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Purification and serological analysis of tomato spotted wilt virus

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Abstract

A modified procedure for the purification of TSWV is described which consists of differential centrifugation, treatment with antiserum against sap from healthy *N. rustica* and density-gradient centrifugation in sucrose gradients. Centrifugation in the zonal-rotor proved efficient for processing large amounts of infected leaves.

Antisera to purified TSWV were produced in rabbits. These sera reacted with healthy *N. rustica* sap. This reaction could be abolished by absorption with either healthy *N. rustica* sap or polysaccharides purified from *N. rustica*. Unabsorbed sera reacted with the major TSWV structural proteins following their electrophoretic separation in polyacrylamide-gels. The positions of the precipitin lines corresponded with those of the structural proteins visualized by staining. The intensity of these lines was greatly diminished after absorption of the antisera with either healthy *N. rustica* sap or polysaccharides. It was concluded that the reaction of TSWV antiserum with the structural proteins is partly due to host-derived polysaccharides covalently bound to the virus proteins.

Introduction

Tomato spotted wilt virus (TSWV) has attracted attention because of its wide host range, unusual physical and chemical properties and vector relationship (Best, 1968). It was the first plant virus shown to contain lipids (Best and Katekar, 1964). Ultrathin sections of infected leaves and negatively stained particles of purified virus, showed that the virus has an envelope (Ie, 1964; Best and Palk, 1964; Van Kammen et al., 1966). The particles are roughly spherical and their diameter varies between 70 and 90 nm. The envelope is covered with an almost continuous layer of projections (Ie, 1970). More detailed information about the composition and structure of the virus particles is not available. This may be owed to the fact that no single purification procedure has gained general acceptance.

Numerous purification methods have been described (Black et al., 1963; Best and Palk, 1964; Martin, 1964; Van Kammen et al., 1966; Best, 1968; Gumpf and Weathers, 1972; Tsakiridis and Gooding, 1972; and very recently Joubert et al., 1974; and Paliwal, 1974). Our attempts to purify TSWV by any of these methods either failed or resulted in unsatisfactorily pure preparations.

This paper describes a modified purification procedure, the preparation of antisera to purified virus, and the serological analysis of the virus. The results had earlier been included in the senior author's dissertation (Tas, 1975).

Material and methods

Virus isolate, plants and growing conditions. An isolate of TSWV (TSWV-S) obtained from an isolate of TSWV producing necrotic as well as faint yellow lesions on *Nicotiana glutinosa*, was used throughout this study. Through repeated excision of and virus transfer from necrotic lesions it was possible to isolate TSWV-S, which produced only necrotic lesions on *N. glutinosa*. The isolate was maintained in *Nicotiana rustica* and in *Tropaeolum majus*.

For purification the virus was propagated in *N. rustica*. Plants were grown in a glasshouse at 21°–24°C and a relative humidity of 60–90%. The plants were inoculated when they had three fully expanded leaves. Systemically infected leaves were harvested 12–14 days after inoculation; inoculated leaves were occasionally used for purification 5–6 days after inoculation. In winter the plants were additionally illuminated by HPI/T400 Watt lamps (Philips) for 16 h per day.

Virus purification. All work was done in a cold room at 3°C. Batches of 60–100 g of infected leaves were vacuum-infiltrated with extraction buffer consisting of 0.01 M Tris, 0.01 M sodium sulfite, and 0.1% cysteine hydrochloride, pH 8.0. The leaves were homogenized for 30 sec in a Waring Blendor at low speed and the homogenate was squeezed through cheese cloth. The resulting extract was centrifuged for 10 min at 10000 *g*. The virus was purified from both the supernatant (LS) and the pellets (LP) as the latter often contained large quantities of virus. The LP was resuspended in 200 ml resuspension buffer consisting of 0.01 M tris, 0.01 M glycine, 0.1% cysteine hydrochloride and 0.01 M sodium sulfite, pH 7.9, and left for 1–2 h. This suspension was then centrifuged for 10 min at 10000 g. The supernatant and the LS supernatant were separately centrifuged for 30 min at 25000 rpm in a Spinco R30 rotor to concentrate the virus. The pellets were resuspended in 20 ml resuspension-buffer followed by the addition of 0.2–0.4 ml antiserum which was produced to extracts from healthy *N. rustica* plants, and left for 1 h. The precipitate was removed by centrifuging for 10 min at 10000 *g*. The supernatant was layered in portions of 6 ml on 3–30% ($^{w}/_{w}$) sucrose gradients in resuspension buffer; the tubes were centrifuged at 23000 rpm for 45 min (SW25 rotor, Spinco). The virus zones were removed from the gradient and centrifuged on a gradient of 25–50% ($^{w}/_{w}$) sucrose for 5 or 16 h at 23000 rpm. The virus was concentrated after dilution with resuspension buffer at 30000 rpm for 1 h.

