SEROLOGICAL REACTIVITY OF SUGARCANE-MOSAIC VIRUS

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INTRODUCTION

Sugarcane mosaic is a widespread disease occuring in many parts of the world. It is a disease of economic importance and has been reported several times in Puerto Rico (1, 2, 3, 4)[2]. Using the precipitin test, Chester (5) reported negative findings on the serological activity of the virus causing this disease. However, in 1935, Desai (6) reported on the antigenic properties of sugarcane-mosaic virus. Since his report, investigations on the serological properties of the virus seem to have remained at a standstill. It thus appeared desirable to reopen the investigation of sugarcane mosaic along these lines with the idea of preparing antisera for use in field surveys of this disease.

Moreover, Summers, Brandes, and Rands (7) had demonstrated the existence of several strains of sugarcane-mosaic virus by the inoculation of differential hosts. Bruehl (8) recently obtained evidence of the existence of several strains of the virus in Puerto Rico. The possibility of studying the relationship between the various strains by means of serological tests (9) therefore remained open.

The present paper is a preliminary report on the serological activity of sugarcane-mosaic virus.

MATERIALS AND METHODS

Preparation of Virus (Antigen) for Inoculation

The sugarcane-mosaic virus was propagated in sugarcane variety B.H. 10–12 grown in the Station grounds. Cane juice was expressed by grinding in a meat chopper at 7°C. The juice was then centrifuged at about 3,800 r.p.m. in a refrigerated International PR-1 centrifuge for 20 minutes. The sediment was discarded and the supernatant liquid was again centrifuged at about 19,000 r.p.m. (25,000 g)[3] for 2 hours using a "multispeed attachment." The sediment thus obtained was resuspended in 0.01-molar phosphate buffer at pH 7.0, using $\frac{1}{10}$ of the original volume of juice. After suspension in the buffer a homogeneous light-green liquid was obtained. This material was then subjected to a second low-speed cycle and,

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[2] Numbers in parentheses refer to Literature Cited, pp. 14–5.

[3] Gravitational force in kilograms per gram.

although a slight dark-green sediment was obtained, the supernatant liquid retained a green opalescent color. This supernatant liquid was then distributed in small vials and kept at −15°C. until used for inoculation or other tests. This is referred to as "concentrate" below. All centrifuging was carried out at temperatures of 4° to 8°C.

INFECTIVE CONTROLS

Qualitative tests for the presence of virus in (1) the expressed juice, (2) the supernatant liquid after high-speed centrifuging, and (3) the concentrate were carried out by rubbing leaves of sorghum, *Holcus sorghum*, using the carborundum method. Ten to twenty plants were used for each control. All controls were positive for virus as shown by the development of characteristic symptoms in the majority of plants from 7 to 12 days after inoculation.

PREPARATION OF ANTISERUM

Young healthy rabbits weighing about 6 pounds each were used in preparing the antiserum. Normal serum was obtained from each rabbit before inoculation. Each animal was then injected once a week with varying amounts of antigen by both the intraperitoneal and intravenous routes. Serum was obtained from the rabbits 1 week after the second inoculation and tested for antibody by the precipitation technique described below. A second series of two injections per week was then given for 2 weeks and the serum retested. The rabbits were then exsanguinated under aseptic conditions and the serum was placed in vaccine bottles and refrigerated until used. The precipitation test described below was used solely for determining the antibody response.

Precipitation Test

PREPARATION OF ANTIGEN

Healthy cane juice from variety B.H. 10–12 plants, as well as infective juice from mosaic-diseased plants of the same lot used for preparing the antigen for inoculation, was expressed as described before and centrifuged at 3,000 r.p.m. for 30 minutes. The supernatant liquids were placed in the icebox overnight. Next day the juices were centrifuged in a Servall SS-2 angle centrifuge at 6,000 r.p.m. (6,000 g) for 15 minutes for final clarification. The antigen prepared by high-speed centrifugation was also used in testing the sera for antibodies, the sera reacting to the same titer as with the fresh antigen.

