Occurrence, Spatial Distribution, and Pathogenicity of Some Phytoparasitic Nematodes on Creeping Bentgrass Putting Greens in Kansas

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ABSTRACT

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Eleven genera of phytoparasitic nematodes, of which *Criconemella*, *Helicotylenchus*, *Tylenchorynchus*, and *Hoplolaimus* were the most abundant, were recovered from soil samples collected from 81 creeping bentgrass (*Agrostis palustris* 'Penncross') putting greens in Kansas. The relationship between population densities of *Criconemella ornata*, *Helicotylenchus pseudorobustus*, and *Hoplolaimus galeatus* and growth and quality of turfgrass was studied in detail on two creeping bentgrass putting greens from 1984 to 1986. Correlations between numbers of *C. ornata* or *H. pseudorobustus* and visible turfgrass injury were not observed; however, turfgrass growth, as measured by dry weight accumulation of foliage clippings, was negatively correlated with numbers of adults plus juveniles per 100 cm^3 of soil for both species in 1985. In contrast, visible ratings of turfgrass damage increased linearly with logarithmic increases in population density of *H. galeatus* in 1985 when temperatures were higher. Populations of *C. ornata* and *H. galeatus* were aggregated on a bentgrass green at three sampling dates (June, July, and August), but the degree of aggregation varied with time and between species.

Phytoparasitic nematodes are indigenous components of the soil microfauna associated with roots of warm- and cool-season turfgrasses used for putting-green surfaces on golf courses. In the southern United States, high population densities of certain parasitic nematode species can detrimentally affect the growth of these turfgrasses (5,9,12,14,22) and result in severe damage to putting greens. While many of these species are associated with cool-season turfgrass putting greens in Kansas and other parts of the northern and central United States (15,17–19), their effect on turfgrass growth and quality is poorly understood.

The purpose of our study was to determine which genera of phytoparasitic nematodes are most commonly associated with creeping bentgrass (*Agrostis palustris* Huds.) putting greens in Kansas, to ascertain the effects of population densities of certain nematode species on turfgrass growth and quality, and to observe spatial and temporal distributions of these nematodes during the summer. Preliminary reports on these findings have been published (20,21).

MATERIALS AND METHODS

Survey. Soil samples were collected from three to five putting greens on each of 20 golf courses throughout Kansas from May through August 1984. All putting greens were composed primarily of creeping bentgrass, although some annual bluegrass (*Poa annua* L.) was also present. Each green was sampled by removing 12 soil cores (2.5-cm diam. × 8-cm deep) at roughly equal intervals in a zig-zag pattern across the green. Soil samples from each green were combined, stored at 10 C, and processed within 4 days of sampling. Nematodes were extracted from 100-cm^3 soil samples with a modification of the Christie-Perry technique (4) as described by Alby (1). Population estimates were adjusted for extraction efficiency by calculating the proportional loss of each nematode species at each step in the extraction procedure. This was accomplished by repeated washings of the same soil sample, multiple seiving, and measurements of relative migration through the filter tissue for each nematode species (6).

Relationship of nematode populations to turfgrass injury. On 27 June 1984, 0.9 × 0.9 m plots were established on a creeping bentgrass Penncross putting green near Clay Center, Kansas (4.4% organic matter, 90% sand, pH 7.9). The green was representative of Kansas golf greens in terms of plant species, soil composition, and indigenous populations of parasitic nematode species. *Criconemella ornata* (Raski) Luc & Raski and *Helicotylenchus pseudorobustus* (Steiner) Golden were the most abundant phytoparasitic nematodes present; initial plot population densities ranged from 3,500 to 5,850 and 63 to 981 nematodes per 100 cm^3 of soil for *C. ornata* and *H. pseudorobustus*, respectively. Plots were left untreated or were treated with the nematicides fenamiphos (Nemacur 10G at 14.6 kg a.i./ha) and ethoprop (Mocap 10G at 22.4 kg a.i./ha) or the insecticide isophenphos (Oftanol 5G at 2.2 kg a.i./ha). In 1985 and 1986, plots (0.9 × 0.9 m) on a second creeping bentgrass green with initial population densities of *Hoplolaimus galeatus* Cobb and *C. ornata* ranging from 220 to 511 and from 7,125 to 12,225 nematodes per 100 cm^3 of soil, respectively, were left untreated or treated with either fenamiphos (Nemacur 10G at 11.2 and 22.4 kg a.i./ha or Nemacur 3E at 14.6 kg a.i./ha) or isophenphos (Oftanol 5G at 2.2 kg a.i./ha). The experimental design on both greens was a randomized complete block with four replicates. Plots were irrigated with 1 cm of water immediately after pesticide application. Four soil cores (2.5-cm diam. × 8-cm deep) were collected near the center of each plot before pesticide application (27 June 1984, 6 June 1985, and 28 May 1986), and again on 7, 23, and 15 July, and 5 September and 29 and 23 August in 1984, 1985, and 1986, respectively. Nematodes were extracted from samples as previously described, and counts were adjusted for extraction efficiency and $\log_{10}$-transformed before analysis to standardize variances. At each sampling date, foliar quality of turfgrass on each plot was rated on the percentage of foliar discoloration where 0% = no visible foliar discoloration to the turfgrass (turfgrass green and healthy) and 100% = turfgrass totally discolored. Symptoms of diseases caused by fungi were not detected on any green at the time ratings were made.

