Further Developments in the Study of the Ratoon Stunting Disease of Sugarcane in Puerto Rico[1]

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ABSTRACT

Small, coryneform, noncultivable xylem-inhabitating bacteria with mesosomes, measuring 1430-1830 nm × 140-165 nm, were observed in negatively stained juice extracted from sugarcane varieties B 49-119, PR 1059 and PR 1140, showing internal symptoms of ratoon stunting disease. Ten sugarcane varieties, PR 1059, PR 1140, PR 61-902, PR 62-258, PR 62-285, PR 64-15, PR 64-610, B 49-119, CB 49-260 and CP 52-43, were tested for resistance to ratoon stunting disease. PR 61-902, inoculated with the causal agent, produced significantly less cane and sugar per acre (approximately 31%) in plant cane, first ratoon and second ratoon crops as compared with those obtained from disease-free, hot water treated nursery stock. Therefore, it is recommendable to use only the ratoon stunting disease-free seedpieces of this variety for commercial planting in Puerto Rico.

INTRODUCTION

Ratoon stunting disease (RSD) of surgarcane has long been assumed to be caused by a virus. However, this concept was altered considerably in 1972 when Plavsic-Banjac and Maramorosch observed the presence of pleomorphic bodies resembling small bacteria or mycoplasma in the xylem of RSD-affected canes (12). In 1973, Maramorosch et al. considered the pleomorphic bodies representing bacteria or rickettsiae (11). In 1974, Liu et al. (8, 9) in Puerto Rico, isolated a bacterium resembling *Xanthomonas vasculorum* from RSD-affected canes while Teakle et al. in Australia observed the presence of a small coryneform bacterium (16, 17). Although this coryneform bacterium has never been isolated in culture, reports from several countries (1, 2, 3, 7, 13, & 16) seemed to support its relationship with the RSD-affected canes. The role of the bacterium in the etiology of the disease was further investigated locally. The purpose of this paper is to present the results of studies on the negatively stained juice extracted from RSD-affected canes. Data obtained from a local RSD variety yield trial are also included.

MATERIALS AND METHODS

The majority of the samples of sugarcane varieties B 49-119, PR 1140, and PR 1059 showing internal symptoms of RSD in electron microscope

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examinations were obtained from a nursery which was established in 1971 at the Gurabo Substation. Ratoon stunting-affected canes were surface-sterilized with 70% alcohol and then cut into small pieces with a sterile knife. The reddish discolored vascular bundle tissues from the nodes of diseased canes were chopped vertically on a 3 × 3 in plate of dental wax with a sterile razor blade in a sterile petri dish containing 100 ml of double distilled water. The suspension was then poured into a test tube. A 0.5% sucrose solution was added to the suspension. The suspensions were stained with a 1.5% solution of phosphotungstic acid (PTA). A carbon coated grid was left floating on the surface of the suspensions for approximately 10 min. The excess water on the grid was removed carefully with a piece of filter paper and the grid examined immediately in a Siemen's electron microscope at 80 KV.

In the yield trial for varietal resistance to RSD, 10 sugarcane varieties, PR 1059, PR 1140, PR 61-902, PR 62-258, PR 62-285, PR 64-15, PR 64-610, B 49-119, CB 49-260, and CP 52-43 were planted in a Fraternidad soil (a very fine montmorillonitic isohyperthermic family of Udic Chromusterts) in December 1972 at the Lajas Substation with seedpieces derived from hot water treated (50.5°C for 2½ h) as well as from the RSD-inoculated nursery stock. This experiment consisted of 80 plots (25 × 25 ft) with a split-plot field design. Each treatment was replicated 4 times. Data on percent sucrose, tonnage of cane and yield (hundredweight of sugar per acre) from plant cane, first ratoon and second ratoon crops were obtained and statistically analyzed. The nursery and the experimental plots were hand-weeded to avoid possible effect by herbicides. Unfortunately, the first ratoon crop was accidentaly burned before harvest. Machetes used in harvesting were disinfected with a 10% lysol solution before passing from one experimental plot to another. The plant cane was harvested in February 1974 at 13½ months of age. The first ratoon crop was harvested in March, 1975 at 13 months of age and the second ratoon in March 1976 at 12 months.

RESULTS

STUDIES ON THE CAUSAL AGENT

Figure 1 shows coryneform bacteria with mesosomes were found in the negatively stained juice extracted from RSD-affected canes of B 49-119, PR 1059 and PR 1140, but not from those extracted from hot water treated canes of the same varieties. The coryneform bacteria measured 1,430-1,830 nm long × 140-165 nm wide and were non-cultivable.

