Evidence that Ratoon Stunting Disease of Sugarcane Is Caused by Virus and Not Mycoplasma

A. G. Gillaspie, Jr.

Research Plant Pathologist, Crops Research Division, ARS, USDA, Houma, Louisiana 70360.

The author thanks Charles Pfizer and Company, Brooklyn, New York, for supplying the tetracycline compounds used. Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the USDA, and does not imply its approval to the exclusion of the other products that may also be suitable.

Accepted for publication 22 April 1970.

ABSTRACT

Treatment of ratoon stunting disease-affected sugarcane (*Saccharum* tri-specific hybrid) with tetracycline compounds did not eliminate the infectious agent from the cane, nor did it affect symptom expression. The evidence presented indicates that a mycoplasma is not the causal agent, and does not contradict previous evidence that a virus is. The results illustrate that not all diseases of obscure etiology involve mycoplasma, and provide another example showing that tetracyclines are ineffective against a disease induced by a virus. Phytopathology 60:1448-1450.

In 1967, Japanese scientists described structures resembling mycoplasma or psittacosis-lymphogranuloma-trachoma bodies in plants infected with diseases previously attributed to viral agents (2). In 1968, pathologists in Taiwan (8) and Japan (11) reported mycoplasmalike bodies associated with white leaf disease of sugarcane. White leaf is heat curable (54 C, 50-min hot water treatment) and is transmitted by a leafhopper (8, 10). Lin & Lee (9) reported the successful culturing and reinoculation of the mycoplasmal agent of white leaf. In their experiments, tetracycline compounds depressed symptom expression.

The tetracycline group of antibiotics reduces (i) the number of mycoplasmalike bodies (7); (ii) the severity of symptom expression (3); or induces (iii) complete symptom remission (1, 5, 12) in several diseases where mycoplasma may be involved. An effect of tetracycline on symptom expression has become accepted as an indication of mycoplasmal etiology.

Ratoon stunting disease (RSD) of sugarcane lacks external symptoms; is heat curable; and, as reported for the alfalfa mosaic virus-mycoplasma complex (6), is mechanically transmissible. RSD is characterized by two types of symptoms present in the interior of the stem of some varieties. These are the juvenile symptoms, which consist of reddish- to salmon-colored discolorations of the area of the young shoots just below the growing point, and the mature nodal symptoms, which consist of reddish to brownish discolorations of the vascular bundles in the node. Other effects of the disease (e.g., reduced growth) are quantitative and cannot be used diagnostically.

The infectious agent of RSD appears to be a virus (4), but it has not been completely characterized. The agent is sensitive to organic solvents, high ionic strengths, and other conditions used in clarifying and purifying virus preparations. The disease is latent at many stages of development, so it is difficult to compare it to the diseases having mycoplasmal etiologies. Since so many of the diseases of questionable etiology are being re-examined, the possibility that this puzzling disease agent is mycoplasmal rather than viral was explored through the use of tetracycline.

MATERIALS AND METHODS.—Five tetracycline compounds were tested separately in this experiment. These were oxytetracycline hydrochloride, tetracycline hydrochloride, methacycline hydrochloride, oxytetracycline amphoteric, and tetracycline base. The solutions were prepared at a concn of 100 ppm. The compounds were dissolved in 0.005 M potassium phosphate buffer, pH 7. Concn up to 1,000 ppm were previously tested; at concn between 500-1,000 ppm, some of the compounds did not fully dissolve.

Infected plants of an interspecific hybrid of sugarcane, variety C.P. 44-101, were used as test plants because of their reliable symptom expression. The diseased sugarcane was treated with tetracycline by one of five methods: (i) single-bud cuttings were dipped in antibiotic solutions for 1-3 hr, then planted in pots in soil; (ii) single-bud cuttings were split lengthwise, aspirated for 10 min in the antibiotic solutions using a water-aspirator, then planted in an upright position in vermiculite in 3-inch peat pots; (iii) single-bud cuttings were grown in vermiculite for 1 month; the vermiculite was removed from the roots, the roots were then dipped in the antibiotic solutions for 10-15 min, and the cuttings were planted in soil in pots (removal of the vermiculite damaged the roots and killed some of the plants); (iv) two-bud cuttings were germinated in an upright position in tumblers of distilled water; when roots had formed (about 10 days) they were dipped in antibiotic solutions for 1 hr and the setts (the original stem cuttings that supported the young shoots) were planted in soil in pots; (v) two-bud cuttings from which the lower bud had been removed were washed in running water for 2 hr, placed in an upright position in tumblers filled with distilled water, and, after roots and buds had begun to grow (10-15 days), the water in the tumblers was replaced by antibiotic solutions which were changed every 3-4 days. For this experiment, the plants were grown in a growth chamber (27 C, 11-hr photoperiod); for the other experiments, the plants were grown under greenhouse conditions.

