Occurrence in Florida of the Bacterium That Causes Bermudagrass Stunting Disease

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ABSTRACT

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Clavibacter xyli subsp. *cynodontis*, the causal agent of bermudagrass stunting disease (BSD), was frequently isolated from bermudagrass in Florida but was not constantly associated with symptoms. When Tifgreen and Tifdwarf bermudagrass (*Cynodon* spp.) plants were experimentally inoculated with *C. xyli* subsp. *cynodontis* from Florida and maintained in a screenhouse under about 75% shade, they developed significantly less shoot biomass than buffer-inoculated controls. Reductions in shoot and root biomass were also observed in plants vegetatively propagated from inoculated plants. When inoculated plants were transferred to better growing conditions with full sunlight, significant reductions in dry weight were not observed, suggesting that such environmental stresses as low light intensity can play an important role in the development of BSD.

A small, coryneform bacterium was isolated recently from bermudagrass (*Cynodon dactylon* (L.) Pers.) plants from Taiwan also infected with a mycoplasmalike organism associated with bermudagrass white leaf disease (2,8). When cuttings of a common type bermudagrass without white leaf disease were inoculated with the bacterium, developing stolons and leaves were stunted (4). Stunting was most severe in regrowth of plants trimmed to the soil line and in plants vegetatively propagated from infected plants. The disease was named bermudagrass stunting disease (BSD). The bacterium causing BSD and a closely related bacterium causing ratoon stunting disease of sugarcane were subsequently named *Clavibacter xyli* subsp. *cynodontis* and *C. xyli* subsp. *xyli*, respectively (3).

Bermudagrass turf is widely used on golf courses in southern Florida and is frequently affected by a disease of unknown etiology commonly referred to as bermudagrass decline (1). The disease occurs predominantly in greens and is of great concern to the golf course industry because of unsightly circular patches of chlorotic and dying grass. During efforts to identify the cause of bermudagrass decline, *C. xyli* subsp. *cynodontis* was found infecting the xylem vessels of bermudagrass. Strains of *C. xyli* subsp. *cynodontis* from Taiwan were morphologically and biochemically indistinguishable from the strains we isolated in Florida (3).

Because Tifgreen and Tifdwarf are the predominant bermudagrass cultivars planted on golf course greens in Florida and because the relationship of *C. xyli* subsp. *cynodontis* to bermudagrass decline in greens was of interest, the incidence and pathogenicity of Florida strains of *C. xyli* subsp. *cynodontis* to Tifgreen and Tifdwarf were examined in an effort to determine the role of the bacterium in disease of bermudagrass turf in southern Florida.

MATERIALS AND METHODS

Bermudagrass turf samples were submitted by golf course superintendents or collected from experimental plots and germ plasm collections at the Ft. Lauderdale Research and Education Center. Internodes from three rhizomes or stolons of each turf sample were used in attempts to isolate *C. xyli* subsp. *cynodontis* that were performed as described previously (2,4), with a few modifications. Internodes in some early experiments were dipped briefly in 95% ethanol, immersed for 2.5 min in 1% sodium hypochlorite (20% commercial bleach), and washed three times in sterile deionized water. Sap was then expressed with forceps from 3- to 5-mm longitudinal portions of the internodes and blotted on the surface of SC medium (2), modified (SCM) by autoclaving glucose and cysteine with the medium instead of adding them as filter-sterilized stock solutions. In later experiments, surface-sterilized internodes were rinsed in running tap water instead of sterile deionized water, and a semiselective medium (SCMS) was then used. The SCMS medium was prepared by adding 10 ml of a filter-sterilized solution containing 0.05 g of cycloheximide, 0.05 g of hymexazol, 0.01 g of colistan, and 0.05 g of polymixin B to 1,000 ml of autoclaved SCM medium at 50 C.