Further amounts of virus were often extracted from pellets obtained after centrifugation of the virus suspension previously incubated with 'healthy' antiserum, and in the first sucrose gradient centrifugation. These pellets had been resuspended in 6 ml resuspension buffer and left overnight at 2–4°C and centrifuged for 10 min at 10000 *g*. The resulting supernatants were then reintroduced into the purification scheme at the first sucrose gradient stage.

Sometimes large amounts of leaf material (> 200 g) were processed. Sucrose density gradient centrifugation was then carried out in a zonal rotor. The first gradient consisted of 15–30% ($^{w}/_{w}$) sucrose in resuspension buffer and made in a B XIV rotor of a MSE50 centrifuge. The virus was centrifuged at 25000 rpm for 1 h. The second gradient was made of 25–50% ($^{w}/_{w}$) sucrose and was run at 25000 for 15 h.

Infectivity assays. Infectivity of the virus suspensions was determined on detached leaves of *Petunia hybrida* cv. Pink Beauty. Following inoculation the leaves were placed on wet filter paper in petri dishes and kept at approximately 22°C. Lesions were visible two days after inoculation and were counted the following day.

Purification of polysaccharides. Polysaccharides of *N. rustica* were purified by a modification of the method of Willers et al. (1964). Leaves of *N. rustica* were homogenized with a Waring Blendor in 2 volumes of 0.1 M Tris-HCl buffer, pH 7.2 and the homogenate was pressed through cheesecloth and centrifuged for 10 min at 10000 *g*. The resulting supernatant was heated for 15 min at 100°C to denature the proteins. The precipitate was removed by filtration or by low speed centrifugation. Following lyophilization of the sap, the powder was dissolved in 10 ml of double distilled water. The polysaccharides were precipitated by adding 1.5 volumes of acetone, pelleted by centrifugation for 10 min at 5000 *g* and dissolved in 1.5 ml double distilled water. An equal volume of 25% trichloric acetic acid was added to this solution. After centrifugation the polysaccharides were again precipitated by adding 10 volumes of acetone. The sediment was dissolved in distilled water and dialysed for 48 h at 4°C against several changes of distilled water. A precipitate, sometimes occurring during dialysis, was removed by low-speed centrifugation.

Preparation of antisera to extracts of healthy N. rustica and to TSWV. Healthy leaf tissue was homogenized in 4 volumes 0.1 M Tris-HCl buffer, pH 8.0, using a Waring Blendor at low speed. Cell debris was removed by low-speed centrifugation for 10 min at 10000 *g*. The supernatant was centrifuged for 30 min at 54000 *g* and the pellet resuspended in 0.01 M Tris, pH 8.0. This suspension was emulsified with Freund's incomplete adjuvant and used for immunization. Rabbits were injected 3 times at 10-day intervals both intramuscularly and subcutaneously on each occasion. The extracts of *N. rustica* injected on the three occasions, were prepared from 2.5, 5 and 10 g of plant material, respectively. The rabbits were bled ten days after the last injection and subsequently at weekly intervals.

TSWV antiserum no. 1 was prepared by three intramuscular injections of purified virus at 10-day intervals. Freund's incomplete adjuvant was used for the second and third injections only. Rabbits were bled two weeks after the final injection. TSWV antiserum no. 2 was prepared by injection of small quantities of purified virus, emulsified in Freund's incomplete adjuvant, into alternate footpads at 10-day intervals. Six weeks after the second footpad injection virus was administered intravenously without adjuvant. Rabbits were bled 10 days after this injection and thereafter at weekly intervals.

Agar gel double-diffusion tests. Antigen-antibody reactions were studied using agar gel double diffusion techniques. The agar gel consisted of 1% agar or agarose in 0.05 M Tris-HCl buffer pH 8.0, containing 0.05 sodium azide.

