Pathogenicity and Interaction of Three Nematode Species on Six Bermudagrasses[1]

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Abstract: The ring nematode (*Criconemoides ornatus*), stunt nematode (*Tylenchorhynchus martini*), and sting nematode (*Belonolaimus longicaudatus*) reproduced readily on six bermudagrasses (Common, 'U-3', 'Tufcote', 'Continental', 'Tiffine', and 'Tifdwarf'). Populations of a single nematode species influenced the population development of a second and third parasitic nematode species on a particular host plant. Activity of most nematodes adversely affected reproduction of other nematode species in mixed cultures. Generally, the number of fibrous roots produced by plants decreased as the number of nematode species in the treatments increased. Tifdwarf bermudagrass appeared to be more tolerant to *C. ornatus* and *T. martini* than other grasses tested. *Key Words:* Interaction, Pathogenicity, *Criconemoides, Tylenchorhynchus, Belonolaimus*, Bermudagrass.

Several reports have implicated species of *Criconemoides Tylenchorhynchus,* and *Belonolaimus* nematodes causing severe injury to turf grasses of home and industrial lawns, turf nurseries and golf course greens (5, 6, 15, 16, 17, 20, 22, 23, 24). *C. ornatus* Raski was found in Georgia (18), Florida (18, 25), and Alabama (25) on several agronomic crops and also occurs frequently in turf producing areas in the Southeastern states (unpublished data). Troll and Tarjan (26) often found large numbers of stunt nematodes, *Tylenchorhynchus* spp., in soil samples from golf courses in Rhode Island. In Georgia, Powell (17) reported that turf infected with *T. maximus* showed a marked improvement when treated with a nematicide. *T. martini* Fielding was reported on sugarcane (1) and rice (8) and is commonly found in turf surveys (unpublished data). *B. longicaudatus* Rau was associated with injury to roots of bermudagrasses in Florida (12, 15), South Carolina (10, 19), and Georgia (19). The probability of only one plant-parasitic nematode being present in a field infestation is extremely low (14). Although some nematode species interaction studies on agronomic crops and cranberry have been reported (2, 3, 4, 7, 12, 13, 21), none have appeared showing the single and combined effects of *C. ornatus, T. martini* and *B. longicaudatus* on turf. The present study was designed to characterize the interactions of these nematode species upon selected bermudagrasses.

MATERIALS AND METHODS

A split-plot statistical design was chosen in which whole plots were the bermudagrass varieties and subplots were the nematode inoculum combinations. Bermudagrasses used were: (i) *Cynodon dactylon* (L) Pers. ('Common'); (ii) three varieties within *C. dactylon* ('U-3', 'Tufcote', and 'Continental'), and (iii) two varieties derived from *C. dactylon* × *C. transvaalensis* ('Tiffine' and 'Tifdwarf'). Nematode-free stocks were obtained by rooting aerial cuttings of stolons from each grass in 5-cm clay pots filled with a 3 : 1 (v/v) mixture of steamed Tifton sandy loam and builder's sand sieved through a 0.64-cm screen. Stolons were held in the greenhouse under moist conditions for 3 weeks to promote root development. Temperature was maintained at 28–35 C.

Received for publication 15 May 1969.

[1] Cooperative investigations of Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture and the University of Georgia College of Agriculture Experiment Station, Georgia Coastal Plain Station, Tifton. Journal Series Paper No. 374.

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TABLE 1. Clipping and root weights of six bermudagrasses 155 days after inoculation with *Criconemoides ornatus, Belonolaimus longicaudatus,* and *Tylenchorhynchus martini* singly and in combination.