Tentative identification of *C. xyli* subsp. *cynodontis* after primary isolation was based on the following criteria: 1) slow growth, taking 5–7 days on the SCM medium or 7–14 days on the SCMS medium for colonies to become readily visible (0.1–1.0 mm in diameter); 2) circular, convex colonies with entire margins; 3) yellow-orange colonies; 4) coryneform cell morphology in wet mounts observed by phase-contrast microscopy (3); 5) culture medium requirements (3) with growth on SCM medium but not on King's medium B (7), 523 medium (6), or YSC medium (5). Preliminary identification was confirmed by indirect fluorescent-antibody (IFA) staining using antiserum to *C. xyli* subsp. *xyli* or *C. xyli* subsp. *cynodontis* (2).

Tifgreen and Tifdwarf bermudagrass (*Cynodon* spp.) cuttings were inoculated by immersion overnight in a suspension containing 1×10^9 cells per milliliter of *C. xyli* subsp. *cynodontis* in 0.01 M phosphate-buffered saline (0.85%) (PBS), pH 7.0, or in PBS alone using previously described methods (4). The FB-1 strain (3) of *C. xyli* subsp. *cynodontis* (isolated in Florida) was used. Buffer-inoculated cuttings were used as controls. When no uninfected source of Tifgreen was found, hot-water treatment was examined as a means of obtaining uninfected nursery plants for inoculation experiments. Stolons about 25 cm long from four separately maintained isolates of Tifgreen were each treated at 51, 53, or 55 C for 30, 60, 120, or 240 min in a water bath. Controls were not treated. Stolons were then divided into single-node cuttings. Cuttings were rooted in a soil mix in flats, then transferred to soil in pots (8 × 8 cm). Two Tifgreen plants from different lines were determined on the basis of repeated isolation attempts to be free of *C. xyli* subsp. *cynodontis* after hot-water treatment for 240 min at 51 C and were used as a source of cuttings for experiments.

In pathogenicity tests, treatments consisted of controls (buffer-inoculated) and inoculated cuttings of the two Tifgreen lines and one Tifdwarf line. There were 20–30 cuttings per treatment. Cuttings were grown individually in plastic pots (8 × 8 cm) containing a soil mix. Replicates of the six treatments were randomized on benches in a screenhouse with about 75% shade. Shoots were harvested, dried, and weighed at 3.0, 5.5, and 8.0 mo after inoculation, and plants were then transferred to full sunlight.

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Plants were harvested and root and shoot dry weights were determined after another 2.5 mo. Isolation attempts were made at each sampling date from plants not previously determined to be infected with *C. xyli* subsp. *cynodontis*. Before drying and weighing at 8 mo after inoculation, one stolon about 25 cm long was taken from each buffer-inoculated plant determined to be uninfected and from inoculated plants determined to be infected. This was done to examine the effects of infections with the bacterium on vegetatively propagated progeny of the two bermudagrass cultivars. Both lines of Tifgreen and the single line of Tifdwarf were used. Twelve replicates were planted in a randomized complete-block design in flats (50 × 35 × 8 cm) containing sand. Cuttings were propagated under intermittent mist in a shadehouse for 1 mo to induce rooting, then placed in full sunlight for 2 wk. Plants were then harvested and shoot and root dry weights determined.

RESULTS AND DISCUSSION

C. xyli subsp. *cynodontis* was frequently isolated from bermudagrass in southern Florida but was not consistently associated with stunting or other symptoms. The bacterium was found in samples from eight of 10 golf courses in scattered urban areas on the lower east and west coasts of Florida. The bacterium was isolated from 14 of 15 samples from the eight courses. Other samples purposely collected on four Ft. Lauderdale golf courses from greens with and without bermudagrass decline symptoms had the bacterium present regardless of the condition of the green. Subsequently, the bacterium was consistently isolated from turf samples from several golf courses and from experimental bermudagrass turf plots with no history of bermudagrass decline.

