Association of a Bacterium with a Disease of Toronto Creeping Bentgrass

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ABSTRACT

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Plants of Toronto creeping bentgrass exhibiting wilt symptoms of unknown etiology were examined ultrastructurally. Bacteria were found in xylem of roots, crowns, and leaves, and were similar in size (0.5 μm × 1.5 μm), morphology (rippled cell wall), and location (xylem) to rickettsialike bacteria found in association with Pierce's disease of grape, plum leaf scald, and phony disease of peach. Our suggested name for the disease is "bacterial wilt" of Toronto creeping bentgrass.

Agrostis palustris Huds. cv. Toronto (C-15 creeping bentgrass) has developed several serious disease problems in the past 15 yr. Originally, a disorder of unknown etiology became known as the "C-15 problem" (9) among turfgrass workers in the north central states. Fungicides gave inconsistent control. More recently, Meyers and Turgeon (7) reported red leaf spot caused by *Helminthosporium erythrospilum* (Drechs.), and Larsen (6) has reported leaf blight and crown rot caused by *Drechslera catenaria* (Drechs.) Ito (= *Helminthosporium catenarium* Drechs.) as important diseases of *A. palustris* cv. Toronto. Fungicides, however, have been effective in controlling each of these diseases (6,7). Recently, an additional disease of unknown etiology was observed on Toronto creeping bentgrass for which common fungicide applications were ineffective. These and perhaps other unidentified problems have been implicated as part of the original C-15 problem, which we believe is more properly termed the "C-15 syndrome" of Toronto creeping bentgrass.

This recently observed disease of unknown etiology occurred on *A. palustris* cv. Toronto putting greens at the Butler National Golf Course in Oak Brook, IL, in August 1979 and again in May 1980. Initially, leaf blades wilted from the tip down. Within several days the entire leaf wilted and became dark green, shriveled, and twisted. Early in disease development the crown and roots generally appeared white and in good health, except for possible slight internal discoloration. Root and crown tissues were in good condition even after the entire leaf was wilted. Eventually, leaf blades turned reddish brown and roots became quite discolored. Decomposition of the entire plant followed.

The disease spread in an irregular manner through the putting green, killing individual plants. *Poa annua* L. and varieties of *A. palustris* other than Toronto appeared unaffected. Large areas of a putting green or the entire green were destroyed within a few days. The disease occurred primarily in the spring and fall and was favored by periods of heavy rainfall followed by cool nights and warm days. Symptoms of the disease and the nature of its spread suggested that an infectious agent was involved. The purpose of this research was to examine that possibility.

MATERIALS AND METHODS

Segments measuring 5–10 mm of root, crown, and leaf tissue from diseased plants were surface sterilized in 0.5% sodium hypochlorite or 0.1% mercuric chloride or were dipped in 95% ethanol and flamed. After three washes in sterile distilled water, the plant segments were plated on potato-dextrose agar (PDA) and 2% water agar for isolation of fungi, on nutrient agar (NA) and sucrose (0.5%) nutrient agar (SNA) for isolation of bacteria, and on JD-3 medium (1) for isolation of certain fastidious bacteria. Surface-sterilized plant pieces were also macerated in sterile distilled water and plated in serial dilution on NA and JD-3 for isolation of bacteria. Plates were incubated at 25 C and observed daily for 2 wk for growth of microorganisms. Isolation attempts were repeated throughout the summer. Symptomless plants were taken through identical sterilization and isolation procedures.

Electron microscopy was performed on samples collected in June and August 1980. Several 1-mm pieces of leaves, crowns, and roots from each of 10 diseased plants exhibiting wilting of the leaf tips were fixed in cold 4% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2, for 2 hr; postfixed overnight in cold 1% buffered osmium tetroxide; and dehydrated in a graded series of ethanol. Specimens were embedded in a 1:1 mixture of epon-araldite and ERL epoxy resin (3). Ultrathin sections were stained with 2% uranyl acetate and subsequently in lead citrate (8). Root and crown segments from five symptomless plants were prepared in the same manner. Sections were examined in a Philips 300 transmission electron microscope.

RESULTS AND DISCUSSION

No fungal pathogens commonly reported from turfgrass nor any other fungi were observed or isolated regularly from diseased plants obtained from Butler National Golf Course. Several bacteria, differing in colony color and morphology, were frequently isolated from diseased plants on PDA, NA and SNA, and JD-3. Pathogenicity tests by either the immersion of injured roots or crown injection, both using pure bacterial broth cultures, were inconclusive after several attempts. Bacteria were not isolated consistently from symptomless plants.

