Properties and Relationships of Two Xylem-Limited Bacteria and a Mycoplasmalike Organism Infecting Bermuda Grass

M. J. Davis, R. H. Lawson, A. G. Gillaspie, Jr., and R. W. Harris

Assistant professor, University of Florida, Agricultural Research and Education Center, 3205 S.W. College Avenue, Ft. Lauderdale 33314; research plant pathologists and microbiologist, respectively, Beltsville Agricultural Research Center, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, MD 20705.

A portion of this research was done while the senior author was in the Department of Plant Pathology, Cook College, Rutgers University, New Brunswick, NJ 08903.

The research was supported in part by USDA/SEA Competitive Research Grant 59-2121-0-1-532-0 and by New Jersey Agricultural Experiment Station Project 11900.

Florida Agricultural Experiment Stations Journal Series Paper 3222.

The technical assistance of Carol A. Davis is gratefully acknowledged.

Accepted for publication 29 July 1982.

ABSTRACT

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.

Properties of two Gram-positive, xylem-limited bacteria and one mycoplasmalike organism (MLO) infecting Bermuda grass were compared. A bacterium originally isolated from Bermuda grass, but not the ratoon-stunting disease (RSD) bacterium from sugarcane, caused severe stunting described herein as Bermuda grass stunting disease (BSD). White leaf disease of Bermuda grass is presumed to be caused by an MLO that alone incites leaf chlorosis, axillary shoot proliferation, and stunting. In combination with the BSD bacterium a more severe disease reaction often developed that caused early death of the plant. Bacteria alone were associated with BSD. Plants with white leaf symptoms contained MLOs alone, but plants with white leaf symptoms combined with early death contained MLOs and the BSD bacterium. Cell wall preparations from cultured RSD and BSD bacteria contained major amounts of 2,4-diaminobutyric acid, glycine, glutamine, alanine, fucose, and rhamnose, indicating a possible relationship to the plant pathogenic corynebacteria. The RSD and BSD bacteria are antigenically related, but apparently neither are related to a number of species of corynebacteria and other Gram-positive bacteria.

Ratoon stunting disease (RSD) of sugarcane has been found in most sugarcane-producing areas of the world (21) and is responsible for considerable reduction of sugarcane yields in many areas. Stunting is the only overt symptom of RSD. The immediate economic importance of RSD in a particular geographic area often depends on the availability of water (21). RSD is commonly spread in vegetatively propagated sugarcane and readily transmitted on blades used in harvesting. Consequently, the disease often becomes more serious in stubble or ratoon crops. In nature, RSD has only been found affecting sugarcane, but the RSD pathogen has been experimentally transmitted to a number of grass species (21).

Bacteria observed in the xylem and in xylem extracts from diseased plants by using light and electron microscopy were consistently associated with RSD (8,17,23). Bacteria generally

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

inhabited the lumen and pit fields of tracheary elements, but also occurred in the cell walls and lacunae of the xylem (10,13,25,26). Bacteria in the tracheary elements were often associated with a matrix material of undetermined origin. Microcolonies of the bacteria were observed in the matrix material extracted from infected stalks (12). Both in situ and in extracts, the bacteria usually measured 0.25–0.50 × 1–4 μm with occasional lengths of 10 μm or greater (8,26). Straight, slightly curved, and swollen rod-shaped cells were seen. Unicellular forms were most prevalent, but paired and filamentous forms with septa were also observed. Filamentous forms with pseudobranching and branching were reported (12,22). Ultrastructurally the cell wall resembles that of Gram-positive bacteria, and mesosomes were frequently present (10,23,25).

Gram-negative, motile *Xanthomonas* spp. were isolated and described as possible etiological agents of RSD (16,24). However, this work was never confirmed. Nayiager et al (18) reported the isolation of four bacteria from sugarcane with RSD in a modified White's tissue culture medium. One strain was thought to reinfect sugarcane following inoculation; however, neither this strain nor any of the other three was conclusively shown to incite RSD. Liao and Chen (15) isolated a bacterium associated with RSD on a modified *Legionella pneumophilia* and mycobacteria medium. The bacterium was nonmotile, nonspore-forming, non-acid-fast, Gram-positive, aerobic, and either filamentous or a clublike rod. The bacterium multiplied in an inoculated sorghum-sudangrass hybrid, an indicator host for RSD, and was reisolated after 6–8 wk. However, symptom development in sorghum-sudangrass and inoculation of sugarcane were not reported.

Koch's postulates were first completed with a bacterium that was consistently isolated from sugarcane with RSD by Davis et al (3). The bacterium was isolated on a medium (SC) developed for it and in addition to sharing similar properties with the bacterium isolated by Liao and Chen (15), the bacterium was catalase-positive, oxidase-negative, and serologically indistinguishable from the bacterium consistently found in sugarcane with RSD (3). The same bacterium was consistently isolated from sugarcane with RSD from the United States, Brazil, South Africa, and Japan.