For tests with virus proteins separated electrophoretically on polyacrylamide gels (Tas et al., 1976) and TSWV antiserum, the gels were cut longitudinally into two halves, and laid flat side down onto 1% agar(ose). Throughs of 1 mm wide were cut at a distance of 5 mm on each side of the gel and filled with serum. Precipitin lines developed in 1 to 2 days at room temperature.

Absorption of TSWV antiserum. Extracts of healthy *N. rustica* plants were prepared by triturating leaves in 0.1 M Tris-HCl, pH 8.0, with a mortar and pestle. Cell debris was removed by low-speed centrifugation. The pellet obtained after centrifugation for 30 min at 54000 *g* was resuspended in TSWV antiserum. The mixture was incubated for 1 h at 37 °C and then overnight at 2–4 °C. The antigen-antibody precipitate and denatured leaf material were removed by centrifugation in a Spinco R 40-rotor for 30 min at 70000 *g*. To absorb TSWV antiserum with polysaccharides an excess was added and after incubation the precipitate was removed by low-speed centrifugation.

TSWV antiserum was absorbed by TSWV by adding twofold dilutions of virus to four different aliquots of serum. After incubation, the precipitate was removed by low-speed centrifugation.

Results

Purification. Preparations with a high purity were obtained during summer with the procedures described. Purity was judged by visual inspection of the final virus pellets, by electron microscopy (Fig. 1) and by the absorption profile after centrifugation on the second sucrose gradient (Fig. 2). The virus preparations obtained from

Fig. 1. Electron micrograph of TSWV particles. The particles were fixed with an equal volume 5% glutaraldehyde in double-distilled water, washed by floating on water and stained with 2% PTA, pH 6.

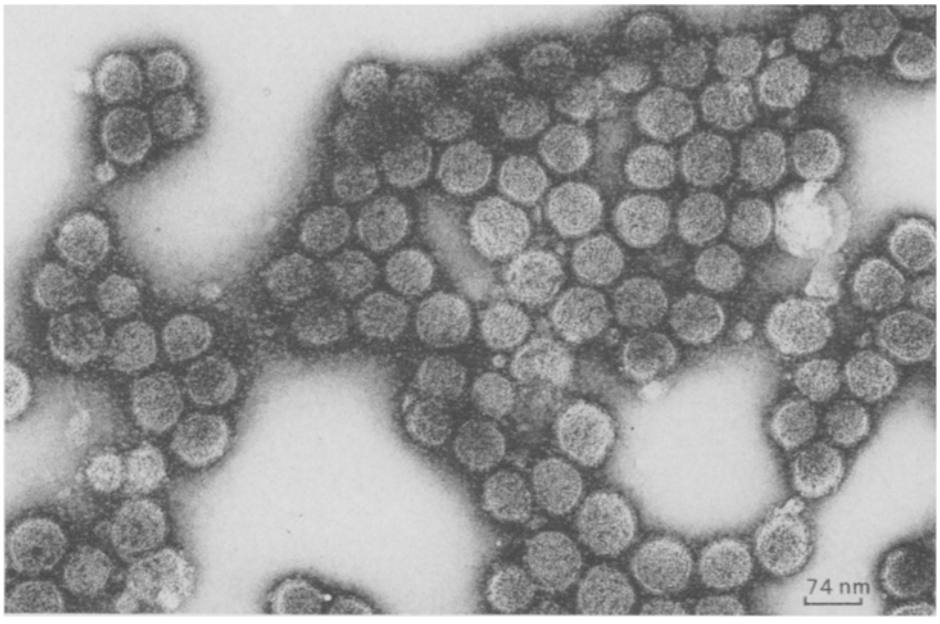

Fig. 1. Elektronenmicroscopische opnamen van TSWV-deeltjes. De deeltjes werden gefixeerd door een gelijk volume 5% glutaaraldehyde in dubbel gedistilleerd water op een preparaatdrager te brengen. Dit werd na 1 min in water gewassen. Het preparaat werd gekleurd met 2% kaliumfosforwolfraamzuur, pH 6,0.

Fig. 2. Distribution of UV-absorbing material (257 nm) and infectivity over a 25–50% ($^{w}/_{w}$) linear sucrose gradient after centrifugation of the virus band from the 15–35% ($^{w}/_{w}$) linear sucrose gradient, overnight at 25000 rpm in zonal rotor B XIV of a MSE 50.

