DELIVERY OF *STEINERNEMA RIOBRAVIS* THROUGH A DRIP IRRIGATION SYSTEM[†]

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RESUMEN

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Se realizaron dos experimentos para examinar la aplicación de *Steinernema riobravis* desde una tubería de riego por goteo de 78 m de longitud. Se recolectaron muestras de agua a las salidas de la linea con el fin de determinar concentraciones de nematodos a varias distancias del punto de inyección. La recuperación de nematodos fue menor en las salidas que estaban a mayor distancia del punto de inyección. La utilizacion del sistema de irrigación durante un periodo más largo de tiempo (1.75 h vs. 0.75 h) resultó en una aplicación de sólo 0.47% más de nematodos. Se desmontaron y lavaron secciones de la línea principal de irrigación, observandose que 34.6% de los nematodos inyectados habían sedimentado antes de alcanzar las salidas más distantes del sistema. La posibilidad de que partículas, tales como nematodos sedimenten, debería considerarse cuando se aplican tales materiales a través de un sistema de irrigación.

Palabras claves: nematodos, nematodos entomopatógenos, *Steinernema riobravis*, tecnología de irrigación.

Steinernematid and heterorhabditid nematodes are beneficial for the management of a variety of soil insects (Kaya, 1993). Efficacy of commercial application depends on a number of ecological conditions, including the maintenance of adequate soil moisture and the prevention of drying of nematodes during application (Kaya, 1993). Nematodes are typically applied with standard spray equipment, but applications through irrigation systems may also be effective (Georgis, 1990; Kaya, 1993). Successful applications have been achieved with furrow (Cabanillas and Raulston, 1996), trickle (Reed *et al.*, 1986), and center-pivot (Wright *et al.*, 1993) irrigation systems. Distribution of *Heterorhabditis* spp. by a trickle irrigation system was relatively uniform, but only 37% to 59% of the total number of nematodes injected into the system were actually recovered (Reed *et al.*, 1986).

Trickle or drip irrigation systems are particularly important in vegetable production systems in Florida (Pitts *et al.*, 1991). The objectives of this study were to observe the distribution of *Steinernema riobravis* by a drip irrigation system under a tomato (*Lycopersicon esculentum*) crop in Florida and to identify any losses of nematodes during the application process.

Two experiments were conducted in a tomato field on Immokalee fine sand (96% sand, 2% silt, 2% clay) at the University of Florida Southwest Florida Research and Education Center in Immokalee, Florida. 'Agriset' tomatoes were transplanted 0.3 m apart on 26 Feb. 1996 into raised

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beds 0.8 m wide and 73 m long. The irrigation system for each bed consisted of a single central line of Netafim[R] Streamline driptape (Netafim Irrigation Inc., Altamonte Springs, FL) with an inside diameter of 16 mm. The irrigation line for each bed was 77.8 m long from an injection port (before the bed) to the end of the bed, and contained 252 emitters, spaced 0.3 m apart (no emitters within 1.0 m of injection port). At the operating pressure of 69.0 kPa, the flow rate was 870 ml/hr from each emitter. A hole deep enough to accommodate a 600-ml beaker was dug beneath selected emitters for collection of water samples.

An injection port adapted to a CO_2-pressurized stainless steel tank was installed before the beginning of the bed for injection of a suspension of *Steinernema riobravis*, obtained from Biosys (Palo Alto, CA). The nematodes were mixed in the tank with water and 2g of a blue tracer dye (FD & C No. 1, Warner-Jenkinson, St. Louis, MO) which served as an indicator to note when injected material reached a particular emitter. The tank was agitated continuously during the injection process to maintain the nematodes in suspension.