RSD VARIETY YIELD TRIALS

Table 1 shows that the tonnage of cane per acre from plant cane, first ratoon, second ratoon and combined crops was significantly reduced by

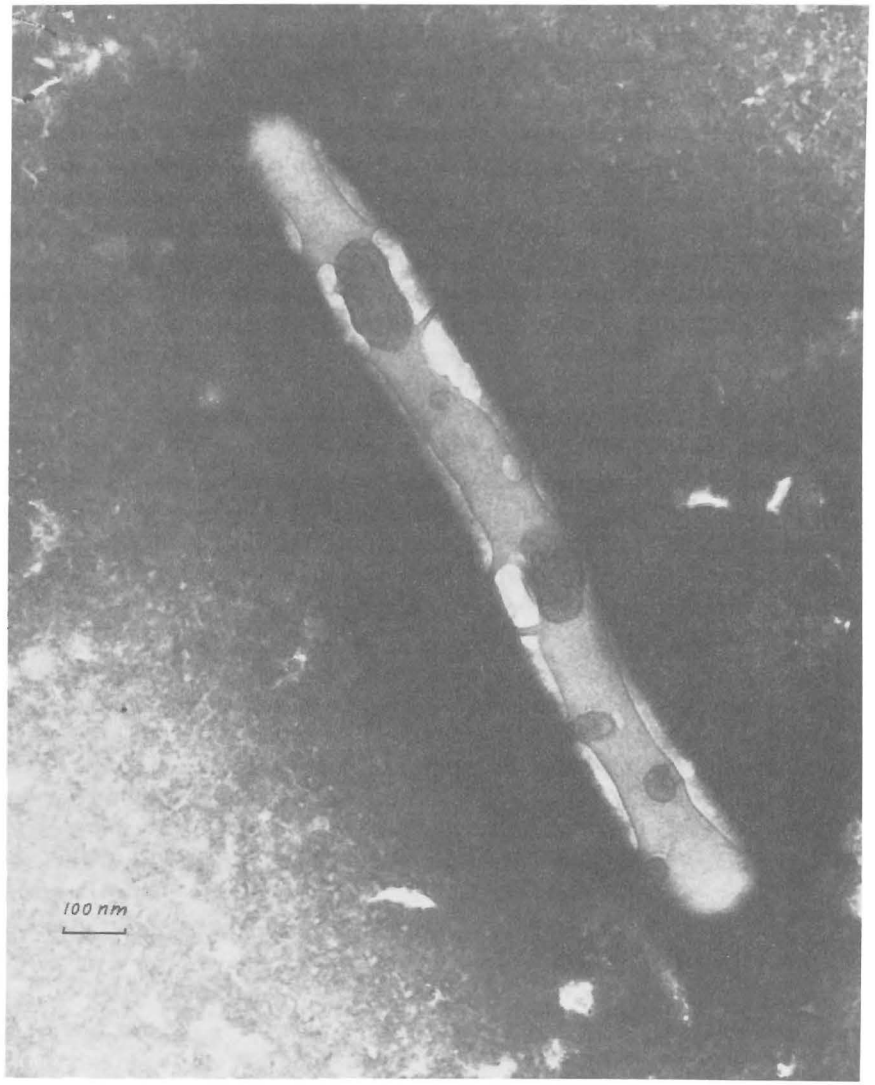

FIG. 1.—Negatively stained, coryneform bacterium observed in the juice extracted from ratoon stunting affected canes. Note the presence of mesosomes. (× 140,000)

inoculation with juice extracted from RSD-affected canes in variety PR 61-902. Production of sugar per acre also was reduced significantly (approximately 31%) in PR 61-902 as a result of reduced tonnage. Neither tonnage of cane per acre nor percent yield was significantly reduced by inoculation on varieties PR 64-15, PR 64-610, B 49-119, CB 49-260, PR 62-258, PR 1059, CP 52-43, PR 62-285 and PR 1140. No statistically

TABLE 1.—*Sucrose percent yield, tons of cane per acre and hundredweights of sugar per acre of the 10 sugarcane varieties inoculated with juice extracted from ratoon stunt affected canes*

