermann funnel at 26 C (15). Juveniles were collected daily and stored in shallow water at 15 C. The nematodes were used within 3 days after collection. Subsamples of nematode inoculum were examined microscopically to determine the number of live juveniles per milliliter. Desired nematode rates were obtained by diluting the suspensions with water.

Experimental design of field studies: All field trials were performed at the University of California South Coast Field Station at Irvine. Soil type was a San Emigdio sandy loam: 75.5% sand, 12.5% silt, 12% clay; 0.45% organic matter; pH 7.2. The nematode delivery system consisted of a low pressure drip irrigation assembly similar to one used for applying nematicides (16) and biological control agents (1). Application cylinders were used to passively inject the liquid fertilizer and the nematode inoculum at separate times into the drip irrigation water stream. Water from each injection cylinder was uniformly distributed to each of five replicate plots, each located in a separate block allowing a randomized complete block design. Polyethylene drip tubing with in-line emitters (Drip In Irrigation Company, Fresno, CA) spaced at 30-cm intervals supplied irrigation water at an hourly flow rate of 2 liters per emitter. The polyethylene emitters were a labyrinth type, self cleaning, turbulent flow dripper with an output uniformity of 98%. Drip lines were buried ca. 3 cm beneath the soil surface. Slow release fertilizer (13-13-13, 9-month formulation, Osmocote, Sierra Chemical Company, Milpitas, CA) was applied in two bands, 10 cm deep and 10 cm off bed center, at a rate of 42 kg/100 m. Seven-week-old UC 82 tomato seedlings were planted next to each of 11 emitters in 2.5-m × 0.55-m beds on 1-m centers. Twice during the following 2 weeks, 2.6 g/liter additional fertilizer (15-30-1 plus micronutrients, Stern's Miracle-Gro Products, Inc., Port Washington, NY) was applied through the drip system. Weed and insect control were according to standard agricultural practice.

Release of nematodes through drip system: Nematode inoculum was applied through the drip system at the following densities per emitter: approximately 7.3×10^4 eggs and 4.7×10^4 J2 of *M. incognita*, 1.5×10^4 J2 of *Acrobeloides* sp., and 1.3×10^5 juveniles of *D. durus*. Solution samples were collected for 1 minute every 5 minutes from the last emitter of the appropriate drip line. The number of eggs or live juveniles were determined in each sample. The experiment was repeated once.

Root-knot nematode infestation trials: To determine a rate that resulted in sufficient root galling, several different densities of nematode inocula were applied. The first year, after beds were planted and irrigated to approximately −0.04 MPa at 30 cm deep, nematode inocula were released at the following concentrations: 6.1×10^3, 3.5×10^4, and 7.3×10^4 eggs of *M. incognita* per emitter, 1.5×10^4 and 4.7×10^4 J2 of *M. incognita* per emitter. Three days after application, 11 soil cores per plot were taken next to the emitter from uninfested and root-knot nematode-infested (3.5×10^4

J2/emitter) treatments to determine the distribution of the nematodes. The cores were divided carefully into 0–10-cm, 10–20-cm, and 20–30-cm samples and placed on modified Baermann funnels (15). After 3 days at 26 C, root-knot nematodes were counted. Tomatoes were harvested after 14 weeks and fruit weight per plot was determined. Root systems were removed from the soil and immediately rated for root-knot galling (7).

In the second year, two of three plots were fumigated with 1 liter metham-sodium (Soil-prep, Wilbur-Ellis, Fresno, CA) per 6.38 m^2 3 weeks before planting to reduce indigenous nematode populations and root-rotting fungi. In addition, the irrigation schedule was controlled by monitoring with tensiometers. The moisture level at 30 cm deep was maintained between −0.01 and −0.03 MPa during the first month. Thereafter, the irrigation was scheduled to keep tensiometer readings at 45 cm deep between −0.01 and −0.05 MPa. In the first trial, nematode inocula were applied at the following densities: 5×10^4, 1×10^5, and 5×10^5 eggs, and 5×10^4 and 1×10^5 J2 of *M. incognita* per emitter. In the other two trials, one of which was not fumigated, nematode plots were infested with 3×10^5 eggs of *M. incognita* per emitter. The trials were harvested after 13 weeks and fruits were weighed. Every root system was carefully recovered, shaken free of soil, and rated for root-knot galling on a scale of 0 to 10 (7).