A bacterium resembling the RSD bacterium and a mycoplasmalike organism (MLO) were reported to dually infect diseased Bermuda grass showing white leaf and witches'-broom symptoms (2). The MLO alone was associated with white leaf symptoms, while the MLO and the bacterium were associated with witches'-broom symptoms. A spiroplasma was isolated from Bermuda grass with witches'-broom symptoms, but was not shown to be the causal agent (2,19). MLO's had previously been observed in Bermuda grass with white leaf disease in Taiwan (1) and a similar, if not the same, disease called "yellow leaf" was reported in Israel (27). The mixed infection with witches'-broom symptoms had not been previously described, nor had symptoms been described for plants singly infected with the bacterium. The bacterium was subsequently isolated in axenic culture (3,15), and reported to incite stunting but not witches'-broom symptoms in inoculated Bermuda grass (3). We have named this condition Bermuda grass stunting disease (BSD).

The BSD bacterium was cultured on the two different media used for the RSD bacterium (3,15). The BSD bacterium morphologically resembled the RSD bacterium in culture as well as in the host and was shown to be serologically related to the RSD bacterium. The BSD bacterium was readily distinguished from the RSD bacterium by a more rapid growth rate and production of yellow-orange, nondiffusable pigments in culture. The BSD bacterium, unlike the RSD bacterium, did not incite RSD-symptoms in sugarcane or wilting of sorghum-sudangrass uprights (3). Shoot growth of Bermuda grass was retarded following inoculation with either bacterium. RSD and BSD bacteria apparently infected their natural hosts and multiplied more readily in these hosts as indicated by reisolation from plants inoculated with bacteria from culture.

The RSD and BSD bacteria have not been taxonomically characterized. Prior to their isolation in axenic culture, attempts were made to identify possible relationships with known taxa by using chemotaxonomic and serological techniques. Several workers (10,12,23,25,26) have suggested, based on morphological and ultrastructural characteristics, that the bacteria were possibly related to the plant pathogenic corynebacteria or the actinomycetes. The occurrence of filamentous, branched forms that were apparently undergoing septate division led to the hypothesis that the bacterium was an actinomycete.

Chemotaxonomic analyses were performed using the RSD bacterium extracted from diseased sugarcane. Analyses of the major amino acids in the cell walls of the RSD bacterium indicated that diaminopimelic acid was absent and that either lysine or ornithine, or both were present (11,25). The cell wall sugars of the RSD bacterium were found to be rhamnose and fucose, and the guanine plus cytosine content of the DNA was reported as 60 mole percent (11). The results of the chemotaxonomic studies agreed with the possible relationship to the actinomycetes. However, the results did not conclusively rule out the possible relationship to the plant pathogenic corynebacteria since the same sugars and amino acids have been found in the cell walls of some corynebacteria and the guanine plus cytosine content was only slightly lower than that of most plant pathogenic corynebacteria, which are usually in the molar ratio range of 60 to 78 mole percent.

Results of immunofluorescent antibody (IFA) staining and Ouchterlony gel double diffusion tests demonstrated a relationship between the RSD and BSD bacterium in tests with antisera produced against the RSD bacterium from sugarcane extracts or from culture (3). However, no relationship between the RSD bacterium and numerous recognized species of corynebacteria and actinomycetes has been demonstrated by either IFA staining (4,9) or microagglutination (4,6,7).

The objectives of this study were to determine the etiological roles of two xylem-limited bacteria, the RSD bacterium and the BSD bacterium, in disease of Bermuda grass, and to determine the serological and chemotaxonomic relationships between the two bacteria and other bacteria. Since the BSD bacterium has only been found in nature associated with white leaf disease of Bermuda grass, the host response to the BSD bacterium alone as well as in mixed infections was examined.

MATERIALS AND METHODS

Source of plant material. Bermuda grass (*Cynodon dactylon* (L.) Pers.) with white leaf symptoms and naturally infected with the BSD bacterium was obtained from T. A. Chen, Department of Plant Pathology, Cook College, Rutgers University, New Brunswick, NJ 08903. Bermuda grass with white leaf symptoms and not infected with the BSD bacterium was obtained from H. J. Su, Department of Plant Pathology and Entomology, National Taiwan University, Taipei, Taiwan. Healthy Bermuda grass was obtained from turf plots at Cook College, Rutgers University.

Inoculation of Bermuda grass. Rhizomes from healthy plants were severed 2 cm on each side of nodes with axillary shoots that were 2–4 cm long. Cuttings were inoculated with the RSD and BSD bacteria from SC medium suspended ($A_{560\ nm}$ = 0.1) in 0.01 M phosphate buffer (pH 6.9). Cut surfaces of these single-node cuttings were immersed for 12 hr in inoculum and then transferred to vermiculite for rooting. Controls were inoculated with buffer alone. After rooting in a mist bench, the plants were transferred to soil in 10-cm-diameter clay pots. All plants were kept in a screened portion of a greenhouse. Five months after inoculation, the plants were cut back to soil level. The presence of the bacteria in the shoots was confirmed by observing expressed sap with a phase-contrast microscope (×1,000) and reisolating the bacteria on the SC medium as described (3). Single-node cuttings from plants shown to be infected by the bacteria and control plants were then propagated for further study.