Variety	Treatments	Sucrose percent yield				Tons of cane per acre				Hundredweights of sugar per acre			
		Plant cane	1st ratoon	2nd ratoon	Combined crops	Plant cane	1st ratoon	2nd ratoon	Combined crops	Plant cane	1st ratoon	2nd ratoon	Combined crops
PR 61-902	HWT	12.37	12.81	12.63	12.60	41.68[1]	48.04[1]	44.04[1]	44.59[1]	102.63[1]	122.48[1]	110.83[1]	111.98[1]
	RSD	12.05	11.30	12.33	11.89	31.40	34.02	30.90	32.08	75.53	76.66	76.71	76.30
PR 64-15	HWT	12.09	10.31	12.01	11.47	45.76[1]	47.63	28.77	40.72	111.03[2]	97.92	70.25	93.07
	RSD	12.27	13.38	11.70	12.45	36.23	40.56	28.00	34.93	88.92	108.13	65.70	87.58
PR 64-610	HWT	12.84	15.05	14.89	14.26	41.66[2]	46.55	39.17	42.46	107.24	137.08	119.13	121.15
	RSD	13.01	15.41	14.92	14.44	35.62	38.37	38.85	37.62	92.51	119.34	116.40	109.42
B 49-119	HWT	11.23	10.77	11.83	11.28	47.94[2]	57.30	52.11	52.45	107.55[1]	123.32	122.85	117.91
	RSD	10.08	10.98	12.24	11.10	40.82	52.54	47.31	46.89	82.36	111.68	115.72	103.25
PR 62-258	HWT	11.77	14.01	11.46	12.41	40.43	46.96	37.86	41.75	95.02	132.73	83.53	103.76
	RSD	11.60	12.53	12.50	12.21	41.35	43.41	39.57	41.25	96.10	107.82	99.22	101.05
PR 1059	HWT	12.83	12.59	13.81	13.08	40.12	39.60	34.63	38.12	103.41	100.73	95.33	99.82
	RSD	12.52	13.85	14.81	13.72	34.73	39.87	35.04	36.55	87.40	109.97	104.28	100.55
CB 49-260	HWT	10.04	10.89	11.12	10.68	43.85	56.48[2]	53.02	51.12	88.87	122.16	117.82	109.62
	RSD	11.32	10.73	11.72	11.25	42.52	46.82	47.06	45.46	96.35	100.65	109.85	102.28
CP 52-43	HWT	12.40	13.41	12.94	12.92	40.20	47.50	47.60	45.10	99.75	126.45	122.99	116.40
	RSD	12.47	14.18	13.10	13.25	35.27	42.60	43.21	40.36	88.02	120.72	113.07	107.27
PR 62-285	HWT	12.20	12.00	12.83	12.34	39.44	52.74	47.27	46.48	96.32	126.64	121.08	114.68
	RSD	12.33	13.07	13.16	12.86	40.67	52.94	49.41	47.67	100.43	139.94	130.30	123.55
PR 1140	HWT	12.99	13.42	14.75	13.72	33.25	41.51	38.62	37.97	86.52	111.91	114.94	104.46
	RSD	12.88	13.74	13.80	13.47	34.54	35.93	37.62	36.03	89.66	98.63	104.42	97.57
Average	HWT	12.08	12.53	12.83	12.48	41.43[1]	48.43[2]	42.31	44.06[1]	99.84[1]	120.14[2]	107.88	109.28[1]
	RSD	12.05	12.91	13.03	12.66	37.31	42.71	39.70	39.90	89.73	109.35	103.57	100.88

[1] Significant at the 1% level.
[2] Significant at the 5% level.
HWT = Canes derived from hot water treated nursery.
RSD = Canes derived from ratoon stunt inoculated nursery.

significant percent sucrose change because of inoculation occurred in any of the varieties tested.

DISCUSSION

Results obtained in this study agree with the findings of Bailey (1), Chen (2), Damann (3, 4, 5), Gillaspie (6, 7), Ricaud (13), and Teakle (16), who reported the presence of coryneform, xylem-inhabitating bacteria in RSD-affected canes. It is interesting to note also that the *Xanthomonas vasculorum*-like organism as reported by Liu et al. (8, 9) could be isolated under local conditions approximately 30% of the time from RSD affected canes showing no gumming disease symptoms. Although fresh cultures of *Xanthomonas vasculorum*-like organisms produced symptoms resembling those of RSD, the infectivity, however, was rather low.

The constant presence of coryneform, small bacterium with mesosomes in the xylem extracted juice of RSD-affected canes as found in this study merits special attention, although the validity of mesosomes in classification of bacteria is still under study by several investigators. Heat as well as phenothyl alcohol induces the formation of mesosomes (14, 15), and gram negative bacteria such as *Xanthomonas* spp., on the other hand, produce no mesosomes after staining with PTA (19). Since this coryneform bacterium is non-cultivable and since its pathogenicity has not yet been established, its etiological role remains to be determined.

Results obtained from the RSD variety yield trial agree also with the findings of López Rosa (10), who reported in 1970 that some of the RSD-affected varieties produced significantly fewer tons of cane and sugar per acre as compared with those derived from hot water treated nursery stock. Since RSD-affected PR 61-902 produced significantly less canes, sugar per acre (approximately 31%) in plant cane, first ratoon and second ratoon crops, it is recommendable to use only the RSD-free seedpieces of this variety for commercial plantings in Puerto Rico.

RESUMEN

Estudios de microscopía electrónica realizados con savia exprimida del xilema de caña de azúcar de las variedades B 49-119, PR 1059 y PR 1140 que presentaban síntomas de enanismo de retoño, demostraron en tinción negativa, la presencia de pequeñas corinebacterias con mesosomas de tamanño 1430-1830 nm × 140-165 nm. Las bacterias fueron no cultivables.

Las siguientes diez variedades, PR 1059, PR 1140, PR 61-902, PR 62-258, PR 62-285, PR 64-15, PR 64-610, B 49-119, CB 49-260 y CP 52-43, fueron sometidas a la prueba de resistencia a la enfermedad del enanismo del retoño. La variedad PR 61-902 infectada, produjo significativamente menos caña y azúcar por acre (aproximadamente 31%) en la plantilla, primero y segundo retoños, en contraste con los resultados obtenidos de las cañas libres de enfermedad cuando se usó semilla tratada con agua caliente. Por lo tanto, para la siembra de esta variedad en plantaciones comerciales de Puerto Rico se recomienda usar semilla tratada.

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