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Influence of Irrigation and *Heterorhabditis bacteriophora* on Plant-parasitic Nematodes in Turf

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Abstract: Daily irrigated, 80% pan replacement, and nonirrigated field plots of Kentucky bluegrass (*Poa pratensis* L.) were inoculated with a mixture of *Steinernema carpocapsae* (All strain) and *Heterorhabditis bacteriophora* (HP-88 strain) in 1988. In 1989, daily irrigated and nonirrigated plots were inoculated with HP-88 alone. The turf and associated soil contained populations of *Tylenchorhynchus dubius, T. nudus, Pratylenchus penetrans, Paratylenchus projectus,* and *Criconemella rustica.* In irrigated plots in 1988, population densities of *Tylenchorhynchus* spp. were lower in plots inoculated with HP-88 plus All compared with that in control plots. The same effect was absent under nonirrigated conditions. In 1989, population densities of *Pratylenchus penetrans* associated with inoculated turf were lower than those recovered from noninoculated turf in irrigated but not under nonirrigated conditions. Population densities of plant-parasitic nematodes were generally higher in the irrigated compared with the nonirrigated environment.

Key words: competition, *Criconemella rustica,* entomopathogenic nematode, *Heterorhabditis bacteriophora,* irrigation, nematode, *Paratylenchus projectus, Poa pratensis, Pratylenchus* spp., *Steinernema carpocapsae, Tylenchorhynchus* spp.

Inundative release of entomopathogenic nematodes is an alternative to chemicals for control of white grubs in turf (7,16). Field trials have been mostly with *Steinernema carpocapsae* or *Heterorhabditis bacteriophora.* Although *S. carpocapsae* is easier to mass produce, it is not as effective as *H. bacteriophora* for control of grubs (6,7,16). In the process of evaluating applications of *S. carpocapsae* and *H. bacteriophora* for control of Japanese beetle larvae, *Popillia japonica,* we observed that inoculation of turf with entomopathogenic nematodes affected population densities of plant-parasitic nematodes.

Although most research with entomopathogenic nematodes has focused on their ability to infect insects, a few studies have noted interactions among entomopathogenic nematodes and plant-parasitic nematodes (2,10). Ishibashi and Kondo (10) found that 10,000 *S. carpocapsae* per 100 cm^3 of sandy soil reduced population densities of tylenchid nematodes by 75–90% for 5 weeks after application. Using entomopathogenic nematodes as a preventive treatment, Bird and Bird (2) suppressed populations of *Meloidogyne javanica* associated with tomato plants and reduced their reproductive capacity by adding 5×10^6 *Steinernema glaseri* to each plant. A similar effect was observed by Ishibashi et al. (11) when application of 10^5–10^6 *S. carpocapsae* per 200 cm^3 soil suppressed galling of tomato roots by *Meloidogyne incognita.* In further work, Ishibashi and Choi (9) found that *S. carpocapsae* was positively attracted to and aggregated around the root tips of tomato.

The objective of this research was to determine the impact of *H. bacteriophora* and *S. carpocapsae* on the population dynamics of plant-parasitic nematodes associated with *Poa pratensis* (Kentucky bluegrass) in irrigated and nonirrigated turf environments.

Materials and Methods

Research plots were established in an irrigation study area at the Hancock Turfgrass Research Center at Michigan State University, East Lansing, Michigan. The site consisted of nine blocks of *P. pratensis* established on a sandy loam soil (68.9% sand, 18.7% silt, 12.4% clay). Each block was 120 m^2 and managed under one of three irrigation regimes: (i) 2.5 mm of water applied daily at noon; (ii) replacement of 80% of the water lost from open pan evaporation (1), applied as irrigation on

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Monday and Friday of each week; or (iii) no supplemental irrigation. All blocks received three applications of 1.36 kg N per year and were mowed twice each week to a height of 6.4 cm.

