Pathogenicity of *Tylenchorhynchus nudus* to Creeping Bentgrass and Annual Bluegrass

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ABSTRACT

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Greenhouse and growth chamber experiments were conducted to determine and compare the effects of *Tylenchorhynchus nudus* on the root growth of bentgrass (*Agrostis palustris*) and annual bluegrass (*Poa annua*), and to examine the relationship between *T. nudus* population levels and maximum root length of bentgrass and annual bluegrass. Root growth was suppressed by *T. nudus* on both bentgrass and annual bluegrass, but more so on bluegrass. Annual bluegrass produced longer roots than bentgrass both in the presence and in the absence of the nematode. Maximum root length was functionally related to *T. nudus* population levels on both grasses.

Additional keywords: stunt, turf

All species of turfgrasses are parasitized by nematodes, and nematodes may be a limiting factor in turfgrass growth (12,13). *Tylenchorhynchus* spp. are believed to be pathogenic to bentgrass (*Agrostis palustris* Huds.) and Kentucky bluegrass (*Poa pratensis* L.) in the midwestern United States (12,13) having been associated with symptoms of root injury that include suppressed growth, discoloration, lesions, and swelling.

Proof of nematode pathogenicity to turf is difficult, but poor turfgrass growth associated with large nematode population densities has been accepted as evidence of pathogenicity (16). Potted grass cultures inoculated with a monospecific culture of nematodes can be used to demonstrate pathogenicity (2–5,8,15,22, 26), but Koch's postulates are difficult to apply in the strictest sense because nematodes are obligate parasites, and symptoms of nematode damage are often indicated only by poor root development (16). Although pathogenicity studies have been done with some nematode species associated with turf, pathogenicity has not been demonstrated unequivocally with controlled experiments.

Taylor et al (24) sampled 26 putting greens from six golf courses in Illinois and determined that *Tylenchorhynchus* spp. were the most abundant plant-parasitic nematodes present. An unidentified *Tylenchorhynchus* sp. was recovered from all putting greens, with an average of 284 nematodes recovered per 125 g of soil. *Tylenchorhynchus claytoni* Steiner was recovered from 27% of bermudagrass (*Cynodon dactylon* (L.) Pers.) putting greens sampled in North Carolina, and the highest nematode density was 10,300 nematodes/500 cm^3 of soil. It was also recovered from 40% of bentgrass putting greens sampled, and the highest density was 7,900 nematodes/500 cm^3 of soil (11). *Tylenchorhynchus* spp. were recovered from 22% of putting greens sampled in an Ohio study (17).

Tylenchorhynchus nudus Allen frequently was associated with Kentucky bluegrass turf in South Dakota and is believed to have a role in summer decline of Kentucky bluegrass (20). Root and clipping weights of Kentucky bluegrass were reduced significantly by *T. nudus* (21). Troll and Tarjan (27) postulated that *Tylenchorhynchus* spp. are major factors in damaging bentgrass. *Pratylenchus penetrans* (Cobb) Filipjev & Schuurmans Stekhoven and *Tylenchorhynchus agri* Ferris, alone and in combination, inhibited root growth of bentgrass (19) but suppressed top growth only when present together. A study in Nebraska (22) associated species of *Helicotylenchus* and *Tylenchorhynchus* with unhealthy bluegrass but did not demonstrate pathogenicity of the nematodes.

A majority of golf course putting greens in Illinois are a mixture of bentgrass and annual bluegrass. The objectives of this study were to determine and compare the effects of *T. nudus* on root weight and root length and to show the relationship between *T. nudus* population levels and root length of bentgrass and annual bluegrass.

MATERIALS AND METHODS

A population of *T. nudus* obtained from a golf course putting green at the University of Illinois was cultured in the greenhouse on bentgrass (cv. Penncross) in 15-cm-diameter clay pots containing a 3:1 mixture of sand and soil. Nematodes were extracted for use as inoculum by the centrifugal-flotation method (6).