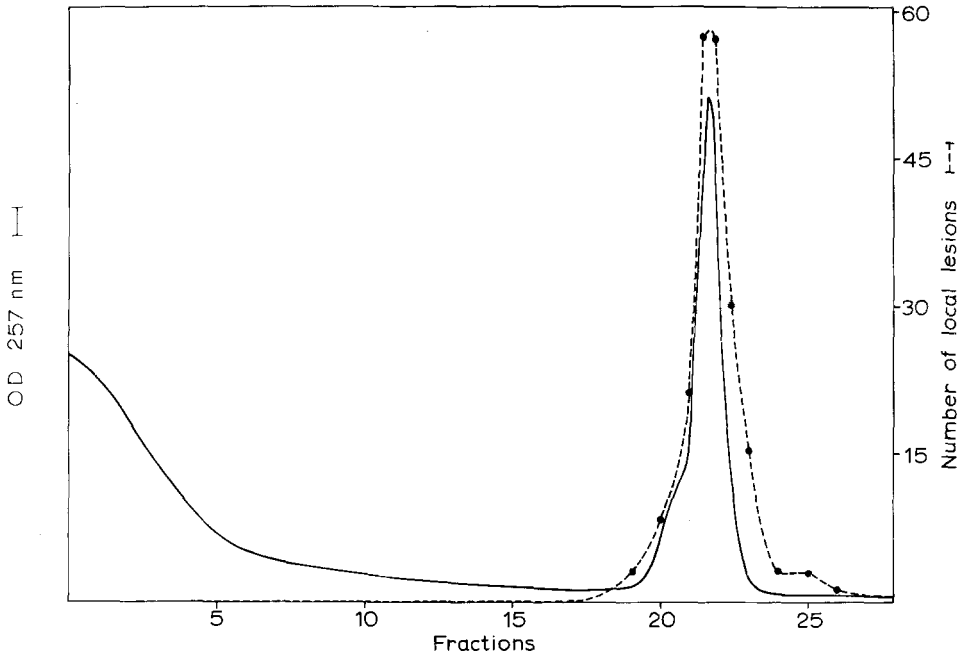

Fig. 2. Verdeling van UV-absorberend materiaal (257 nm) en infectiositeit in een 25–50% ($^{w}/_{w}$) lineaire saccharosegradiënt na centrifugering bij 25000 rpm in een B XIV-rotor in een MSE 50 ultracentrifuge gedurende 16 uur. Het virus was eerst gecentrifugeerd in een lineaire gradiënt van 15–30% ($^{w}/_{w}$) saccharose.

the LP pellets were preferred to those from the LS supernatant since the former contained no detectable impurities.

The distribution of virus after the first low-speed centrifugation over the LP pellet and LS supernatant varied with each batch of infected plants. It was not influenced by any buffer tested, such as those used by Black et al. (1963), and Best (1968), and was not affected by Mg^{++} in the extraction buffer.

Virus yield and infectivity of the purified extracts differed markedly between the various purifications. In summer the infected leaves contained much virus as shown by infectivity tests. The virus could then be purified easily and was not or hardly contaminated with plant material. In winter infectivity was low and purification was difficult or impossible. Then, a hardly discernible band was obtained after the first gradient centrifugation and greenish plant material was scattered throughout the gradient. Additional amounts of antiserum did not improve the purity.

Attempts to enhance the virus concentration and to improve virus purification by varying temperature, day length, light intensity and relative humidity were unsuccessful. Temperature did affect the intensity of symptoms, especially necrosis, on *N. rustica*, but not the virus concentration in the plant.

Results were less satisfactory with the purification methods of Black et al. (1963),

Martin (1964), Best (1968) and Joubert et al. (1974). No virus was obtained with chromatography on calcium phosphate columns, and with filtration over celite pads (Peters and Kitajima, 1970). With zone electrophoresis, the virus was faded over a wide band containing greenish plant material also. Zone electrophoresis after fixation of the virus with glutaraldehyde as done by Joubert et al. (1974) was not studied. Such fixation may attach covalently impurities to the virus (Tas and Peters, unpublished observations).

Vacuum-infiltrated leaf material was homogenized for 30 sec. Longer periods resulted in more impurities in the purified preparations, and may have increased also the likelihood of virus inactivation.