Electron microscopy. Ultrathin sections were made from Bermuda-grass leaves, nodes, and internodes of plants that were either healthy, showing white leaf symptoms (received from T. A. Chen and H. J. Su), or had been inoculated with the BSD bacterium. The tissue pieces were fixed for 2 hr in a mixture of 2% glutaraldehyde and 1.5% acrolein buffered in 0.05 M phosphate

(pH 7.2). Samples were postfixed in cold, buffered 1% OsO_4 for 2 hr, dehydrated in a graded ethanol series, and embedded in Epon 812 by using propylene oxide. Sections were stained with aqueous uranyl acetate and lead citrate.

Cell wall analysis. The procedures of Lechevalier and Lechevalier (14) for determining the amino acid and sugar composition of the cell walls of bacteria were adapted for analysis of the RSD and BSD bacteria. Cells were grown in stationary cultures for 10–14 days in 500 ml of S8 broth (3) in 2,000-ml Erlenmeyer flasks and harvested in late log phase by centrifugation. Cells harvested at different times were washed twice in sterile distilled water, autoclaved, and stored frozen at −8 C until 3 g of moist cells had been collected. The cells were ground by hand with a glass homogenizer in 30–40 ml of 0.1 M phosphate buffer, pH 8.0, and the unbroken cells were removed by centrifugation at 4,400 *g* for 3 min. The cells were further broken by sonic oscillation for 2 min. Purification of the cell walls including saponification and enzyme digestion, and analysis of the amino acid and sugar composition of the walls by paper and thin-layer chromatography were conducted as described (14).

Serology. Antiserum against the RSD bacterium from culture was produced (3). Indirect fluorescent antibody (IFA) staining was performed as described (9), except the bacteria to be examined were grown on SC medium (3), suspended in 0.01 M phosphate buffer, 0.85% saline, and air-dried smears were fixed on fluorescent antibody slides with 95% EtOH for 1 min. Serological comparisons were made in a one-way test with antiserum against the RSD bacterium. For the most part, type strains were used; these were obtained from the American Type Culture Collection (ATCC), Rockville, MD 20852, and National Collection of Plant Pathogenic Bacteria (NCPPB), Plant Pathology Laboratory, Harpenden, England. The following species in addition to the RSD and BSD bacteria, were examined: *Agromyces ramosus* (ATCC 25173), *Arthrobacter flavescens* (ATCC 25091), *Corynebacterium betae* (NCPPB 374), *C. fascians* (ATCC 12974), *C. flaccumfaciens* (NCPPB 1446), *C. ilicis* (ATCC 14264), *C. insidiosum* (NCPPB 1109), *C. iranicum* (NCPPB 2253), *C. michiganense* (NCPPB 2979), *C. nebraskense* (NCPPB 2981), *C. oortii* (ATCC 25283), *C. poinsettiae* (ATCC 9682), *C. rathayi* (NCPPB 2980), *C. sepedonicum* (ATCC 33113), *C. tritici* (ATCC 11403), *Mycobacterium fortuitum* (ATCC 14467), and *Nocardia uniformis* ssp. *tsuyamanensis* (ATCC 21806).

