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Effect of Soil Depth and Moisture on the Vertical Distribution of *Steinernema riobrave* (Nematoda: Steinernematidae)

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Abstract: The effect of soil moisture on the distribution of *Steinernema riobrave* in a sand column was determined. Larvae of *Pectinophora gossypiella* were used to detect *S. riobrave* infective juveniles (IJ) in each 2.5-cm section of 30-cm-long soil columns. Soil moisture was determined for each section and related to the numbers of nematodes recovered from infected insect baits. Infective juveniles of *S. riobrave* applied on the sand column surface showed some degree of positive geotaxis. IJ in soil columns with a consistent moisture gradient grouped in the upper 12.7 cm within a water potential range of $^{-}$40 to $^{-}$0.0055 MPa (2% to 14% moisture). Nematodes in sand columns that were gradually dehydrating moved down the soil column, aggregating on the 28th day between 15–23 cm in depth. Nematode redistribution over time allowed IJ to remain within a water potential range of $^{-}$0.1 to $^{-}$0.012 MPa (5.2% to 9.5% moisture).

Key words: Entomopathogenic nematodes, movement, soil moisture, spatial distribution, *Steinernema riobrave,* vertical distribution.

Entomopathogenic nematodes in the family Steinernematidae (Rhabditida) are able to infect, kill, and reproduce in many insect orders. The infective juvenile (IJ) is a soil-dwelling, non-feeding stage that carries a symbiotic bacterium of the genus *Xenorhabdus* (Bird and Akhurst, 1983). On contacting an insect, the IJ penetrate the host through natural body openings (Georgis and Hague, 1981) and move into the insect hemocoel. The IJ releases bacteria, which multiply and cause septicemia. Steinernematid IJ develop into amphimictic adults that mate and produce progeny. When the nutrients inside the insect cadaver become depleted, dauers or IJ are formed. The IJ exit the cadaver into the soil to infect a new host.

Womersley (1990) discussed IJ associations with the environmental spaces of the soil, the water dynamics of which vary depending upon moisture availability. At field capacity, nematode movement can be restricted due to the lack of surface tension forces necessary for movement. Molyneux and Bedding (1984) found different moisture ranges for optimum host infection depended upon the experimental media used. Water potential values are therefore reported in the present study for comparison with other studies.

Kung (1990) reported that *Steinernema carpocapsae* (Weiser) and *Steinernema glaseri* (Steiner) persistence was greater at surprisingly low moisture levels-2% and 4%, respectively-compared with higher levels of 8% and 16%. However, Townsend et al. (1998) monitored mortality of green June beetles, *Cotinis nitida* (L.) larvae by *S. carpocapsae,* and report the optimum soil moisture by weight as 30%. Clearly, the optimum moisture level for persistence of original IJ nematodes within a system is much lower than the optimum moisture level for insect infection.

Active dispersal of entomopathogenic nematodes is an important characteristic that affects their efficiency as biological control agents (Kaya and Gaugler, 1993). Kennedy (1961) presented the idea that insect migration results from the evolution of specialized behavior, which involves direct linear movement allowed by the suppression of activities such as feeding and reproduction. The same concept can be applied to many organisms both more and less complex than the phylum Nemata.

Nematode movement is restricted in soils

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that have inadequate or excessive moisture levels (Wallace, 1958). Dispersal is also influenced by other factors, including temperature (Molyneux, 1985), host presence (Georgis and Poinar, 1983), phoretic dispersal (Epsky et al., 1988), IJ storage time and conditions (Westerman, 1992), sex (Grewal et al., 1993), and behavioral differences between species and strains (Lewis et al., 1992). Steinernematid nematodes are reported to be capable of moving several centimeters within an 8-hour period (Westerman and Godthelp, 1990). The current experiment elucidates the vertical dispersal pattern of the entomopathogenic nematode *Steinernema riobrave* Cabanillas, Poinar and Raulston with reference to sand depth and moisture.

Materials and Methods

Steinernema riobrave was obtained from J. R. Raulston (USDA, ARS, Weslaco, TX) and was originally isolated from soil samples collected from the Lower Rio Grande Valley, Texas. Infective juvenile *S. riobrave* were obtained by in-vivo culturing (Kaya and Stock, 1997) in late-instar *Galleria mellonella* L. (Lepidoptera: Pyralidae). At 27 °C, infective juveniles were extracted with White traps (White, 1927) and stored in distilled water at 15 °C. Nematodes were stored for no longer than 4 days prior to use.