Greenhouse experiment. The four treatments used in the greenhouse experiment were nematodes and associated microorganisms, surface-disinfested nematodes, nematode-associated microorganisms without nematodes, and a nontreated control (no nematodes and no microorganisms). Each treatment was applied to both bentgrass and annual bluegrass. Nematodes were surface-disinfested with $HgCl_2$ (0.10 mg/ml) and streptomycin (1.0 mg/ml) (9). Surface-disinfested nematodes were placed onto potato-dextrose agar plates to demonstrate that they were axenic. Following extraction of nematodes from soil, microorganisms extracted with the nematodes were segregated by first allowing nematodes to settle to the bottom of a beaker for 60 min and then passing the supernatant through a 25-μm pore sieve to try to ensure removal of all nematodes from suspension. No nematodes were observed in the supernatant after sieving.

The experimental design was a 2 × 2 × 2 factorial with grass type, nematodes, and nematode-associated microorganisms as factors in a randomized complete block design. Pots in the greenhouse experiment were 9-cm diameter and contained 300 cm^3 of a sterile 3:1 sand-soil mix. The soil was series Watseka (sandy, mixed, mesic Aquic Hapludolls), and sand was purchased from a builder's supply company. Pots were seeded with approximately 2,350 viable seeds, and 9,600 nematodes were added to the surface of the soil 23 days after seeding. Dry root weight and final nematode populations for each pot were determined 68 days after seeding. Roots were collected by sieving, and nematodes were collected by the centrifugal-flotation method (6).

Growth chamber experiments. Two experiments with bentgrass and two with annual bluegrass were conducted in a growth chamber at 28 C with a 12-hr photoperiod. Each experiment employed a 2 × 2 factorial arrangement of treatments in a completely randomized design with six replications. Treatments were nematodes and associated microorganisms, surface-disinfested nematodes, nematode-associated microorganisms

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without nematodes, and a nontreated control (no nematodes and no microorganisms). Nematode inoculum level in one experiment on each grass was 240 nematodes/cm^3 of soil and 120 nematodes/cm^3 of soil in a second experiment on each grass.

These experiments used a modified version of the Cone-Tainer assay described previously (28). Cones in this study were 11.3-cm long by 1.5-cm diameter and contained 10 cm^3 of the 3:1 sand-soil mixture. Approximately eight seeds were placed into a cone and allowed to germinate. Seedlings were culled until each cone held four seedlings. Nematode inoculum was added to the surface of the soil 6 days after seeding, and root length was recorded 16 days after seeding. Root length was determined by pushing the column of soil and roots out of the cone, gently separating roots from soil, and measuring the maximum root length of each seedling. This measurement is referred to herein as root length.

Two replications of an experiment using Cone-Tainers were conducted to compare the effect of *T. nudus* on bentgrass and annual bluegrass. A 2 × 2 factorial arrangement of treatments included nematodes on bentgrass, bentgrass without nematodes, nematodes on annual bluegrass, and annual bluegrass without nematodes. Nematodes at a rate of 120 nematodes/cm^3 soil were added to the surface of the soil 6 days after seeding, and root length was recorded 16 days after seeding.

Relating nematode populations to root length. Two replications of a Cone-Tainer experiment were conducted to show the relationship between *T. nudus* population levels and root length on bentgrass, and two similar runs were conducted on annual bluegrass. Treatments were the initial population levels (0, 10, 20, 40, 80, and 120 nematodes/cm^3 of soil) of surface-disinfested *T. nudus* added to the surface of the soil. General linear models procedures (10) were used to fit least squares regression curves to the data sets.

Analysis of variance procedures and least squares regression procedures were performed on the cell means in each of the cone tests. Cell means were calculated as the mean root length of the four seedlings (subsamples) in each cone. All differences reported herein are significant at $P = 0.05$ unless otherwise specified.

