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Root Growth of Bentgrass and Annual Bluegrass as Influenced by Coinfection with *Tylenchorhynchus nudus* and *Magnaporthe poae*[1]

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Abstract: A study was conducted in growth chambers to examine main factor and interaction effects of *Tylenchorhynchus nudus* and *Magnaporthe poae* on creeping bentgrass and annual bluegrass at 24, 28, and 30 C. A 2 × 2 factorial arrangement of treatments was employed with presence and absence of *T. nudus* and *M. poae* as factors with each temperature run separately for 14 or 18 days. *Tylenchorhynchus nudus* decreased bentgrass and annual bluegrass root length at all three temperatures. *Magnaporthe poae* had no effect on bentgrass root length at 24 C, increased root length at 28 C, and suppressed root growth at 30 C. *Magnaporthe poae* had no effect on annual bluegrass root length at 24 and 28 C but suppressed root growth at 30 C. A significant interaction between *M. poae* and *T. nudus* occurred only on bentgrass at 28 C and 30 C; at these two temperatures, *M. poae* did not act independently of *T. nudus*.

Key words: *Agrostis palustris*, annual bluegrass, bentgrass, disease complex, grass, interaction, *Magnaporthe poae*, nematode, *Poa annua*, turf, *Tylenchorhynchus nudus*.

Complex ecological relationships exist among organisms in nature, and numerous interactions between fungi and nematodes have been documented (8,20). Root-knot nematodes (*Meloidogyne* spp.) can increase the severity of diseases caused by weakly pathogenic fungi by predisposing plants to fungal infection (21). *Tylenchorhynchus dubius* (Butschli) Filipjev increases damage on *Poa pratensis* L. caused by *Fusarium roseum* Link:Fr. (28). Leaves of Kentucky bluegrass coinfected by *Panagrolaimus* sp. and *Helminthosporium vagans* Drechs. typically are damaged more than leaves infected solely by the fungus (18). Vesicular-arbuscular (VA) mycorrhizae have been reported to improve plant vigor, decrease disease severity, and suppress nematode reproduction (4,9,11,22, 25). Diederichs (2) reported that *Gigaspora margarita* Becker & Hall and *Glomus manihotis* Howeler, Sieverding & Schenck significantly improved the growth of chickpea (*Cicer arietinum* L.) and suppressed *Meloidogyne javanica* (Treub) Chitwood reproduction.

Magnaporthe poae Landschoot & Jackson, an ectotrophic parasite, has been recovered from roots of bentgrass (*Agrostis palustris* Huds.) and annual bluegrass (*Poa annua* L.) (12) and has been implicated as the cause of summer patch disease (24). *Tylenchorhynchus nudus* Allen, an ectoparasite, is pathogenic to bentgrass and annual bluegrass (1). Since *T. nudus* and *M. poae* can compete for niches on turf roots, a biological interrelationship (and a statistical interaction between their effects) may be expected. The objective of this study was to characterize and quantify the main factor and interaction effects of *T. nudus* and *M. poae* on the root length of creeping bentgrass and annual bluegrass to determine if these factors act independently of each other.

Materials and Methods

A population of *T. nudus* obtained from a golf course putting green at the University of Illinois, Urbana, Illinois, was cultured in the greenhouse on bentgrass (cv. Penncross) in 15-cm-diameter clay pots

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containing a 3:1 mixture of sand from a building supply company and soil from an agricultural field (series Watseka [sandy, mixed, mesic Aquic Hapludolls]; 2% organic matter). Nematodes were extracted by the centrifugal-floatation method (10) for use as inoculum. All nematodes were surface disinfested with $HgCl_2$ and streptomycin (13) to reduce populations of contaminating organisms associated with the nematodes.

Magnaporthe poae (isolate WF841) was isolated from *P. annua* roots from a golf course in Skokie, Illinois. The fungus was grown for 5 weeks on twice autoclaved millet seeds (*Panicum miliaceum* L.). Colonized seeds were spread into shallow pans covered with paper and air dried for 2 days in a laminar flow hood. Seeds were stored in the dark at approximately 24 C until used. Inoculum was stored less than 3 months, and whole seeds were used as inoculum. Twenty randomly selected colonized seeds were placed on potato dextrose agar and incubated for 7 days to determine viability of *M. poae* and purity of inoculum. *Magnaporthe poae* was recovered from 95% of the seeds.

Separate experiments were conducted in controlled environment chambers with a 12-hour photoperiod (40 $\mu E \cdot m^{-2} \cdot s^{-1}$) and constant temperatures of 24, 28, or 30 C ($\pm$0.5). A 2 $\times$ 2 factorial arrangement of treatments in a completely randomized design with six replicates included *T. nudus* inoculum levels of 0 and 60 nematodes/cm^3 of soil and *M. poae* inoculum levels of two colonized millet seeds/10 cm^3 of soil and two uncolonized, sterilized millet seeds.