Polyvinyl chloride pipe of 2.5-cm diam. was cut into 2.5-cm-long sections. Twelve individual sections were joined together with waterproof tape to form a 30-cm-long tube. The bottom of each tube was sealed with Plexiglas. Soil columns were constructed by filling the tubes with sterilized, sand-based medium (92% sand, 6% silt, 2% clay, 0% organic matter, pH 6.8). The soil medium contained 20% water (weight: dry weight of soil) throughout the columns.

Steinernema riobrave were acclimated at 27 °C for 4 hours, and then 500 IJ in 1 ml of distilled water were pipeted onto the top of each column. The tops of the columns were sealed with Parafilm (American National Can™) to minimize water evaporation. The columns were maintained at 27 °C in an upright position. Columns were weighed daily, and water was added to the tops to maintain a constant weight. Columns were sampled on days 0, 5, 7, and 9, and the number of nematodes was estimated in each section of each column using a baiting technique (see below). Column replication was 12-fold for each time period.

It was noted that the use of Parafilm to seal the columns was insufficient, as the seals would work loose over a 24-hour period. Once the film had pulled away from the slides, the columns could dehydrate from the top to some extent. As the experiment was conducted at 27 °C and the incubator used employed a fan to circulate air, dehydration was significant. Therefore, it was decided to continue the experiment rather than alter the cycle of dehydration and rehydration at this point.

The number of nematodes in column sections was determined with a live-bait method modified from Fan and Hominick (1991). The columns were partitioned into 12 sections; each section was weighed (to determine relative water-to-sand content by comparison of wet and dry weights) and placed in a small plastic container (50-ml capacity). Distilled water was added to return sections to 20% water content, where necessary. Sections with higher than 20% water content were allowed to lose excess water by evaporation at room temperature. Four late-instar *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) were then added to the containers. The sections of PVC tubing forming the cylinder were not removed, as this may have removed nematodes from the system. The containers were sealed with Parafilm and then incubated for 96 hours at 27 °C. After incubation, insects were rinsed 3 times in distilled water and dissected under a stereomicroscope in quarter-strength Ringer's solution. The numbers of nematodes present within each larva were counted. The total number of IJ infecting *G. mellonella* from each column section consisted of the sum of nematodes found in all four insects.

On day 9 the Parafilm tops on half of the cylinders were removed. These cylinders

were allowed to dry out naturally for the remaining experimental period, while the remaining cylinders with Parafilm lids were weighed daily and maintained at overall 20% moisture. Columns from the rehydrated and the dehydrating group were removed on days 12, 14, 16, 19, 21, 23, and 28. The numbers of nematodes in column sections were determined on each sampling day using the live-bait method described above. On day 28, the column sections from the dehydrating group were weighed prior to baiting with *P. gossypiella* so the final moisture content of each section could be determined. Column replication was 6-fold for each time period within each of the two groups.

Extra columns were used to establish a water-release curve for the sand media using the method described by Hamblin (1981) (Fig. 1). Moisture percentages could then be converted to water potentials for comparison with other studies using different experimental media.

Factorial analysis of variance was used to test for significant differences among main treatment means. Comparative nematode survival over time was analyzed among different water treatments. The numbers of nematodes in sections along columns were then related to soil moisture. The Student-Newman-Keuls test was used to partition means into significant ranges when a significant F value was determined by analysis of variance. The 1% level of probability was used in all statistical tests (CoStat, Cohort Software, Minneapolis, MN).

Parts of the above experiment were repeated in time, but the data presented here are taken from a single run of the entire experiment where all parts of the assay were conducted at the same time. Trends observed where parts of the experiment were run independently resulted in similar data trends and the same conclusions.

Results

Infective juveniles of *S. riobrave* applied at the soil column surface appeared to show some degree of positive geotaxis. In soil columns with a consistent moisture gradient, IJs were detected most frequently in the upper 12.7 cm within a water potential range of $^{-}40$–$^{-}0.0055$ MPa (2–14% water, weight: dry sand weight) (Fig. 2). Nematodes in sand columns that were gradually dehydrating moved down the soil column, aggregating on day 28 between 15–23 cm in depth (Fig. 2). Nematode redistribution over time allowed IJ to remain within a water potential range of $^{-}0.1$–$^{-}0.012$ MPa (5.2–9.5% water) (Table 1).