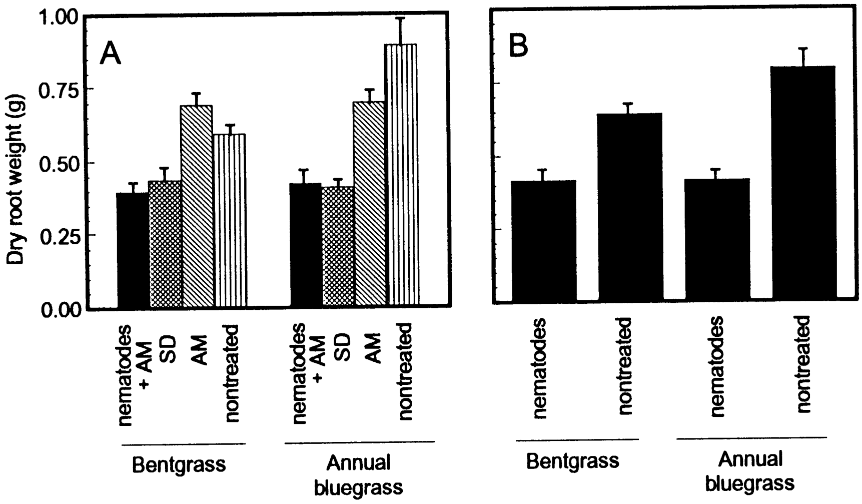

Fig. 1. Effect of *Tylenchorhynchus nudus* (surface-disinfested [SD] and not disinfested) and associated microorganisms (AM) on dry root weight of bentgrass and annual bluegrass in a greenhouse experiment. **(A)** Data were analyzed as a 2 × 2 × 2 factorial with type of grass, nematodes, and AM as factors. Type of grass and nematodes were significant ($P = 0.05$) factors, but AM was not. **(B)** Data were analyzed as a 2 × 2 factorial with type of grass and nematodes as factors. Type of grass and nematodes were significant ($P = 0.05$) factors and there was a significant ($P = 0.10$) grass × nematode interaction.

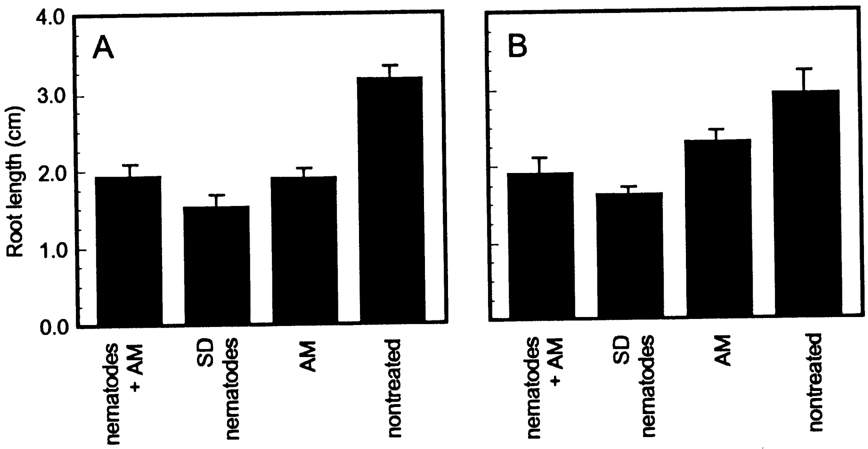

Fig. 2. Effect of *Tylenchorhynchus nudus* (surface-disinfested [SD] and not disinfested) and associated microorganisms (AM) on maximum root length of bentgrass grown in Cone-Tainers in growth chambers. Data were analyzed as a 2 × 2 factorial with nematodes and AM as factors. The effect of nematodes was significant ($P = 0.05$), and there was a significant nematode × AM interaction. **(A)** Experiment 1 with 2,400 nematodes. **(B)** Experiment 2 with 1,200 nematodes.

RESULTS

Greenhouse experiment. Factorial analysis of variance indicated that the microorganisms did not affect dry root weight and were not involved in any significant interactions so that factor was dropped from the model (Fig. 1A). Reanalysis of the data as a 2 × 2 factorial with grass type and nematode presence as the only factors indicated significant differences between treatments with nematodes and treatments without nematodes for both bentgrass and annual bluegrass (Fig. 1B). In the absence of nematodes, annual bluegrass produced more dry root weight than bentgrass, but the two grasses produced the same dry root weight in the presence of nematodes. There were no differences among the number of nematodes recovered from pots to which nematodes were applied. Surface-disinfestation had no effect on the viability of *T. nudus*. Nematode levels recovered from bentgrass at the end of the test were 1,584/100 cm^3 for nondisinfested nematodes and 1,631/100 cm^3 for surface-disinfested nematodes. The levels recovered from annual bluegrass were 1,583/100 cm^3 for nondisinfested nematodes and 1,538/100 cm^3 for surface-disinfested nematodes.