Important for obtaining pure virus was the use of antiserum to an extract from healthy leaf material. The amount needed differed for each batch of serum and was emperically determined. Addition of serum led to considerable clarification of the virus suspension. Especially, cellular fragments co-sedimenting with the virus in the first sucrose gradient were efficiently removed. Some virus was lost by this treatment as found in infectivity tests, and by thin sectioning of pellets. The serum titres were 8 to 16 and amounts of 0.2 to 0.4 ml were usually added. In some cases more serum was added to enhance purity of the virus preparations.

However, the serum treatments did considerably reduce the infectivity of virus suspensions. This cannot be explained solely by precipitation of virus by antibodies to healthy *N. rustica* components since it occurred also to the same extent with pre-immune serum. The drop in infectivity might be due to an inhibitory activity present in the sera. Infectivity was partly restored by centrifugation on sucrose gradient.

Fig. 3. Distribution of UV-absorbing material (260 and 280 nm) and infectivity following centrifugation of the virus suspension on a 3–30% ($^{w}/_{w}$) sucrose gradient in resuspension buffer. Centrifugation was for 45 min at 23000 rpm in a Spinco SW-25 rotor (with brake).

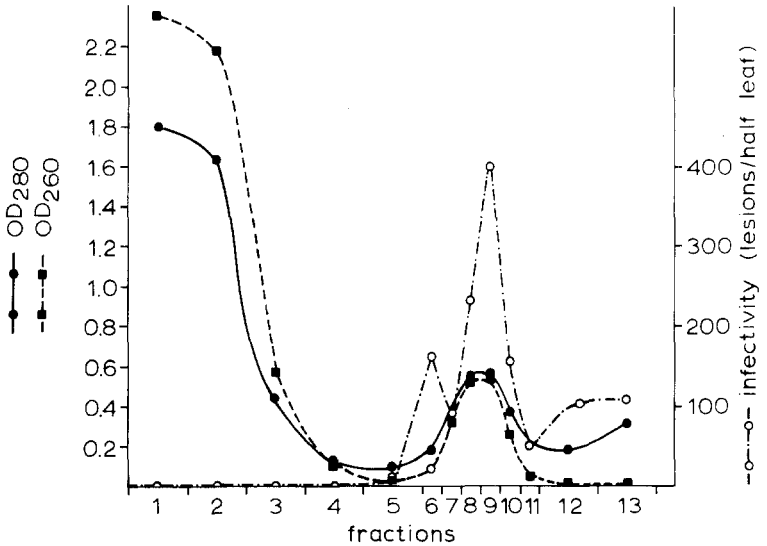

Fig. 3. Verdeling van UV-absorberend materiaal (260 en 280 nm) en infectiositeit na centrifugering van een virussuspensie in lineaire gradiënt van 3–30% ($^{w}/_{w}$) saccharose in resuspensiebuffer.

The distribution of infectivity in the gradient corresponds well with the position of maximum absorbance at 280 and 260 nm (Fig. 3). In several experiments two closely spaced light-scattering bands were observed on the first gradient, but this was not reflected in the absorption profile. The infectivity distribution appeared also to be splitted in two peaks (Fig. 3); as also found by Best (1968) and Tsakirides and Gooding (1972). This phenomenon, although it merits further attention, was not studied.

Persistence of infectivity. The virus isolate (TSWV-S) lost its infectivity in approximately 1 h at 18 °C in crude *N. rustica* sap, its dilution end point was between 10^{-4}–10^{-5} and it was inactivated between 42 and 46 °C. When purified and stored in about 45% sucrose the virus had a much longer longevity. The infectivity at 0 °C was then even retained for approximately 18 days; at 18 °C some infectivity was still left after 6 h and thermal inactivation point was between 50–55 °C. This observation is at variance with that of Joubert et al. (1974) who claimed that the virus was rapidly inactivated in 40% sucrose.