The mean numbers of IJ from entire columns indicated overall nematode recovery over time per column (Fig. 3). The largest reduction in nematode numbers occurred

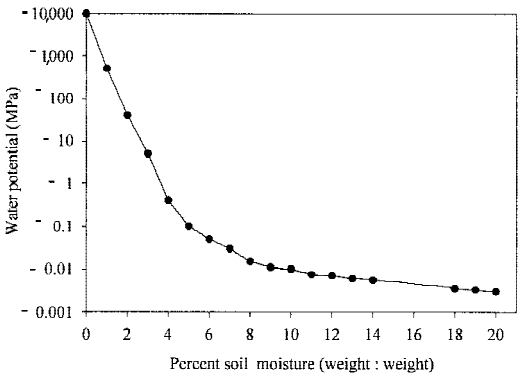

Fig. 1. Moisture release curve for the sand-based media used in the present study.

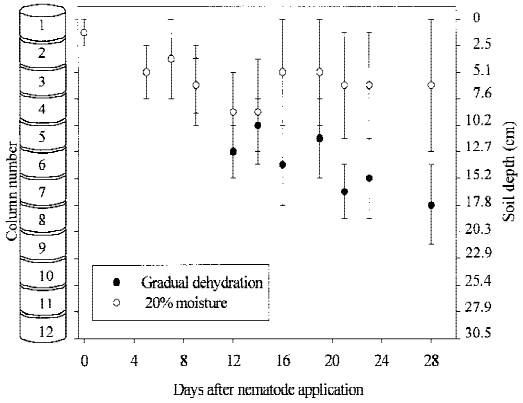

Fig. 2. Bars indicate the column range (and soil depth) within which 75% of the originally applied *Steinernema riobrave* IJ could be recovered. Symbols show the center of the range. After day 9, half the columns were allowed to dehydrate while the remaining columns were rehydrated daily.

TABLE 1. Water potential (MPa) and percent water content in relation to column section and column depth, for rehydrated and dehydrated columns.

Column section	Depth (cm)	Rehydrated columns–means for days 5, 7, and 9		Dehydrated columns ≠ day 28	
		Percent water	Water potential (MPa)	Percent water	Water potential (MPa)
1	0–2.5	2	–40	0	–10,000
2	2.5–5.1	2	–40	0	–10,000
3	5.1–7.6	6.6	0.035	0.2	–5,400
4	7.6–10.2	7	–0.03	1.4	–100
5	10.2–12.7	14	–0.0055	3.9	–0.4
6	12.7–15.2	19	–0.0033	5.2	–0.1
7	15.2–17.8	19	–0.0033	6.2	–0.05
8	17.8–20.3	19	–0.0033	7.1	–0.03
9	20.3–22.9	19	–0.0033	9.5	–0.01
10	22.9–25.4	20	–0.003	21.1	–0.003
11	25.4–27.9	21	–0.003	21.2	–0.003
12	27.9–30.5	21	–0.003	21.2	–0.003

within the first 8 days. Analysis of data from days 12–28 indicated differences in nematode survival over time ($P < 0.001$), in different cylinder sections ($P < 0.001$), and among different water treatments ($P = 0.006$) (Fig. 2). Greater numbers of IJ were detected in rehydrated sand. A significant interaction between column section and time occurred ($P = 0.001$).

Rehydrated columns resulted in significant differences in the numbers of nematodes recovered over time ($P < 0.001$), and within column sections ($P < 0.001$). The highest detection occurred within sections 1–4 (Fig. 2). Dehydrated columns showed significant differences in the numbers of nematodes over time ($P < 0.001$), and within column sections ($P < 0.001$). The highest detection occurred within sections 4 to 6. The number of nematodes recovered within soil columns at different moisture contents was highest at low moisture levels and low water potentials (Fig. 4).