Examination of 331 bermudagrass clones in a germ plasm collection at Ft. Lauderdale resulted in isolation of the bacterium from 275 (83%) of the clones. Preliminary identification of the bacterium was based only on colony morphology and colony color on the SCMS medium. Forty-five isolates preliminarily identified as *C. xyli* subsp. *cynodontis* were examined by IFA staining, and the identities of 44 (98%) were verified. Seventeen isolates that resembled *C. xyli* subsp. *cynodontis* were regarded as questionable because they formed pale yellow colonies instead of the yellow-orange colonies characteristic of the subspecies. When 14 of the questionable isolates were examined further, five (36%) were identified as *C. xyli* subsp. *cynodontis* by IFA staining. The cell morphology of the IFA-negative bacteria with pale yellow colonies resembled that of *Arthrobacter* spp. when viewed with phase-contrast microscopy (×1,200) in wet mounts.

Several sources of uninfected Tifdwarf were found; however, all turf samples of Tifgreen, including two from commercial turf nurseries, were infected with *C. xyli* subsp. *cynodontis*. Hot-water treatment was used to obtain uninfected Tifgreen for pathogenicity tests (Table 1). Hot-water treatment for 2 hr at 51 C, used to cure sugarcane of *C. xyli* subsp. *xyli* (9), was not completely effective in curing Tifgreen of *C. xyli* subsp. *cynodontis*. Although treatments of 51 C for 240 min, 53 C for 120 min, and 53 C for 240 min were consistently therapeutic, too few cuttings survived to suggest that the treatments can be relied on for therapy. At 55 C for 30 or 60 min, survival of the cuttings was greatly impaired (<5%) without killing *C. xyli* subsp. *cynodontis*.

Like strains from Taiwan (4), *C. xyli* subsp. *cynodontis* from Florida caused stunting of bermudagrass. The average dry weight of shoots from Tifgreen and Tifdwarf bermudagrass inoculated with the FB-1 strain of *C. xyli* subsp. *cynodontis* from Florida was significantly less than that of buffer-inoculated shoots of the same clones after 5.5 mo and again after 8 mo when the plants were grown in a screenhouse under about 75% shade (Table 2). When the same plants were placed in direct sunlight after 8 mo, no difference in dry weights between treatments was observed in the regrowth of shoots 2.5 mo later. However, infected plants propagated under mist during the same period, then transferred to full sunlight for 2 wk, developed significantly less shoot and root biomass compared with identically treated uninfected plants (Table 3). Apparently, bermudagrass is more likely to express symptoms under low light levels than under the more favorable conditions of normal light intensity.

Table 1. Therapeutic effects of different temperatures and durations of hot-water treatment on systemic infections with *Clavibacter xyli* subsp. *cynodontis* in Tifgreen bermudagrass

Duration of treatment (min)	Temperature (C)					
	51			53		
	Cuttings treated[a]	Survival[b] (%)	Infected[c] (%)	Cuttings treated[a]	Survival[b] (%)	Infected[c] (%)
60	156	53.2	100.0	145	17.9	73
120	144	16.7	12.5	152	3.9	0
240	150	7.3	0.0	154	1.9	0

[a] An approximately equal number of stolons of four separately maintained lines of Tifgreen bermudagrass were subjected to each treatment, then the stolons were cut into single-bud cuttings and planted.
[b] Survival of untreated cuttings was 93.9% (155/165).
[c] A plant was considered infected if *C. xyli* subsp. *cynodontis* was isolated from any of three shoots 3 mo after treatment.

Table 2. Dry weights of Tifgreen and Tifdwarf bermudagrass shoots as affected by inoculation with the FB-1 strain of *Clavibacter xyli* subsp. *cynodontis* from Florida

Cultivar	Treatment[a]	No. of plants	Infected plants[b] (%)	Average dry weight of shoots (g)[c]	
				Grown in shade	Grown in sunlight
Tifgreen	Inoculated	54	87.0	2.47 (2.40)*	4.03 (4.06) NS
	Control	56	0.0	3.11*	4.16 NS
Tifdwarf	Inoculated	21	57.1	1.59 (1.42)**	2.38 (2.02) NS
	Control	19	0.0	2.65**	2.85 NS

[a] Treatments consisted of single cuttings inoculated with the FB-1 strain of *C. xyli* subsp. *cynodontis* from Florida and buffer-inoculated controls. Data from two Tifgreen lines were combined after no significant difference ($P = 0.05$, t test) between similarly treated lines was observed.
[b] A plant was considered infected if any isolation attempt 6 or 8 mo after inoculation was positive.
[c] Plants were grown 8 mo in a screenhouse, then moved to full sunlight. Dry weights were measured for shoots regrown 2.5 mo after trimming just above the soil level. Dry weights are averages of all plants. Values in parentheses are for infected plants. Significant differences (t test) of dry weights between inoculated and control shoots are indicated by asterisks: * = $P = 0.05$, ** = $P = 0.01$, and NS = not significant.