Serology. TSWV antisera no. 1 and no. 2 had titres of 32 and 32–128, respectively. Both sera were compared for their reaction with 1) purified TSWV, 2) the proteins of TSWV after denaturation of the virus with SDS and ME and electrophoresis in polyacrylamide gel (Tas et al., 1977), 3) sap from healthy *N. rustica* and 4) polysaccharides purified from healthy *N. rustica*. In agar gel double-diffusion tests, antiserum no. 1 formed 2 precipitin and antiserum no. 2 formed 3 lines with purified virus (Fig. 4). The antisera had two precipitin lines in common (a and c), antiserum no. 2 formed an additional line between a and c. As more than one precipitin line is found, it is clear that TSWV does not diffuse in agar as intact particles. Complete particles should diffuse very slowly in the agar because of their size (Ackers and Steere, 1962). Other large enveloped viruses such as lettuce necrotic yellows virus (Lin and Campbell, 1972) also give more than one precipitin line in double diffusion tests with their homologous antisera. McLean et al. (1971) explain the occurrence of more lines by breakage of the virus particle into small globular particles which reaggregate to 'rosettes'.

The reaction of the antisera with electrophoretically separated proteins is illustrated in Fig. 5. Antiserum no. 1 gave a moderate reaction with proteins 1, 2+3 and 4 and

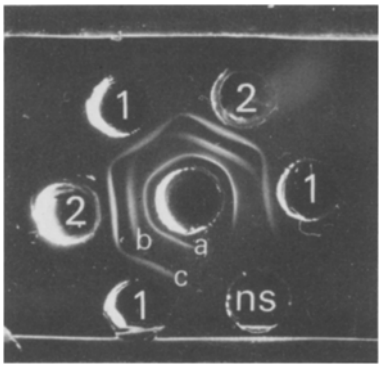

Fig. 4. Double-diffusion test in 1% agar with purified TSWV stored at −20 °C) and antisera no. 1 and 2 and pre-immune serum (ns), respectively. Central well contained TSWV, the peripheral wells antisera no. 1 (1), no. 2 (2) and pre-immune serum (ns). Precipitin lines are labeled a, b and c with increasing distance from the antigen well.

Fig. 4. Reactie van TSWV (bewaard bij −20 °C) met antiserum no. 1 en no. 2 en pre-immuun serum (ns) in 1% agar. Het putje in het centrum bevatte TSWV, de omliggende putjes de antisera. De precipitatielijntjes zijn van binnen naar buiten aangegeven met a, b en c.

Fig. 5. Double-diffusion test in 1% agar between antiserum no. 1 and 2 and electrophoretically separated proteins of TSWV on 7.5% polyacrylamide gels. The electrophoresis front is f and glycolipid band is gb. The conditions for electrophoresis are described by Tas et al. (1976).

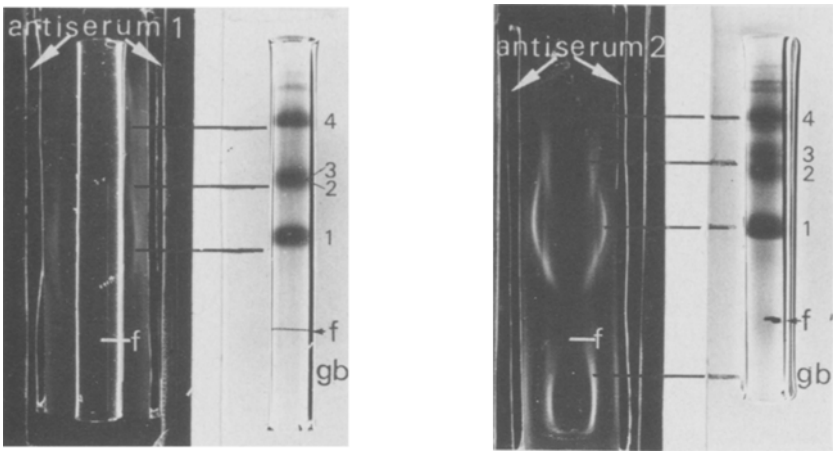

Fig. 5. Reactie van antiserum no. 1 en no. 2 met eiwitten van TSWV, die door polyacrylamide-electroforese gescheiden waren, in 1% agar. Het front is aangeduid met f, en de glycolipiden met gb. De omstandigheden voor deze elektroforese wordt beschreven in Tas et al. (1976).

not al all with the glycolipid band (Tas et al., 1977). Antiserum no. 2 gave a clear line with protein 1, a vague line with protein 2+3 and a very weak reaction with protein 4. A clear reaction occurred with the glycolipid band (gb in Fig. 5).