Experiments utilized cone-shaped containers (30) called conetainers (Conetainer Corp., Canby, OR) which were 11.3 cm long with a diameter of 1.5 cm and contained 10 cm^3 of soil. Grass seeds were placed on the soil surface, and the conetainers were covered with moistened cheesecloth until seeds germinated. Seedlings were thinned to four per conetainer. For tests conducted at 24 and 28 C, nematode and fungus inoculum was added 6 days after seeding. For tests conducted at 30 C, soil was infested 5 days after seeding. Root length was determined at 18 days after seeding in trials conducted at 24 and 28 C and 14 days after seeding in trials conducted at 30 C. Roots were gently separated from soil, and the maximum length of the root system of each plant was measured. This measurement is referred to herein as root length. Neither discoloration of roots nor foliar symptoms of damage occurred during these tests. Root weights were not determined because the root systems were very small with little difference in dry weight.

Two runs of the experiment were conducted at each temperature for each grass species. Tests at the three temperatures were done separately. An analysis, which combined data from similar tests at each temperature (5), was performed with SAS general linear models procedures (14) to identify main factors and interactions whose average effect in repeated experiments was stable and significant. In the combined analysis of variance, the factor identifying the two runs for each grass at each temperature was treated as a random effect. Analysis of variance procedures and single degree of freedom contrasts (individual treatment comparisons) were performed on the cell means for each test. Cell means were calculated as the mean root length of the four seedlings (subsamples) in each conetainer.

The 2 $\times$ 2 factorial arrangement of treatments employed in these experiments allows analysis of main factor effects and of statistical interaction between factors. When describing treatment effects, "*T. nudus*" and "*M. poae*" refer to main factor effects in the factorial analysis of variance while "*T. nudus* alone" and "*M. poae* alone" (or similar statements) refer to individual treatments. In this paper, the term interaction refers exclusively to a significant statistical interaction (P = 0.05 unless otherwise specified).

Results and Discussion

Root length of both bentgrass and annual bluegrass was reduced significantly by

T. nudus (main factor effect) at all three temperatures tested (24, 28, and 30 C) (Table 1, Fig. 1). On both grasses, *M. poae* (main factor effect) suppressed root growth only at 30 C (Table 1, Fig. 1). At all three temperatures on both grasses, plants inoculated with both *T. nudus* and *M. poae* had significantly shorter roots than plants inoculated with only *M. poae* (individual treatment comparisons), and plants inoculated with *T. nudus* alone had significantly shorter roots than the uninoculated control (individual treatment comparisons). On both bentgrass and annual bluegrass at 30 C, plants inoculated with *M. poae* alone had significantly shorter roots than the uninoculated control (individual treatment comparisons).

Summer patch disease on bentgrass and annual bluegrass in Illinois occurs when summer temperatures are high. This high temperature effect has been documented on *Poa pratensis* (24) and by our research showing that annual bluegrass and bentgrass root length was reduced at 30 C by *M. poae*. *Magnaporthe poae* had no significant effect on bentgrass or annual bluegrass at 24 C and no effect on annual bluegrass at 28 C. Bentgrass root length increased significantly when colonized by *M. poae* at 28 C, but was reduced significantly at 30 C.

The term "interaction" has been used both qualitatively and quantitatively in the nematology literature (23,29). Nonstatistical, qualitative interactions should be called interrelationships to distinguish them from statistical, quantitative interactions (19,27). To determine if statistical interactions exist, a factorial experimental design must be used. If a factorial design is ignored in the analysis, a significant interaction effect cannot be established; a separation of means procedure establishes treatment differences but not interactions (26). Previous nematological research has described pathogen–pathogen interactions in both qualitative (18) and quantitative (2,3,15,16,28) terms. Much of the previous work describing quantitative interactions employed factorial arrangements of treatments (2,3,15,28) but failed to analyze the data with factorial analyses of variance.

A statistical interaction effect in a 2 × 2 factorial experiment is defined as the difference between a treatment mean and the value that would be expected if the two factors had additive effects (17). A nonzero interaction effect indicates that the factors tested were not acting independently. The factorial arrangement of treatments employed in these tests allows discussion of main-factor effects or of the individual treatment effect of *T. nudus* alone, *M. poae* alone, and *T. nudus* plus *M. poae* on bentgrass and annual bluegrass. The statistical interaction of *T. nudus* and *M. poae* also may be discussed.

