

Relationship between Cultural Factors and Nematodes on Merion Kentucky Bluegrass¹

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Abstract: A 2-year study was conducted on Merion Kentucky bluegrass turf (*Poa pratensis*) to identify potential relationships among seasonal population dynamics of nematodes, chemical applications, thatch, tillering, dollar spot caused by *Sclerotinia homoeocarpa*, clipping weight, and other factors. Numbers of *Tylenchorhynchus maximus* determined during June were inversely related to the wet weight of grass from May. One or more monthly counts of *Paratylenchus hamatus*, *Criconebella rusium*, and *T. maximus* negatively correlated with the numbers of spring tillers. Applications of benomyl, used for dollar spot control, decreased numbers of *T. maximus* and free-living nematodes, and this chemical was associated with acidification of the thatch. *Hoplolaimus galeatus* levels were associated with an estimated 8% increase in the severity of dollar spot.

Key words: *Criconebella rusium*, dollar spot, *Hoplolaimus galeatus*, Kentucky bluegrass, nematode, *Paratylenchus hamatus* *Poa pratensis*, *Sclerotinia homoeocarpa*, turfgrass, *Tylenchorhynchus maximus*.

Many species of nematodes are found in Merion Kentucky bluegrass turf, *Poa pratensis* L., but neither their pathogenic nor economic significance has been adequately analyzed. Kentucky bluegrass turf, probably the most economically important grass planted in New Jersey, is established on golf course fairways, lawns, parks, sports fields, and elsewhere. Laughlin (11) reported that the vegetative growth of bentgrass and Kentucky bluegrass were suppressed by *Tylenchorhynchus dubius*, and that damage increased with elevated temperature. The clipping weight of Kentucky bluegrass parasitized by *T. nudus* was 28% lower than that of healthy grass (18). The potential damage to Kentucky bluegrass by New Jersey's indigenous nematode population is unknown.

Plant-parasitic, predatory, microbivorous, and mycophagous nematodes exist together in a dynamic interaction in this bluegrass habitat. Babu (1) studied population density in relation to season, soil

moisture, and vertical distribution of nematodes in Kentucky bluegrass plots in New Jersey. Additional associations may exist among cultural procedures, edaphic factors, thatch, turfgrass diseases such as dollar spot, and nematodes.

The current study relates the seasonal population dynamics of soilborne nematodes in Merion Kentucky bluegrass turf during a 2-year period with chemical applications, thatch removal, tillering and top growth, dollar spot, and edaphic factors.

MATERIALS AND METHODS

Merion Kentucky bluegrass was seeded in sandy loam soil (Table 1). The grass was maintained at a height of 3.2 cm, clippings were removed from the site, and irrigation was applied as needed. Three years later, the turf was inoculated by broadcasting oat-grain cultures of *Sclerotinia homoeocarpa* Bennett, the causal agent of dollar spot. Because tests showed the site had a low nitrogen level, urea was added each year to increase nitrogen levels by 98.6 kg/ha-N. The turf received no lime, herbicides, or insecticides during the subsequent 2-year experiment.

Thirty-six plots, each measuring 2.29 × 4.05 m (9.3 m²), were established in a 3 × 12 block. The block was made up of four 3 × 3 latin squares, with each latin square consisting of three replications of two fungicide treatments and an untreated con-

Received for publication 8 January 1991.

¹ New Jersey Agricultural Experiment Station Publication D-11285-1-91. Supported by New Jersey State and U.S. Hatch Act Funds.

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The authors thank Dr. Richard J. Trout, Department of Statistics and Computer Science, New Jersey Agricultural Experiment Station, for his assistance with statistical designs and computations.

TABLE 1. Soil analyses of Merion Kentucky bluegrass plots during the first year (May).

Parameter	Mean ($\bar{x} \pm sd$)	Range
Sand (%)	56 \pm 2	54–60
Silt (%)	27 \pm 2	22–30
Clay (%)	16 \pm 1	14–18
NO ₃ -N (kg/ha)	49 \pm 5	36–53
NH ₃ -N (kg/ha)	6 \pm 2	3–11
P (kg/ha)	146 \pm 15	122–180
K (kg/ha)	135 \pm 12	115–161
Ca (kg/ha)	1,776 \pm 128	1,558–1,985
Mg (kg/ha)	625 \pm 38	549–681

tol. Every column contained 12 plots. The fungicides benomyl and iprodione were applied twice weekly at rates of 1.8 g a.i./m² and 0.64 g a.i./m², respectively. The fungicides were suspended in water and sprayed (1.2 L/m²) on individual plots. Chemical applications started the first year on June 11 and on May 22 the second year.