In double-diffusion reactions antiserum no. 1 formed precipitin lines with sap from healthy *N. rustica* as well as with polysaccharides purified from *N. rustica* (Fig. 6). The reaction of antiserum no. 1 with healthy *N. rustica* sap could be abolished by absorption with polysaccharides purified from *N. rustica*. Antiserum no. 2, however, did not react with healthy *N. rustica* sap and reacted only weakly with polysaccharides from healthy *N. rustica*. The reactions of antisera no. 1 and 2 are summarised in Table 1.

Absorption of antisera no. 1 and 2 with either healthy sap or polysaccharides from *N. rustica* led to a greatly reduced reaction with intact virus in double-diffusion tests, absorption with polysaccharides being the most efficient.

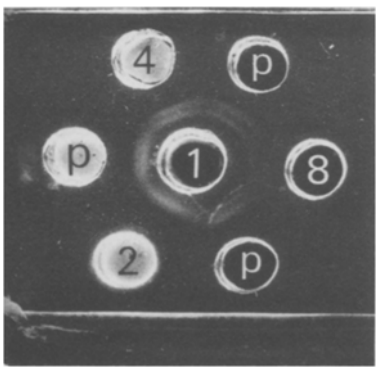

Fig. 6. Double-diffusion test in 1% agar with antiserum no. 1 and dilutions of *N. rustica* sap (1:2, 1:4 and 1:8) and polysaccharides from *N. rustica* (p). The central well contains antiserum no. 1.

Fig. 6. Serologische reactie van antiserum no. 1 tegen een drietal twee-voudige verdunningen van sap van N. rustica en polysacchariden uit N. rustica in 1% agar. Het centrale putje bevatte het antiserum.

Table 1. Comparison of the reactions of antiserum no. 1 and no. 2 with proteins 1, 2+3, 4 and glycolipid following separation on SDS gels. The reactions with *N. rustica* sap and polysaccharides from *N. rustica* were determined by gel double diffusion. The intensity of the precipitin lines is evaluated as – no reaction, + slight reaction, ++ moderate, +++ strong and ++++ very strong reaction.

Antigen	Antiserum 1	Antiserum 2
protein 1	++	++++
protein 2+3	++	++
protein 4	++	+
glycolipid	–	+++
N. rustica sap	++	–
polysaccharides	++	+

Tabel 1. Vergelijking van de reacties van antiserum no. 1 en no. 2 met de TSWV-eiwitten 1, 2, 3 en 4, en de glycolipiden uit dit virus na scheiding op polyacrylamide-gels. De reacties met sap van N. rustica en polysacchariden uit N. rustica werden bepaald met de dubbele-diffusiemethode. Geen reactie: –; zwakke reactie: +; milde reactie: ++; sterke reactie: +++; en zeer sterke reactie: ++++.

No reaction was found between absorbed antiserum no. 1 and electrophoretically separated proteins of TSWV, although a weak reaction with protein 1 remained in the case of antiserum no. 2. From these observations several general conclusions can be drawn. Antisera produced with purified TSWV react either with components in healthy sap or with polysaccharides. This reaction can be abolished by absorption with polysaccharides extracted from healthy plants. Thus, the virus preparation used for immunization a rabbit must have contained polysaccharides either as contaminants or as components of the virus particle. Furthermore absorption with both healthy sap and polysaccharides decreased the intensity of the reaction with purified virus either intact or SDS-disrupted. Hence it may be concluded that the reaction of the antisera against TSWV with healthy sap is by polysaccharide components which may also occur covalently linked to the structural proteins of the virus particle.

When four aliquots of antiserum no. 2, were absorbed with two-fold dilutions of intact TSWV, and tested with purified virus only precipitin line a was found (Fig. 7). This line must be due to antibodies, which could not be neutralized by intact virus. It seems impossible that this line was caused by reaction of an antigen on the outside

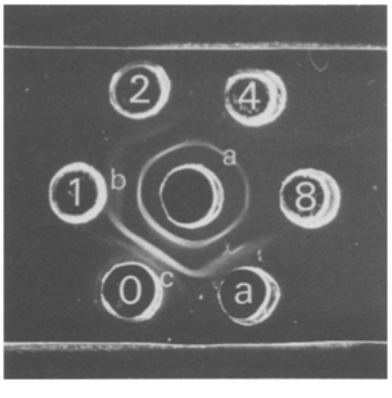

Fig. 7. Double-diffusion test in 1% agarose between untreated TSWV (central well) and antiserum no. 2 absorbed with increasing amounts of intact TSWV (resp. 0, 1, 2, 4 and 8 ml intact TSWV) and antiserum no. 2 absorbed with *N. rustica* sap (a). Precipitin lines are labeled a, b and c with increasing distance from the antigen well.