Approximately 2 mo after pesticides were applied (7 September 1984, 29 August 1985, and 23 August 1986), a 10-cm-diameter × 10-cm-deep core containing turfgrass and soil was removed from the center of each field plot and placed in a 10-cm-diameter plastic pot. Additional space in the pots was filled with pasteurized sand. Pots were then placed on greenhouse benches in a randomized complete block design and irrigated as needed. Temperatures ranged from 20 to 30 C. After approximately 1 and 2 mo, foliage in each pot was clipped to a height of 1 cm. Clippings were collected, dried at 50 C for 5 days, and weighed. After the second clipping, soil in each pot was processed to determine population densities of parasitic nematodes. Clipping weights were then compared to population estimates of nematode species at the

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time the turf plugs were removed from field plots and with final densities in the greenhouse. Population estimates and clipping weights were also compared among various pesticide treatments.

To determine whether pesticides alone were responsible for a reduction in turfgrass growth, creeping bentgrass Penncross was seeded into 10-cm-diameter plastic pots containing pasteurized sand. The turfgrass was irrigated and fertilized as needed. After 1 mo, fenamiphos (Nemacur 10G at 22 kg a.i./ha) or isophenphos (Oftanol at 2.2 kg a.i./ha) was applied to pots with a hand-held shaker. Control pots did not receive a pesticide application. Ethoprop was not included because it was phytotoxic in field experiments. The experimental design was a randomized complete block with four replicates. Turfgrass in each pot was clipped 1 and 2 mo after pesticide application and processed as described previously.

All greenhouse and field data were subjected to analysis of variance with least significant difference mean separation tests and regression analysis.

Spatial and temporal distribution. In 1986, the second putting green was divided into an 8 × 7 rectangular grid with 3-m intervals between grid intersections. On 28 May, 15 July, and 23 August, four soil cores (2.5-cm diam. × 8-cm deep) were removed from 34 grid intersections and were processed for nematodes as described previously. Not all grid intersections (56 total) were sampled, but all areas of the grid were represented. The same grid intersections were sampled at each date. Sample frequency distributions of *C. ornata* and *H. galeatus* were tested for goodness of fit by χ^2 analysis to the Poisson, Thomas double Poisson, negative binomial, and Neyman type A distributions (8). Frequency class intervals for population densities of nematode species at each sampling date were determined with a MINITAB program (Minitab, Inc., State College, PA). The number of frequency classes ranged from eight to 13.

A measure of spatial dependence or aggregation of nematode populations within the sampling grid was obtained with Lloyd's index of mean crowding (11) and a geostatistical software program developed by Kark (10). The use of geostatistics in the analysis of spatial patterns of plant pathogens is described by Chellemi et al (3). In our study, spatial variability was measured by determining the average semivariance for all pairs of sample points at increasing grid intervals at orientations of 0°, 45°, 90°, and 135° to the grid axis. Semivariance values for each direction were fitted to linear or quadratic regression models. An increase in the population variance between paired groups at increasing intervals indicates spatial dependence or aggregation while relatively little change in variance implies no spatial dependence or a sampling interval too large to detect dependence.