Juvenile symptoms were checked within 2 months (about the 5-6 leaf stage) (4). Some plants were grown for about 6 months to check mature nodal symptoms. The infectious agent of RSD was assayed

TABLE 1. Symptom expression of ratoon stunting disease after treatment with tetracycline compounds

Treatment solution	Tetracycline applied by: Dipping cuttings — Symptoms observed: Juvenile[c]	Dipping cuttings — Symptoms observed: Nodal[d]	Aspirating cuttings — Symptoms observed: Juvenile	Dipping roots A[a] — Symptoms observed: Juvenile	Dipping roots A[a] — Symptoms observed: Nodal	Dipping roots B[b] — Symptoms observed: Juvenile	Dipping roots B[b] — Symptoms observed: Nodal
0.005 M PO_4, pH 7	10/10[e]	5/5	16/16	2/2	7/7	10/10	4/5
Oxytetracycline amphoteric	10/10	10/11	14/14	—	3/3	7/7	4/5
Tetracycline hydrochloride	9/9	9/9	16/16	9/9	8/8	6/6	6/7
Oxytetracycline hydrochloride	7/9	9/9	21/21	9/9	6/7[f]	7/7	5/6
Tetracycline base	9/9	6/6	20/20	10/10	10/10	6/6	7/7
Methacycline hydrochloride	9/9	7/9	15/15	8/8	2/2	6/6	5/6

[a] Cuttings grown in vermiculite, vermiculite removed, and roots dipped.
[b] Cuttings germinated upright in water, roots dipped, and planted in soil.
[c] Symptom is a reddish- to salmon-colored discoloration of young stem just below the growing point.
[d] Symptom is a reddish to brownish discoloration of the vascular bundles in the node.
[e] Number positive per number surviving; plants not positive were either too small or questionable.
[f] One plant showed no nodal symptoms but was found to contain infectious agent when assayed.

on stem cuttings of healthy C.P. 44-101 (4). Juice for assay was prepared by grinding immature stalks in a mortar and pestle in water and by grinding mature stalks in a laboratory mill and extracting in a Carver press. The juice was applied to the freshly cut surface of healthy cuttings. Symptoms were read after 1-2 months of growth.

RESULTS AND DISCUSSION.—The results (Table 1) show no evidence of inhibition in juvenile or nodal RSD-symptom expression in the treated cane. One mature stalk showing nodal symptoms was taken from each treatment and ground, and the juice assayed on healthy C.P. 44-101. The assay results show the agent to be present in each case. Similar results were obtained in tests in which cuttings were dipped in other concn of antibiotic. In some tests, higher concn of 500-1,000 ppm were phytotoxic. Tetracycline thus had not repressed symptoms nor eliminated the infectious agent.

When sugarcane cuttings were grown directly in the tetracycline solutions (v), the phytotoxicity of these compounds, at the concn used, affected the growth of the young shoots and roots adversely. The roots became browned and appeared dead. Only a few of the treated plants showed typical juvenile symptoms (Table 2). The plants without symptoms were assayed as a group for each tetracycline compound used. The infectious agent was shown to be present in all the samples of the setts and in two of the five samples of young shoots. These results reflect the difficulties of working with this disease. Unless shoot growth is adequate, the frequency with which juvenile symptoms are expressed becomes reduced and the symptoms lessen. The concn of the infectious agent in the young portions of the stem is low, and as a consequence, assay results are uncertain.