On 20 May 1988, two 0.1-m^2 plots were established in each of the nine irrigation blocks. The plots were contained with metal lawn edging sunk to a depth of 15 cm to prevent movement of entomopathogenic nematodes. One of the plots in each block was inoculated with a mixture of 2.47×10^9/ha living *H. bacteriophora* (HP-88 strain) plus 2.08×10^9/ha living *S. carpocapsae* (All strain), and the other was maintained as a noninoculated control. Both nematodes were obtained from Biosys, Inc. (Palo Alto, CA). They were shipped by overnight courier as a semisolid nematode concentrate on sponges in an insulated package. Nematodes were stored in an incubator at 4C for 1 week prior to the test. Turf plots were inoculated by spraying the nematode suspension with a hand-held CO_2 sprayer. Soil was collected from each of the 18 plots after 5 weeks (26 June) with a 10.0-cm-deep by 2.0-cm-d soil probe. Six cores were taken at random from each plot. The soil was mixed and the nematodes were extracted from 100-cm^3 subsamples with a modified centrifugation–sugar flotation procedure (12). Plant-parasitic and entomophathogenic nematodes were identified and counted.

In 1989, the irrigation plot design was changed to six blocks of each regime, with each block being 60 m^2, half the size of the 1988 blocks. The soil type and management practices were the same as in the 1988 test.

In the 1989 test, the irrigation treatments consisted of only daily irrigation with 2.5 mm of water applied at noon or no irrigation. Each plot was either inoculated with *H. bacteriophora* (HP-88) at the rate of 2.47×10^9 live nematodes/ha on 25 May or was not inoculated. The two nematode treatments were applied to daily irrigated and nonirrigated plots in a 2 × 2 factorial design with six replications of each treatment. Each plot was sampled for nematodes immediately before inoculation and 1, 3, 5, and 8 weeks afterward. Soil samples were collected and processed, and nematodes were identified and counted as previously described.

Results and Discussion

Four weeks after inoculation with *H. bacteriophora* and *S. carpocapsae* in 1988, detectable population densities of entomopathogenic nematodes ranged from 0.3–0.8/100-cm^3 soil, compared with 50–200 plant-parasitic nematodes/100-cm^3 soil. *Tylenchorhynchus* spp. (a mixture of *T. dubius* and *T. nudus*) were the most abundant nematodes present in all irrigation treatments (Table 1). Population densities of *Tylenchorhynchus* spp. recovered from daily irrigated or 80% replacement plots that were inoculated with entomopathogenic nematodes were variable but lower than those recovered from the noninoculated environment (Table 1). Similar differences due to inoculation with entomopathogenic nematodes were not observed in the nonirrigated environment. Populations of *Pratylenchus penetrans* were low in all treatments (0.3–1.7 per 100-cm^3 soil). *Paratylenchus projectus* was also found in irrigated control plots and nonirrigated control plots. The mean densities ±SE per 100 cm^3 of soil for *P. projectus* in irrigated and nonirrigated plots were 6.3 ± 5.9 and 10.0 ± 10.2, respectively. Inoculation with entomopathogenic nematodes had no effect on *P. projectus* populations.

In July and August of 1989, thatch and soil moisture levels, respectively, averaged 48 and 22% in irrigated plots, and 33 and 16% in nonirrigated plots (13). Populations of *P. penetrans* recovered from soil were consistently higher in irrigated control plots compared with nonirrigated control plots (Table 2). Inoculation with *Heterorhabditis bacteriophora* (HP-88) reduced populations of *P. penetrans* in irrigated plots 1 week after inoculation (Table 2). Inoculation with HP-88 did not affect populations of *P. penetrans* in nonirrigated

TABLE 1. Influence of inoculation with entomophathogenic nematodes (HP-88 + All) and irrigation on populations of plant-parasitic nematodes associated with *Poa pratensis* in 1988.