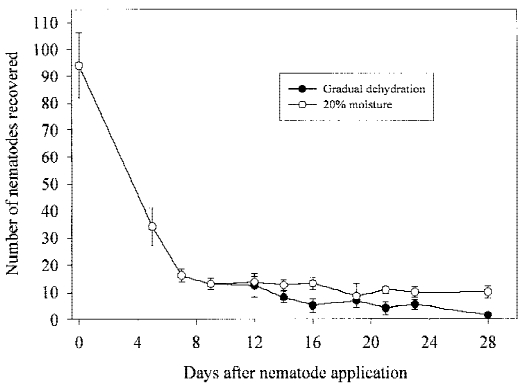

FIG. 3. Mean numbers (±SE) of *Steinernema riobrave* recovered in sand columns (total of all sections) over 28 days. Sand columns were either rehydrated daily to an overall 20% moisture level or allowed to dehydrate after 12 days. Nematodes were applied at 500 infective juveniles/column.

DISCUSSION

Nematode persistence within sections of the columns was used to relate a numerical nematode count with percent moisture levels. Optimum persistence occurred between

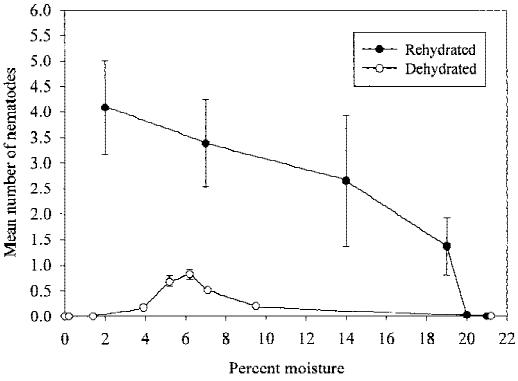

FIG. 4. Mean numbers (±S.E.) of *Steinernema riobrave* recovered in sand columns, at different percent moistures. Nematode distribution is plotted with reference to the percent moisture of the sand media. Sand columns were either rehydrated daily to an overall 20% moisture level (date averaged from days 5, 7, and 9) or allowed to dehydrate (date averaged from columns sampled on day 28). Nematodes were applied at 500 infective juveniles/column.

2 to 7% water (−40–−0.03 MPa) in columns that were rehydrated daily and between 1.4 and 5.2% water (−100–−0.1 MPa) in columns that were dehydrating. Rehydrated columns may have had improved persistence at higher moisture level due to the continuous drainage of water added to the top of the columns aiding the downward movement of IJ from upper sections of the column. Our results are similar to those reported by Duncan et al. (1996), in which *S. riobrave* survived best at 2 to 4% moisture compared with lower (0.5–1.0%) and higher (4.0–12.0%) moisture levels (water potentials were not included with their results). Duncan and McCoy (1996) also reported *S. riobrave* surviving and infecting insect hosts at depths of 0–15 cm, where soil moisture fluctuated between 1–6% throughout the year.

Migration of the nematodes followed a positively geotropic tendency toward more suitable soil moisture levels. Duncan et al. (1996) reported that *S. riobrave* exhibited a positive geotropism in experimental tubes and also mentioned that, irrespective of the depth at which they inoculated their microcosms, most IJ *S. riobrave* were recovered at soil moistures between 2–4%. Villani and Wright (1990) observed that many soil-inhabiting animals migrate vertically to avoid the damaging effects of temperature extremes or lack of humidity. The vertical redistribution of IJ in sand columns allowed *S. riobrave* nematodes to remain within a suitable moisture range of 5.2–9.5% water (−0.1–−0.012 MPa). However, observations indicating movement could also include effects from increased survival of nematodes within the same zone.

The results discussed pertain to the ability of nematodes to maintain infectivity at different soil moisture levels. They do not address total nematode persistence or infection of a host under different moisture regimes.

Literature Cited

Bird, A. F., and R. J. Akhurst. 1983. The nature of the intestinal vesicle in nematodes of the family Steinernematidae. International Journal for Parasitology 13:599–606.

Duncan, L. W., D. C. Dunn, and C. W. McCoy. 1996. Spatial patterns of entomopathogenic nematodes in microcosms: Implications for laboratory experiments. Journal of Nematology 28:252–258.

Duncan, L. W., and C. W. McCoy. 1996. Vertical distribution in soil, persistence, and efficacy against citrus root weevil (Coleoptera: Curculionidae) of two species of entomopathogenic nematodes (Rhabditida: Steinernematidae; Heterorhabditidae). Environmental Entomology 25:174–178.