ABSORPTION OF SERUM

After the healthy cane juice had been centrifuged at 3,000 r.p.m. for 30 minutes it was mixed with antiserum in the proportion of 3 parts of juice

to 1 part of serum and incubated at 37°C. for 2 hours. The serum-juice mixture was then removed from the incubator and placed in the icebox overnight. Next day any precipitate formed was thrown down by centrifuging at 2,000 r.p.m. for 20 minutes. The absorbed sera were then ready for use.

TITRATION OF ANTISERUM

The absorbed serum was diluted in buffered saline of pH 7 in twofold steps in 75 × 10 mm. test tubes. An equal volume of antigen (infective cane juice) was added to each tube. Dilutions were made in duplicate and after shaking the mixtures, one set of dilutions was placed in a 37°C. incubator and another in a 40°C. water bath. All tubes were examined for the presence of precipitate at intervals of 15, 30, 45, 60, and 120 minutes after the start of the incubation period. The mixtures were then removed from the bath and incubator and placed in the icebox at 8° to 10°C. overnight. Next day the reaction was read again after the tubes reached room temperature.

For reading, the tubes were placed in such a way as to receive oblique illumination from a fluorescent lamp. Titers were read as the final serum dilution where definite and easily perceptible precipitation was observed. The occurrence of nonspecific reactions was controlled by subjecting the following mixtures to the same incubation periods and conditions: (1) Healthy cane juice plus saline, (2) healthy cane juice plus antiserum, (3) infective cane juice plus saline, and (4) infective cane juice plus normal rabbit serum. In these controls sera and juices were mixed in the stronger concentrations used in the actual titration.

RESULTS

Results from a representative experiment of duplicate tests carried out both at 37°C. and 40°C. and read before and after placing in the cold are shown in table 1. Three different experiments gave essentially the same results.

From table 1 it can be seen that incubation in a 40°C. water bath hastened precipitation. At 37°C. both sera reacted to a titer of $\frac{1}{16}$ when read after 60 minutes while at 40°C. both sera reacted to a titer of $\frac{1}{32}$. The reaction was also more intense at 40°C. during the first interval. However, at the end of the incubation period (2 hours) the sera reacted to the same titers whether incubated at 37°C. or 40°C., and the reaction was of comparable intensity. A definite two- to fourfold rise in titer was observed with both sera after they were placed in the cold. Final titer obtained were $\frac{1}{128}$ for rabbit No. 1 serum and $\frac{1}{256}$ for rabbit No. 2 serum.

The above data confirm the fact that sugarcane-mosaic virus is antigenic

TABLE 1.—*Data on titration of antisera to sugarcane-mosaic virus*

Temperature	Sera from—	Incubation period	Reaction[1] of final serum dilution indicated—						
			1/8	1/16	1/32	1/64	1/128	1/256	1/512
Test runs									
37° C.	Rabbit No. 1	30 min.	−	−	−	−	−	−	−
		60 min.	+	+	−	−	−	−	−
		90 min.	+	+	+	−	−	−	−
		120 min.	+	+	+	+	+	−	−
		19 hr.[2]	+	+	+	+	+	−	−
	Rabbit No. 2	30 min.	−	−	−	−	−	−	−
		60 min.	+	+	−	−	−	−	−
		90 min.	+	+	+	+	−	−	−
		120 min.	+	+	+	+	−	−	−
		19 hr.[2]	+	+	+	+	+	+	−
40° C.[3]	Rabbit No. 1	30 min.	−	−	−	−	−	−	−
		60 min.	+	+	+	−	−	−	−
		90 min.	+	+	+	−	−	−	−
		120 min.	+	+	+	+	−	−	−
		19 hr.[2]	+	+	+	+	+	−	−
	Rabbit No. 2	30 min.	−	−	−	−	−	−	−
		60 min.	+	+	+	−	−	−	−
		90 min.	+	+	+	+	−	−	−
		120 min.	+	+	+	+	−	−	−
		19 hr.[2]	+	+	+	+	+	+	−