	Treatments							
Variety	Check	C.o.[a]	B.l.	T.m.	C.o. + B.l.	C.o. + T.m.	B.l. + T.m.	C.o. + B.l. + T.m.
				Mean clipping weights (g)[b]				
Common	186.3	161.8	177.6	161.3	162.3	156.1*[c]	173.8	151.5*
U-3	168.6	144.8	166.3	163.8	145.0	116.8*	142.8	133.5*
Continental	170.3	168.5	173.3	151.5	170.5	166.1	178.0	184.8
Tufcote	208.6	168.8*	165.1*	146.1*	174.0*	159.6*	165.3*	168.1*
Tiffine	177.5	153.0	157.1	164.0	176.8	166.3	135.6*	129.5*
Tifdwarf	130.6	151.0	119.6	133.5	127.8	128.0	124.8	131.5
				Mean root weights (g)[d]				
Common	109.0	90.6	85.0	73.0*[c]	98.0	70.6*	85.0	61.6*
U-3	109.6	86.0	99.3	87.3	86.6	69.0*	73.6*	66.3*
Continental	96.6	84.3	68.6	61.0*	54.6*	63.0*	65.0*	45.0*
Tufcote	123.0	97.0	95.0	84.0*	83.0*	89.6*	80.3*	88.3*
Tiffine	106.0	78.0	74.0*	68.0*	74.6*	67.6*	44.6*	51.3*
Tifdwarf	78.0	64.3	62.0	51.3	48.3*	63.6	61.6	56.3

[a] C.o., B.l., and T.m. = *C. ornatus, B. longicaudatus,* and *T. martini,* respectively.
[b] LSD for two treatment means in same variety: .05 = 27.7.
[c] * = Difference from treatment check significant at .05 level.
[d] LSD for two treatment means in same variety: .05 = 28.8.

Nematodes used were: (i) ring (*C. ornatus*), (ii) sting (*B. longicaudatus*), and (iii) stunt (*T. martini*). They were handpicked from soil samples from turf grasses and increased for stock cultures in the greenhouse on 'Tifgreen' bermudagrass, millet (*Pennisetum typhoideum*), and corn (*Zea mays*), respectively. Inocula were obtained from stock cultures using Jenkin's centrifugal-flotation method (11) and were surface-disinfested 30 min in 0.001% 8-hydroxyquinolin sulfate and rinsed with tap water. Inocula were calibrated by counting nematodes from 1-ml aliquants replicated six times. Approximate numbers of nematodes were measured with a pipette from water suspensions of inocula. Inoculum levels were approximately 1000, 350, and 1800 per pot of ring, sting, and stunt, respectively. A lower level of sting nematodes was used because of an insufficient amount of inoculum available.

Nematode treatments were as follows: (i) control-no nematodes; (ii) *C. ornatus* alone; (iii) *B. longicaudatus* alone; (iv) *T. martini* alone; (v) *C. ornatus* + *B. longicaudatus*; (vi) *C. ornatus* + *T. martini*; (vii) *B. longicaudatus* + *T. martini* and (viii) *C. ornatus* + *B. longicaudatus* + *T. martini.* Each treatment was replicated three times.

After 15.2-cm clay pots were filled with the soil-sand mixture, measured quantities of calibrated nematode suspensions were pipetted into (5 cm × 5 cm) depressions in the potted soil. Plants, selected for uniformity were transferred with pot ball intact from 5-cm clay pots, into the depressions, and soil was gently washed in around the roots. A complete nutrient solution (700 g of a commercial fertilizer, VHPF,®[3] 123 g of KNO_3, and 227 g of $MgSO_4$ in 83.27 liters

[3] Miller Chemical Company, Baltimore, Maryland. VHPF contains 6% nitrogen, 25% available phosphoric acid, 15% potash and minor elements. Mention of trademark name or proprietary product does not constitute a guarantee or warranty of the product by the USDA, or recommendation of it to the exclusion of other products that may be suitable.

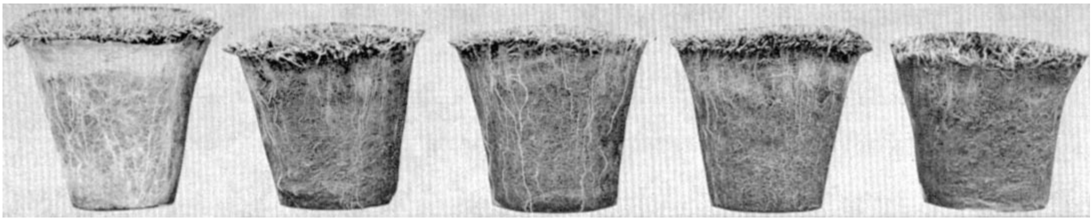

FIG. 1. Effect of *C. ornatus, B. longicaudatus,* and *T. martini* on root growth of Tiffine bermudagrass. Left to right—Control, *C. ornatus, B. longicaudatus, T. martini,* and three species combined.

