Factors Associated with Populations of Plant-Parasitic Nematodes in Bentgrass Putting Greens in Oklahoma

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ABSTRACT

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Numerous genera of plant-parasitic nematodes are frequently present at high populations in golf course putting greens. The objectives of this research were to identify and quantify plant-parasitic nematodes from Oklahoma bentgrass putting greens and to characterize specific soil physical and chemical features and management factors that may account for differences in or associated with the observed nematode populations. In the fall of 2000, nematodes were identified from 99 individual bentgrass putting greens sampled from 46 different locations in Oklahoma. In addition to green age and bentgrass cultivar, soil pH, nitrate-nitrogen ($\text{NO}_3\text{-N}$), plant-available phosphorus and potassium, organic matter (OM), bulk density, and particle size distribution were determined for each green. Expenditures on management activities, including fertilizer, herbicide, fungicide, and insecticide, were determined for 20 of the sampled courses and compared with nematode populations. Nematodes from seven genera were found in Oklahoma greens, with *Criconebella* spp. being the most common. *Paratrichodorus* spp., *Tylenchorhynchus* spp., and *Helicotylenchus* spp. also were common. The logarithmic populations of all nematodes combined increased with greater green age ($r = 0.37$). A negative relationship was observed between soil bulk density and all nematodes combined ($r = -0.29$). Soil $\text{NO}_3\text{-N}$, plant-available K, and OM increased with combined logarithmic populations of all plant parasitic nematodes ($r = 0.23, 0.28, \text{ and } 0.37$, respectively). A four-factor model accounted for 75% of the total variation in the data and permitted groupings of all variables into four uncorrelated factors. Total logarithmic nematode populations increased with fungicide and herbicide expenditures ($r = 0.31$). This study suggests that populations of plant-parasitic nematodes may be influenced by the putting green abiotic soil environment and possibly indirectly by management practices on putting greens.

Additional keywords: *Agrostis palustris*, bulk density, *Criconebella*, *Helicotylenchus*, *Hoplolaimus*, *Paratrichodorus*, *Tylenchorhynchus*

Creeping bentgrass (*Agrostis palustris* Huds.) is an important component of putting greens in the southern United States. To achieve a high-quality putting surface, bentgrass is maintained at very low heights (<4 mm) and is grown in a root zone predominantly composed of sand or a sand-peat mixture (19). Although tolerant of low mowing heights, bentgrass green quality can be reduced by hot temperatures, resulting in a reduction in root growth and subsequent recovery from wear or disease. To

maintain a high-quality putting surface, greens are intensely managed with pesticides, fertilizer, and irrigation to prevent disease, damage from wear, and deficiencies in fertility and moisture, and to minimize deleterious effects due to extremes in environmental conditions. In spite of efforts to maintain turf density, vigor, quality, and disease suppression, greens often fail to respond to management inputs and to maintain a desirable putting surface. This often occurs during warm periods, when use is frequent and environmental conditions are conducive for disease. These conditions often are present when plant-parasitic nematodes are present at their highest populations (17).

Plant-parasitic nematodes are important yield-reducing pests of most agronomic crops worldwide. Nematodes typically feed on subterranean plant tissues, including roots, tubers, and rhizomes. Feeding rarely causes plant death; however, nematodes can arrest root growth and cause lesions, necrosis, or impaired root function. For agronomic crops, affected plants are

chlorotic, stunted in growth, have lower yields, and often are more susceptible to diseases caused by facultative microbes. Turf grass growth can be stunted by nematodes and stands of turf can become less dense. For specific nematode-turf combinations, the affected turf grass may die. In addition, large numbers of plant-parasitic nematodes in a bentgrass putting green may interfere with the effectiveness of management inputs, such as nutrient uptake by plants.