RESULTS

Eleven genera of phytoparasitic nematodes were recovered from soil samples collected from 81 creeping bentgrass golf greens in Kansas (Table 1). The most frequently occurring and abundant genera were *Criconemella*, *Helicotylenchus*, *Tylenchorynchus*, and *Hoplolaimus*. Several other genera were found only on a small percentage of greens sampled or occurred at low populations.

In 1984, application of ethoprop, fenamiphos, and isophenphos reduced ($P < 0.05$) population densities of *H. pseudorobustus* but not *C. ornata* on a creeping bentgrass green 1 mo after pesticide application. Midsummer population estimates on all plots ranged from 27 to 1,600 *H. pseudorobustus* and from 950 to 2,600 *C. ornata* per 100 cm^3 of soil. All pesticides continued to supress *H. pseudorobustus* 3 mo after application. Ethoprop caused extensive foliar burning within 48 hr of application, but the turfgrass recovered after 10 days.

In 1985, none of the pesticides significantly reduced population densities of *C. ornata*, *H. pseudorobustus*, or *H. galeatus*; however, there was a general trend toward decreased numbers of nematodes in plots treated with fenamiphos. In 1986, all rates of fenamiphos reduced ($P < 0.05$) numbers of *H. pseudorobustus*, whereas only the high fenamiphos rate decreased ($P < 0.05$) numbers of *C. ornata* and *H. galeatus*. The high rate of fenamiphos continued to suppress ($P < 0.05$) *H. galeatus* but not *C. ornata* 2 mo after pesticide application. Ranges in midsummer population densities for 1985 and 1986 were 450–13,650 *C. ornata*, 0–311 *H. pseudorobustus*, and 53–798 *H. galeatus* per 100 cm^3 of soil.

Population estimates of *C. ornata*, *H. pseudorobustus*, and *H. galeatus* or total parasitic nematode population densities in pesticide-treated plots were not correlated with foliar damage to creeping bentgrass in 1984 or 1986. All plots had <15 % of the turf discolored in both July and August, even though relatively high numbers of these nematode species were present in some plots. In 1985, turfgrass damage ratings in July increased linearly ($R^2 = 0.70$) with logarithmic increases in numbers of *H. galeatus* (Fig. 1). The model predicted a threshold for visible turfgrass injury of approximately 120 *H. galeatus* per 100 cm^3 of soil.

There were no significant differences in foliar clipping weights of turfgrass cores removed from pesticide-treated or nontreated field plots in 1984 or 1986; however, there was a general trend toward increased clipping weights in fenamiphos-treated cores. Similarly, there were no correlations between initial or final population densities of *C. ornata*, *H. galeatus, H. pseudorobustus*, or total parasitic nematode numbers and clipping weights. In 1985, all fenamiphos rates

Table 1. Frequency of occurrence and population densities of phytoparasitic nematodes in soil samples from creeping bentgrass putting greens in Kansas

Genera	Courses infested (%)[a]	Greens infested (%)[b]	Nematodes/100 cm^3 soil Mean[c]	Nematodes/100 cm^3 soil Maximum[d]
Helicotylenchus	80	55	425	1,044
Hoplolaimus	75	47	81	364
Criconemella	70	52	900	5,300
Tylenchorhynchus	60	38	242	1,498
Xiphinema	20	8	22	42
Paratylenchus	10	6	194	616
Longidorus	10	2	14	14
Paratrichodorus	10	2	35	42
Hemicycliophora	5	5	116	210
Heterodera	5	1	28	28
Pratylenchus	5	1	28	28

[a] Percentages of golf courses with at least one infested putting green. Percentages based on 20 golf courses with three to five putting greens sampled on each golf course.
[b] Percentages based on a total of 81 greens with three to five creeping bentgrass greens sampled from each course.
[c] Mean population density from creeping bentgrass greens in which the nematode was found.
[d] Maximum population found in composite samples from individual creeping bentgrass greens.

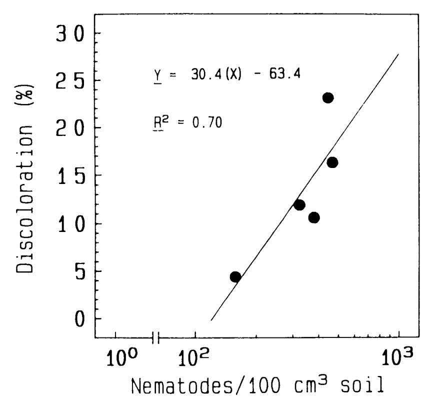

Fig. 1. Relationship between visual damage of creeping bentgrass in 1985 and population estimates ($\log_{10}$-transformed) of *Hoplolaimus galeatus*. Turf discoloration ratings based on a percent scale where 0% = no turf discoloration and 100% = turf completely discolored.