The first experiment, conducted on 9 May, involved four different beds, which served as four replications. On each bed, four emitters spaced 18.3 m apart were established as collection points. These emitters were located at distances of 18, 37, 55, and 73 m from the injection port. A suspension of 67.2 million *S. riobravis* in 4 L water was injected into the irrigation line of each bed at a pressure of 103.5 kPa. At the system operating pressure of 69.0 kPa, an average of 110 sec was required to inject this volume of suspension. Preliminary calibration under similar conditions, except that only the blue dye was injected without nematodes, revealed that 36 min were required for injected dye to reach the most distant emitter. Therefore, to ensure adequate time for nematode collections, the beakers for collecting water samples were kept in place for 45 min after injection of nematodes. After that time, water samples ranging in size from 520 to 590 ml were removed from the collection points. Each sample was mixed well, and a 10-ml aliquot was removed for counting of nematodes. Count data were converted to numbers of nematodes released from an emitter during the 45-min collection period. Resulting data were subjected to analysis of variance, and differences in mean nematode recovery with emitter distance were compared by Duncan's multiple-range test, using MSTAT-C software (Freed et al., 1991).

The second experiment was conducted on a single bed on 10 July 1996. Collection stations were established at 17 emitters in this bed, with a spacing of 4.6 m between collection sites. A suspension of 178 million *S. riobravis* in 10 L water was injected at a pressure of 65.5 kPa into the irrigation line. The system pressure in this irrigation line measured 62.1 kPa. An injection time of 18 min was required at this volume and pressure. Preliminary calibrations revealed that, under these conditions, water flow was relatively uniform, with 124 to 136 ml water collected in 10 min at each of the 17 emitters measured. Additional calibrations with blue dye revealed that 28 min were required for injected material to reach the most distant emitter. For the experiment, collection beakers were kept in place for 46 min (18 min injection time plus 28 min travel time), after which time, water samples ranging in size from 544 to 600 ml were removed from the emitters. The beakers removed from each collection site were immediately replaced with a second set of beakers, and collection of water samples continued for an additional 60 min. After the second set of water samples was obtained, water flow was shut off, and

17 sections of the main drip line, each 1.83 m in length, were clamped at each end to seal any water and other material within the section. The locations of these sections were the 1.83 m immediately proximal to (i.e., injector side) each of the 17 emitter collection sites. The clamped sections were then cut out of the drip line, the contents (ca. 400 ml) were drained, and the tube segments were flushed with water at a velocity of >0.6 m/sec to obtain a total volume of 1000 ml for each sample. Thus, three sets of samples were available: a first and second set of water samples from each emitter, and samples washed from the drip line sections. A 10-ml aliquot was removed from each of these for counting. Additional 10-ml aliquots were obtained from the original tank mixture before injection and from the material (97 ml) remaining in the tank after injection. All count data were then converted to the appropriate units (nematodes per 46 min, per 60 min, per 1.83-m of drip tube, or per tank).

In the first experiment, far fewer nematodes were obtained from the most distant emitter than from the other three emitters during the 45-min collection period (Table 1). Based on the 67.2 million nematodes available for injection into one bed and 252 emitters in the drip line for each bed, an even distribution of nematodes should result in 267,000 nematodes available from each emitter. Nematode numbers obtained from the first three emitters (Table 1) averaged 253 000, only 5% below the theoretical estimate, assuming even distribution of all nematodes injected. However, severe losses occurred at the fourth emitter. It is possible that a longer flow time was needed to move nematodes to this distant emitter, or that nematodes settled out of suspension in the drip line and did not reach this collection station. In a similar experiment, Reed *et al.* (1986) observed that only 37% to 59% of total *Heterorhabditis* spp. injected into a drip irrigation system were actually recovered, and speculated that some of the nematodes lost may have settled in the drip tubing. The second experiment was designed to test these hypotheses.

In the second experiment, the numbers of nematodes recovered per emitter during the first collection time decreased greatly at emitters ≥64.0 m from the injection point (Table 2). These results were similar to those obtained in the first experiment. Collecting for an additional 60 min (second collection) resulted in more nematode recovery from some of the more distant emitters (Table 2). However, total nematode recovery in this second collection was very low, only 0.47% of the num-

Table 1. Numbers of *Steinernema riobravis* collected from emitters located at various distances from the injection point in a drip irrigation system, during 45 min following injection, 9 May 1996.

Distance from injection point to emitter (m)	Nematodes collected per emitter[z]
18.3	205 600 ± 23 390 a
36.6	252 300 ± 18 440 a
54.9	302 600 ± 116 300 a
73.2	4 274 ± 1 632 b

[z]Data are means ± standard errors of four replications. Means in column followed by the same letter are not different ($P = 0.01$) according to Duncan's multiple-range test.

