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SEROLOGICAL AND ELECTRON MICROSCOPICAL INVESTIGATIONS OF THE RELATIONSHIP BETWEEN *SORGHUM* RED STRIPE VIRUS AND SUGAR CANE MOSAIC VIRUS[1]

*Met een samenvatting: Serologisch en elektronenmicroscopisch onderzoek naar de verwantschap tussen het „*Sorghum *red stripe" virus en het suikerrietmozaïekvirus*

BY

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INTRODUCTION

A disease of *Sorghum* causing mosaic symptoms and red necrotic stripes on the leaves was described by GOIDANICH in 1938 and 1939 under the name of "arrossamento striato" (red stripe). This author suspected that the disease was due to a virus.

In 1953 the disease again came under investigation. Two years later GRANCINI found the disease occurring also on maize from fields in the surroundings of Milan, but the symptoms were here weaker than on *Sorghum vulgare*. In addition it appeared possible to transmit the mosaic from the maize plants to various healthy *Sorghum* plants and vice versa. *Sorghum* so infected, developed mosaic and red spots on the leaves. In 1957 GRANCINI and LOVISOLO published independently of each other extensive papers on the mosaic disease of maize and the "arrossamento striato" of *Sorghum*.

GRANCINI decided that *Sorghum* red stripe and maize mosaic were due to the same virus because of the fact that artificial infections from *Sorghum* with the characteristic symptoms of the red stripe produced the same symptoms on *Sorghum* in the greenhouse as did the virus taken from maize.

In the same article the author also pointed out that there is apparently a close relationship between sugar cane mosaic virus (S.C.M.V.) and *Sorghum* red stripe virus (S.R.S.V.). He mentioned in this connection a publication of BRANDES (1920) in which the identity of a mosaic disease of maize and the S.C.M.V. disease was established. GRANCINI investigated the in vitro characteristics of the maize mosaic virus and found that the data obtained were comparable with those of the S.C.M.V. (thermal inactivation point 53 °C, dilution end-point 10^{-3}–10^{-4}, longevity in vitro 7–15 h. in the case of the mosaic virus from maize). The above mentioned author found also considerable agreement with S.C.M.V. regarding host range.

LOVISOLO suggested also a relationship between the *Sorghum* virus and S.C.M.V. on the grounds of certain in vitro characteristics (thermal inactivation point 46–50 °C, dilution end-point 10^{-3}, longevity in vitro 2 h. 40 min.–4 h. 50 min. in

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the case of S.R.S.V.). DEAN & COLEMAN (1959) reported the occurrence of a severe necrosis of *Sorghum* leaves in Mississippi (U.S.A.) caused by S.C.M.V.

In the present publication the relationship between S.R.S.V. from *Sorghum* and maize, and S.C.M.V. from sugar cane is investigated serologically. In addition an electron microscopical examination was made of S.R.S.V. from *Sorghum* and maize, and S.C.M.V. from sugar cane and maize. In view of differences in length given in the literature for S.C.M.V. (620–670 mμ, GOLD & MARTIN, 1955) and S.R.S.V. (750 mμ, BRANDES, 1959), the present authors determined at Wageningen under conditions that were as far as possible uniform, the length of S.C.M.V. and S.R.S.V. particles obtained from the above mentioned host plants.

SEROLOGICAL INVESTIGATION

Materials and methods

The serological tests were carried out using sap from mosaic diseased leaves of *Sorghum halepense* from Italy, from mosaic diseased maize plants inbred line Lo 3 (the seed of which was obtained from Italy, sown in Wageningen and the plants then inoculated with S.R.S.V.) and from sugar cane leaves with S.C.M.V.

Diseased sugar cane sets were obtained from Puerto Rico and subsequently grown at Wageningen. Infective sap from sugar cane, *Sorghum halepense* and maize and healthy sap from maize were expressed through cheese-cloth after grinding in a mortar and centrifuged at 5,000 r.p.m. for 30 minutes. Antisera against S.C.M.V. came from Puerto Rico and had been prepared by injecting rabbits with sap from mosaic diseased maize and sugar cane, according to the method described by PÉREZ & ADSUAR (1954). According to these authors the first lot of antiserum had a titre of 1 : 16 (from sugar cane) and the second of 1 : 8 (from maize).

For the absorption healthy maize sap was mixed with antiserum in the proportion 4 : 1 and kept at room temperature overnight. Next day the suspension was centrifuged at 5,000 r.p.m. for 30 minutes to eliminate any precipitate formed.

Normal serum was treated in the same way for comparison. The sera were then ready for use. In the experiments 2, 3 and 5 antiserum was used without prior absorption.

The serological tests were carried out by means of micro-reaction method under paraffin oil, the so-called microprecipitation reaction (VAN SLOGTEREN, 1955). The following tests were carried out:

S.R.S.V. from *Sorghum* with antiserum against S.C.M.V.