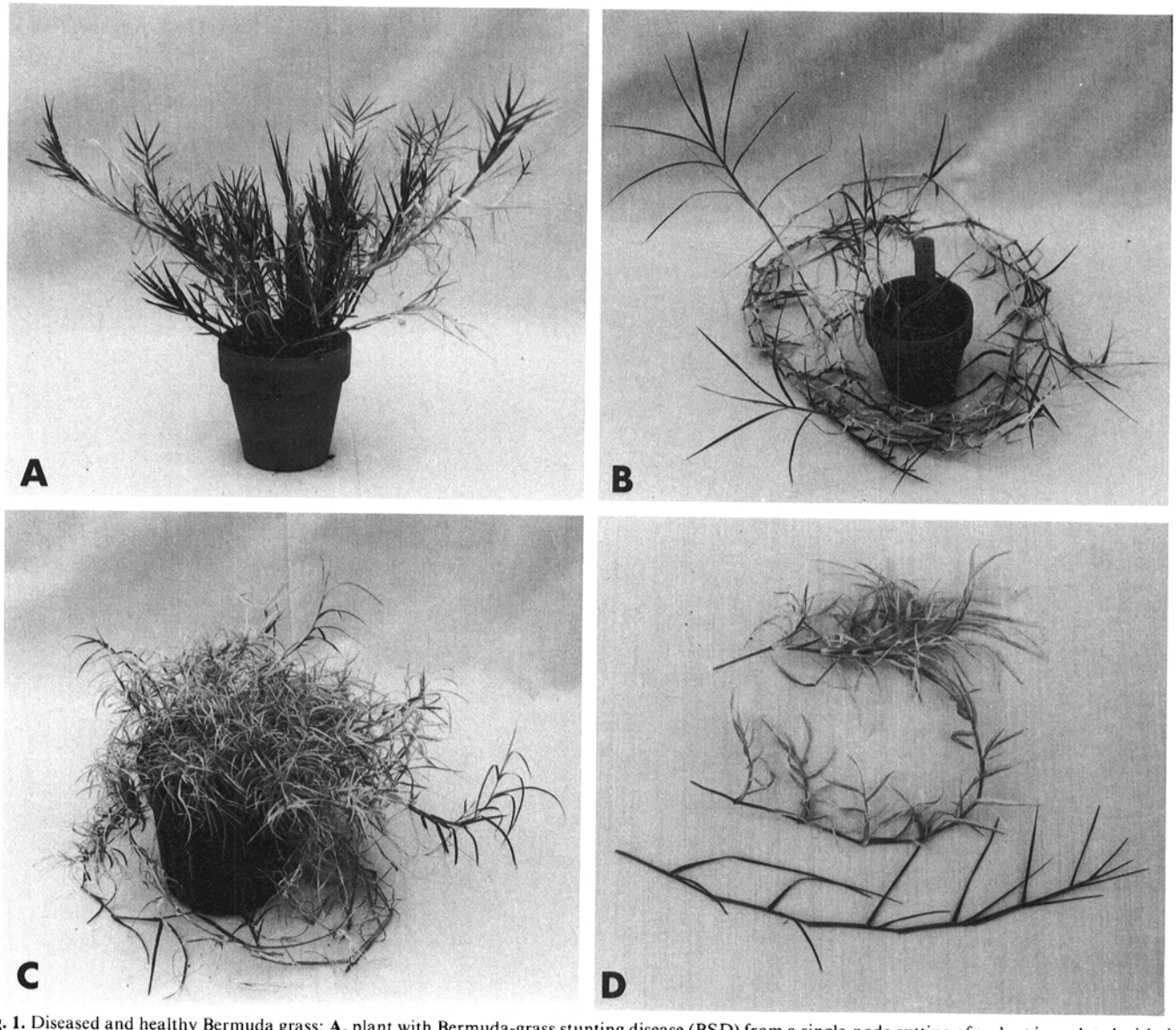

Fig. 1. Diseased and healthy Bermuda grass; **A,** plant with Bermuda-grass stunting disease (BSD) from a single-node cutting of a plant inoculated with the BSD bacterium; **B,** healthy plant with stolons from a single-node cutting of a plant inoculated with phosphate buffer alone; **C,** plant with white leaf disease from H. J. Su; **D,** comparison of a healthy shoot (bottom) to moderately (middle) and extensively (top) diseased shoots with white leaf disease. Plants from T. A. Chen with a mixed infection of the BSD and MLO and propagated at the same time as plants with the MLO alone (white leaf plants) died before photographs were taken.

RESULTS

Etiology of Bermuda grass stunting disease. Bermuda grass plants inoculated with the BSD bacterium became stunted (Fig. 1). The stunting was greater in shoots that developed after infected plants were cut back to the soil level. Plants that developed from cuttings infected with the BSD bacterium were also severely stunted. Internodes were shorter and leaf elongation was inhibited. In contrast, control plants and plants inoculated with RSD bacterium did not become stunted when similarly treated but developed shoots that elongated normally and formed stolons with axillary shoots. Plants inoculated with the BSD bacterium and control plants showed no chlorotic shoots typical of white leaf disease. Severely stunted plants consistently had more shoots that had developed at or below the soil level, but did not develop stolons or have lateral branches on aboveground shoots. One year after cuttings were planted, the average fresh weights of the developing shoots were significantly less for plants from cuttings infected with the BSD bacterium than for control plants and plants grown from cuttings infected with the RSD bacterium (Table 1). Also, the BSD bacterium, but not the RSD bacterium, was consistently observed by phase-contrast microscopy (×1,000) of the expressed sap of plants from infected cuttings (Table 1).

MLOs were observed in phloem of plants (from H. J. Su and T. A. Chen) with white leaf symptoms (Table 2). Plants infected with MLOs from both sources had chlorotic shoots, many axillary shoots, small leaves, and short stolons (Fig. 1). Dieback of extensively symptomatic areas of the plants was often observed. Plants (from T. A. Chen) with the BSD bacterium and MLO (Fig. 2) remained more stunted and died more quickly when shoots with white leaf symptoms were propagated. White leaf plants from both

TABLE 1. Fresh shoot weights of Bermuda grass and the frequency with which the ratoon stunting disease (RSD) and the Bermuda grass stunting disease (BSD) bacteria were observed in expressed sap[y]

Parent infection	Propagated plants (no.)	Plants with bacteria (no.)	Average fresh shoot weight (g)[z]
None	12	0	21.1 a
RSD bacterium	16	3	20.5 a
BSD bacterium	26	24	13.3 b

[y] Progeny of plants infected with the RSD or the BSD bacterium and of buffer-inoculated plants were examined. Data were taken 1 yr after propagation.

