T.Pl.ziekten 66 (1960): 102–106

SEROLOGICAL RELATIONSHIP OF SOME EUROPEAN, AMERICAN, AND CANADIAN ISOLATES OF THE WHITE CLOVER MOSAIC VIRUS[1]

Met een samenvatting: Serologische verwantschap tussen enige Europese, Amerikaanse en Canadese isolaties van het witte-klavermozaïekvirus

BY

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INTRODUCTION

BOS, DELEVIĆ & VAN DER WANT (1959) recently published the results of their investigations on a mosaic virus of white clover in The Netherlands. On the basis of symptomatology, host range and physical properties, they assumed the Dutch virus to be identical with the white clover mosaic virus which has been incompletely described in the U.S.A. by ZAUMEYER & WADE (1935, 1936) and by PIERCE (1935). WEISS (1939) designated the virus as *Trifolium virus* 1 ZAUMEYER & WADE. According to the literature review of BOS e.a. the virus occurs in the U.S.A., The Netherlands and Germany. Recently SCOTT & GOLD (1959) reported the occurrence of the virus in California.

During 1959, the present authors contacted each other. It appeared that more or less independently, they were doing research on this virus in The Netherlands (BOS & MAAT), the U.S.A., Indiana (BANCROFT)[8], California (GOLD & SCOTT), Maryland (SCOTT), and in Canada (PRATT). Recently in Germany, the virus was again isolated (QUANTZ). This isolate is very similar to that described by this author (QUANTZ, 1956). To establish the relationships between their isolates, the authors exchanged antisera and, where possible, also virus isolates. The present note deals with the results of serological comparisons.

EXPERIMENTS AND RESULTS

The relationships established among the isolates at the various laboratories are only qualitative when interlaboratory comparisons are made since different investigators used different preparative and serological procedures. The results of these qualitative comparisons are diagrammed in fig. 1. Each arrow indicates

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[8] This isolate was originally found by Dr. J. TUITE in 1957 in white clover in Indiana.

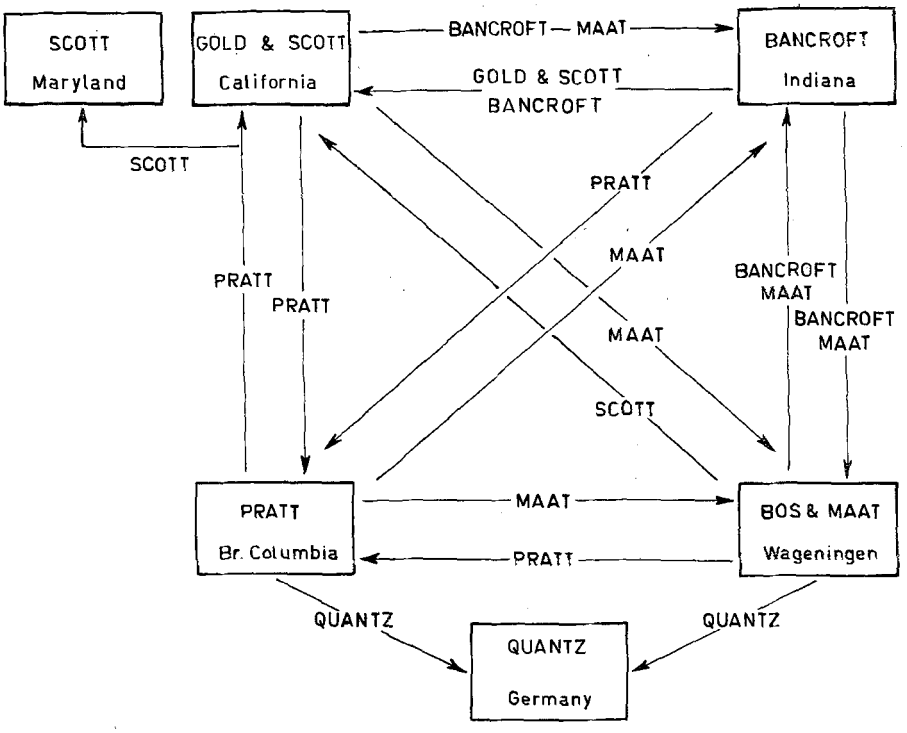

FIG. 1. Survey of the relationship between some European, American and Canadian isolates of the white clover mosaic virus, as established in different laboratories (explanation in the text).

