Symptom Suppression with Oxytetracycline of a Toronto Creeping Bentgrass Disease of Presumed Bacterial Etiology

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ABSTRACT

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Symptom suppression of a Toronto creeping bentgrass disease of unknown etiology was achieved with 1.0 and 1.5 g/L solutions of oxytetracycline applied as drench treatments at the rate of 2 L/m^2. Streptomycin sulfate and cupric hydroxide did not reduce disease development. Scanning electron micrographs revealed many bacteria in the xylem of untreated plants and of those treated with streptomycin sulfate and cupric hydroxide, whereas no bacteria were found in oxytetracycline-treated plants.This evidence supports our previous findings that a bacterium is the presumable incitant of this disease of Toronto creeping bentgrass.

In a recent report we presented evidence for the association of bacteria with a Toronto (C-15) creeping bentgrass disease (7) previously known as the "C-15 problem" (8). The bacteria were limited to xylem vessels, except in advanced stages of the disease, and were similar in morphology (rippled cell wall) and size (≃0.5 × 1–1.5 μm) to rickettsialike bacteria found in association with Pierce's disease of grapevines and alfalfa dwarf (1), phony peach disease (3), and plum leaf scald (5). Pierce's disease has been effectively suppressed with tetracycline antibiotics (4,6). The effects of streptomycin, oxytetracycline, and a copper compound as drench treatments for suppressing the C-15 problem in Toronto creeping bentgrass are summarized in this paper.

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MATERIALS AND METHODS

Field trials were established at two Illinois locations that had experienced the C-15 problem the previous year—the St. Charles Country Club in St. Charles (location A) and Village Links Golf Course in Glen Ellyn (location B). The test chemicals were oxytetracycline (Mycoshield: 17% WP, Pfizer Chemicals Division), streptomycin sulfate (Agrimycin 17: 21.2% WP, Pfizer Chemicals Division), and cupric hydroxide (Kocide 101: 77% WP, Kocide Chemical Corporation).

At location A, 1.0 and 1.5 g/L solutions of each chemical were applied as drench treatments with a sprinkling container at the rate of 2 L/m^2 (5 gal/100 ft^2) to 0.93-m^2 plots of turf. At location B, 1.0 g/L solutions of the three test chemicals were applied at the rate of 2 L/m^2 (5 gal/100 ft^2) to 1.86-m^2 plots. A randomized complete block design (including untreated check plots) with three replicates was used at each location. At location A, two applications were made 1 wk apart starting 20 May 1981, as symptom development was becoming apparent. At location B, applications were made weekly for 6 wk starting 5 May 1981, before symptoms were evident.

In an experiment at location B that was not replicated, one application of 1 g/L solutions of oxytetracycline and streptomycin were each applied 23 April 1981 to adjacent halves of a golf green at the rate of 2 L/m^2 (5 gal/100 ft^2).

Disease severity was assessed 25 June 1981 after a severe outbreak of disease. Estimates of the percentage of disease on a scale of 1 (0–10%) to 10 (91–100%) were made of all treatments. The data were statistically analyzed by analysis of variance and Duncan's multiple range tests.

For scanning electron microscopy (SEM) studies, three Toronto creeping bentgrass plants were selected from each treatment and washed in running tap water for 2 hr. Crowns were removed, fixed in 4% glutaraldehyde, postfixed in 1% osmium tetroxide, dehydrated in a graded series of ethanol, and freeze fractured in liquid nitrogen (2). Following critical point drying and sputter-coating, crown pieces were observed in a JEOL JSM-35C scanning electron microscope.

RESULTS

At location A, two applications of oxytetracycline at 1.0 and 1.5 g/L significantly (P = 0.01) reduced the severity of the C-15 problem when compared with the untreated check, streptomycin, and cupric hydroxide treatments (Table 1). Small areas of decaying turf and many individual wilted plants with characteristic shriveled, blue green leaf tips were noted in the check, streptomycin, and cupric hydroxide plots, whereas all plants in the oxytetracycline plots remained healthy with no symptom expression (Fig. 1). Streptomycin and cupric hydroxide test plots were not significantly different from check plots, and no differences were noted between the 1.0 and 1.5 g/L levels of any treatment.