Plant-parasitic nematodes from numerous genera often are present in putting greens. *Helicotylenchus*, *Criconebella*, *Hoplolaimus*, and *Tylenchorhynchus* spp. were found in 55, 52, 47, and 38%, respectively, of 81 greens from 20 Kansas golf courses (18). Mean nematode populations in the study were 425, 900, 81, and 242/100 cm^3 soil for *Helicotylenchus*, *Criconebella*, *Hoplolaimus*, and *Tylenchorhynchus* spp., respectively. Similar results were found in an earlier study conducted in New York in which *Criconebella*, *Tylenchorhynchus*, *Hoplolaimus*, and *Helicotylenchus* spp. were present in 78, 71, 55, and 39%, respectively, of 295 samples collected from 16 golf courses; however, mean population values were not reported (11).

Fluctuations or distribution differences of nematode populations are common in turfgrass ecosystems. Fluctuating populations of *Belonolaimus longicaudatus* parasitizing Bermudagrass in California were correlated with soil temperatures ($r^2 = 0.40$) (2). In Illinois, *Tylenchorhynchus nudus*, *Helicotylenchus cornutus*, and *Criconebella curvata* were more frequently recovered from the upper 2.5 cm of a bentgrass putting green than from 2.5- to 5.0-cm depths (4). However, in New England, nematode populations were not found to be consistent within a green over time (23).

The effect of individual and combined populations of plant-parasitic nematodes on bentgrass root and shoot growth has been characterized. In greenhouse studies, only mixed populations of *Hoplolaimus* spp. and *T. dubius* reduced shoot growth and caused bentgrass leaf chlorosis (9). A similar reduction in shoot growth was observed for mixed populations of *Pratylenchus penetrans* and *T. agri*, but not for individual nematode species (15). The effects of plant-parasitic nematodes on bentgrass plants in situ has been more dif-

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ficult to characterize due to variable factors such as temperature, damage from other diseases, and green construction.

Effective management of plant-parasitic nematodes in existing bentgrass greens is limited to applications of organophosphorous pesticides. Concerns about organophosphorous pesticide safety and fate in the environment could potentially limit their use on golf courses. Older greens that do not provide acceptable turfgrass quality are either fumigated or rebuilt using new root zone media. These disruptive management options usually are reserved until the accumulation of organic matter or presence of plant pathogens are thought to be too high to be managed by standard management practices such as the removal of numerous, small soil cores (aerification) prior to the application of sand to the surface of the green.

The sand-based root zone, continual presence of plants, and absence of fluctuating soil water content likely encourage an increase in populations of plant-parasitic nematodes. However, communities of plant-parasitic nematodes in bentgrass greens from the same region can differ dramatically (21). Previous studies of nematode distribution in bentgrass greens have not examined factors which could account for differences in nematode populations. The objectives of this research were to identify and quantify plant-parasitic nematodes from Oklahoma bentgrass putting greens and to characterize specific soil physical and chemical features and maintenance expenditures that may account for differences in or are associated with the observed nematode populations in the greens sampled.

MATERIALS AND METHODS

From September through the first week of November 2000, soil samples were collected from 99 bentgrass putting greens from 43 golf courses and 3 research facilities in Oklahoma. Samples, one from each green, were collected with a cup cutter (10.8 cm in diameter) to a depth of about 10 cm. Green age and bentgrass cultivar were recorded at the time of sampling for each green. Soil samples were stored (<2 weeks) at 4°C and thatch was removed

prior to analysis. Nematodes were extracted from a 100-cm³ subsample of soil by sieving and centrifugal floatation (1). Plant-parasitic nematodes were identified to genus, and total populations from 100 cm³ of soil were determined using a compound light microscope.

A portion of each soil sample (>200 cm³) assayed for nematodes was air dried on a greenhouse bench for a minimum of 1 week, ground with a rotary impact grinder, and sieved (<2 mm). All samples were analyzed for pH, nitrate-nitrogen (NO₃-N), plant-available phosphorus (P) and potassium (K), and soil organic matter (OM). Soil pH was measured by glass electrode in a 1:1 soil:water suspension (16). Soil NO₃-N was extracted with 1 M KCl solution and quantified by the cadmium reduction method (7). Plant-available P and K were extracted using the Mehlich 3 solution (10). Soil OM was determined from organic carbon (C) using a dry combustion C analyzer (12) and a conversion factor of 1.724 (based on organic matter containing 58% C).