The area of each plot covered by dollar spot was estimated visually by three individual investigators during each August. The mean percentage of dollar-spot coverage was then calculated for each plot.

Six randomly selected rows in the block were mechanically dethatched (vertically mowed, Ryan Ren-O-Thin mower) during October of the first year and again the following May. Mowing of top growth was done on a weekly basis at a height of 3.2 cm, and the clippings were removed. During the second year, the fresh weight of bluegrass clippings from just 1 week's growth was determined during June, September, and October. Four, 10.2-cm-d turf plugs were taken randomly from each plot during May of the second year following vertical mowing, and the total numbers of individual tillers were counted. The thickness of uncompressed thatch was measured from turf plugs in June and September the first year, and in the final year of the experiment during May and November.

Soil pH was determined each year (14). During the second year of the experiment, the pH of thatch was taken after tissue-grinding the air-dried thatch to ensure

uniformity. A suspension of thatch in water, 4:1 v/v, from every plot was incubated for 30–45 minutes before pH was measured with a standard pH meter.

Soil analyses were done during May of the first year on each plot. The proportions of sand, silt, and clay were obtained using the sedimentation method (3). NO₃-N and NH₃-N were extracted (12) and analyzed using hydrazine reduction (9) for NO₃-N, and the indophenol method (2) for NH₃-N in a Technicon Auto Analyzer I. P, K, Ca, and Mg were extracted from soil using the method of Flannery and Markus (6). K and Ca levels were obtained with an Auto Analyzer II, whereas P was colorimetrically determined by a modification of the vanadomolybdophosphoric method. Mg content was determined by the modified lake procedure (6).

In the first year, nematodes were sampled on May 30, August 27, and November 19; in the second year, they were sampled monthly (May 19, June 16, July 15, August 15, September 15, and October 21). Most of the data reported herein were taken during the final year. All soil cores were to a depth of about 10 cm, using 1.9-cm-d soil probes. Nine soil cores from each plot were collected, combined, and mixed well before 250 cm³ were removed for processing by the centrifugation (sugar) flotation method using a 38- μ m sieve (8). *Tylenchorhynchus maximus* Allen, *Paratylenchus hamatus* Thorne and Allen, *Hoplolaimus galeatus* Cobb, *Criconebella rusium* (Khan, Chawla, & Saha) Luc & Raski, *Tylenchus* sp., and free-living nematodes were counted. Free-living nematodes consisted of microbivorous, mycophagous, and predatory nematodes. Occasionally, other species of plant-parasitic nematodes, numbering less than 25/250 cm³, occurred in some soil samples.

Data were analyzed using various SAS programs (15). Means and standard deviations ($\bar{x} \pm sd$) were calculated. Correlation coefficients (r), and the probability of greater correlation coefficients ($P > r$) were determined. The GLM procedure

for ANOVA and Duncan's multiple-range test, were selected to test means ($P = 0.05$). Stepwise regression (backward) analysis was used to model the dependent variable dollar spot (August) from second year data. The plant-parasitic nematode populations from July and August were used as the independent variables to estimate their impact on dollar spot. All population numbers remaining in the equation were at $P \leq 0.05$.

RESULTS

Soil analyses indicated uniform nutrient distribution and no deficiencies of P, K, Ca, and Mg, while N levels remained low (Table 1). There was a consistent negative correlation ($P \leq 0.05$) between *H. galeatus* and $\text{NH}_3\text{-N}$ ($r = -0.33$ to $r = -0.54$) in 7 of 9 sampling months over the 2-year period. The mean numbers of *H. galeatus* were correlated ($P \leq 0.05$) with P during August ($r = 0.53$) and November ($r = 0.57$) the first year, and also the following May ($r = 0.36$). The mean numbers of *T. maximus* were correlated the first year with Mg (June, $r = -0.34$). The *T. maximus* levels were correlated ($P \leq 0.05$) with Ca ($r = -0.39$, $r = -0.48$), K ($r = 0.32$, $r = 0.56$), Mg ($r = -0.39$, $r = -0.41$), and P ($r = -0.36$, $r = -0.45$) during July and August of the second year, respectively. The *P. hamatus* population levels were correlated ($P \leq 0.05$) the first year with Mg ($r = -0.44$), Ca ($r = -0.41$), and NO_3 ($r = -0.34$) during June, $\text{NH}_3\text{-N}$ during August ($r = -0.53$), and P during November ($r = 0.37$).