Table 3. Dry weights of Tifgreen and Tifdwarf bermudagrass stolons after propagation from uninfected plants and plants infected with *Clavibacter xyli* subsp. *cynodontis* from Florida

Cultivar	Treatment	No. of plants	Average dry weight (g)[a]		
			Shoots	Roots	Total
Tifgreen	Infected	24	0.36	0.04	0.40
	Uninfected	24	0.55	0.07	0.62
Tifdwarf	Infected	12	0.39	0.09	0.48
	Uninfected	12	0.84	0.20	1.04

[a] Differences between dry weights of infected and comparable uninfected materials were significant ($P = 0.05$, t test).

The stunting symptoms of BSD, previously reported for an unidentified type of bermudagrass inoculated with strains of the BSD bacterium from Taiwan (4), were not as severe in Tifgreen and Tifdwarf bermudagrass inoculated with a Florida strain of the BSD bacterium in these tests. Because environmental conditions and host and pathogen sources all differed from the original work (4), it is not possible to determine why severe stunting was not observed. Judging from the stunting that did occur in shaded plants, turf infected by *C. xyli* subsp. *cynodontis* may be predisposed to additional environmental and biological stresses. The lack of constant association of the bacterium with bermudagrass decline suggests that the BSD bacterium is not a primary cause of that disorder.

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LITERATURE CITED

1. Davis, M. J., Augustin, B. J., and Atilano, R. A. 1982. Bermudagrass decline. Grounds Maintenance. Oct. 42, 44.
2. Davis, M. J., Gillaspie, A. G., Jr., Harris, R. W., and Lawson, R. H. 1980. Ratoon stunting disease of sugarcane: Isolation of the causal bacterium. Science 210:1365-1367.
3. Davis, M. J., Gillaspie, A. G., Jr., Vidaver, A. K., and Harris, R. W. 1984. *Clavibacter*: A new genus containing some phytopathogenic coryneform bacteria, including *Clavibacter xyli* subsp. *xyli* sp. nov., subsp. nov. and *Clavibacter xyli* subsp. *cynodontis* subsp. nov., pathogens that cause ratoon stunting disease of sugarcane and Bermudagrass stunting disease. Int. J. Syst. Bacteriol. 34:107-117.
4. Davis, M. J., Lawson, R. H., Gillaspie, A. G., Jr., and Harris, R. W. 1983. Properties and relationships of two xylem-limited bacteria and a mycoplasmalike organism infecting Bermuda grass. Phytopathology 73:341-346.
5. Dye, D. W., and Kemp, W. J. 1977. A taxonomic study of plant pathogenic *Corynebacterium* species. N.Z. J. Agric. Res. 20:563-583.
6. Kado, C. I., and Heskett, M. G. 1970. Selective media for isolation of *Agrobacterium, Corynebacterium, Erwinia, Pseudomonas,* and *Xanthomonas*. Phytopathology 60:969-976.
7. King, E. O., Ward, M. K., and Raney, D. E. 1954. Two simple media for demonstration of pyocyanin and fluorescein. J. Lab. Clin. Med. 44:301-307.
8. Laio, C. H., and Chen, T. A. 1981. Isolation, culture, and pathogenicity to Sudan grass of a corynebacterium associated with ratoon stunting of sugarcane and with Bermuda grass. Phytopathology 71:1303-1306.
9. Todd, E. H. 1960. The ratoon stunting disease of sugarcane and its control in Florida. U.S. Dep. Agric. Crops Res. ARS 34-12. 7 pp.