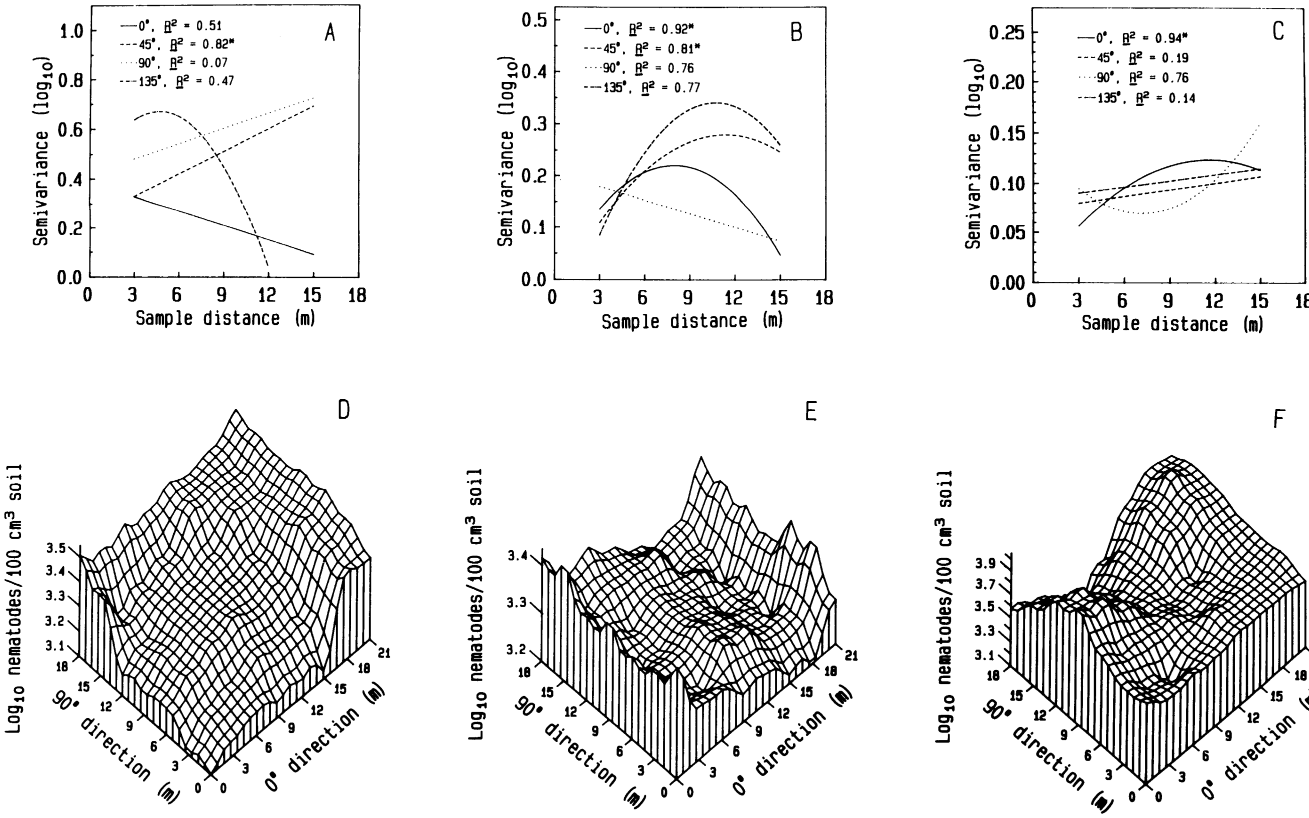

Fig. 2. Regressions of directional semivariograms at 0°, 45°, 90°, and 135° for population estimates of *Criconemella ornata* on a creeping bentgrass green at increasing intervals between sampling points in **(A)** June, **(B)** July, and **(C)** August 1986, and **(D-F)** surface maps of spatial distributions for each month. Significant regressions ($P < 0.10$) are indicated by an asterisk.