Some nematode counts were correlated ($P \leq 0.05$) with the sand, silt, or clay content of the soil. Numbers of *T. maximus* ($r = 0.34$) and *C. rusium* ($r = -0.43$) were correlated during June of the first year with the sand content of soil. Levels of *C. rusium* (June, $r = -0.38$ and August, $r = -0.41$) and *H. galeatus* (June, $r = -0.43$) were negatively correlated with the silt content. *H. galeatus* also was correlated ($r = 0.46$) with the clay content of soil during June. The mean numbers of *C. rusium* from the

first year's counts were correlated with the clay content of soil ($r = 0.35$).

Soil pH was 6.7 ± 0.1 (6.5–6.9) and 5.5 ± 0.2 (5.2–5.8) for the first and second year, respectively. During the first year, the mean population of *T. maximus* was correlated with soil pH ($r = -0.51$). Otherwise, numbers of nematodes showed little relationship to soil pH.

Mean dollar-spot severity in the 36 turfgrass plots was estimated at $30 \pm 22\%$ (2–70%) and $30 \pm 23\%$ (3–79%) during the first and second year, respectively. Results of chemical control of dollar spot with benomyl and iprodione have been reported (7,19).

During the first year, benomyl suppressed ($P \leq 0.05$) the mean population levels of *T. maximus* to 39 ± 20 from $85 \pm 60/250 \text{ cm}^3$ soil (untreated control-August), and to 30 ± 20 from $61 \pm 47/250 \text{ cm}^3$ soil (untreated controls-November). Benomyl suppressed an increase of *T. maximus* for 3 of 6 months the second year, free-living nematodes over the full 6 months, and the total number of nematodes for 2 of the 6 months (Table 2). Higher numbers of *H. galeatus* and *C. rusium* were detected in the benomyl-treated plots during June. Iprodione had little effect on nematode levels.

Thatch was more acid ($P = 0.05$) in benomyl-treated plots (pH 5.6 ± 0.8), than in the control (pH 6.1 ± 0.2). The pH ranged from 3.7 to 6.4 ($\bar{x} = 6.0 \pm 0.2$). Positive correlations ($P \leq 0.05$) existed between thatch pH and free-living nematodes for 5 of 6 sample months ($r = 0.49$ to $r = 0.56$), but during the first year there were no correlations between thatch pH and free-living nematodes.

Thatch thickness averaged 17 ± 4 mm during the first year and 15 ± 3 mm the following year. Thatch thickness the second year was 17 ± 3 mm and 13 ± 1 mm for the nonmowed and vertically mowed plots, respectively. Numbers of *T. maximus* were higher in vertically mowed plots as compared with those not vertically mowed during 5 out of 6 months (Table 3). *Tylenchus* sp. developed higher numbers in non-

TABLE 2. Numbers of nematodes per 250 cm³ of soil from fungicide-treated and nontreated plots of Merion Kentucky bluegrass during the second year.

Sampling month	Control	Benomyl	Iprodione	Control	Benomyl	Iprodione
<i>Tylenchorhynchus maximus</i>			<i>Paratylenchus hamatus</i>			
May	144 a†	41 b	105 ab	225 a	211 a	386 b
June	208 a	98 b	128 ab	327 a	324 a	386 a
July	339 a	119 a	219 a	304 a	289 a	304 a
August	275 a	171 a	190 a	184 a	182 a	92 a
September	112 a	46 b	69 b	451 a	536 a	362 a
October	118 a	75 a	76 a	243 a	229 a	270 a
<i>Hoplolaimus galeatus</i>			<i>Criconemella rusium</i>			
May	57 a	55 a	64 a	206 a	239 a	246 a
June	144 a	242 b	143 a	825 a	1,442 b	1,000 ab
July	198 a	235 a	207 a	1,132 a	1,254 a	1,250 a
August	231 a	332 a	208 a	582 a	882 a	688 a
September	416 a	602 a	594 a	831 a	842 a	1,187 a
October	533 a	542 a	564 a	909 a	1,160 a	1,103 a
<i>Tylenchus</i> sp.			Free-living			
May	462 a	330 a	484 a	1,112 a	328 b	949 a
June	1,642 a	1,568 a	1,404 a	1,333 a	676 b	1,332 a
July	2,428 a	1,883 a	2,038 a	2,032 a	1,299 b	2,087 a
August	1,820 a	1,414 ab	1,258 b	2,516 a	1,409 b	2,499 a
September	1,718 a	1,588 a	1,581 a	1,717 a	759 b	1,281 a
October	1,057 a	818 a	809 a	1,782 a	925 b	1,563 a

† Population values in a row followed by the same letter do not differ ($P = 0.05$) by Duncan's multiple-range test.

vertically mowed plots (Table 3). A significant suppression of dollar spot occurred the second year in vertically mowed plots ($10\% \pm 6$), compared with those that did not receive this treatment ($50\% \pm 17$). Interactions between thatch thickness and dollar spot were previously reported (7,19).