Controls[4]

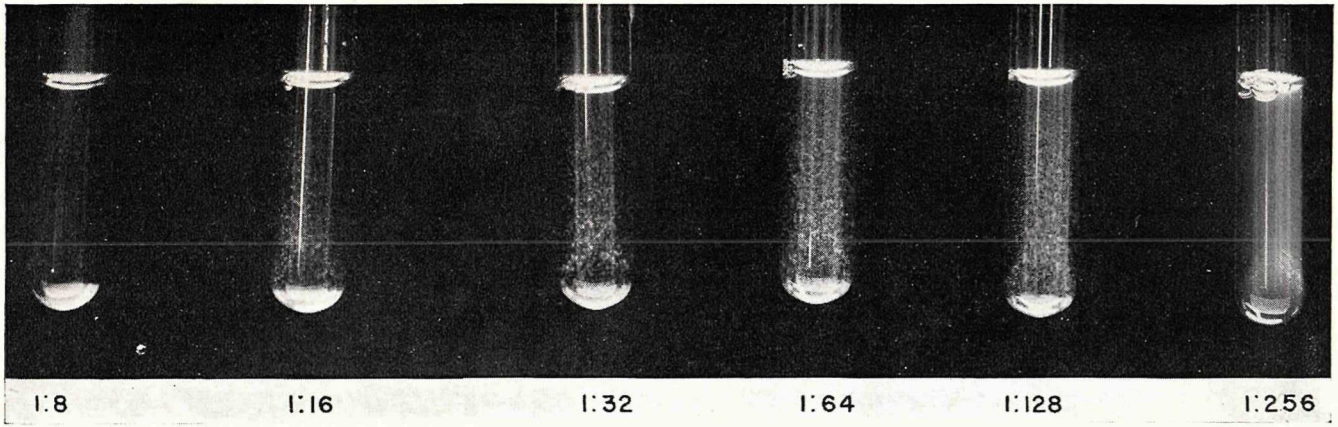
Temperature	Incubation period	Undiluted healthy juice plus saline	Undiluted healthy juice plus rabbit No. 1, serum 1/4	Undiluted healthy juice plus rabbit No. 2, serum 1/4	Undiluted infective juice plus saline	Undiluted infective juice plus normal rabbit serum 1/4
37° C.	30 min.	−	−	−	−	−
	60 min.	−	−	−	−	−
	90 min.	−	−	−	−	−
	120 min.	−	−	−	−	−
	19 hr.[2]	−	−	−	−	−
40° C.[3]	30 min.	−	−	−	−	−
	60 min.	−	−	−	−	−
	90 min.	−	−	−	−	−
	120 min.	−	−	−	−	−
	19 hr.[2]	−	−	−	−	−

[1] Readings were made as either + (precipitate formed) or − (no precipitate).

[2] Icebox.

[3] Water bath.

[4] In the control mixtures, healthy and infective juice were added undiluted to absorbed antisera or normal rabbit serum in a dilution of 1/4.



