The Role of Tylenchorhynchus dubius in the Development of Fusarium Blight of Merion Kentucky Bluegrass

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ABSTRACT

The possible role of *Tylenchorhynchus dubius*, a stylet nematode, in the development of Fusarium blight of turfgrasses on *Poa pratensis* 'Merion' was investigated. These studies suggest an interaction between the fungus *Fusarium roseum* and the stunt nematode *T. dubius* in development of symptoms associated with Fusarium blight of turfgrasses. *T. dubius* appeared to be the dominant pathogen in this relationship. The possibility of controlling the disease with nematicides and the effect of benomyl, a known control of Fusarium blight, on *T. dubius* are discussed.

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Fusarium blight of turfgrass incited by *Fusarium roseum* (Lk.) emend. Snyd. & Hans. f. sp. *cerealis* and *F. tricinctum* (Cda.) Snyd. & Hans. f. sp. *poae* was initially characterized by Couch & Bedford (4). In their study, the pathogenicity of *Fusarium* was established by inoculation of the turfgrass foliage with conidia suspensions and incubation in dew chamber for 2-5 days. Bean (1) postulated that the important phase of the disease involved the destruction of crown areas of the grass plant. He felt that crop debris played an important role in the development of the disease by allowing *F. roseum* to grow as a saprophyte, and that moisture stress and high light intensity were the keys to allowing *F. roseum* to become pathogenic and gain entrance to the plant. His attempts to inoculate the crown regions, however, were unsuccessful. Troll (13) demonstrated that in addition to moisture stress, interrelationships with other microorganisms in the soil, especially other fungi, might be important in disease development.

The present authors initiated a survey to determine if factors other than moisture stress might account for predisposition of the plant to attack by *F. roseum*. A survey showed that Merion Kentucky bluegrass plants growing in blighted areas had poorly developed root systems. These plants, especially in the outer edge of the blighted circles, were severely stunted as compared to the healthy-appearing grass plants around them. All turf areas exhibiting the symptoms of the Fusarium blight disease had high counts (200-1,500/100 cc of soil) of *Tylenchorhynchus dubius* (Butschli) Filipjev.

Fusarium-nematode interactions, primarily involving the endoparasites *Meloidogyne* spp., are common in many crops (3, 6, 7, 12). There is also evidence for the involvement of ectoparasites in *Fusarium*-nematode interactions. Labruyere et al. (9) found that both *Hoplolaimus uniformis* Thorne and *F. oxysporum* f. *pisi* must be present for the complete disease expression of "early yellowing" of peas. Kisiel et al. (8) showed that the interaction of *Tylenchus agricoli* and *F. roseum* resulted in increased penetration of the vascular stele by the fungus, but *Tylenchorhynchus claytoni* Steiner did not appear to enhance fungal penetration or disease development.

We conducted the following study to determine whether a *Fusarium-Tylenchorhynchus* interaction exists in the etiology of Fusarium blight of turfgrass, a disease previously thought to be incited by the fungus alone.

MATERIALS AND METHODS.–Merion Kentucky bluegrass (*Poa pratensis* L.) seed was planted in rows in sterilized white sand and grown until seedlings reached a height of between 0.5-1 inch. *Fusarium roseum* cultures were maintained on potato-dextrose agar (PDA) in a controlled temperature cabinet at 24 C. The cultures were transferred periodically (every 3 weeks) to Merion Kentucky bluegrass and reisolated to avoid losses of pathogenicity from continuous culture on artificial medium. Macrospore counts were adjusted with 1% Bio Cert TM PDA to ca. 200,000/ml. *T. dubius* was obtained from infested soils using a modified Baermann method. Ten plants were removed from the sand culture and placed in 7.6-cm plastic pots containing a sand-loam-peat (1:1:1) soil mixture, previously steam-pasturized. Treatments were added to the root zones at this time. They were: (i) 1 ml of 200,000 macrospores of *F. roseum* in 1% PDA/pot; (ii) 300 *T. dubius*/pot; (iii) nematode-fungus combination (treatments a + b combined); and (iv) a distilled water control. Pots were placed in washed sand in rectangular 20 × 35 cm plastic tubs which were submersed to within an inch of the top in a temperature controlled water tank. The pots were maintained in 16 ± 1 C for the first 14 days, then raised to 21 ± 1 C for the duration of the experiment. Clippings were harvested ca. 1 month after receiving the treatments, and their fresh and dry weights determined. After clipping, the plants were maintained at 21 ± 1 C for an additional 60 days, at which time root fresh weights were measured.