Growth chamber experiments. Factorial analysis of variance with relevant contrasts indicated significant differences in bentgrass root length (Fig. 2) in both experiments between treatments with and without nematodes. Both experiments also indicated significant differences in root length between the surface-disinfested nematode treatment and the nontreated control. In the first experiment (Fig. 2A), the treatment using nematode-associated microorganisms only (microorganisms associated with 240 nematodes/cm^3 of soil) suppressed root growth as much as the surface-disinfested nematodes. The surface-disinfested nematode treatment retarded root growth the same amount as the nematode plus associated microorganisms treatment. In the second experiment, microorganisms associated with 120 nematodes/cm^3 of soil (without nematodes) did not significantly affect root length, but root length in this treatment (nematode-associated microorganisms) was not significantly different from root length in the surface-disinfested nematode treatment. The effects of the nematode and the associated microorganisms were not additive.

Factorial analysis of variance with relevant contrasts for each experiment indicated significant differences between the treatments containing and not containing nematodes (Fig. 3). Both experiments indicated a significant difference between the surface-disinfested nematode treatment and the nontreated control. In the first experiment (Fig. 3A), the nematode-associated microorganisms (microorganisms associated with 240 nematodes/cm^3 of soil) reduced root length as much as the surface-disinfested nematodes, but the presence or absence of these microorganisms did not change the effect of the nematodes; the surface-disinfested nematode treatment suppressed root growth the same amount as the nematode plus associated microorganisms treatment. In the second experiment, microorganisms associated with 120 nematodes/cm^3 of soil did not affect root growth. The effects of the nematodes and the associated microorganisms were not additive.

Annual bluegrass produced a longer root system than bentgrass in both the presence and absence of nematodes (Fig. 4). Root length of bentgrass and annual bluegrass was affected by nematodes. The effect of *T. nudus* on bentgrass and annual bluegrass was statistically similar in one test (Fig. 4A) (indicated by no significant grass × nematode interaction in the factorial ANOVA) but statistically different in the replicate test with annual bluegrass being affected more severely than bentgrass (Fig. 4B).

Relating nematode populations to root length. Least squares regression models were fit to each data set, and in both runs the regression of bentgrass root length on nematode number was linear, and the error variance terms were similar. The method described by Neter et al (14) was used to test for homogeneity of slope and intercept values, and the slope and intercept terms for the two data sets were not significantly different. The data were then pooled, and a new regression was estimated that required both data sets to have the same slope and intercept values (Fig. 5).

Least squares regression models were fit to each annual bluegrass data set, and it was determined that second-order polynomial models described the data. The models were tested for homogeneity of intercept, linear coefficient, and quadratic coefficient values; the linear and quadratic coefficients were not significantly different, but the intercept values were different. The data were reanalyzed using a model that gave both data sets the same linear and quadratic terms but allowed different intercept values (Fig. 6).

DISCUSSION

T. nudus suppressed root growth of both bentgrass and annual bluegrass and therefore *T. nudus* must be accepted as a pathogen of these grasses. Most of the work presented in this study used maximum root length to estimate damage caused by *T. nudus*, whereas the work of others primarily used root weight of other grasses (1,7,21,23). Todd and Tisserat (25) used a turf quality rating (percent discoloration) to measure damage to bentgrass. For our growth chamber tests, root length was measured as the dependent variable. Because measurements were made on individual grass plants, measuring maximum root length was easy and accurate. Since the dry weight of a single plant's root system is very small, measuring root length of individual grass plants in millimeters may be more reliable than measuring dry root weight in micrograms.

Surface-disinfested nematodes were used in some tests to ensure that whatever damage occurred was caused by the nematodes rather than by other microorganisms associated with the nematodes. In these studies, nematode-associated microorganisms did not influence the damage caused by *T. nudus* to either grass type. Surface-disinfested nematodes were not included in the two Cone-Tainer experiments that had both grass types, since the purpose was to compare the effect of *T. nudus* on the two grasses, and it had already been demonstrated that the nematode-associated microor-