of tap water) was added 100 ml per pot each week for two weeks and 100 ml per pot biweekly thereafter until the experiment was terminated 155 days after inoculation. Tap water was applied as needed. Grasses were clipped to uniform heights and clipping weights recorded four times during the experiment.

At the conclusion of the experiment each plant was removed from the pots, and roots were submerged and washed thoroughly in six liters of water, blotted, and weighed. Water containing soil and root washings was roiled vigorously, and nematodes separated from a liter aliquant by the centrifugal-flotation method. Samples were then placed in calibrated counting dishes and nematodes in 1/10 the total area were counted. The counts were multiplied by 60 to give a population estimate for each pot.

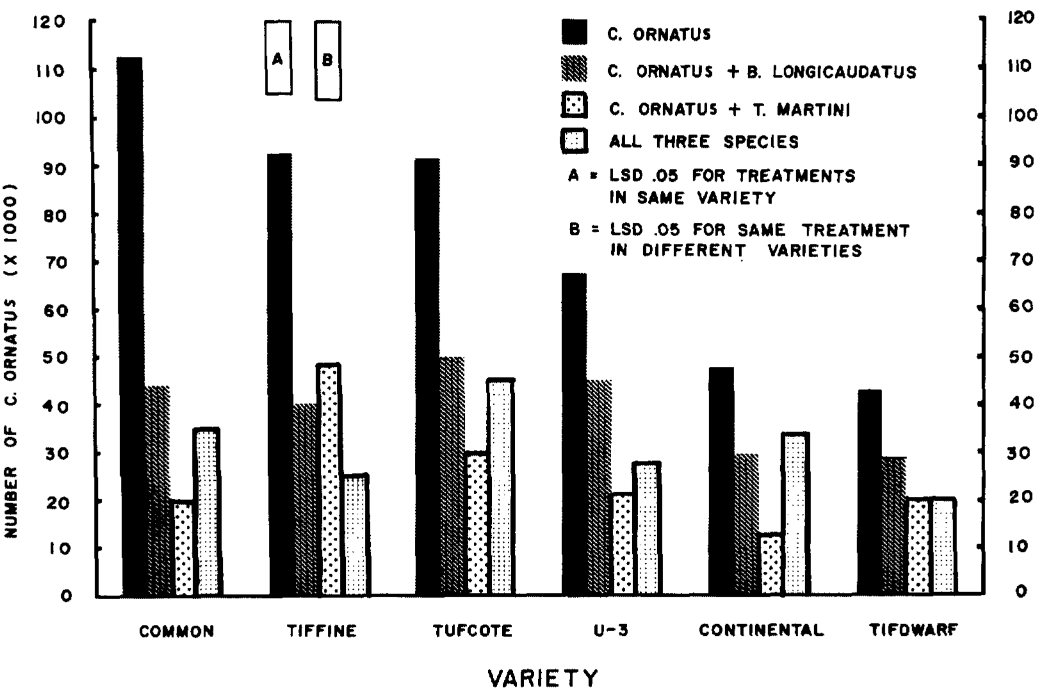

FIG. 2. Population of *C. ornatus* for each of six varieties of bermudagrass after 155 days with *C. ornatus, B. longicaudatus,* and *T. martini* singly and combined.