Overzicht van de verwantschap tussen enige Europese, Amerikaanse en Canadese isolaties van het witte-klavermozaïekvirus, zoals werd aangetoond in verschillende laboratoria (uitleg in de tekst).

a positive reaction. The arrowhead points to the source of the virus tested. The tail indicates the source of the antigen used for producing the antiserum and the place where this antiserum had been produced. Alongside the arrow, the person who performed the test is mentioned. All reactions were positive. This figure clearly demonstrates the mutual relationships among the Dutch, American and Canadian isolates. In the most recent experiments, QUANTZ tested the Dutch and Canadian antisera against a newly obtained German isolate with positive results.[1]

At Wageningen, MAAT made some more detailed experiments by means of the microreaction method under paraffin oil (VAN SLOGTEREN, 1955). Four different antisera were compared, viz. a Dutch one, and antisera sent by BANCROFT, GOLD and PRATT. They were tested against two virus isolates, viz.

[1] After this paper had been sent to the editors, a publication was received written by Dr. P. R. FRY, Plant Disease Division, Department of Scientific and Industrial Research, Auckland, New Zealand, on "A clover mosaic virus in New Zealand pastures" (N.Z.J. agric. Res. 2, 1959: 971–981). The antiserum against this virus was kindly sent to Vancouver and to Wageningen and tested against two Canadian and one Dutch isolate of the white clover mosaic virus, in all cases with positive results.

TABLE 1. Serological test[1] with four antisera, viz. the Dutch 8.4.1, the American antisera of BANCROFT and of GOLD and the Canadian antiserum of PRATT, against two isolates of the virus, viz. the Dutch W.K.V. and the American isolate of BANCROFT (W.C.M.V.).

Serologische proef[1] met vier antisera, te weten het Nederlandse 8.4.1, de Amerikaanse antisera van BANCROFT *en van* GOLD *en het Canadese antiserum van* PRATT, *tegen twee isolaties van het virus, te weten het Nederlandse W.K.V. en de Amerikaanse isolatie van* BANCROFT *(W.C.M.V.).*

Dilutions of antisera *Verdunningen van de antisera* / Dilutions of antigen *Verdunningen van het antigeen*		8.4.1					BANCROFT					GOLD			PRATT			Normal serum *Normaal serum*
		1/4	1/16	1/64	1/256	1/1024	1/1	1/4	1/16	1/64	1/256	1/1	1/4	1/16	1/4	1/16	1/64	1/4
W.K.V.	1/2	+++	++	+	+	—	++	+	+	±	—	+	±	—	+	±	—	—
	1/8	++	+	+	±	—	±	±	±	±	—	±	±	—	—	±	—	—
	1/32	±	±	±	±	—	—	—	—	—	—	—	±	—	—	—	—	—
	1/128	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
W.C.M.V.	1/2	+++	++	+	+	—	++	+	+	±	—	+	±	—	++	+	—	—
	1/8	++	++	+	+	—	+	±	±	—	—	±	±	—	±	±	—	—
	1/32	+	±	±	±	—	—	—	—	—	—	±	±	—	—	—	—	—
	1/128	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Healthy *Gezond*	1/2	—	—	—	—	—	—	—	—	—	—	±	—	—	—	—	—	—

[1] The results of the microreaction method under paraffin oil after two hours at room temperature.
De resultaten der microreactie-methode onder paraffine olie na twee uren bij kamertemperatuur.

the Dutch isolate W.K.V. (of Bos e.a., 1959) and an American isolate of BANCROFT. Both isolates were multiplied in Beka-beans *(Phaseolus vulgaris* L.) under the same conditions. Healthy Beka-beans were tested as a control. The results are summarized in table 1. They indicate a serological relationship among the four viruses against which the antisera had been prepared. This is in agreement with the results summarized in fig. 1. Moreover, this experiment demonstrated no quantitative difference in serological characters between the Dutch virus and BANCROFT's isolate.