Table 2. Numbers of *Steinernema riobravis* collected at various distances from the injection point of 178 million nematodes in a drip irrigation system, 10 July 1996.

	First collection		Second collection		Residue from drip line	
Distance from injection point to emitter (m)	Nematodes collected per emitter (46 min)	Total nematodes from all emitters in section[y]	Nematodes collected per emitter (60 min)	Total nematodes from all emitters in section[y]	Nematodes per 1.83-m of drip tube	Nematodes per 4.58-m section of drip tube[z]
4.6	527 800	6 334 000	239	2 860	6 400	16 000
9.2	612 000	8 549 000	820	7 940	1 300	3 250
13.7	460 100	8 041 000	370	8 920	100	250
18.3	473 700	7 003 000	150	3 900	0	0
22.9	626 400	8 251 000	82	1 740	100	250
27.4	565 100	8 936 000	557	4 780	100	250
32.0	558 100	8 424 000	636	8 940	500	1 250
36.6	596 700	8 661 000	462	8 240	0	0
41.2	606 900	9 027 000	385	6 350	200	500
45.8	665 900	9 546 000	689	8 050	200	500
50.3	691 800	10 183 000	1 650	17 540	100	250
54.9	821 300	11 348 000	785	18 260	8 800	22 000
59.5	621 600	10 822 000	6 070	51 400	61 300	153 200
64.0	120 750	5 568 000	8 780	111 300	10 350 000	25 880 000
68.6	87 700	1 563 000	11 970	155 600	12 200 000	30 500 000
73.2	98 800	1 399 000	3 680	117 400	3 690 000	9 225 000
77.8	6 530	790 000	710	32 900	10 600	265 000
Total	8 141 000	124 400 000	38 020	566 100	26 420 000	66 060 000

[y]Total nematodes recovered from all 15 emitters in 4.58-m section of drip tube. Calculated as 15 × (average of total nematodes from the two emitters on either side of the section). Value for first section calculated as 12 × total from the first emitter (only 12 emitters in first section of tube).
[z]Calculated as 2.5 × value in previous column.

bers recovered during the first collection (46 min duration). Thus, it does not appear that low recovery at distant emitters was due to insufficient time for nematodes to travel to these points, since recovery could not be improved much by increasing the irrigation time.

Many nematodes settled out of suspension in the more distant sections of the drip tube, and were recovered by washing out the sections. The numbers of nematodes recovered from these sections of drip line at distances of ca. 64 m and beyond corresponded to decreases in numbers recovered from the emitters (Table 2). When all nematodes obtained from all samples were considered (including the 1.4 million which remained behind in the 97 ml water left in the spray tank), a total of 192.5 million nematodes were recovered. This total is 8.1% higher than the estimate of 178 million nematodes in the original suspension prior to injection. A sampling error of ≤10% is probably acceptable for most research studies (Southwood, 1978), and the similarity of these two estimates suggests that the nematodes applied were accounted for. The 66 million nematodes which settled in the drip tube and were not delivered by the irrigation system represented 34.6% of the total inoculum injected (191.1 million).

The irrigation system used here was inadequate for distribution of nematodes through the last 20% of the emitters. The hydraulic characteristics of a liquid moving through a tube change as the closed end of the tube is approached, resulting in reduced velocity and momentum of the moving liquid, thus increasing the opportunity for suspended particles to settle due to reduced turbidity. Perhaps undesirable settling of nematodes could be reduced by using a control valve at the end of the tube to maintain flow rate, by maintaining 20% excess drip line beyond the end of the bed, by using smaller-diameter tubing near the end of the bed, or by other means. Hydraulic characteristics would change if improved flow could be maintained near the end of the tube, and so it would be necessary to recheck consistency of nematode delivery from all emitters. Regardless of the mechanisms involved, the dynamics of delivery of particulate matter, such as nematodes, is quite different from the delivery of materials which are soluble in water, and should be examined carefully when attempting to deliver such materials through an irrigation system.

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