Data analysis: All data were subjected to analysis of variance and Fisher's least significant differences (LSD).

Results

Release of nematodes through drip system: The release pattern of juveniles of different nematode species and the egg inoculum of *M. incognita* was similar (Fig. 1). More than 90% of eggs and J2 of *M. incognita*, and juveniles of *Acrobeloides* sp. passed through the system in less than 20 minutes. The release of juveniles of *D. durus* took slightly longer probably because of the greater inoculum level. Microscopic examination of nematodes in the samples did not reveal any visible damage or viability loss due to the passage through the application system.

Root-knot nematode infestation trials: Three days after application of root-knot nematode J2, the majority recovered were from the top 10 cm of soil (Fig. 2); however, about one-fourth of the nematode population was extracted from depths below 10 cm.

Root systems were severely affected by root-rotting fungi by harvest time of the first-year field trial. Only the highest inoculation level with J2 resulted in root galling (3.6 ± 0.3) and in a significant yield reduction of 13% relative to the uninfested control (Table 1). Variability of galling within and among replicated plots was less than 10%.

In the second-year field trial with different inoculum levels, root galling was heavy in plots inoculated with eggs or J2 (Table 2). Significantly more galling occurred at the 10^5 than at the 5×10^4 eggs per emitter level. There was no significant difference in galling between the two highest inoculum levels. Galling was generally heavier when J2 were applied than with the same inoculum density of eggs. No significant differences were observed between 5×10^4 and 10^5 J2 per emitter. The variability in galling within and among replicated plots was very small at all inoculum levels. After about 2 months, approximately 20% of the plants showed foliar symptoms of tomato spotted wilt disease, accounting in part for the large variability in yield data.

Trials three and four confirmed that an inoculum level of 3×10^5 eggs of *M. incognita* per emitter consistently achieved heavy root-knot galling on tomato roots (Table 3). Again, galling was very uniform.

Plots treated with metham-sodium had less root rot and had reduced background nematode infection in the controls.

Discussion

The drip system for delivering nematodes has numerous advantages over con-

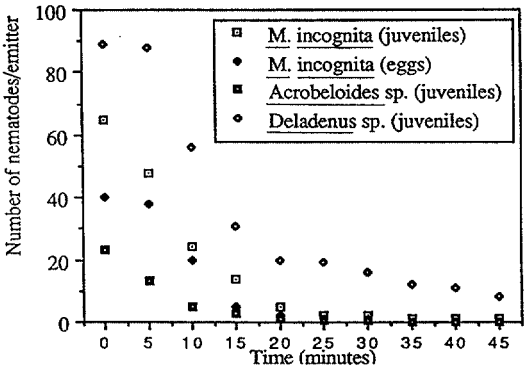

FIG. 1. Release of nematodes through a drip delivery system using the passive camistes method.

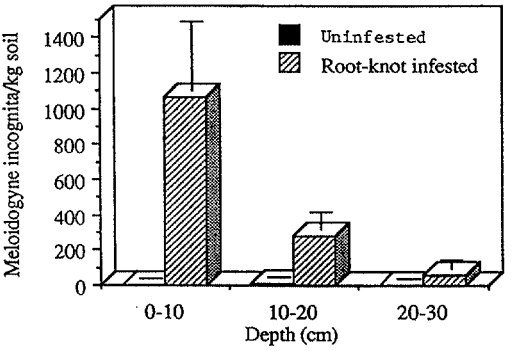

FIG. 2. Vertical distribution of *Meloidogyne incognita* in soil 3 days after field infestation with second-stage juveniles through a drip delivery system.

ventional methods for infesting field plots. High-pressure release such as in overhead irrigation systems may harm nematodes (10). Low-pressure release through drip emitters did not harm the nematode inoculum. Furthermore, there was an even distribution of the inoculum and small variability in root galling of tomato plants within and between replications. The delivery of defined initial population densities of a single nematode species increases precision in field studies. Moreover, nematode-infested field sites were immediately accessible for biological experiments, thereby eliminating the delay of building up populations with susceptible crops to achieve sufficient pest pressure for testing nematode-resistant cultivars, nematicides, or biological control agents. There is also a likelihood of uneven build-up if a build-up period is necessary. The drip system method of delivery allows more flexibility in deciding when to commence an experiment.