To determine soil bulk density and the distribution of sand particle sizes, a separate soil subsample (31 cm³) was dried for 24 h at 70°C in a forced-air oven and then weighed. Samples then were heated to 510°C in a furnace for 24 h to remove organic matter. Dry sand fractions were separated into United States Department of Agriculture-defined limits for basic soil textural classes by vibrating for at least 1 min on nested sieves of 2,000, 1,000, 500, 250, 106, and 53 µm over a pan (3). Sand fractions collected on each sieve and silt with clay fraction collected inside the pan were weighed.

For all samples, nematode populations were transformed to a log₁₀ (X + 1) scale for analysis and the relationship between population densities for individual and total nematodes and soil factors were assessed by correlation analysis using SAS (SAS Institute, Cary, NC). The relationship between golf course expenditures (5) on fertilizer, herbicide, fungicide, insecticide, adjuvant, sand applications, and nematode populations was assessed by correlation analysis for a subsample of 20 different golf courses from which 43 greens were

sampled. These 20 courses varied in maintenance expenditures. Only significant correlation coefficients ($P \leq 0.05$) are presented. To determine the effect of bentgrass cultivar on nematode populations, samples containing only the cultivars Penncross (Tee-2-Green Corp., Canby, OR) and SR1020 (Seed Research of Oregon Inc., Corvallis, OR) were selected for analysis of variance. To identify underlying factors responsible for correlations among variables, factor analysis on the soil variables was performed using SAS/FACTOR procedure (SAS Institute). Principle component analysis was first used for factor extraction and all factors with an eigenvalue greater than one were subjected to orthogonal rotation using varimax rotation (SAS Institute). The variables used were pH, NO₃-N, plant-available P and K, OM, bulk density, percent sand, and particle size distributions.

RESULTS

The two most common bentgrass cultivars planted were Penncross and SR1020, which were grown on 34 and 31% of the greens sampled, respectively. Bentgrass mixtures, Pennlinks (Tee-2-Green Corp.), and Penn A-4 (Tee-2-Green Corp.) were present on 8, 6, and 6% of the greens, respectively. Five additional cultivars were present on the remaining 15% of greens.

Plant-parasitic nematodes from seven genera were identified in Oklahoma bentgrass putting greens (Table 1). *Cricone-mella* spp. were found most frequently and had the greatest population densities in greens where this nematode was present. In 28% of the greens with *Cricone-mella* spp., its populations were above 1,000/100 cm³ of soil. *Paratrichodorus* was the second most frequent nematode genus found in samples. However, population densities of this nematode genus were generally the lowest of those nematodes present in greens. *Tylenchorhynchus* spp. also were present in nearly half of the greens sampled. Although *Helicotylenchus* spp. were present in only 32% of greens, population densities were usually high.

The average age of putting greens sampled was 12.8 years. Total combined population densities of plant-parasitic nematodes increased with green age ($r = 0.37$). Individual populations of *Cricone-mella*, *Tylenchorhynchus*, and *Helicotylenchus* spp. also increased with green age (Table 2). NO₃-N, plant-available P and K, and OM in the soil also increased with increasing green age ($r = 0.34, 0.31, 0.26$, and 0.74 , respectively).

Soil pH ranged from 5.6 to 8.8, with an average of 7.4. However, pH was not correlated with individual or combined nematode population densities. Numbers of *Cricone-mella* and *Tylenchorhynchus* spp. increased with increasing NO₃-N content of greens. Populations of *Cricone-mella*, *Tylenchorhynchus*, and *Helicotylenchus*

Table 1. Plant-parasitic nematode genera present in bentgrass putting greens in Oklahoma

Genera ^a	Greens (%) ^b	Mean/100 cm ³ soil ^c	SEM ^d
<i>Cricone-mella</i>	59.2	968	167
<i>Tylenchorhynchus</i>	47.6	460	94
<i>Paratrichodorus</i>	57.3	89	13
<i>Helicotylenchus</i>	32.0	727	355
<i>Pratylenchus</i>	16.2	126	43
<i>Hemicycliophora</i>	20.4	177	55
<i>Hoplolaimus</i>	5.8	164	92

^a Nematodes were collected from 99 greens at 46 locations in the fall of 2000. Populations were determined for 100 cm³ of soil by sieving and centrifugal floatation.