[z] Values followed by a different letter are different according to the chi-square test ($P = 0.01$).

TABLE 2. Identification of Bermuda grass stunting disease (BSD) bacterium and white leaf disease (WLD) mycoplasmalike organism in Bermuda grass with single and mixed infections examined by electron microscopy[a]

Disease	Sample source	Sample condition	Frequency of[b] BSD bacterium	Frequency of[b] MLO
BSD	Inoculated[c] plant	Symptomatic	0/5[d]	0/5
WLD	H. J. Su	Symptomatic	0/3	3/3
		Nonsymptomatic	0/2	1/2
BSD+WLD	T. A. Chen	Symptomatic	5/7	6/7
		Nonsymptomatic	0/5	0/5

[a] Node, internode, and in some instances leaf tissues were examined for the presence of the BSD bacterium in the xylem and a mycoplasmalike organism (MLO) in the phloem. Symptomatic and nonsymptomatic portions of some plants were examined.

[b] Frequencies are given as the number of samples with the organism present per the number of samples examined.

[c] Plant was propagated using a single-node cutting from a plant inoculated 1 yr earlier with the BSD bacterium.

[d] Previous sampling from nodes, but not internodes, confirmed the presence of the bacteria, but not MLOs.

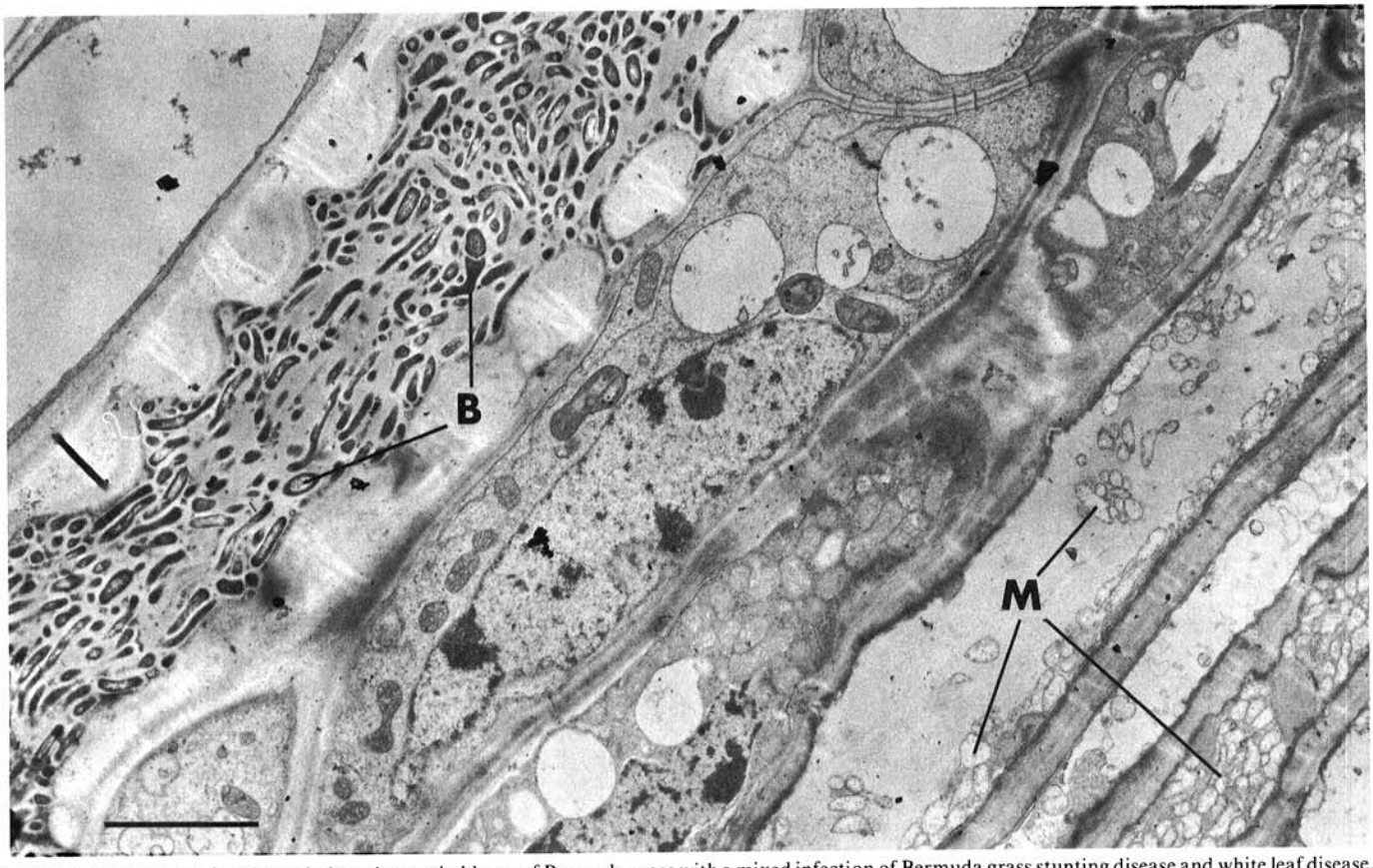

Fig. 2. Longitudinal section through the xylem and phloem of Bermuda grass with a mixed infection of Bermuda grass stunting disease and white leaf disease. The BSD bacterium (B) is shown in a vessel element and the mycoplasmalike organism (M) is shown in the sieve tubes. Scale bar = 10 μm.

sources showed remission of white leaf symptoms during the winter months, when temperatures were lower in the greenhouse. No chlorosis, dieback, or plant death could be attributed solely to infections by the BSD bacterium.

In addition to the MLO, the BSD bacterium was readily observed in the xylem when internode, leaf, or node samples from symptomatic portions of the dually infected plants were examined by electron microscopy (Table 2). MLOs were also readily found in plants (from H.J.Su) with white leaf disease and were more readily found in the nodes than in leaves or internodes. However, at the time these data were taken the BSD bacterium was not found in ultrathin sections of the plants from T. A. Chen. In an earlier microscopy experiment, samples from the nodes of the plants had contained the BSD bacterium.

Immunofluorescence. Only cells of the RSD and BSD bacteria strongly fluoresced when reacted with antiserum prepared against cells of the RSD bacterium extracted from sugarcane confirming previously reported results (3). No specific fluorescence was observed when RSD antiserum was reacted with the recognized species examined in the genera *Agromyces*, *Arthrobacter*, *Corynebacterium*, *Mycobacterium*, and *Nocardia*.