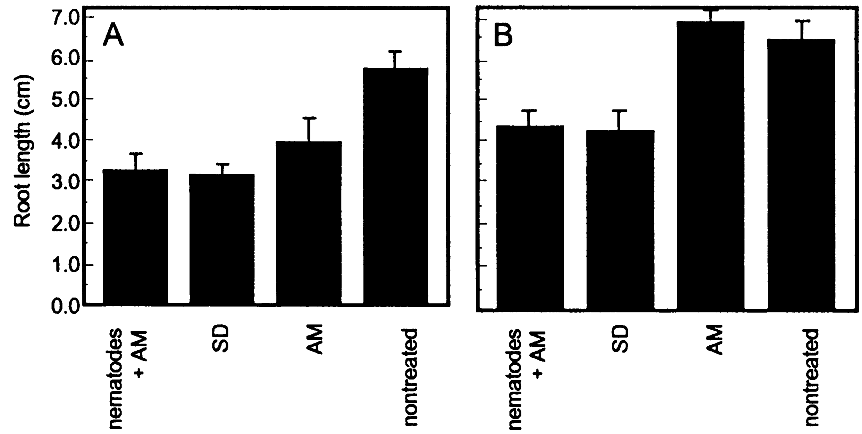

Fig. 3. Effect of *Tylenchorhynchus nudus* (surface-disinfested [SD] and not disinfested) and associated microorganisms (AM) on maximum root length of annual bluegrass grown in Cone-Tainers in growth chambers. Data were analyzed as a 2 × 2 factorial with nematodes and AM as factors. The effect of nematodes was significant ($P = 0.05$). **(A)** Experiment 1 with 2,400 nematodes. There was a significant nematode × AM interaction. **(B)** Experiment 2 with 1,200 nematodes.

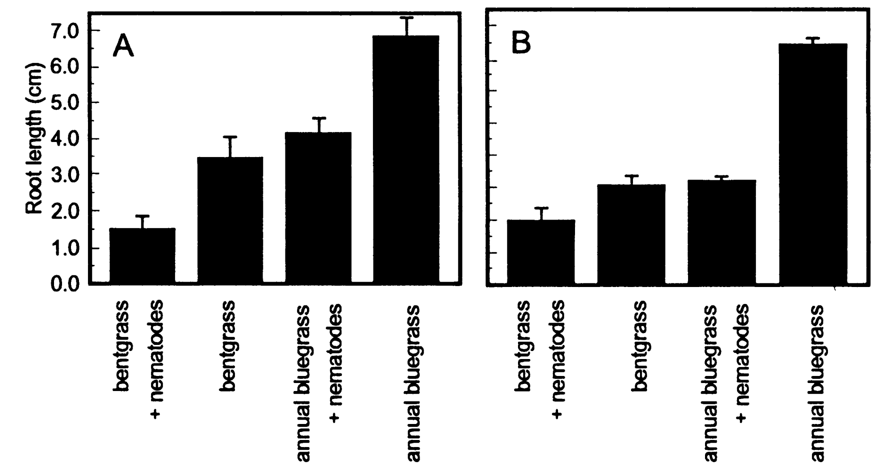

Fig. 4. Effect of *Tylenchorhynchus nudus* (not disinfested) on maximum root length of bentgrass and annual bluegrass grown in Cone-Tainers in growth chambers. Data were analyzed as a 2 × 2 factorial with nematodes and type of grass as factors. The effect of nematodes was significant ($P = 0.05$). **(A)** Run 1. **(B)** Run 2. There was a significant ($P = 0.05$) grass type × nematode interaction.

ganisms would not influence the effect of *T. nudus*. Although nematode-associated microorganisms did not affect damage caused by *T. nudus*, there is evidence that the associated microorganisms retarded root growth and that their concentration influenced their effect. The microorganisms associated with 240 nematodes/cm^3 soil significantly suppressed root growth of bentgrass and annual bluegrass, whereas the microorganisms associated with 120 nematodes/cm^3 soil did not significantly affect the root growth of either grass.

Creeping bentgrass and annual bluegrass usually coinhabit putting greens in Illinois. Although annual bluegrass produced longer roots and greater root weight than bentgrass whether nematodes were present or not, *T. nudus* may be more damaging to annual bluegrass.