Only juveniles of *P. hamatus* were observed during May and June, whereas adults were first observed during late summer. Other species of stylet-bearing nematodes had adults present during all monthly observations. During the first year, *Pasteuria penetrans* was observed parasitizing *T. maximus*, but this was not seen the following year.

Clipping weights taken in May of the second year from plots not vertically mowed correlated ($P \leq 0.05$) with the June population of *T. maximus* ($r = -0.65$). In plots not vertically mowed, the population of *H. galeatus* for 3 out of 6 months had positive correlations with the clipping weights taken during September (May, $r =$

0.59; June, $r = 0.51$; September, $r = 0.66$) and October (June, $r = 0.51$; July, $r = 0.48$; September, $r = 0.73$). Clipping weights for May correlated with an increased incidence of dollar spot (August, $r = 0.86$) and thatch thickness (May, $r = 0.73$). The mean clipping weight in May was 110 ± 89 g (9–188 g), 426 ± 221 g (131–1,046 g) in September, and 602 ± 324 g/plot (178–1,475 g/plot) during October. Mean clipping weights in May were less in vertically mowed plots: 34 ± 22 g as compared to 186 ± 62 g/plot ($P = 0.05$) for nonvertically mowed plots. Mean weights of turf grass clippings from nonvertically mowed plots were slightly less than the clipping weight from the vertically mowed plots during both September and October (ns).

The mean numbers of spring tillers/10.2 cm-d plug/plot of Merion Kentucky bluegrass were correlated ($P \leq 0.05$) with the populations of *C. rusium* (June, $r = -0.36$), and *T. maximus* (July, $r = -0.38$; August, $r = -0.37$). Similarly, the popu-

TABLE 3. Mean numbers of nematodes per 250 cm³ of soil from heavily thatched (+) and vertically mowed (-) plots of Merion Kentucky bluegrass during the second year.

Nematode	May		June		July		August		September		October	
	+	-	+	-	+	-	+	-	+	-	+	-
<i>Tylenchorhynchus maximus</i>	86 a†	108 a	114 a	175 a	173 a	278 a	127 a	298 b	79 a	73 a	79 a	101 a
<i>Paratylenchus hamatus</i>	235 a	313 a	319 a	372 a	212 a	285 a	149 a	156 a	468 a	432 a	331 a	164 b
<i>Hoplolaimus galeatus</i>	51 a	66 a	176 a	177 a	236 a	191 a	280 a	234 a	576 a	499 a	597 a	495 a
<i>Criconemella rusium</i>	202 a	259 a	881 a	1,296 a	970 a	1,454 b	708 a	726 a	838 a	1,069 a	1,016 a	1,099 a
<i>Tylenchus</i> sp.	412 a	440 a	1,568 a	1,508 a	2,295 a	1,937 a	1,778 a	1,217 b	1,623 a	1,635 a	1,029 a	760 b
Free-living	900 a	692 a	1,414 a	812 b	2,273 a	1,339 b	2,516 a	1,767 b	1,223 a	1,282 a	1,717 a	1,129 b
Total number	1,885 a	1,887 a	4,455 a	4,339 a	6,259 a	5,605 a	5,602 a	4,400 b	4,808 a	5,117 a	4,769 a	3,749 b

† Population values in a row followed by the same letter for each month do not differ ($P = 0.05$) by Duncan's multiple-range test.

lation levels of *P. hamatus* during the second year correlated ($P \leq 0.05$) with spring tillers during 2 of the 6 sampling months (May, $r = -0.36$; October, $r = 0.39$). Spring tillers numbered $78 \pm 20/\text{plug}$ (7–101). The mean numbers of spring tillers were correlated ($P \leq 0.05$) with clipping weight (May, $r = 0.74$), thatch thickness (May, $r = 0.66$), free-living nematodes (August, $r = 0.31$; October, $r = 0.35$), total nematodes (August, $r = 0.32$; October, $r = 0.66$), and *Tylenchus* sp. (August, $r = 0.34$; October, $r = 0.33$). The first-year count of fall tillers did not correlate either with nematode numbers or other parameters.