Researchers can infest plots before planting and any time thereafter with single or multiple applications without disturbance of the growing crop. In addition, both eggs and J2 of *M. incognita* can be delivered through the drip system and result in heavy root galling. Use of J2 has some advantages for precision purposes because the actual infectious units are used as inoculum. These plant parasites attack roots immediately, whereas nematode eggs are exposed for a longer time to environmental and biological factors which influence survival and development. Also, there is no way of knowing which eggs will hatch. Delivery of egg inoculum, however, may more closely simulate natural conditions which can be important for experiments where the con-

TABLE 1. Infestation of tomato field plots with *Meloidogyne incognita* eggs and second-stage juveniles (J2) delivered through a drip irrigation system.

Treatment	Root gall rating†	Yield (kg/bed)
Uninfested control	0.1 ± 0.1a	11.5 ± 0.6a
Eggs (7.3×10^4/emitter)	0.3 ± 0.3a	11.5 ± 0.4a
J2(4.7×10^4/emitter)	3.6 ± 0.3b	10.0 ± 0.5b

Numbers followed by the same letter are not significantly different according to Fisher's LSD ($P = 0.05$).
† Rating scale 0–10 (7).

TABLE 2. Infestation of tomato field plots with *Meloidogyne incognita*, eggs and second-stage juveniles (J2) delivered through a drip irrigation system. Soil treated 3 weeks before planting with 1 liter methamsodium/6.38 m².

Treatment	Root gall rating†	Yield (kg/bed)
Uninfested control	1.9 ± 0.6	44.0
Eggs per emitter		
5×10^4	6.2 ± 0.3	35.1
1×10^5	7.2 ± 0.2	36.1
5×10^5	7.3 ± 0.2	39.6
J2 per emitter		
5×10^4	7.8 ± 0.1	40.5
1×10^5	8.1 ± 0.1	37.0
LSD ($P = 0.05$)	0.8	8.0

† Root gall rating 0–10 (7) ± standard error.

TABLE 3. Infestation of tomato field plots with *Meloidogyne incognita* eggs delivered through a drip irrigation system.

Treatment	Trial 3†		Trial 4	
	Root gall rating‡	Yield (kg/ bed)	Root gall rating‡	Yield (kg/ bed)
Uninfested control	0.3	43.3	2.4	37.1
Eggs (3×10^5/emitter)	6.1	36.5	6.9	34.7
LSD ($P = 0.05$)	0.8	2.8	3.5	2.3

† Treated 3 weeks before planting with 1 liter metham-sodium/6.38 m².
‡ Rating scale 0–10 (7).

trol target is the egg or the hatching process. Also, there may be logistical difficulties in obtaining freshly hatched J2 for large field trials. Extended storage periods of the J2 may be necessary which could affect their infectivity if not aerated.

The lack of highly significant yield differences in the field trial can be attributed to a reduced stress of nematode-infected plants grown under drip irrigation and slow release fertilizer.

These results suggest that it takes about 30,000 J2 per emitter to produce a consistent severe gall rating on tomato roots in field soil. This is higher than the number of J2 used per pot in greenhouse tests and may suggest a higher degree of mortality than was observed in the nematodes after passing through the system. Further studies are needed to determine percentage of infectivity before and after passing through the system. Inoculation in the field involves much greater soil masses than does inoculation in greenhouse pots. Losses due to parasitization may be much higher in field soil than in sterilized potting soil. Considering all the factors that might affect the survival and infectivity of J2 injected into field soil using this delivery method, 30,000 J2 seem reasonable for field studies with low variability between plants and between replicates.