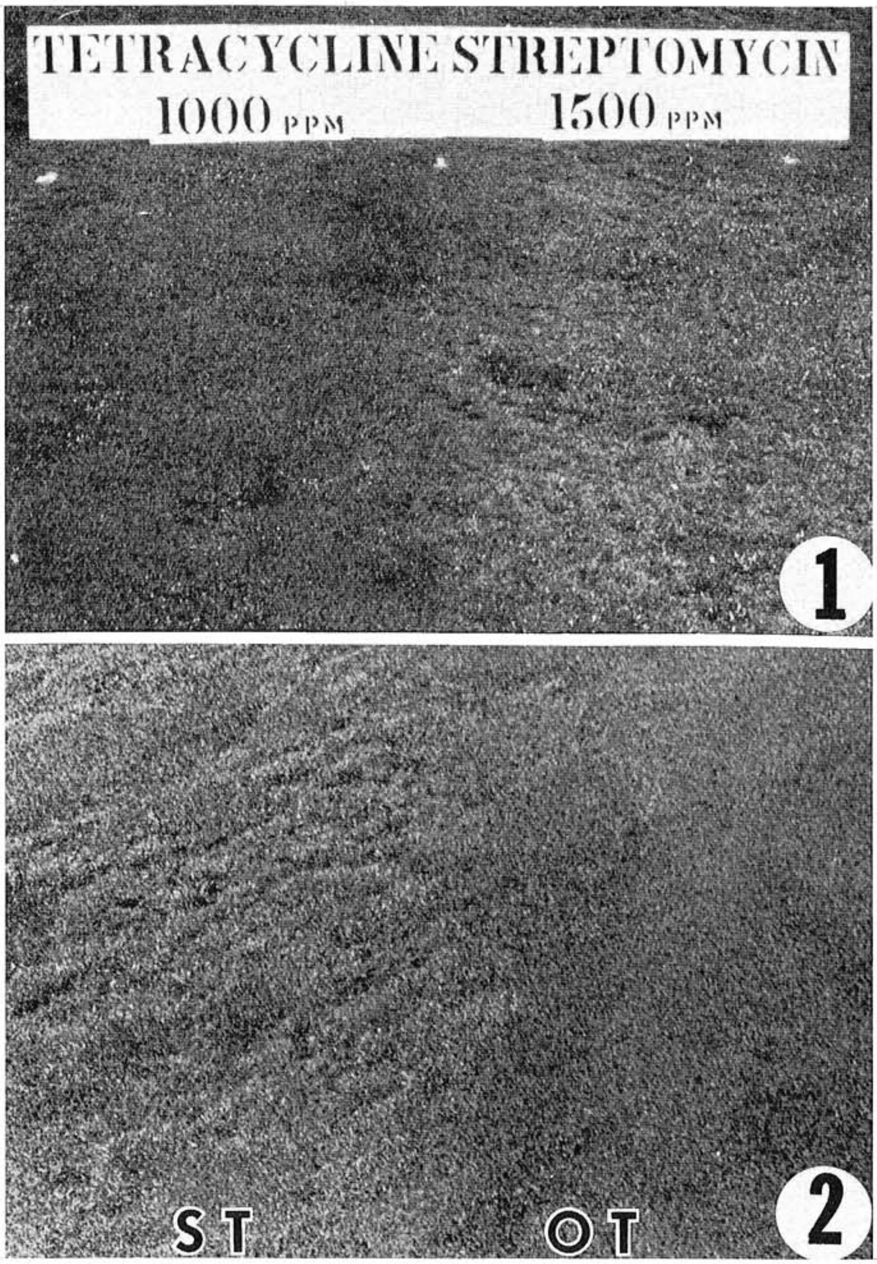
A marked reduction in disease severity was also obtained at location B after six applications of oxytetracycline (Table 1). Streptomycin sulfate and cupric hydroxide did not reduce disease severity. In the single application experiment at location B, disease developed on the streptomycin-treated half of the green 6 wk after the single application; however, complete symptom suppression was observed on the oxytetracycline half of the green (Fig. 2).

Some yellowing of turf in oxytetracycline-treated areas was noted when the chemical was applied during the high light-intensity portion of the day when temperatures exceeded 23 C. The yellowing was not evident on plots treated in the early morning or late afternoon.

Examination of bentgrass plants by SEM revealed many bacteria in xylem vessels of all plants from untreated check plots (Fig. 3). Similarly, bacteria were also found in xylem vessels in plants from the plots treated with cupric hydroxide and streptomycin sulfate. No bacteria were found in any plants from the oxytetracycline-treated plots (Fig. 4), and no differences were discerned between any of the treatment levels.

DISCUSSION

Excellent symptom suppression of the C-15 problem was achieved by two applications of oxytetracycline at location A and six applications at location B. Because antibiotic applications were made after symptom development