The greenhouse test supported results from growth chamber experiments even though different dependent variables were used. It is reasonable that the two dependent variables, maximum root

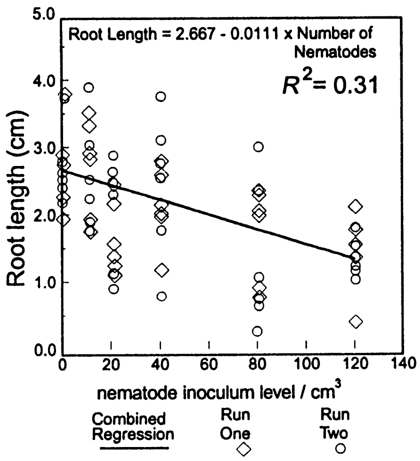

Fig. 5. Bentgrass root length. Regression of maximum root length on *Tylenchorhynchus nudus* inoculum levels on bentgrass grown in Cone-Tainers in a growth chamber at 28 C with a 12-hr photoperiod.

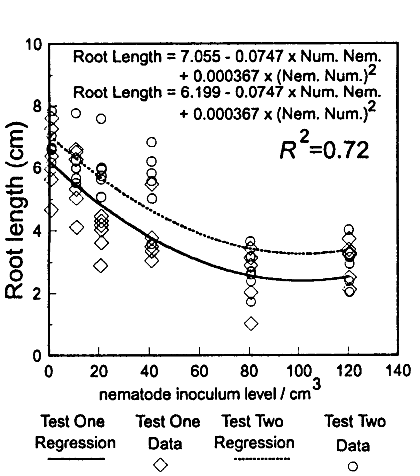

Fig. 6. Annual bluegrass root length. Regression of maximum root length on *Tylenchorhynchus nudus* inoculum levels on annual bluegrass grown in Cone-Tainers in a growth chamber at 28 C with a 12-hr photoperiod.

length and root weight, could be correlated and that a reduction in maximum root length might mean a reduction in root weight. This relation may not exist if a reduction in root length is accompanied by an increased production of secondary and tertiary roots, but this type of increased root production did not appear to have occurred in this study. Microorganisms associated with 32 nematodes/cm^3 of soil did not decrease root weight in the greenhouse test. This lack of effect was consistent with the Cone-Tainer tests in which the nematode-associated microorganism inoculum level was relatively low (microorganisms associated with 120 nematodes/cm^3 soil).

In this study, the regression equation relating nematode numbers to root length on bentgrass was linear, which shows that the effect of *T. nudus* on bentgrass is additive with inoculum levels between 0 and 120 nematodes/cm^3 soil. There was much variation in these data, resulting in a low multiple correlation coefficient ($R^2 = 0.31$). The regression was significant and there was no pattern to a plot of the residual values indicating that the model is appropriate.

Seinhorst (18) stated that below a certain nematode density (the tolerance limit) the yield should not be affected. Furthermore, a graph relating nematode population levels and yield will assume a characteristic shape with two asymptotes: one represents the yield when nematode populations are below the tolerance limit, and the other represents a minimum yield that remains unaffected even at the highest nematode densities. The regressions presented herein do not show this type of relationship because the data were used to determine descriptive models rather than forcing the data to fit a preconceived paradigm. If the data were less variable or if a different range of nematode populations had been used, perhaps a higher order regression which is more consistent with Seinhorst's (18) predictions could be justified. The inoculum level needed to reach Seinhorst's "minimum yield" may have been reached in the factorial growth chamber experiments on bentgrass where inoculum levels of 120 and 240 nematodes/cm^3 appear to have caused the same amount of damage. This was not observed on annual bluegrass.

The regression equations relating nematode numbers to root length on annual bluegrass were quadratic in nature. The two equations had the same linear and quadratic components, but they had different intercept values. Because the curves have the same shape, it may be concluded that this regression relationship is constant although the intercept values may vary slightly. The nature of this relationship indicates that as the number of nematodes increases, the effect of each additional nematode will be slightly less than the effect of the nematodes that preceded it for inoculum levels between 0 and 120 nematodes/cm^3 soil. There was less variation in these data ($R^2 = 0.72$) than in the data for the regression on bentgrass ($R^2 = 0.31$). There was no pattern in a plot of residual values that supports the contention that quadratic models adequately describe the data.

This study demonstrated that *T. nudus* can damage both bentgrass and annual bluegrass. It is unknown how environmental and nutritional stresses or inter-nematode species competition would affect the pathogenicity of *T. nudus*. Additional studies will explore the development of a damage threshold in naturally infested golf course putting greens. Damage thresholds are needed before modification of current management strategies can be justified.

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