The model equation, dollar spot % (August) = $39.9 - 0.015 (C. rusium, \text{July}) + 0.032 (H. galeatus, \text{August})$ ($R^2 = 0.23, P \leq 0.05$), predicts the percentage of dollar spot in August in relation to the population of plant-parasitic nematodes present during the corresponding time period of July and August. There were too few observations when dollar spot was sorted by either treatment or vertical mowing parameters to produce other significant model equations. According to the equation, the mean numbers of *C. rusium* ($\bar{x} = 1,212$) are associated with an 18% suppression of dollar spot, whereas *H. galeatus* ($\bar{x} = 257$) is associated with an 8% increase in dollar spot.

DISCUSSION

Factors that influence nematodes in their soil environment include soil particle size, moisture, mineral nutrition of host plants, temperature, and population interactions. These factors often correlate with each other and with nematode populations, making such data difficult to interpret (7,19). After identifying correlations among variables, additional research is necessary on the nature of these relationships.

Correlations between nematode numbers and mineral content of soil, as occurred in this study, have been documented previously (13,18). Nematode

populations seemed unaffected by the small variations in soil pH among plots. The importance of the inverse relationship between $\text{NH}_3\text{-N}$ and the numbers of *H. galeatus*, during 7 of 9 sampling months over the 2-year period, should be further investigated.

The model equation suggests that for every increase of 100 *H. galeatus*/250 cm^3 of soil, the severity of dollar spot increases by 3.2%. Although regression analysis does not indicate a cause-effect relationship, a hypothesis could be made that *H. galeatus* injures Merion Kentucky bluegrass so that the severity of the disease caused by *S. homoeocarpa* increases. Similarly, with an increase of 100 *C. rusium*/250 cm^3 of soil, the severity of dollar spot decreases by 1.5%. The phenomenon might be associated with an attenuation of Merion Kentucky bluegrass density from an increased severity of disease.

Tylenchorhynchus maximus was inversely related to clipping weight taken in May, whereas *H. galeatus* was positively correlated with October's and November's clipping weights. These species should be examined to determine if they have a pathogenic effect on turf growth. Chastagner and McElroy (4) reported that *T. maximus*, in conjunction with other genera, was correlated with stress damage to annual bluegrass, *Poa annua*. *Tylenchorhynchus nudus* suppressed top and root growth of Kentucky bluegrass, and pathogenicity was aggravated by nutrient and water stress (18). Population density of *T. maximus* increased in vertically mowed plots, possibly reflecting the stimulation of tillering caused by this treatment. Rooting and tillering at plant nodes create new plants, and this additional biomass should eventually enhance nematode build-up.

Thatch is an intermingled layer of dead and living shoots, rhizomes, and roots lying between the green shoots and the soil. Accumulation of thatch occurs when cultural or environmental factors stimulate plant growth or retard tissue decomposition. When removing thatch by vertical mowing, some dollar-spot mycelia and

sclerotia also are removed. Rhizome breakage and wounding results in growth stimulation. In vertically mowed plots, population density of *T. maximus* increased in August, *P. hamatus* increased in October, and *C. rusium* increased in July. Increased nematode populations following thatch removal were probably due to stimulated root growth.

Lower pH in the benomyl-treated plots creates an environmental condition that deters bacterial breakdown of organic matter and produces increased thatch thickness. Although benomyl might be directly toxic to free-living nematodes, a reduction in the bacterial food supply of microbivorous nematodes by acidification of soil might explain how benomyl applications resulted in reduced numbers of free-living nematodes. Acidified soil, microbial control, and the subsequent increased thatch following application of benomyl and other fungicides has been previously reported (16,17). Dernoeden et al. (5) found no change in pH or thatch accumulation in turf plots where benomyl or iprodione were applied, but their dosage rates were approximately 50% of that used in our research. Our results agree with those of Laughlin and Vargas (10), in which benomyl had a slight nematicidal effect. Some monthly counts of *T. maximus* and free-living nematodes showed significant declines. Field experiments (5) did not indicate that benomyl affected nematode numbers. *Hoplolaimus galeatus* and *C. rusium* slightly increased in numbers in the benomyl treated plots, probably due to greater turf coverage. Neither benomyl nor iprodione effectively controlled nematodes.

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