^b Percent of greens containing respective nematode.

^c Mean number of nematodes based on greens having the respective nematode.

^d Standard error of the mean.

spp. increased with greater plant-available P and K, and OM content. A negative relationship was observed between soil bulk density (0.85 to 1.75 g/cm³, 1.21 g/cm³ average) and populations of *Paratrichodorus*, *Helicotylenchus*, and combined populations of plant parasitic nematodes ($r = -0.28, -0.27$, and -0.29 , respectively). In addition, only NO₃-N, plant-available K, and OM increased with population densities of all plant-parasitic nematodes combined ($r = 0.23, 0.28$, and 0.37 , respectively).

Soil samples were composed primarily of sand (85.2 to 98.9%, 94.7% average) and particle size distribution was primarily limited to particles less than 1 mm in diameter and greater than 106 µm in diameter (>2 mm, 0.95%; 1 mm to 2 mm, 6.5%; 500 µm to 1 mm, 24.3%; 250 to 500 µm, 40.1%; 106 to 250 µm, 20.4%; 53 to 106 µm, 2.4%). Population densities of *Hoplolaimus* spp. were negatively correlated with the percentage of sand fraction between 500 µm and 1 mm ($r = -0.22$). Populations of *Hoplolaimus* and *Cricone-mella* spp. increased as the percent of sand frac-

tion between 106 and 250 µm in diameter increased ($r = 0.20$ and 0.21 , respectively). Total populations of plant-pathogenic nematodes decreased as sand fractions greater than 1 mm increased ($r = -0.26$ [1 mm] and -0.21 [2 mm], respectively).

Populations of *Cricone-mella* spp., *Tylenchorhynchus* spp., and all combined plant-parasitic nematodes were greater in greens planted with cv. Pennncross ($P \leq 0.001$). However, the age of greens planted with the bentgrass cv. Pennncross was, on average, 12.6 years older than greens planted with SR1020 bentgrass ($P \leq 0.0001$).

In the factor analysis, eigenvalues for the first four factors were greater than one and accounted for 75% of the total variation for the soil variables (Table 3). The first factor accounted for 33.8% of the total variation in the soil variables, and had high positive factor loading on the 106- to 250-µm sand fraction grouping, moderate loadings for N and K, and negative loadings for the fraction groupings between 0.5 and 2 mm in diameter (Table 3). The second factor accounted for 17.9% of the total

variation for the soil variables and had moderate to high positive loadings for pH, plant-available P and K, bulk density, and OM. The third factor accounted for 13.8% of the total variation for the soil variables and had high positive loadings for percent sand and sand fraction between 250 and 500 µm in diameter and a high negative loading for the fraction between 53 and 106 µm. The fourth factor accounted for 9.5% of the total variation in the soil variables and had high positive loading for only the fraction greater than 2 mm. In regard to communalities (the percent of the variance of each variable accounted for by the factor analysis), the four-factor model explained nearly 80% or better the variance for percent sand and all sand fraction distribution groups (Table 3). However, the four-factor model only explained 53 to 68% of the variance for the soil factors, NO₃-N, plant-available P and K, pH, bulk density, and OM.

Factor analysis separated the set of highly correlated soil characteristic variables into four uncorrelated (orthogonal) factors. Using the factor scores associated with these four factors, a regression analysis was performed to model the populations of plant-parasitic nematodes from golf course putting greens based on the information in the soil characteristic variables. Each of the four factors was termed cpg1 (factor 1), cpg2 (factor 2), cpg3 (factor 3), and cpg4 (factor 4), where the respective factors were described previously. By using the factor scores associated with these factors in a regression analysis, multicollinearity problems inherent in the data are avoided. The resulting regression equation was obtained: $\text{Log population} = 2.36 + 0.208\text{cpg1} + 0.368\text{cpg2} - 0.225\text{cpg4}$ ($r^2 = 0.25$).