RESULTS.–*T. dubius* alone and in combination with *F. roseum* significantly reduced top growth (dry weight basis) when compared to nontreated controls. However, no significant difference was observed between *T. dubius* or *F. roseum* only treatments. On a fresh weight basis, only the *T. dubius-F. roseum* combination significantly reduced the top growth over the nontreated controls (Table 1, Fig. 1).

TABLE 1. Fresh and oven-dry foliar weights of Merion Kentucky bluegrass clippings, inoculated with *Fusarium roseum* and/or *Tylenchorhynchus dubius*, 1 month after inoculation (Trial 1)

Treatment	Fresh weight (g)[a]		Oven-dry weight (g)[a]	
	Trial 1	Trial 2	Trial 1	Trial 2
T. dubius-F. roseum combination	.43 a	.22 a	.13 a	.04 a
T. dubius alone	.61 ab	.35 ab	.18 ab	.07 a
F. roseum alone	.76 b	.40 b	.21 b	.08 ab
Untreated control	.94 b	.59 c	.27 c	.13 b

[a]Each value is a mean of four replications. Means not followed by the same letter are significantly different at the 5% level according to Duncan's multiple range test.

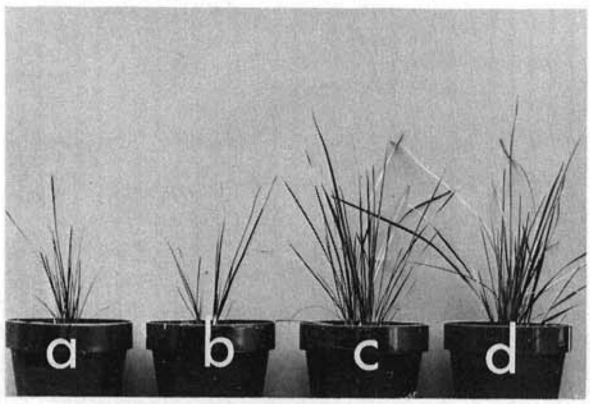

Fig. 1. Top growth of Merion Kentucky bluegrass plants which received **a)** 300 *Tylenchorhynchus dubius* adults; **b)** 300 *T. dubius* and 200,000 *Fusarium roseum* macrospores; **c)** 200,000 *F. roseum* macrospores; **d)** untreated controls.

In a second trial, there were no significant differences in the amount of top growth reduction, on a dry weight basis, among plants inoculated with *T. dubius* alone, *F. roseum* alone, or the *T. dubius-F. roseum* combination. Only the *T. dubius* alone and the *T. dubius-F. roseum* combination significantly reduced the top growth as compared to the nontreated control. On a fresh weight basis, the *T. dubius-F. roseum* combinations significantly reduced the top growth compared to the *Fusarium* alone treatment and the nontreated control (Table 1).

Fresh root weights were taken at the end of 90 days. The results were similar for both experiments, and representative data are given in Table 2. There was a significant reduction in fresh root weights of plants inoculated with *T. dubius* alone and with the *T. dubius-F. roseum* combination as compared with those inoculated with *Fusarium* alone and the nontreated control. The stunted root systems of plants inoculated with *T. dubius* only and of those with *T. dubius* in combination with *F. roseum* can be seen in Fig. 2.

TABLE 2. Fresh weights of Merion Kentucky bluegrass roots inoculated with *Fusarium roseum* and/or *Tylenchorhynchus dubius* after 90 days

Treatment	Fresh weight (g)[a]
T. dubius-F. roseum combination	.31 a
T. dubius alone	.36 a
F. roseum alone	.83 b
Untreated control	1.10 b

[a]Each value is a mean of four replications. Means not followed by the same letter are significantly different at the 5% level according to Duncan's multiple range test.