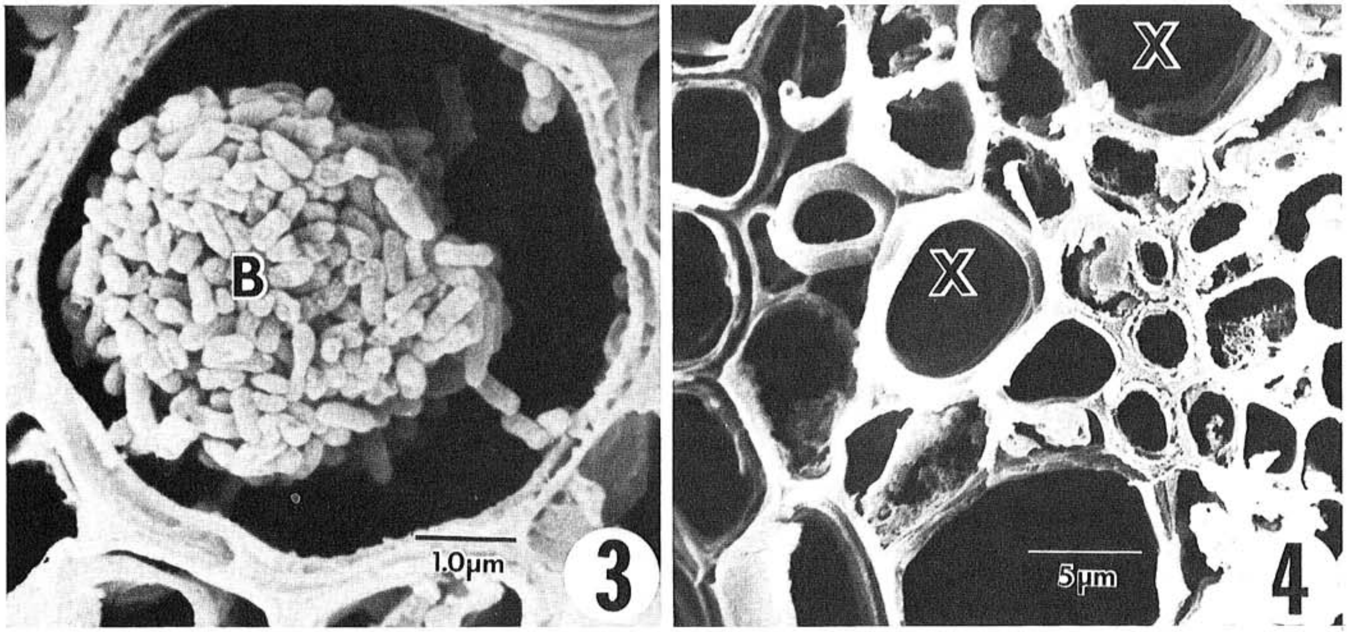
Figs. 1–2. Toronto creeping bentgrass treated with antibiotics: **(1)** After two applications, symptoms were suppressed in the oxytetracycline-treated plots (tetracycline at 1,000 ppm = 1 g/L), whereas disease developed in the streptomycin-treated plots (1,500 ppm = 1.5 g/L). **(2)** After single applications (1 g/L at 190 L/93 m^2) of each antibiotic to adjacent halves of a golf green, disease developed on the streptomycin-treated (ST) half, whereas no disease developed on the oxytetracycline-treated (OT) half even after 6 wk.

Table 1. Effects of two antibiotics and a copper compound on a disease of Toronto creeping bentgrass

	Disease rating[z]		
	Location A		Location B
Treatment[y]	1.0 g/L	1.5 g/L	1.0 g/L
Oxytetracycline	1.0 a	1.0 a	1.0 a
Streptomycin	6.0 b	5.6 b	3.6 b
Cupric hydroxide	5.6 b	5.3 b	4.3 b
Check	5.6 b		4.6 b

[y]Two treatments at location A and six at location B were applied as 1.0 g/L and 1.5 g/L solutions at the rate of 2 L/m^2.

[z]On a scale of 1 (0–10%) to 10 (91–100%). Each number represents the mean of three replicates. Means followed by different letters are statistically significant according to Duncan's multiple range test at P = 0.01. Locations A and B were analyzed separately.



Figs. 3–4. Scanning electron microscopy of crown freeze-fractures of Toronto creeping bentgrass: **(3)** Xylem vessel from untreated plant containing many bacteria (B). **(4)** Vascular tissue from plant treated (1.0 g/L) with oxytetracycline. Xylem vessels (X) contain no bacteria.

at location A but before symptom development at location B, both curative and preventive control appeared to be possible with oxytetracycline. The drench method was used because our 1980 field tests with light spray applications (0.6 g/L at 0.2 L/m^2) proved ineffective (*unpublished*). In the present study, oxytetracycline at 1.0 g/L was as effective as at 1.5 g/L. Although these high rates have given excellent control, lower rates may be sufficient for control of this disease. Streptomycin and cupric hydroxide had no effect on the disease at either rate.

Our findings at location A indicated that no more than two applications of oxytetracycline were necessary for disease control for a 4-wk period. The single application experiment at location B indicated that oxytetracycline appeared to persist and be effective for an extended period of time. Further testing with regard to timing and application frequency may be necessary before practical management schemes are feasible. Because tetracycline antibiotics are frequently used in treating human diseases, precautionary measures must be seriously considered before registration as a turfgrass pesticide is recommended.

Because of its high resolution and magnification capabilities, electron microscopy has proven to be an important tool in diagnosis of this disease (7) and has also been useful in this study for determining whether bacteria are present within the xylem of treated plants. The absence of bacteria in oxytetracycline-treated plants, as ascertained by SEM, presumably resulted from antibiotic effects of oxytetracycline upon the bacteria.

The C-15 problem has existed for many years, but little is known about its etiology (8). The association of bacteria with diseased C-15 as ascertained by SEM and the suppression of disease symptoms by oxytetracycline in this study support our previous findings that a bacterium, perhaps a rickettsialike one, is the incitant of the C-15 problem.

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