S.R.S.V. from maize with antiserum against S.C.M.V.

S.C.M.V. from sugar cane with antiserum against S.C.M.V.

At the same time the necessary control tests were conducted with normal serum, healthy sap and physiologic saline solution. The reactions were judged after 2–6 h. at 37 °C.

Results

The results of the serological tests are given in Table 1. It is evident that in addi tion to the strong reaction of S.C.M.V. with its homologous antiserum there was also a positive reaction by S.R.S.V. from *Sorghum* and maize against S.C.M.V. antiserum. Since the titre of the S.C.M.V. in experiment 1 differed

TABLE 1. Serological test with S.R.S.V.[1] from *Sorghum* and maize, and S.C.M.V.[2] from sugar cane against S.C.M.V. antiserum.
Serologische proef met S.R.S.V.[1] uit Sorghum *en mais en S.C.M.V.[2] uit suikerriet tegen S.C.M.V.-antiserum.*

Dilutions of antigen / *Verdunningen van het antigeen* — Dilutions of antiserum / *Verdunningen van het antiserum*	S.R.S.V. from *Sorghum halepense* / *S.R.S.V. uit* Sorghum halepense											
	1/1		1/2		1/4		1/8		1/16		1/32	
No. of experiment — *Proefnummer*	1	2	1	2	1	2	1	2	1	2	1	2
S.C.M.V. 1/1	+	−	+	−	+	−	++	−	−	−	−	−
1/2	+	+	+	+	−	+	+	−	−	−	−	−
1/4	−	++	−	+	−	+	−	−	−	−	−	−
1/8	−	++	−	+	−	+	−	+	−	−	−	−

Dilutions of antigen / *Verdunningen van het antigeen* — Dilutionsofantiserum / *Verdunningen van het antiserum*	S.R.S.V. from maize / *S.R.S.V. uit mais*											
	1/1		1/2		1/4		1/8		1/16		1/32	
No. of experiment — *Proefnummer*	3	4	3	4	3	4	3	4	3	4	3	4
S.C.M.V. 1/1	+	+	+	+	−	−	−	−	−	−	−	−
1/2	+	+	−	−	−	−	−	−	−	−	−	−
1/4	+	−	−	−	−	−	−	−	−	−	−	−
1/8	−	−	−	−	−	−	−	−	−	−	−	−
1/16	−	−	−	−	−	−	−	−	−	−	−	−
1/32	−	−	−	−	−	−	−	−	−	−	−	−

Dilutions of antigen / *Verdunningen van het antigeen* — Dilutions of antiserum / *Verdunningen van het antiserum*	S.C.M.V. from sugar cane / *S.C.M.V. uit suikerriet*											
	1/1		1/2		1/4		1/8		1/16		1/32	
No. of experiment — *Proefnummer*	5	6	5	6	5	9	5	6	5	6	5	6
S.C.M.V. 1/1	−	+	−	+	−	−	−	−	−	−	−	−
1/2	++	+	+	−	−	−	−	−	−	−	−	−
1/4	+++	−	++	−	+	−	+	−	−	−	−	−
1/8	+++	−	+	−	+	−	+	−	−	−	−	−
1/16		−		−		−		−		−		−
1/32		−		−		−		−		−		−

[1] *Sorghum* red stripe virus.
[2] Sugar cane mosaic virus.

The antiserum had a titre of 1:8 in the experiments 2–6 and of 1:16 in experiment 1. Healthy sap and normal serum did not give any specific reaction.

Het antiserum had een titer van 1:8 in de proeven 2–6 en van 1:16 in proef 1. Gezond sap en normaal serum gaven geen enkele specifieke reactie.

from that in experiments 2–6, since the concentration of antigen varied with the host and since the antiserum in experiments 2, 3 and 5 was not absorbed, it is not to be expected that the reactions would be quantitatively comparable. Because of the results obtained in experiment 1, the antiserum used in experiments 2–5 was diluted to $^1/_8$ concentration.

The results showed that in spite of the titres given by PÉREZ & ADSUAR perhaps further dilutions could have been applied. Consequently the antiserum in subsequent experiments was diluted to 1/32. The control tests with normal serum, healthy sap and physiologic saline solution in most cases gave a slight milkiness but never a clear flocculent precipitate.

Discussion

The above mentioned experiments show that there is a serological relationship between S.C.M.V. and S.R.S.V. In view of the differences in concentrations of antigen in the different host plants and the dissimilarity of the two antisera lots, it is difficult to decide whether or not a single strain is involved.