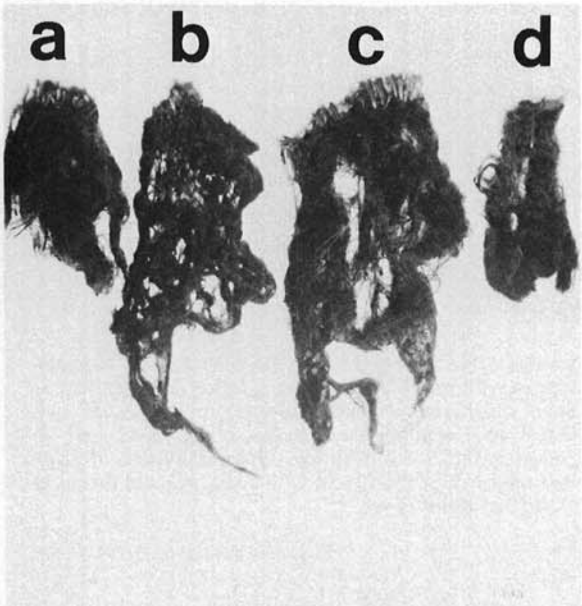

Fig. 2. Root development of Merion Kentucky bluegrass plants 90 days after the following treatments: **a)** 300 *Tylenchorhynchus dubius* adults; **b)** 200,000 *Fusarium roseum* macrospores; **c)** untreated control; **d)** 300 *T. dubius* and 200,000 *F. roseum* macrospores.

Isolations were made from selected roots of plants in all the treatments. *F. roseum* was recovered from plants inoculated with *F. roseum* and from those inoculated with *F. roseum* and *T. dubius* combination. *F. roseum* was not isolated from the plants inoculated with *T. dubius* alone or the nontreated controls. The soils were checked for the presence of *T. dubius* via the sugar method, and showed a 2.5- to 3-fold increase in nematode numbers during this 90-day period in both treatments receiving nematodes. *T. dubius* was not found in the other treatments. The recovered nematodes were primarily juveniles compared to the mature adults which were added 90 days earlier.

DISCUSSION.—In originally describing this disease, Couch & Bedford (4) made reference to the presence of nematodes in some of the Fusarium-blighted turfs, but found no correlation between them and the disease. The present study, however, suggests a positive correlation between the presence of *T. dubius* and the development of Fusarium blight.

In our studies, the *Fusarium* fungus by itself was not sufficient to explain the severe stunting of the top growth and root system shown by diseased grass plants growing in mildly infected turf or in turf recovering from severe infection. Only in the presence of *T. dubius* does this severe reduction in top and root growth occur. The nematode, therefore, may be predisposing the bluegrass to attack by *F. roseum* which under the proper environmental condition can result in the destruction of the grass plant.

The data herein show that the nematode is the dominant pathogen in the disease interaction involving *T. dubius* and *F. roseum*. This may help to explain why fungicides that are normally effective against other Fusarium diseases have failed to control this disease (2, 14). Conversely, a preliminary study with two nematicides, Bay 68138 (Nemacur) [Ethyl 4-(methylthio)-m-tolyl isopropylphosphoramidate] and DuPont 1410 [S-methyl 1-(dimethylcarbamoyl)-N-(methyl carbamoyloxy) thioformimidate] has shown a suppression of the Fusarium blight symptoms (J. M. Vargas, Jr. & C. W. Laughlin, *unpublished data*). A fungicide, benomyl, recently has been reported as a control of Fusarium blight (5, 11, 14). Preliminary evidence indicates that at least part of this control may be attributed to the direct effect of benomyl on the nematode *T. dubius* (10).

Our results indicate that the disease called Fusarium blight of turfgrass,, previously believed to be incited solely by either *Fusarium roseum* or *F. tricinctum*, involves an interaction with *T. dubius*.

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