The results of all experiments indicate that the tetracyclines did not eliminate the infectious agent from sugarcane, and that the tetracyclines did not affect symptom expression for the two types of symptoms available unless they also damaged the growth of the plant. There is no evidence that the causal agent of RSD is a mycoplasma rather than a virus. The results illustrate that not all diseases of puzzling or obscure etiology involve mycoplasma, and provide another example of inefficiency of tetracyclines against disease induced by a virus.

LITERATURE CITED

1. DAVIS, R. E., R. F. WHITCOMB, & R. L. STEERE. 1968. Remission of aster yellows disease by antibiotics. Science 161:793-795.
2. DOI, Y., M. TERENAKA, K. YORA, & H. ASUYAMA. 1967. Mycoplasma- or PLT group-like microorganisms found in the phloem elements of plants infected with mulberry dwarf, potato witches' broom,

TABLE 2. Symptom expression of ratoon stunting disease in infected plants grown in tetracycline and in assay plants

Treatment solution	Positive	Questionable	Negative	Assay of parts[a] from plants negative for symptoms: Setts	Assay of parts[a] from plants negative for symptoms: Shoots
0.005 M PO_4, pH 7	10	1	0	+	+[b]
Oxytetracycline amphoteric	2	5	5	+	—
Tetracycline hydrochloride	0	5	7	+	+
Oxytetracycline hydrochloride	1	5	5	+	—
Tetracycline base	3	5	4	+	—
Methacycline hydrochloride	0	2[c]	2	+	+

[a] Tetracycline-treated plants which were symptomless at the time when symptoms were read were pooled by treatment. The original, infected stem cuttings (setts) and their roots were ground and assayed on healthy C.P. 44-101; and the 0.5 inch of the young shoot closest to the sett was ground and assayed.
[b] With the buffer control positives were tested since no negatives were present.
[c] Seven dead.

aster yellows, or paulownia witches' broom. [In Japanese, English summary.] Ann. Phytopathol. Soc. Japan 33:259-266.

3. Freitag, J. H., & S. H. Smith. 1969. Effects of tetracyclines on symptom expression and leafhopper transmission of aster yellows. Phytopathology 59: 1820-1823.
4. Gillaspie, Jr., A. G., J. E. Irvine, & R. L. Steere. 1966. Ratoon stunting disease virus. Assay technique and partial purification. Phytopathology 56:1426-1427.
5. Granados, R. R. 1969. Chemotherapy of the corn stunt disease. Phytopathology 59:1556 (Abstr.).
6. Hampton, R. O., J. O. Stevens, & T. C. Allen. 1969. Mechanically transmissible mycoplasma from naturally infected peas. Plant Dis. Reptr. 53:499-503.
7. Ishiie, T., Y. Doi, K. Yora, & H. Asuyma. 1967. Suppressive effects of antibiotics of tetracycline group on symptom development of mulberry dwarf disease. [In Japanese, English summary.] Ann. Phytopathol. Soc. Japan 33:267-275.
8. Lin, S. C., & C. S. Lee. 1968. Mycoplasma or mycoplasma-like microorganism in white leaf disease of sugarcane. Taiwan Sugar Exp. Sta. Annu. Rep., 1967-1968. 17-22.
9. Lin, S. C., & C. S. Lee. 1969. White leaf disease of sugarcane. The culturing and successful re-inoculation of the causal Mycoplasma. Sugarcane Pathol. Newsletter 3:2-3.
10. Matsumoto, T., C. S. Lee, & W. S. Teng. 1968. Studies on sugar cane white leaf disease of Taiwan, with special reference to the transmission by a leafhopper, *Epitettix hiroglyphicus* Mats. Int. Soc. Sugar Cane Technol. Proc. 13:1090-1099.
11. Shikata, E., K. Maramorosch, K. C. Ling, & T. Matsumoto. 1968. On the mycoplasma-like structures encountered in the phloem cells of American aster yellows, corn stunt, Philippine rice yellow dwarf and Taiwan sugar cane white leaf diseased plants. Ann. Phytopathol. Soc. Japan 34:208-209 (Abstr.).
12. Story, G. E., & R. S. Halliwell. 1969. Association of mycoplasma-like organism with the bunchy top disease of papaya. Phytopathology 59:1336-1337.