As suggested earlier (12), our results indicate that the drip delivery system is an ideal method for applying beneficial nematodes to target sites. Even the relatively large juveniles of *D. durus* were readily delivered through the emitters. Several *Deladenus* spp. are parasitic on siricid woodwasps (4,5) and have been used as biological control agents (5). Our results also show that neither nematode morphology nor movement influences their release through the emitters, at least within the range of organisms tested. This work and one other (1) illustrate that drip irrigation systems offer new possibilities for applying biological control agents. In an analogy to chemigation, the term for application of agrochemicals through irrigation systems (11), we propose the term biogation for the application of biological units through irrigation systems.

LITERATURE CITED

1. Bahme, J. B., M. N. Schroth, S. D. Van Gundy, A. R. Weinhold, and D. M. Tolentino. 1988. Effect of inocula delivery systems on rhizobacterial colonization of underground organs of potato. Phytopathology 78:534–542.

2. Ball, D. A., and H. Ferris. 1982. A technique for inoculating field sites with *Meloidogyne* eggs. Journal of Nematology 14:420–422.

3. Becker, J. O., M. Monson, and S. D. Van Gundy. 1988. The use of a drip irrigation system for the infestation of field plots with nematodes. Journal of Nematology 20:627 (Abstr.).

4. Bedding, R. A. 1967. Parasitic and free-living cycles in entomogenous nematodes of the genus *Deladenus*. Nature 214:174–175.

5. Bedding, R. A., and R. J. Akhurst. 1974. Use of the nematode *Deladenus siricidicola* in the biological control of *Sirex noctilio* in Australia. Journal of the Australian Entomological Society 13:129–135.

6. Bird, G. W., R. A. Davis, P. W. Johnson, D. MacDonald, M. McKenry, R. B. Malek, C. Orr, W. M. Powell, R. M. Riedel, J. L. Ruehle, and J. Townshend. 1978. Site selection procedures for field evaluation of nematode control agents. Pp. 108–113 *in* E. I. Zehr, G. W. Bird, K. D. Fisher, K. D. Hickey, F. H. Lewis, R. F. Line, and S. F. Rickard, eds. Methods for evaluating plant fungicides, nematicides, and bactericides. St. Paul, MN: American Phytopathological Society Press.

7. Bridge, J., and S. L. J. Page. 1980. Estimation of root-knot nematode infestation levels on roots using a rating chart. Tropical Pest Management 26: 296–298.

8. Fortnum, B. A., R. E. Currin III, and J. P. Krausz. 1987. Water absorbent polymer aids in the infestation of field sites with *Meloidogyne* eggs. Journal of Nematology 19: 135–137.

9. Goodell, P. B., and H. Ferris. 1980. Plant-parasitic nematode distribution in an alfalfa field. Journal of Nematology 12: 136–141.

10. Heald, C. M., and A. W. Johnson. 1969. Sur-

vival and infectivity of nematodes after passing through an overhead sprinkler irrigation system. Journal of Nematology 1:290 (Abstr.).

11. Johnson, A. W., J. R. Young, E. D. Threadgill, C. C. Dowler, and D. R. Sumner. 1986. Chemigation for crop production management. Plant Disease 70: 998–1004.

12. Kaya, H. K. 1985. Entomogenous nematodes for insect control in IPM systems. Pp. 283–302 *in* M. A. Hoy, and D. C. Herzog, eds. Biological control in agricultural IPM systems. New York: Academic Press.

13. McClure, M. A., T. H. Kruk, and I. Misaghi. 1973. A method for obtaining quantities of clean *Meloidogyne* eggs. Journal of Nematology 5:230.

14. McSorley, R., and J. L. Parrado. 1982. Estimating relative error in nematode numbers from single soil samples composed of multiple cores. Journal of Nematology 14: 522–529.

15. Thirugnanam, M. 1978. Evaluation of nematicides for systemic eradication of root-knot nematodes. Pp. 103–104 *in* E. I. Zehr, G. W. Bird, K. D. Fisher, K. D. Hickey, F. H. Lewis, R. F. Line, and S. F. Rickard, eds. Methods for evaluating plant fungicides, nematicides, and bactericides. St. Paul, MN: American Phytopathological Society Press.

16. Van Gundy, S. D., and S. Garabedian. 1984. Application of nematicides through drip irrigation systems. Mededelingen van de Faculteit Landbouwwetenschappen Rijksuniversiteit Gent 49/2b: 629–634.