Cell wall analysis. The major amino acids of the cell walls of the RSD and BSD bacteria were 2,4-diaminobutyric acid, glutamic acid, glycine, and alanine. The cell walls of the bacteria also contained fucose and rhamnose. Two strains of the BSD bacterium from Taiwan and strains of the RSD bacterium from the United States, Brazil, and South Africa produced identical results upon cell wall analysis.

DISCUSSION

Both the RSD and BSD bacteria caused stunting of Bermuda grass and a reduction of fresh weight as noted 5 mo after inoculation (3). The stunting was not evident without the buffer-inoculated controls for comparison. However, after cutting the plants back to the soil, regrowth of plants infected with the BSD bacterium, but not the RSD bacterium, showed a severe stunting disease. Similar to RSD, BSD can cause more damage in the ratoon crops than in the plant crop. Microscopic observation of expressed sap revealed that the BSD bacterium readily multiplied in the inoculated plants and their vegetatively propagated progeny, whereas the RSD bacterium did not. Perhaps the RSD bacterium is capable of causing some reduction in the growth of Bermuda grass, but does not survive well in the plants. Another possibility is that some factor, presumably introduced during the inoculation procedure, affected the development of the Bermuda grass inoculated with the RSD bacterium. Our studies indicate that the ability of the RSD and BSD bacteria to incite disease is not limited as much by an active mechanism of host resistance to infection as by the pathogen's capacity to extensively multiply within a particular host plant. This may be a general phenomenon among fastidious, xylem-limited bacteria that results in the wide host range, mostly symptomless, of some organisms such as the RSD bacterium (21) and the Pierce's disease bacterium (5).

The symptomatologies of BSD and white leaf disease of Bermuda grass are distinct. Stunting manifested by shortened internodes and leaves is the most distinct symptom of BSD, whereas white leaf disease is manifested by extensive chlorosis of the plant and proliferation of axillary shoots. No proliferation of shoots was observed in BSD except at or below the soil level. Unlike white leaf disease, no chlorosis and dieback of symptomatic shoots occurs with BSD. White leaf symptoms predominate in dually infected plants; however, the pathogens appeared to be synergistic in their debilitating effects on the plant. Dually infected plants died more frequently and, therefore, vegetative propagation was more difficult.