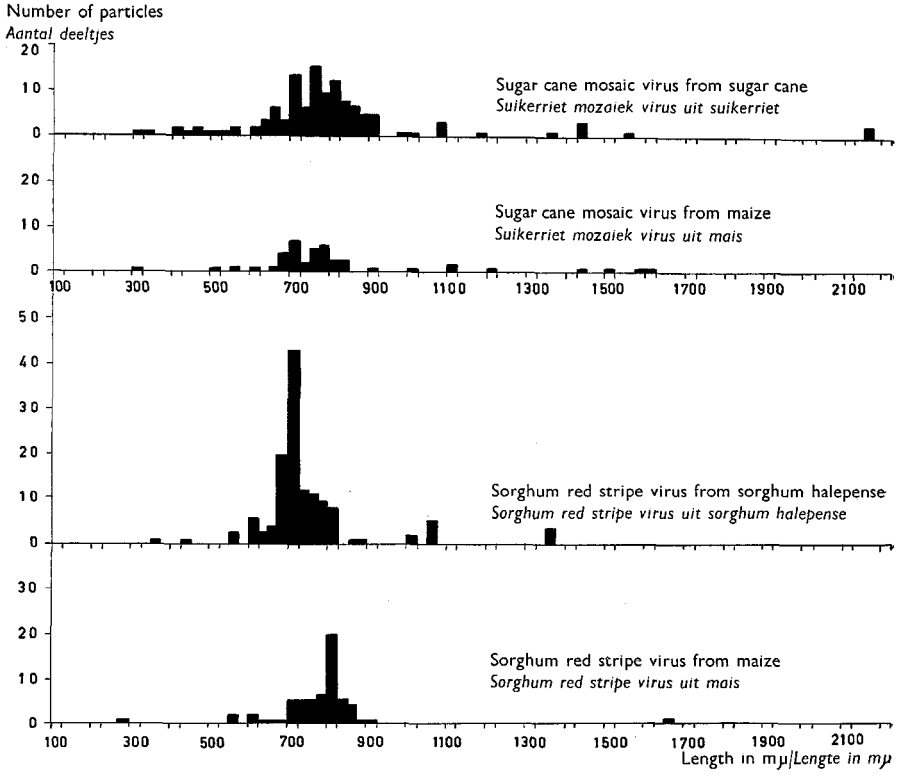

Fig. 2. Frequency diagrams of particle lengths.
Frequentie-diagrammen van de lengten der deeltjes.

ELECTRON MICROSCOPICAL INVESTIGATION

Materials and method

In making the preparations for electron microscopy the mosaic diseased *Sorghum halepense*, maize and sugar cane material used was the same as for the serological tests. The preparations were all made according to the "dipping method" of BRANDES (1957). We examined the preparations with an electromagnetic electron microscope (Philips EM 100) at an electron optical magnification of 2,000. The measurements were made at a total magnification of 128,000 and the particle lengths divided into classes of 25 mμ.

Results

All preparations of the mosaic diseased leaf material contained flexible virus rods (Fig. 1). These particles were absent from healthy material. Frequency diagrams of the particle lengths show the following distribution (Fig. 2) with the mean lengths:

S.R.S.V. from *Sorghum halepense:* 700 mμ,
S.R.S.V. from maize: 775 mμ,
S.C.M.V. from sugar cane: 750 mμ,
S.C.M.V. from maize: 750 mμ.

Although maize plants inoculated with S.R.S.V. developed clear symptoms, it was nevertheless difficult to obtain preparations with a high concentration of virus particles. The number of particles measured was consequently less than in the case of *Sorghum* and sugar cane.

Discussion

From a comparison of the observed lengths of the two viruses obtained from various host plants it can be concluded that there is no demonstrable difference between the average lengths of S.C.M.V. and S.R.S.V.

In view of the fact that the electromagnetic electron microscope used in our experiments permits an experimental error of 10%, the absolute lengths obtained are less reliable than those obtained by BRANDES (1959), using an electrostatic electron microscope. Since in our experiments we were only concerned with comparing the two viruses it is possible to say that the particles of S.C.M.V. and S.R.S.V. are identical with regard to their length.

SUMMARY

Sorghum red stripe virus (S.R.S.V.) from Italy and sugar cane mosaic virus (S.C.M.V.) from Puerto Rico were investigated at Wageningen serologically and with the electron microscope.

By means of the microprecipitation test (VAN SLOGTEREN, 1955) a qualitative relationship was established between the two viruses (Table 1). Electron microscopical investigations showed that with regard to their length there was no difference between the two viruses (Fig. 1 and 2).

SAMENVATTING

„*Sorghum* red stripe" virus (S.R.S.V.), afkomstig uit Italië, en suikerrietmozaïekvirus (S.C.M.V.) uit Portorico zijn te Wageningen serologisch en elektronenmicroscopisch onderzocht. Met behulp van de microprecipitatietoets (VAN SLOGTEREN, 1955) kon een kwalitatieve verwantschap tussen de beide virussen aangetoond worden (tabel 1). Elektronenmicroscopisch onderzoek wees uit, dat er wat betreft de lengte der virusdeeltjes geen verschil bestaat tussen de twee genoemde virussen (fig. 1 en 2).

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