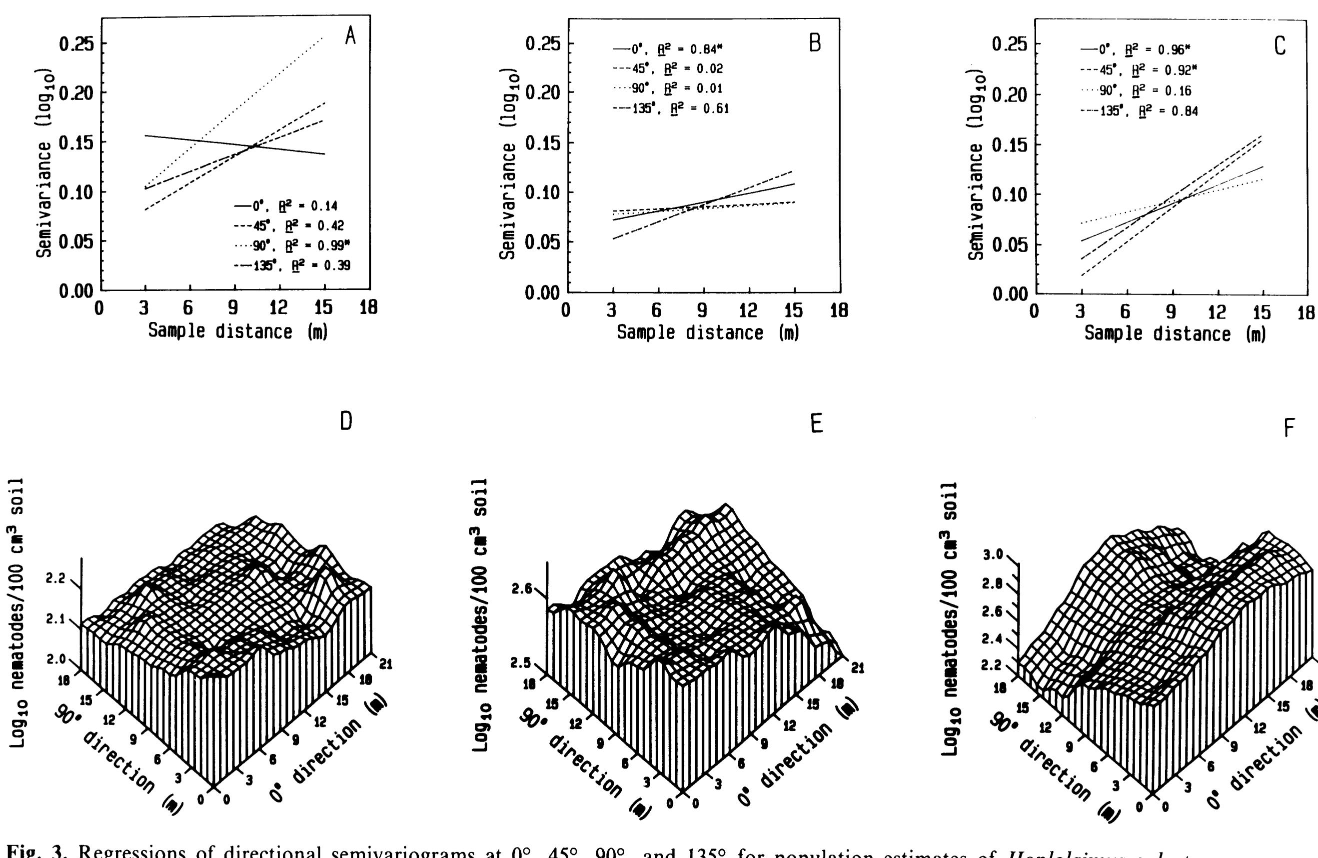

Fig. 3. Regressions of directional semivariograms at 0°, 45°, 90°, and 135° for population estimates of *Hoplolaimus galeatus* on a creeping bentgrass green at increasing intervals between sampling points in **(A)** June, **(B)** July, and **(C)** August 1986, and **(D-F)** surface maps of spatial distributions for each month. Significant regressions ($P < 0.10$) are indicated by an asterisk.

increased (P <0.05) foliar clipping weights compared to control plants. Clipping weights after 2 mo for all treatments (including controls) were negatively correlated with numbers of *C. ornata* ($r = -0.38$, P <0.05) and *H. pseudorobustus* ($r = -0.63$, P <0.05) at the time cores were removed from the field (29 August), but not with final populations in cores placed in the greenhouse for 2 mo. Application of fenamiphos or isophenphos to creeping bentgrass grown in pasteurized sand in the greenhouse did not significantly influence foliar clipping weights when compared to nontreated plants.