Irrigation treatment	Nematode treatment	Plant-parasitic nematodes per 100-cm^3 soil ± SE†	
		Tylenchorhynchus spp.	*Pratylenchus penetrans*
Daily	HP-88 + All	47.6 ± 39.2	0.7 ± 0.7
	None	116 ± 67.4	0.7 ± 0.7
80% replacement	HP-88 + All	42.7 ± 9.2	0.3 ± 0.3
	None	85.3 ± 29.0	1.7 ± 1.2
Nonirrigated	HP-88 + All	60.7 ± 21.4	2.0 ± 1.0
	None	65.0 ± 15.6	0.3 ± 0.3
Analysis of variance:			
Source	df	Mean squares‡	
Irrigation	2	705	0.78
Nematode	1	6,728	0.06
Irrigation × nematode	2	1,586	6.78

Data are means of three replications at 5 weeks after inoculation.
† Five weeks after inoculation with *Heterorhabditis bacteriophora* (HP-88) (2.5×10^9/ha) plus *Steinernema carpocapsae* All (2.1×10^9/ha).
‡ Nematode effect was significant at $0.05 < P \leq 0.10$.

plots. Population densities of *Tylenchorhynchus* spp. were much greater in daily irrigated plots compared with nonirrigated plots throughout the test (Table 3). Inoculation with HP-88 had no effect on populations of *Tylenchorhynchus* spp. in 1989. *Criconemella rustica* was also much more abundant in irrigated turf compared with nonirrigated turf. However, populations were so variable among replications that the irrigation factor was not significant ($0.10 > P > 0.05$, in a 2 × 2 factorial ANOVA, Table 4). Inoculation with *H. bacteriophora* had no effect on populations of *C. rustica*. *Paratylenchus projectus* was recovered in lower numbers, similar to the populations reported for the 1988 study.

Overall, adequate soil moisture provided by daily irrigation of turf supported greater population densities of plant-parasitic nematodes than in drier soil in nonirrigated turf. While studying populations of *Tylenchorhynchus dubius* associated with the grass, *Lolium perenne*, Den Toom (4) found *T. dubius* populations to decrease under moisture stress to the host plant sys-

TABLE 2. Influence of irrigation and entomopathogenic nematodes on densities of *Pratylenchus penetrans* associated with *Poa pratensis*, 1989.

Irrigation treatment	Nematode treatment	Nematodes per 100-cm^3 soil ± SE†				
		0	1	3	5	8
Daily Irrigated	HP-88	5.3 ± 3.2	1.8 ± 0.8	2.5 ± 1.1	3.3 ± 1.8	4.5 ± 2.1
	None	9.2 ± 4.4	8.7 ± 2.1	8.7 ± 3.2	12.0 ± 5.6	7.7 ± 3.0
Nonirrigated	HP-88	3.2 ± 1.9	1.5 ± 1.0	1.0 ± 0.7	1.5 ± 0.7	2.0 ± 0.9
	None	0.0 ± 0.0	2.3 ± 1.2	1.5 ± 0.7	0.0 ± 0.0	2.8 ± 1.3
Analysis of variance:						
Source	df	Mean squares‡				
Irrigation	1	193	66.7*	113*	287*	80.7
Nematode	1	0.7	88.2**	66.7	77.0	24.0
Irrigation × nematode	1	73.5	54.0*	48.2	155	8.2

† Data are means of six replications at 0, 1, 3, 5, or 8 weeks after inoculation with *Heterorhabditis bacteriophora* (HP-88).
‡ Asterisks (*, **) indicate a significant effect at $P < 0.05$ (*) or $P < 0.01$ (**).

TABLE 3. Influence of irrigation and entomopathogenic nematodes on densities of *Tylenchorhynchus dubius*/*T. nudus* associated with *Poa pratensis*, 1989.

Irrigation treatment	Nematode treatment	Nematodes per 100-cm³ soil ± SE†				
		0	1	3	5	8
Daily	HP-88	167 ± 26.8	261 ± 60.0	168 ± 18.4	151 ± 39.6	65.0 ± 16.6
Irrigated	None	124 ± 33.5	243 ± 52.6	136 ± 23.6	110 ± 14.2	52.7 ± 12.7
Nonirrigated	HP-88	109 ± 28.7	114 ± 33.0	75.8 ± 30.9	33.7 ± 8.4	69.2 ± 11.0
	None	64.8 ± 18.0	68.8 ± 28.1	46.3 ± 8.8	31.5 ± 4.9	79.8 ± 17.8
Analysis of variance:						
Source	df	Mean squares‡				
Irrigation	1	20,475*	155,204**	49,413**	58,017**	1,473
Nematode	1	11,310	5,953	5,673	2,773	4.2
Irrigation × nematode	1	3.4	1,040	9.4	2,243	794