Fertilizer, insecticide, adjuvant, and coring or sand application expenditures did not correlate with total population densities of plant-parasitic nematodes (*data not shown*). Total plant-parasitic nematode populations increased with fungicide and herbicide expenditures ($r = 0.31$ and 0.43 , respectively). Numbers of *Cricone-mella* spp. increased with expenditures on adjuvant, fungicide, coring or sand application, and herbicide (Table 4). Populations of *Tylenchorhynchus* spp. increased only with increases in herbicide expenditures, whereas populations of *Paratrichodorus* spp. increased with all expenditures. In contrast, numbers of *Helicotylenchus* spp. were not correlated with any golf course expenditures.

DISCUSSION

Plant-parasitic nematodes were common in Oklahoma bentgrass putting greens. These results are consistent with those from other studies throughout the United States (4,8,11,13). *Cricone-moides* spp. were the most common nematodes found in these studies, as was the case in New

Table 2. Values and correlation coefficients for soil factors and nematode populations from bentgrass putting greens in Oklahoma

	Green age (years)	Factors (kg/ha)			
		NO ₃ -N ^a	Mehlich 3, P ^b	Mehlich 3, K ^b	Organic matter (%) ^c
Mean ^d	12.8	14.3	105.1	167.6	1.51
Minimum	0.5	3.4	12.5	43.1	0.07
Maximum	60	104.4	452.9	808.1	7.37
Correlation coefficients ^e					
<i>Cricone-mella</i>	0.24	0.27	0.36	0.33	0.36
<i>Tylenchorhynchus</i>	0.31	0.30	0.46	0.45	0.50
<i>Helicotylenchus</i>	0.33	NS	0.39	0.30	0.34

^a Determined by extraction with 1 M KCl solution and quantified by the cadmium reduction method; NO₃-N = nitrate-nitrogen.

^b Determined by extraction using Mehlich 3 solution; P = phosphorus, K = potassium.

^c Determined using a dry combustion carbon analyzer.

^d Nematodes were collected from 99 greens at 46 locations in the fall of 2000. Populations were determined for 100 cm³ of soil by sieving and centrifugal floatation and transformed prior to analysis $\log_{10}(X + 1)$.

^e r values significant at $P \leq 0.05$, NS = not significant.

Table 3. Rotated factor loadings and communalities of a four-factor model for physical and chemical soil properties of golf course putting greens and plant-parasitic nematode populations

Soil variable	Factor loadings				Communalities
	1	2	3	4	
pH	0.25	-0.75	0.01	0.19	0.66
Nitrogen	0.54	0.37	0.18	0.30	0.55
Phosphorus	0.26	0.62	-0.10	0.25	0.53
Potassium	0.50	0.53	-0.39	0.01	0.68
Organic matter	0.22	0.76	-0.11	0.01	0.64
Bulk density	0.20	0.73	-0.01	-0.19	0.61
Percent sand	0.07	-0.28	0.84	0.23	0.84
Fraction					
>2 mm	-0.21	-0.09	0.05	0.86	0.79
1-2 mm	-0.75	-0.09	-0.15	0.59	0.93
0.5-1 mm	-0.84	-0.15	0.10	0.34	0.85
250-500 µm	0.12	-0.003	0.81	-0.50	0.92
100-250 µm	0.90	0.11	-0.20	-0.09	0.87
50-100 µm	0.46	-0.05	-0.81	-0.06	0.87
Eigenvalues	4.38	2.34	1.79	1.23	...
Proportion of total S ²	33.8	17.9	13.8	9.5	...

York and Ohio putting greens (11,14). Nematode populations have been reported to vary among golf courses and greens from a single course (21). Nematode populations also varied considerably among the greens sampled in this study (*data not shown*). Greenhouse studies with the bentgrass cvs. Penncross, SR1020, and Pennlinks (70% of the cultivars sampled) indicated that population densities of *Tylenchorhynchus* spp. were not affected by turf grass cultivar (22). However, population densities of nematodes were different for the two prominent cultivars sampled in this study. This probably is due to the greater age of Penncross-planted greens, not differences in bentgrass cultivars.