Epsky, N. D., D. E. Walter, and J. L. Capinera. 1988. Potential role of nematophagous microarthropods as biotic mortality factors of entomogenous nematodes (Rhabditida: Steinernematidae, Heterorhabditidae). Journal of Economic Entomology 81:821–825.

Fan, X., and W. R. Hominick. 1991. Efficiency of the *Galleria* (wax moth) baiting technique for recovering infective stages of entomopathogenic rhabditids (Steinernematidae and Heterorhabditidae) from sand and soil. Revue de Nématologie 14:381–387.

Georgis, R., and N. G. M. Hague. 1981. A neoaplectanid nematode in the larch sawfly *Cephalcia lariciphila* (Hymenoptera: Pamphiliidae). Annals of Applied Biology 99:171–177.

Georgis, R., and G. O. Poinar, Jr. 1983. Effect of soil texture on the distribution and infectivity of *Neoaplectana carpocapsae* (Nematode: Steinernematidae). Journal of Nematology 15:308–311.

Grewal, P. S., S. Selvan, E. E. Lewis, and R. Gaugler. 1993. Male insect-parasitic nematodes: A colonizing sex. Experientia 49:605–608.

Hamblin, A. P. 1981. Filter-paper method for routine measurement of field water potential. Journal of Hydrology 53:355–360.

Kaya, H. K., and R. Gaugler. 1993. Entomopathogenic nematodes. Annual of Entomology 38:181–206.

Kaya, H. K., and S. P. Stock. 1997. Techniques in insect nematology. Pp. 281–324 *in* L. Lacey, ed. Manual of techniques in insect pathology. San Diego, CA: Academic Press.

Kennedy, J. S. 1961. A turning point in the study of insect migration. Nature 186:348–350.

Kung, S-P. 1990. Abiotic factors affecting the persistence of two entomopathogenic nematodes, *Steinernema carpocapsae* and *Steinernema glaseri* (Nematoda: Steinernematidae), in the soil. PhD dissertation, Rutgers University, NJ.

Lewis, E. E., R. Gaugler, and R. Harrison. 1992. Entomopathogenic nematode host finding: Response to host contact cues by cruise and ambush foragers. Parasitology 105:309–315.

Molyneux, A. S. 1985. Survival of infective juveniles of *Heterorhabditis* spp. and *Steinernema* spp. (Nematoda: Rhabditida) at various temperatures and their subsequent infectivity for insects. Revue de Nématologie 8: 165–170.

Molyneux, A. S., and R. A. Bedding. 1984. Influence of soil texture and moisture on the infectivity of *Heterorhabditis* sp. D1 and *Steinernema glaseri* for larvae of the sheep blowfly, *Lucilia cuprina*. Nematologica 30:358–365.

Townsend, M. L., D. T. Johnson, and D. C. Strinkraus. 1998. Laboratory studies of the interactions of environmental conditions on the susceptibility of

green June beetles (Coleoptera: Scarabaeidae) grubs to entomopathogenic nematodes. Journal of Entomological Science 33:40–48.

Villani, M. G., and R. J. Wright. 1990. Environmental influences on soil macroarthropod behavior in agricultural systems. Annual Review of Entomology 35:249–269.

Wallace, H. R. 1958. Movement of eelworms. I. The influence of pore size and moisture content on the migration of larvae of the beet eelworm, *Heterodera schachtii* Schmidt. Annals of Applied Biology 46:74–85.

Westerman, P. R. 1992. The influence of time of storage on performance of the insect parasitic nematode, *Heterorhabditis* sp. Fundamental and Applied Nematology 15:407–412.

Westerman, P. R., and J. M. Godthelp. 1990. The host-searching ability of the insect parasitic nematode *Heterorhabditis* sp. in sand columns. Mededelingen Faculteit Landbouwkundige, University of Gent 55:691–698.

White, G. F. 1927. A method for obtaining infective nematode larvae from cultures. Science 66:302–303.

Womersley, C. Z. 1990. Dehydration survival and anhydrobiotic potential. Pp. 117–137 *in* R. Gaugler and H. K. Kaya, eds. Entomopathogenic nematodes in biological control. Boca Raton, FL: CRC Press.