In contrast to the consistent association of bacteria in sap extracts observed by light microscopy, our examination of ultrathin sections of Bermuda grass infected with the BSD bacterium showed that bacteria are irregularly distributed in the xylem. Bermuda grass from H. J. Su with white leaf consistently showed mycoplasmalike organisms in the phloem of portions of the plant with symptoms but not in symptomless tissue. This result is consistent with the pattern of irregular distribution and the low concentration or absence of MLOs in symptomless tissue. No bacteria were ever observed in the plants from H. J. Su. In contrast, the apparent synergistic effect of the Bermuda-grass bacterium and the MLO on severe disease development in plants from T. A. Chen was correlated with a high concentration of both disease agents in symptomatic tissue. It is possible that the combined infection may stimulate increased multiplication of bacteria. However, a more likely explanation is that the severe stunting induced by the MLO reduces leaf size and internode length and thereby increases the possibility that the bacteria will be observed in samples taken from plants with a greatly reduced surface and sampling area.

Our strains of the RSD and BSD bacteria and those isolated by Liao and Chen (15) were not compared; however, from the preliminary descriptions of the bacteria, the same ones seem to have been isolated. Although our SC medium and the medium of Liao and Chen have considerably different formulations, the essential nutritional requirements of the RSD and BSD bacteria are apparently satisfied by both media. Our preliminary attempts to define these nutritional requirements have shown that growth does not occur in the SC medium without hemin, which is provided by the hemoglobin in the medium of Liao and Chen. Cysteine, also contained in the Isovitalex solution used by Liao and Chen, promotes growth but another sulfur-containing amino acid, methionine, may be substituted. Other possible requirements, such as vitamins, have not yet been defined. The little that is known about the nutritional requirements of the RSD bacterium suggests that the bacterium could not be isolated and maintained in culture on the medium used by Nayiager et al (18), and our preliminary culture data support this conclusion. However, a detailed comparison of the bacteria isolated by the different laboratories as well as the media used would be necessary to conclusively prove this point.

The RSD and BSD bacteria appear to be members of the plant pathogenic corynebacteria based on morphology and cell wall analysis. Our results on the sugar composition of purified cell walls of the RSD bacterium agree with those of Kao et al (11). Rhamnose and fucose have been found by both groups. Our results differ from those of previous reports (11,25) in respect to the amino acid composition of the cell walls. Reports that lysine, ornithine, or both amino acids were found may have arisen as a result of contamination with host or other material from diseased sugarcane extracts. Also, the inherent difficulty of separating some diamino acids by chromatographic methods may have led to misinterpretation of the results. Our results using purified cell walls from cultured bacteria clearly identified diaminobutyric acid and not ornithine or lysine as a major amino acid in the cell walls of both the RSD and BSD bacteria. Diaminobutyric acid is an unusual cell wall amino acid generally found only in some saprophytic and plant pathogenic *Corynebacterium* spp. The RSD and BSD bacteria may have a tetrapeptide containing diaminobutyric acid, glutamine, glycine, and alanine that corresponds to peptidoglycan type B2γ of Schleifer and Kandler (20) and is exemplified by the peptidoglycan in *C. michiganense* and *C. insidiosum*.

Although some data are consistent with the interpretation that the RSD and BSD bacteria are members of the plant pathogenic corynebacteria, IFA staining using antiserum to the RSD bacterium has not revealed any serological relationships between the RSD or BSD bacteria and several Gram-positive bacterial genera, including *Corynebacterium*. However, reciprocal serological tests have not been done and relationships may, therefore, still be found. Further characterization of the RSD and BSD bacteria is needed to taxonomically classify the organisms. Since the bacteria are slow growing and nutritionally fastidious, most standard biochemical tests are of little use without major modification. Chemotaxonomic analyses, such as analysis of DNA homologies, electrophoretic banding patterns of proteins, and cell wall compositions, may hold more promise for the characterization

of these organisms. Axenic culture of the RSD and BSD bacteria makes it possible to further characterize these bacteria and the diseases they cause.