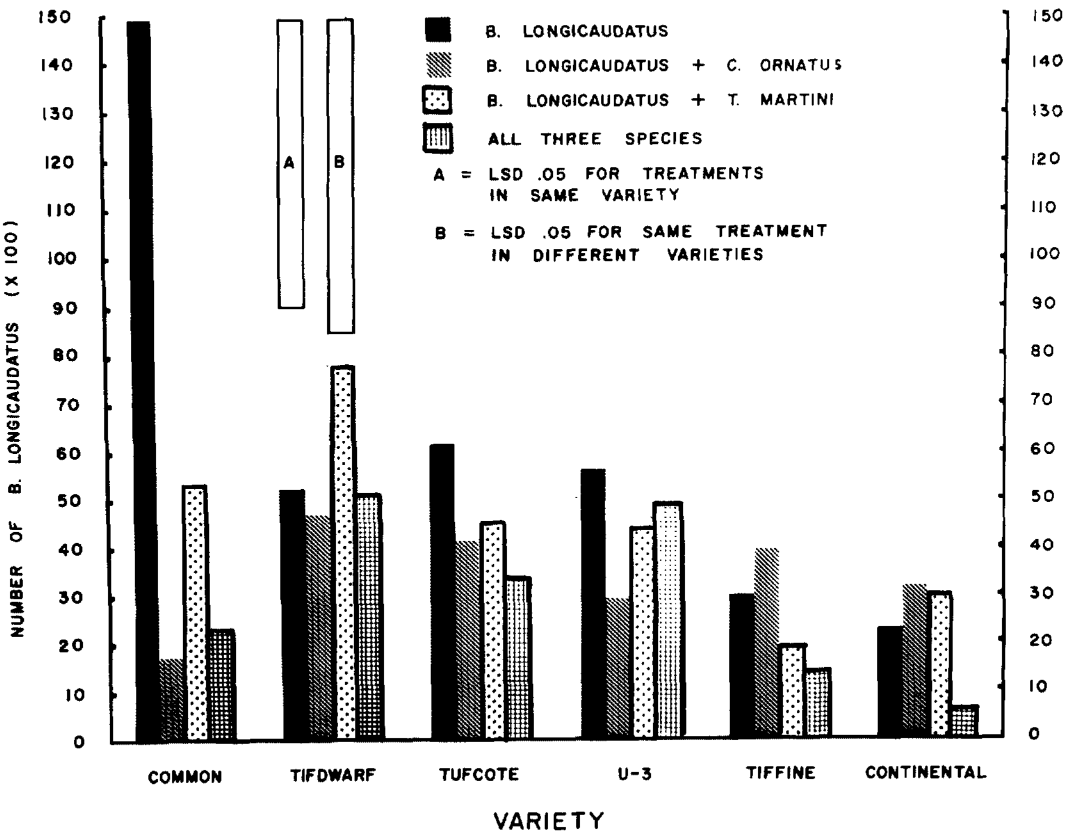

FIG. 3. Population of *B. longicaudatus* for each of six varieties of bermudagrass after 155 days with *B. longicaudatus*, *C. ornatus*, and *T. martini* singly and combined.

RESULTS AND DISCUSSION

Most plants inoculated with nematodes showed less growth than control plants (Table 1). Varietal differences were evident in fresh root and clipping weights. This varietal difference may be attributed partly to morphological variability between varieties and also to the effects of the nematodes feeding on roots of these plants. The variety vs. nematode treatment interaction was not significant in terms of fresh root weight, demonstrating that each variety responded similarly to nematode attack.

Roots of plants infested with *C. ornatus* usually weighed less than those from nematode-free soil but were not statistically different (Table 1). Visual symptoms of nematode attack were generally more apparent on roots inoculated with single nematode species than root weight differences indicate. Roots of control plants in all cases were more dense and fibrous than those of inoculated plants. Generally, the number of fibrous roots decreased as number of nematode species and total number of nematodes in the treatments increased (Fig. 1).