On bentgrass, *M. poae* was involved in a significant interaction with *T. nudus* (main factor interaction) at 28 and 30 C. At 28 C, *M. poae* increased bentgrass root length in the absence of *T. nudus* but had no effect in the presence of *T. nudus*. (individual treatment comparison). At 30 C, *M. poae* decreased bentgrass root length in the absence of *T. nudus* but had no effect in the presence of *T. nudus* (individual treatment comparison). *Magnaporthe poae* was not involved in a significant interaction with *T. nudus* (main factor interaction) at 24 C on either grass or at 28 or 30 C on annual bluegrass. When a significant interaction between *T. nudus* and *M. poae* was indicated, the effect of *M. poae* on root length

TABLE 1. Summaries of combined analyses of variance for bentgrass and annual bluegrass at 24, 28, and 30 C.

Source	Level of significance (P-value)		
	24	28	30
Bentgrass			
Run	.7043	.1651	.7872
T. nudus	.0002	.0001	.0001
M. poae	.2242	.6207	.0002
T. nudus * *M. poae*	.2210	.0239	.0008
Annual Bluegrass			
Run	.8379	.2637	.5204
T. nudus	.0001	.0001	.0001
M. poae	.4187	.5353	.0404
T. nudus * *M. poae*	.3711	.1881	.1158

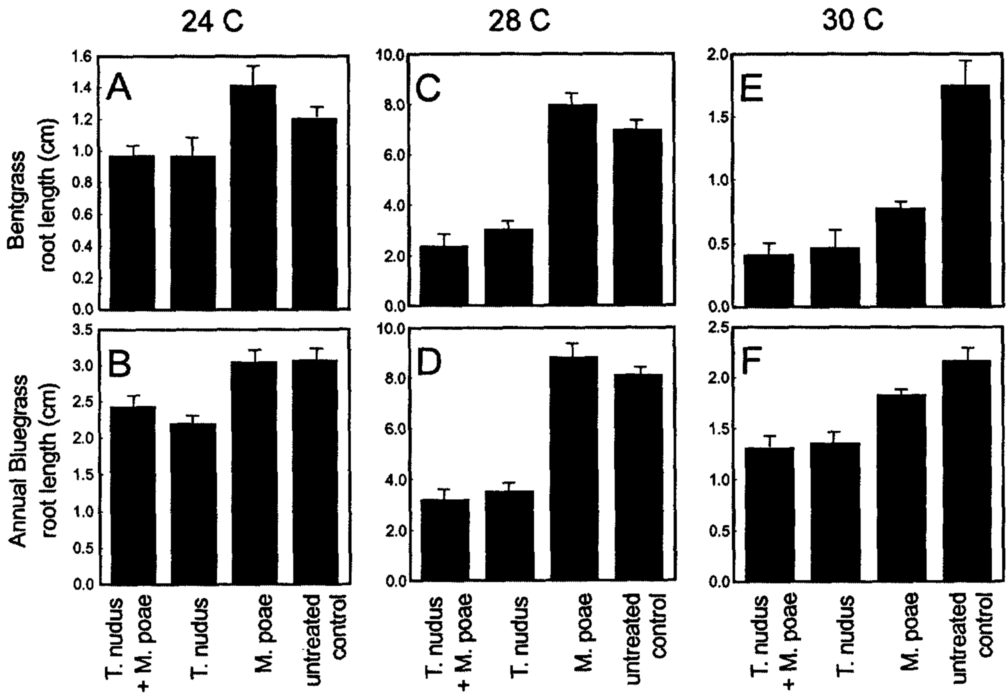

FIG. 1. Effect of *Tylenchorhynchus nudus*, *Magnaporthe poae*, and both on root length of grass. Vertical bars represent one standard error of the mean. A) Bentgrass at 24 C. B) Annual bluegrass at 24 C. C) Bentgrass at 28 C. D) Annual bluegrass at 28 C. E) Bentgrass at 30 C. F) Annual bluegrass at 30 C.

was different in the presence of *T. nudus* than in the absence of *T. nudus*: *M. poae* had no effect if *T. nudus* was present. The effect of *M. poae* in the absence of *T. nudus* depended on the type of grass and the temperature.

When a significant interaction between *T. nudus* and *M. poae* occurred, it was a negative interaction. A negative interaction is one in which the combined effect of the factors is less than the sum of the effect of each factor alone (29). Traditionally, this has been called antagonism, but Wallace (29) suggests that negative interaction is an adequate term, which is less likely to carry unintended connotations. A more thorough understanding of the interaction would be gained by including multiple levels of each factor (6,7). Additional research is needed to determine the longer-term effects of this interaction, but this study provides evidence that these turfgrass pathogens do not act independently of each other.

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