LITERATURE CITED

1. Chen, C. T., Lee, C. S., and Chen, M. J. 1972. Mycoplasmalike organisms in *Cynodon dactylon* and *Brachiaria distachya* affected by white leaf disease. Rep. Taiwan Sugar Exp. Stn. 56:49-55.
2. Chen, T. A., Su, H. J., Raju, B. C., and Huang, W. C. 1977. A new spiroplasma isolated from Bermudagrass (*Cynodon dactylon* L. Pers.). (Abstr.) Proc. Am. Phytopathol. Soc. 4:171.
3. 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 240:1365-1367.
4. Davis, M. J., Whitcomb, R. F., and Gillaspie, A. G., Jr. 1981. Fastidious bacteria of plant vascular tissue and invertebrates. Pages 2172–2188 in: The Prokaryotes. M. P. Starr, H. Stolp, H. G. Truper, A. Balows, and H. G. Schlegel, eds. Springer-Verlag, Berlin, Heidelberg, and New York.
5. Freitag, J. H. 1951. Host range of the Pierce's disease virus of grape, as determined by insect transmission. Phytopathology 41:921-934.
6. Gillaspie, A. G., Jr. 1978. Ratoon stunting disease of sugarcane: Serology. Phytopathology 68:529-532.
7. Gillaspie, A. G., Jr. 1978. An antiserum to the ratoon stunting disease-associated bacterium. Sugarcane Pathol. Newsl. 20:5.
8. Gillaspie, A. G., Jr., Davis, R. E., and Worley, J. F. 1973. Diagnosis of ratoon stunting disease based on the presence of a specific microorganism. Plant Dis. Rep. 57:987-990.
9. Harris, R. W., and Gillaspie, A. G., Jr. 1978. Immunofluorescent diagnosis of ratoon stunting disease. Plant Dis. Rep. 62:193-196.
10. Kamiunten, H., and Wakimoto, S. 1976. Coryneform bacteria found in the xylem of the ratoon stunting diseased sugarcane. Ann. Phytopathol. Soc. Jpn. 42:500-503.
11. Kao, J., Blakeney, E. W., Gerencser, M. A., and Damann, K. E., Jr. 1980. Cell wall composition, percent GC in the DNA, and serotyping of the bacterium associated with ratoon stunting disease of sugarcane. (Abstr.) Phytopathology 70:568.
12. Kao, J., and Damann, K. E., Jr. 1978. Microcolonies of the bacterium associated with ratoon stunting disease found in sugarcane xylem matrix. Phytopathology 68:545-551.
13. Kao, J., and Damann, K. E., Jr. 1980. In situ localization and morphology of the bacterium associated with ratoon stunting disease of sugarcane. Can. J. Bot. 58:310-315.
14. Lechevalier, M. P., and Lechevalier, H. A. 1980. Chemotaxonomy of Actinomycetes. Pages 227–291 in: Actinomycete Taxonomy. A. Dietz and D. W. Thayer, eds. Society for Industrial Microbiology. Spec. Publ. 6. Arlington, VA.
15. Liao, C. H., and Chen, T. A. 1981. Coryneform bacteria in ratoon-stunted sugarcane and in white leaf-diseased Bermudagrass: Isolation, cultivation, and pathogenic role. (Abstr.) Phytopathology 71:236.
16. Liu, L. J., Cortes-Momllov, A., Maramorosch, K., Hirumi, H., Pérez, J. E., and Bird, J. 1974. Isolation of an organism resembling *Xanthomonas vasculorum* from sugarcane affected by ratoon stunting disease. Proc. Int. Soc. Sugar Cane Technol. 15:234-240.
17. Maramorosch, K., Plavsic-Banjac, B., Bird, J., and Liu, L. J. 1973. Electron microscopy of ratoon stunted sugar cane: Microorganisms in xylem. Phytopathol. Z. 77:270-273.
18. Nayiager, M. P., Oellermann, R. A., and Roth, G. 1980. The isolation, culture and morphology of the bacteria associated with ratoon stunting disease of sugarcane. Phytopathol. Z. 99:273-281.
19. Raju, B. C., and Chen, T. A. 1980. Growth, morphology and ultrastructural studies of a spiroplasma associated with Bermudagrass showing witches'-broom symptoms. J. Plant Dis. Prot. 87:37-45.
20. Schleifer, K. H., and Kandler, O. 1972. Peptidoglycan types of bacterial cell walls and their taxonomic implications. Bacteriol. Rev. 36:407-477.
21. Steindl, D. R. L. 1961. Ratoon stunting disease. Pages 433–459 in: Sugarcane Diseases of the World. Vol. 1. J. P. Martin, E. V. Abbott, and C. G. Hughes, eds. Elsevier Publ. Co., Amsterdam. 542 pp.
22. Teakle, D. S., Kontze, D., and Appleton, J. M. 1979. A note on the diagnosis of ratoon stunting disease of sugarcane by negative-stain electron microscopy of the associated bacterium. J. Appl. Bacteriol. 46:279-284.
23. Teakle, D. S., Smith, P. M., and Steindl, D. R. L. 1973. Association of a small coryneform bacterium with the ratoon stunting disease of sugarcane. Aust. Agric. Res. 24:869-874.
24. Tokeshi, H., Sanguino, A., and Akiba, F. 1974. *Xanthomonas albilineans*, provavel agente causal de raqiutismo da soqueira e escaldadura de cana-de-acucar. Brasil Acucareiro 84(6):564-576.
25. Weaver, L., Teakle, D. S., and Hayward, A. C. 1977. Ultrastructural studies on the bacterium associated with the ratoon stunting disease of sugar-cane. Aust. J. Agric. Res. 28:843-852.
26. Worley, J. F., and Gillaspie, A. G., Jr. 1975. Electron microscopy in situ of the bacterium associated with ratoon stunting disease in sudangrass. Phytopathology 65:287-295.
27. Zelcer, A., Bar-Joseph, M., Fleischer, Z., Klein, M., Cohen, S., and Loebenstein, G. 1972. Mycoplasmalike organisms associated with plant disease in Israel. Page 13 in: Summ. 3rd Israel Cong. Plant Pathol. 21–22 February, Rehovot. 94 pp.