All bermudagrasses supported high populations of *C. ornatus*, *B. longicaudatus*, and *T. martini*. *C. ornatus* were greatly reduced at the termination of the experiment if other

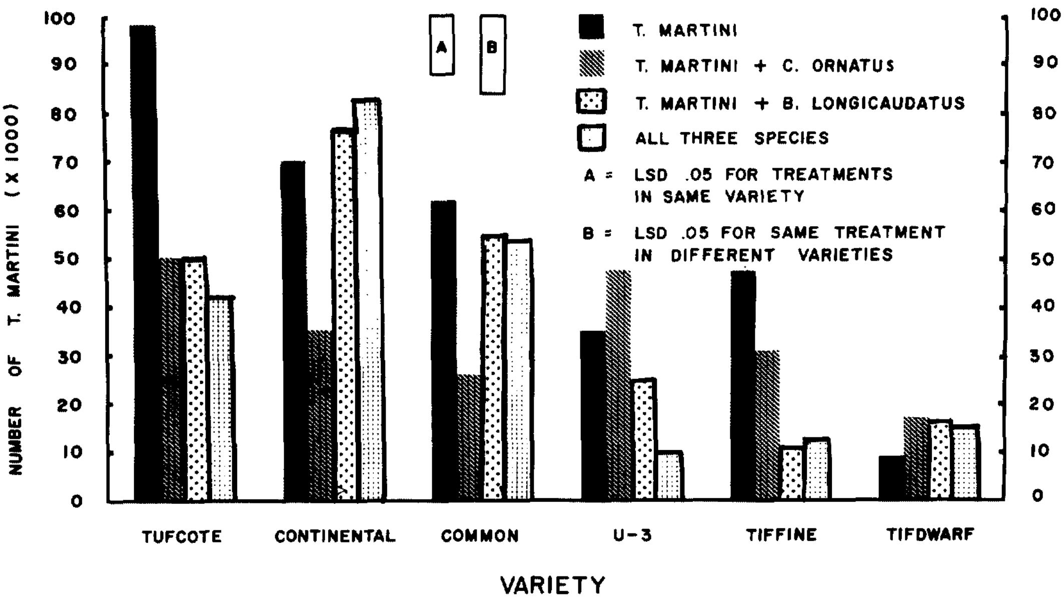

FIG. 4. Population of *T. martini* for each of six varieties of bermudagrass after 155 days with *T. martini*, *C. ornatus*, and *B. longicaudatus* singly and combined.

species were also present on most grasses. *B. longicaudatus* in combination with *C. ornatus* suppressed numbers of the latter on most grasses but less than *T. martini* in combination with *C. ornatus* (Fig. 2). The only significant suppressions noted in *B. longicaudatus* population levels were on 'Common' in the 2- and 3-species treatment combinations (Fig. 3). *C. ornatus*, *B. longicaudatus*, and a combination of both species significantly suppressed *T. martini* populations on 'Tufcote' and 'Tiffine'; however, no nematode combination adversely affected *T. martini* on 'Tifdwarf' (Fig. 4).

Generally, the activity of most nematodes adversely affected reproduction of other nematode species in mixed cultures. In most cases populations of *C. ornatus* and *T. martini* were suppressed more than *B. longicaudatus* by activity of other nematodes, indicating *B. longicaudatus* may be a better competitor than *C. ornatus* or *T. martini*.

C. ornatus or *T. martini* reproduction was least on 'Tifdwarf'. Either alone or with *B. longicaudatus* these nematodes did not significantly decrease clipping yield; and only *C. ornatus* + *B. longicaudatus* measurably reduced root weight of that variety. These observations suggest 'Tifdwarf' may be more tolerant to *C. ornatus* and *T. martini* than the other grasses in this experiment.

B. longicaudatus, *C. ornatus*, and *T. martini* are ectoparasites that feed on roots in all stages of development, but usually prefer younger, succulent roots. These nematodes probably damage roots by destroying cells, injecting digestive secretions, and removing cellular contents. They feed on lateral roots as soon as they are formed, causing further root pruning, decreased water absorption and mineral uptake, and depressed plant growth. Nematodes feeding at root tips arrest root elongation and result in severe necrosis and root pruning. This would account for the extensive reduction of fibrous roots of depressed plants grown in infested soil. Consequently, functional root surface area and nematode food supply and feeding sites would be reduced. It is logical to assume that the root damage caused by *C. ornatus*, *T. martini*, or *B. longicaudatus* may be unfavorable for other nematodes and adversely affect reproduction.

Result presented include a demonstrated interaction between 2- and 3-way mixtures of plant-parasitic nematodes on the same host plant.

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