To study the relationships between the two isolates just mentioned more carefully, a cross-absorption test was made at Wageningen. Pea plants *(Pisum sativum* L.*)* grown under the same conditions were used as the source of both viruses, whereas healthy peas were tested as a control. The plant material was ground in a mortar together with an equal quantity of phosphate citric acid buffer pH 7, filtered through cheesecloth, and centrifuged for 20 minutes at 6,000 r.p.m. Cross absorption of the antisera with heterologous viruses took place as follows: the antisera were mixed with the heterologous virus suspensions obtained as described above (1 : 5). The mixtures were incubated for 1 hour at 37 °C, stored for 1 night at 3 °C and then centrifuged for 20 minutes at 15,000 r.p.m. Antiserum 8.4.1 saturated with sap of healthy peas was added as a control. The results obtained are given in table 2. After cross absorption with the heterologous virus, both antisera gave no visible reaction with their homologous virus. This experiment furnished further evidence for a quantitative serological similarity between the Dutch and Indiana viruses.

TABLE 2. Cross-absorption test of the Dutch antiserum 8.4.1 and the American antiserum of BANCROFT with their homologous and heterologous viruses.

Wederkerige verzadiging van het Nederlandse antiserum 8.4.1 en het Amerikaanse antiserum van BANCROFT *met de homologe en heterologe virussen.*

Antisera with final dilutions of 1/24 / *Antisera met eindverdunningen 1/24* — Antigen / *Antigeen*		8.4.1 saturated 1:5 with W.C.M.V. / *8.4.1 verzadigd 1:5 met W.C.M.V.*	BANCROFT saturated 1:5 with W.K.V. / BANCROFT *verzadigd 1:5 met W.K.V.*	8.4.1[1] saturated 1:5 with healthy (control) / *8.4.1[1] verzadigd 1:5 met gezond (controle)*
W.K.V.	1/4	–	–	+
	1/16	–	–	+
W.C.M.V.	1/4	–	–	+
	1/16	–	–	+
Healthy / *Gezond*	1/4	–	–	–
	1/16	–	–	–

[1] The antiserum of BANCROFT had a high titer, similar to 8.4.1 (see table 1), and was not added as a control.

Het antiserum van BANCROFT *had evenals 8.4.1 een hoge titer (zie tabel 1) en werd niet als controle toegevoegd.*

Discussion

Serology is the most informative and objective single method by which virus relationships may be determined. If the type of co-operative approach reported here is widely used, synonomy will be curtailed and needless confusion will be prevented. The use of antisera also often obviates the need of virus exchange and thus avoids the introduction of dangerous viruses into new areas.

Antisera exchange, however, allows only qualitative relationships to be established. This means that no strain differences can be definitely established because differences in titer of antisera prepared at different places (cf. table 1) may be due not only to strain differences, but also to differences in virus source, local conditions and preparative and serological methods. Since plant, climate and animal are involved here, no accurate standardization is possible. Therefore, without the exchange of virus isolates no exact quantitative relationships can be studied.

Summary

By means of the exchange of antisera a qualitative relationship could be established among a Dutch, some American, a Canadian and a German isolate of the white clover mosaic virus (fig. 1). An exact comparison of two isolates, viz. the Dutch W.K.V. and the American W.C.M.V. (of Bancroft), with a number of different antisera (table 1), and in a cross-absorption test (table 2), revealed no quantitative differences between these isolates. For such quantitative tests the exchange of virus isolates is inevitable.

Samenvatting

Door middel van de uitwisseling van antisera kon een kwalitatieve verwantschap worden aangetoond tussen een Nederlandse, enige Amerikaanse, een Canadese en een Duitse isolatie van het witte-klavermozaïekvirus. Een nauwkeurige vergelijking van twee isolaties, te weten het Nederlandse W.K.V. en het Amerikaanse W.C.M.V. (van Bancroft), met behulp van een aantal verschillende antisera (tabel 1) en in een proef, waarbij de antisera met wederzijdse virussen werden verzadigd (tabel 2), bracht geen kwantitatieve verschillen (stam-verschillen) aan het licht. Voor dergelijke kwantitatieve proeven is de uitwisseling van virusisolaties onvermijdelijk.

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