† Data are the means of six replications at 0, 1, 3, 5, or 8 weeks after inoculation with *Heterorhabditis bacteriophora* (HP-88).
‡ Asterisks (*, **) indicate a significant effect at $P < 0.05$ (*) or $P < 0.01$ (**).

tem. This effect was attributed to a decrease in root mass, and therefore in food supply. Goodell and Ferris (8) also found that moderate moisture stress decreased hatch, whereas severe moisture stress completely inhibited hatch of *Meloidogyne incognita* eggs.

In both 1988 and 1989, the impact of entomopathogenic nematodes on plant-parasitic nematodes was observed in irrigated, but not in nonirrigated environments. This may be due to the moisture dependency of the infective stages of *H. bacteriophora* and *S. carpocapsae* (14). Although interactions between entomopathogenic and plant-parasitic nematodes were observed in both years of this study, the plant-parasitic species involved was different each year. Ishibashi and Kondo (10) reported a similar inconsistency. They observed a reduction of all tylenchid nematodes after application of *S. carpocapsae* the first year of their study, but not in the following year. The suppression of *P. penetrans* populations following inoculation with *H. bacteriophora* supports the hypothesis that inoculation with large population densities of entomopathogenic nematodes may temporarily suppress populations of some plant-parasitic nematodes. However, we found this effect to be dependent on adequate soil moisture. This is the first report of this phe-

TABLE 4. Influence of irrigation and entomopathogenic nematodes on densities of *Criconemella rustica* associated with *Poa pratensis*, 1989.

Irrigation treatment	Nematode treatment	Nematodes per 100-cm³ soil ± SE†				
		0	1	3	5	8
Daily	HP-88	12.0 ± 7.1	16.5 ± 6.4	17.7 ± 7.8	13.5 ± 5.6	19.3 ± 16.6
Irrigated	None	71.0 ± 51.8	69.0 ± 40.1	58.0 ± 35.9	46.8 ± 32.8	28.0 ± 13.9
Nonirrigated	HP-88	6.8 ± 5.5	3.2 ± 3.2	4.3 ± 3.4	4.3 ± 2.1	4.8 ± 2.0
	None	5.3 ± 2.9	3.3 ± 3.3	0.0 ± 0.0	0.82 ± 0.33	1.8 ± 0.8
Analysis of variance:						
Source	df	Mean squares‡				
Irrigation	1	7,526	9,361	7,633	4,537	2,480
Nematode	1	4,959	4,160	1,944	1,350	48.2
Irrigation × nematode	1	5,490	4,108	2,992	2,166	204.2

† Data are means of six replications at 0, 1, 3, 5, or 8 weeks after inoculation with *Heterorhabditis bacteriophora* (HP-88).
‡ Irrigation effect was significant at $0.05 < P \leq 0.10$.

nomenon under field conditions. Previous studies were conducted in pots, polvinylchloride tubes, or sealed plastic bags. In the current studies, *H. bacteriophora* and *S. carpocapsae* were used at population densities 20-fold less than in the previous investigations (2,3,10,11). The inoculation rate of 2.5×10^9 nematodes per ha was selected because it is the labeled rate for use of entomopathogenic nematodes for control of insects associated with turfgrass. More research is needed to confirm our observation that populations of *P. penetrans* may be temporarily suppressed following inoculation with entomopathogenic nematodes.

The suppressive effect of entomopathogenic nematodes on plant-parasitic nematodes may be due to a competition for space or habitat in the rhizosphere (2,9,10, 11). Ishibashi and Kondo (10) did not detect any suppressive effect of a filtrate from an *S. carpocapsae* suspension (5×10^6/ 50 ml) on plant-parsitic nematodes. Another possible mechanism deserving investigation is the stimulation or suppression of nematode predators and pathogens (5,15).

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