Frequency data for populations of *C. ornata* and *H. galeatus* on all grid-sampling dates in 1986 were best fitted to a negative binomial distribution (P >0.05), except for *C. ornata* in July. All nematode species-sampling date combinations had high indices of patchiness (Lloyd's index of 1.3–2.1). Populations of *C. ornata* and *H. galeatus* generally remained stable or increased through the summer (Figs. 2 and 3); however, spatial patterns varied between species and with time. In June, population densities of *C. ornata* increased in the 45° direction to the grid axes (Fig. 2A and D) but showed no spatial dependence in other directions. In July and August, aggregation was greatest in the 0° direction. Semivariance values were characterized generally by quadratic models. Variability increased up to sample distances of 9–12 m, then declined (Fig. 2B and C). Spatial dependence for *H. galeatus* increased in the 0°, 45°, and 135° directions throughout the sampling period and was described by linear regression models (Fig. 3).

DISCUSSION

Our survey results agree with previous reports (15,18,19) that indicate a diversity of parasitic nematode genera are associated with creeping bentgrass putting greens in the northern region of the United States. Nevertheless, only species of *Criconemella*, *Helicotylenchus*, *Tylenchorynchus*, and *Hoplolaimus* appear to be relatively abundant on putting greens in Kansas.

A cause-effect relationship between phytoparasitic nematode population densities and damage to cool season turfgrasses in the northern United States has been difficult to demonstrate (15, 18,19). We found foliar damage ratings to creeping bentgrass were positively correlated with midsummer population densities of *H. galeatus* in 1985. Turfgrass discoloration was severe (ratings >15%) at population densities exceeding 300 *H. galeatus* per 100 cm^3 of soil. High numbers of *C. ornata* and *H. pseudorobustus* also resulted in decreased growth of turfgrass in 1985 but were not correlated with visible injury to the putting green. Damage or growth reduction to creeping bentgrass was not observed in 1986, even though densities of *H. galeatus* and *C. ornata* were similar to or greater than those in 1985. These results suggest that relatively high populations of these nematode species are necessary to cause damage to creeping bentgrass and that damage thresholds may fluctuate from year to year (or even month to month), depending on environmental factors. Creeping bentgrass on putting greens is maintained at near-optimum growing conditions by daily irrigation, frequent fertilization, and routine applications of pesticides to control diseases and insects. High maintenance may ameliorate detrimental effects of nematode feeding to the turfgrass in most circumstances; however, factors such as prolonged high air and soil temperatures, which occurred in midsummer 1985, or a reduction in maintenance may result in suboptimum turfgrass growth and predisposition to injury caused by nematode feeding.

Soilborne pathogens, including nematodes, are often found to be aggregated spatially (2,7,16). We found that populations of *H. galeatus* and *C. ornata* were aggregated on a creeping bentgrass putting green and that spatial patterns varied between species and with time. Generally, population densities and spatial aggregation increased for both species through the summer. This could influence the optimal time to sample greens for nematode populations. We found highest nematode populations in late summer, but midsummer (July) populations were more closely correlated with visible damage or growth reduction of the turfgrass. Therefore, the best time to collect soil samples from putting greens in Kansas would be in late June or July. These results are in agreement with Lucas et al (13), who also recommended sampling creeping bentgrass putting greens in North Carolina in June or October during peak population densities of certain parasitic nematodes.

Factors that may influence spatial association of soilborne pathogens include species-specific characteristics, interspecific associations, physical and biological factors, and environmental influences (2). It is unlikely that physical properties of the soil were responsible for the aggregated distributions we observed because putting greens consisted of an artificially mixed soil of uniform composition. Also, the green was composed of genetically similar perennial plants and received uniform irrigation, pesticide application, fertilization, and other cultural practices. This suggests that other biological factors, such as species-specific or interspecific relationships, play an important role in spatial distribution of phytoparasitic nematodes.

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