Transmission electron microscopy was used to ascertain the presence of microorganisms in affected tissues because the symptomatology suggested a vascular disorder. Examination of ultrathin sections from all 10 diseased creeping bentgrass plants revealed large numbers of rod-shaped bacteria in the roots, crowns, and leaves. The bacteria were limited exclusively to the xylem vessels (Figs. 1 and 2) and measured approximately 0.5 μm in diameter × 1–1.5

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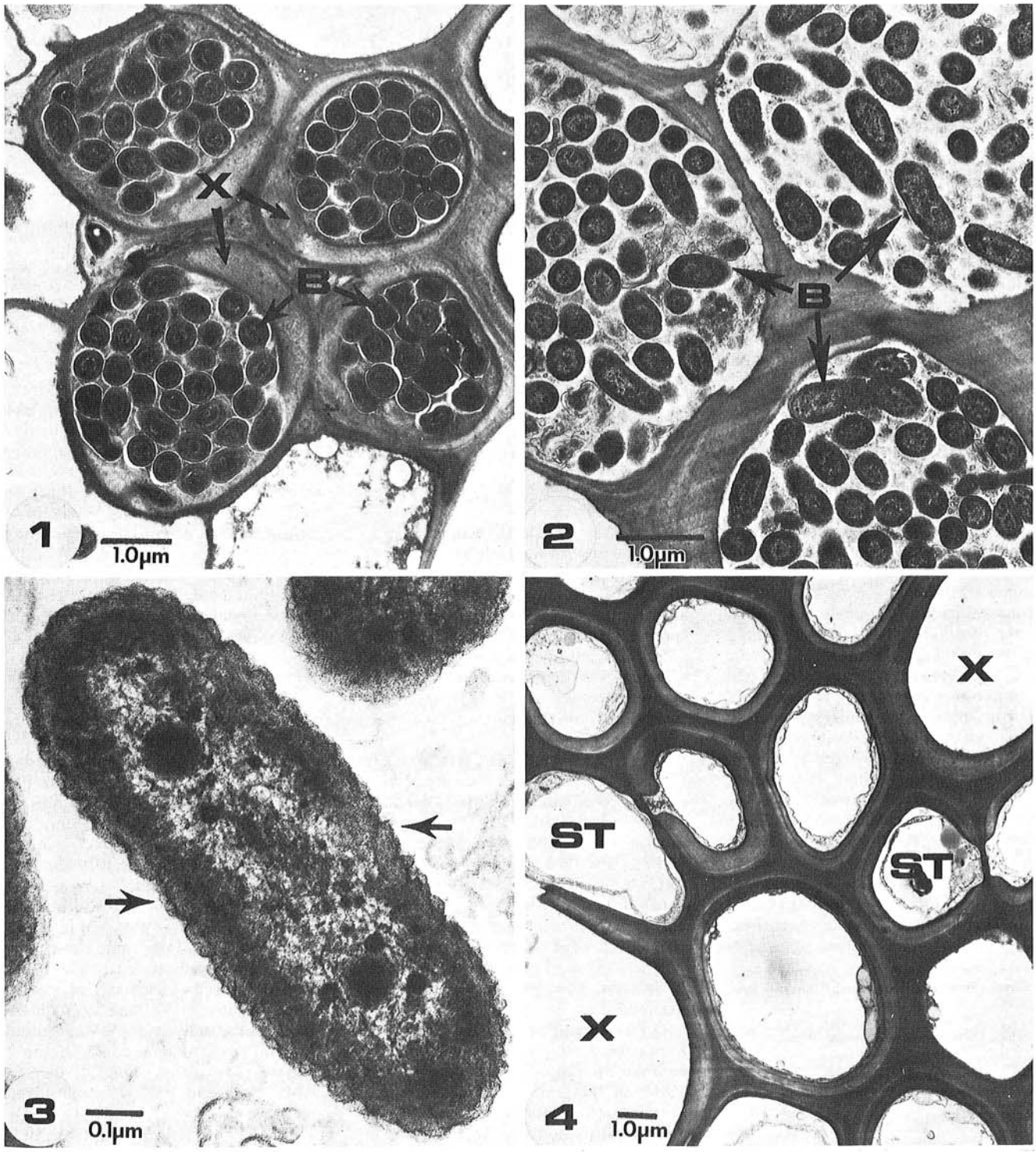
μm in length. In longitudinal section, the bacterial cell wall appeared rippled (Fig. 3). These bacteria were similar in size, morphology, and location within the plant to the rickettsialike bacteria associated with plum leaf scald (5), phony disease of peach (4), Pierce's disease of grapevines (2), alfalfa dwarf (2), and in symptomless johnsongrass (*Sorghum halepense*), which has been implicated as a possible reservoir of rickettsialike bacteria for phony peach disease (10). No bacteria were found in any ultrathin sections from symptomless plants (Fig. 4).

The discovery of bacteria within the xylem vessels of wilted plants suggests a possible causal agent for the disease that destroyed the Toronto greens at the Butler National Golf Course. In addition, the association of bacteria with the disease may represent a significant breakthrough in the unsolved portion of the C-15 syndrome of Toronto creeping bentgrass, for which no effective fungicidal control has been found.

Bacterial diseases of turfgrass have not been reported. Although bacterial diseases are common for other genera of the Gramineae, the bacterium association with Toronto creeping bentgrass represents the first bacterial disease encountered on a turfgrass species. We propose that the disease be named "bacterial wilt" of Toronto creeping bentgrass. Attempts to transmit these organisms from diseased to healthy plants and isolation of the causal bacterium are currently under way in our laboratory.



Figs. 1–4. Ultrathin sections of diseased **(1–3)** and symptomless **(4)** Toronto creeping bentgrass. **(1)** Leaf cross section with four xylem vessels (X) containing cross sections of bacteria (B). **(2)** Xylem of root-crown interface with longitudinal and cross sections of bacteria (B). **(3)** Single bacterium with rippled cell wall (arrows). **(4)** Root cross section from symptomless plant. Xylem (X) with no bacteria present. Sieve tubes (ST) shown for reference.

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