Increasing nematode populations, OM, and plant-available P and K were correlated with greater green age. The correlation of these variables with age probably is a result of applications of fertilizers which contain P and K and plant growth which results in the accumulation of organic matter in greens over time. In Oklahoma, new greens usually are constructed of sand which may contain a few free-living but rarely plant-parasitic nematodes. The greater nematode populations found in older greens is probably a result of nematodes first becoming established and, subsequently, populations increasing over time.

Soil texture has been documented to influence plant-parasitic nematode populations. Numerous studies conducted with soils differing in texture have demonstrated the influence of soil physical characteristics on nematode population density (20,22). For example, *Rotylenchulus reniformis* populations were higher than *Meloidogyne incognita* for soils having less sand (6). The mechanism of influence was suggested to be not a result of nematode exclusion but rather one which favors populations that have a competitive advantage over another species (6). The uniform sand mixture contained in a putting greens does not permit studies of soil texture but rather soil particle size, bulk density, and chemical differences as possible factors in the differences in nematode populations. A limited influence of sand particle size on

nematode populations was found in this study. Although negative relationships were present between nematode population densities and fractions greater than 1 mm, this represents less than 10% of the total soil particles present in greens in this study and can be considered to have a limited effect on nematode populations.

The results of this study indicate that bulk density appears to have an adverse impact on nematode populations. Differences in bulk density could be attributed to the particle size distribution of sands used in green construction and an increase over time as a result of compaction. The mechanism by which bulk density adversely affects nematode population density in greens is not known, but it may be caused by a greater water-holding capacity of the soil, resulting in a decrease in nematode activity.

Factor analysis, a method to identify underlying factors that are responsible for the correlation among variables, can be used to identify relationships and make interpretations not resulting from univariate analysis. Because the factors are orthogonal to each other, it is possible to identify the independent effects each abiotic variable has on nematode populations. The loading of all soil factors with the exception of particle size on two factors suggests their importance in affecting nematode populations. However, communalities estimated the portion of variance from each variable that was explained by the four-factor model, and the communalities for these variables were low, which indicates that much of the variable's variance is not explained. In contrast, the particle size grouping for sand loaded on all factors; however, these loadings were very high and the communalities indicate that most of the variance was explained.

Golf courses vary greatly in the intensity of greens maintenance due to budgetary allowances. It has been suggested that the high irrigation, pesticide, and fertilizer inputs that bentgrass putting greens receive may reduce the detrimental effects of nematodes on turf grasses (11,18). The correlation between nematode densities and expenditures on herbicide and fungicide might support this suggestion, given

the assumption that the greater the financial expenditure the greater the intensity of greens maintenance and ability of the manager to react to the nematode damage. The correlation between $\text{NO}_3\text{-N}$ and nematode densities suggests that the higher $\text{NO}_3\text{-N}$ content in green soils is the result of greater N use by the greens manager to offset the effects of low-vigor greens containing high nematode populations. However, it is difficult to determine if management activities, such as fertilization, are in response to high nematode populations or attributed to high nematode populations, or if management activities possibly aid plants in supporting higher nematode populations without a reduction in turf grass quality.

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Table 4. Correlation coefficients (*r*) for golf course maintenance expenditures and plant-parasitic nematode population densities.

Genera ^a	Expenditures					Sand ^b
	Adjuvants	Fertilizer	Fungicide	Herbicide	Insecticide	
	Correlation coefficients ^c					
<i>Criconeimoides</i>	0.35	NS	0.40	0.51	NS	0.34
<i>Tylenchorhynchus</i>	NS	NS	NS	0.37	NS	NS
<i>Paratrichodorus</i>	0.51	0.51	0.43	0.36	0.36	0.47
<i>Helicotylenchus</i>	NS	NS	NS	NS	NS	NS

^a Nematodes were collected from 43 greens at 20 locations in the fall of 2000. Populations were determined for 100 cm³ of soil by sieving and centrifugal floatation and transformed prior to analysis $\log_{10}(X + 1)$.

^b Aeration prior to application of sand to the surface of the green.

^c *r* values significant at $P \leq 0.05$, NS = not significant.

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