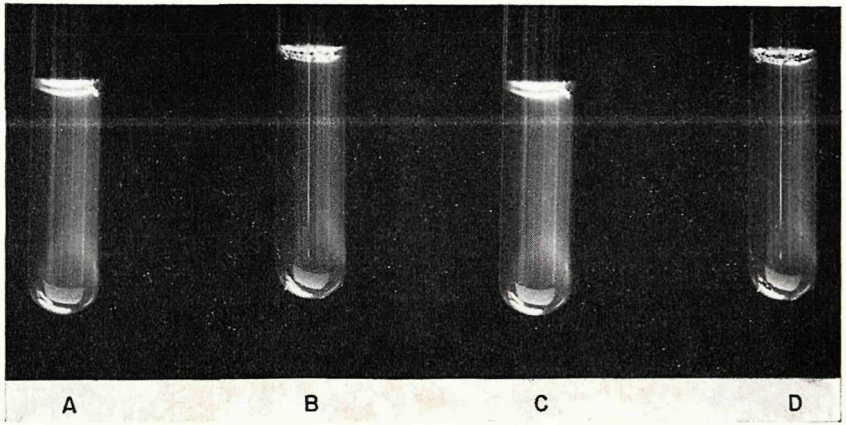


FIG. 1.—Precipitation reaction of sugarcane-mosaic virus with antiserum at various dilutions. Dilutions of antiserum mixed with infective cane juice: A, B, C, and D = control mixtures; A = healthy cane juice plus saline, B = healthy cane juice plus antiserum, C = infective cane juice plus saline, D = infective cane juice plus normal rabbit serum.

and suggest the possibility of using the precipitin reaction in testing for its relationships.

Figure 1 is a photograph of the reaction obtained with rabbit serum No. 1 after 2 hours incubation at 37°C.

SUMMARY

Sugarcane-mosaic infective juice was obtained from infected sugarcane plants of the variety B.H. 10–12. The juice was centrifuged at high speed and the sediment was suspended in phosphate buffer and inoculated into rabbits by the intravenous and intraperitoneal routes. Specific antibodies were demonstrated when the rabbit sera were absorbed with healthy cane juice and had titers of $1/128$ or $1/256$ when tested against mosaic-infected juice by the precipitation technique. The antisera did not react with healthy cane juice after absorption. Normal rabbit serum did not react with the healthy or infective juice used in the tests.

RESUMEN

Se obtuvo jugo infeccioso de plantas de caña de azúcar, variedad BH 10–12, enfermas con el virus del mosaico de la caña. Se centrifugó el jugo a alta velocidad y el sedimento se suspendió en solución de fosfato amortiguador e inoculóse en conejos por vía intravenosa e intraperitoneal.

Se probó la existencia de anticuerpos específicos cuando después de la absorción de los sueros de conejo con jugo de caña sana, se obtuvieron títulos de 1/128 y 1/256 frente al jugo de caña infectado con mosaico Usóse en estas pruebas el método de precipitación.

Los antisueros no reaccionaron con el jugo de caña sana después de la absorción. No se obtuvo reacción alguna del suero de conejos normales frente al jugo infectado o al jugo de caña sana.

LITERATURE CITED

1. Stevenson, J. A., An epiphytotic of cane disease in Puerto Rico, *Phytopathol.* **7** (6) 418–25 1917.
2. Adsuar, J., Preliminary report of a mosaic disease of the resistant sugarcane variety Mayagüez-336, Tech. Paper 7 p. 9, illus., Agrl. Exp. Sta., Univ. of P. R., 1950.
3. Jensen, J. H., The present sugarcane disease situation in Puerto Rico, *Agr. Notes*, Mayagüez, P. R., No. 69, 1936.
4. Sein, F., Jr., Artificial transmission and other studies on sugarcane mosaic, *Proc. Int. Soc. Sugar Cane Tech.*, Fourth Congress, San Juan, P. R., Bull 84. p. 6, 1932.
5. Chester, K. S., Serological studies of plant viruses, *Phytopathol.* **27** (9) 903–12, 1937.
6. Desai, S. V., The antigenic properties of the sugarcane mosaic virus, *Current Sci.*, p. 18, January 1935.

7. Summers, E. M., Brandes, E. W., and Rands, R. D., Mosaic of sugarcane in the U. S., with special reference to strains of the virus., Tech. Bull. 995, pp. 44–99, U.S.D.A., Washington, D. C., 1948.
8. Bruehl, G. W., Strains of sugarcane mosaic in Puerto Rico, *Plant Dis. Rptr.*, **37** (9) 1953.
9. Bawden, F. C., Serological reactions of plant viruses in: Plant viruses and virus diseases, Chronica Botanica Co., Waltham, Mass., 3d edition, pp. 126–48, 1950.