Fig. 7. Serologie van TSWV met antiserum no. 2, dat eerst met toenemende hoeveelheden intact TSWV en met sap van N. rustica (a) was verzadigd. De precipitatielijntjes zijn van binnen naar buiten met de letters a, b en c aangegeven.

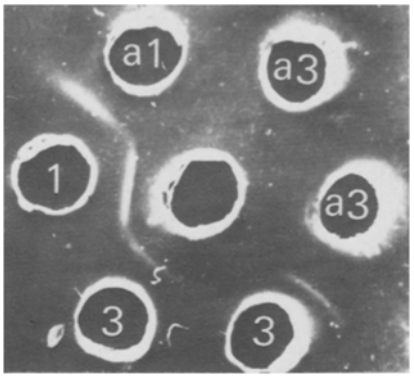

Fig. 8. Double-diffusion test in 1% agar between virus denatured with 6.5 M guanidine-HCl (central well) and antiserum 1 (1), antiserum no. 1 absorbed with polysaccharides from *N. rustica* (a 1), antiserum to *N. rustica* sap (3) and antiserum to *N. rustica* sap absorbed with polysaccharides from *N. rustica* sap (a 3).

Fig. 8. Serologie van TSWV, dat met guanidine-HCl (putje in het centrum) werd behandeld met antiserum no. 1 (1), antiserum no. 1 verzadigd met polysacchariden van N. rustica (a1), antiserum tegen sap van N. rustica (3) en antiserum tegen sap van N. rustica verzadigd met polysacchariden van N. rustica (a3).

of the membrane masked from reaction with antibodies to TSWV in view of the lactoperoxidase-catalysed iodination of intact TSWV (Tas et al., 1977). The results of this experiment indicated that precipitin lines c and b may be due to antigens associated with the outside of the membrane, whereas precipitin line a may be due to an antigen present within the virus envelope, and becoming available for reaction when the virus disrupts upon entering the gel.

In view of the possible occurrence of polysaccharides in purified TSWV, it was interesting to test antiserum to *N. rustica* sap with virus. When TSWV was denatured with 6.5 M guanidine-HCl, a weak reaction with antiserum to *N. rustica* sap was found. This reaction did not occur when this antiserum had been absorbed with polysaccharides (Fig. 8). The weak reaction partly explains why antiserum to healthy *N. rustica* components can be used for purification although the virus contains host polysaccharides.

The weak reaction between antiserum raised against healthy sap and disrupted virus supports the conclusion that the virus proteins contain host derived antigens.

Discussion

The antisera to TSWV were not completely virus specific, since they also reacted with healthy *N. rustica* sap. Absorption with *N. rustica* sap or with polysaccharides extracted from *N. rustica* resulted in a specific antiserum. Absorption also resulted in a four-fold decrease in titre. An antiserum, probably specific for protein 1, was obtained by absorbing antiserum no. 2 with excess intact virus. A number of authors have prepared antisera to the virus (Best and Hariharasubramanian, 1967; Tsakiridis and Gooding, 1972; Joubert et al., 1974; and Paliwal, 1974), but only Paliwal has reported a reaction of TSWV antiserum with healthy sap.

Several observations suggested the occurrence of host polysaccharides in TSWV. Firstly, the reaction of TSWV antiserum with healthy *N. rustica* sap was abolished by absorption with polysaccharides from *N. rustica*. This implies that the virus preparation used for immunization contained polysaccharides. Secondly, the precipitin lines between TSWV antiserum and the elctrophoretically separated proteins of TSWV were clearly diminished after absorption with either healthy *N. rustica* sap or polysaccharides from *N. rustica*. Since the position of the precipitin lines was exactly in correspondence with the positions of proteins 1, 2+3 and 4, there is no reason to

assume that the virus preparations contained plant antigens as contaminants. Thirdly, the weak precipitin line observed between antiserum to *N. rustica* components and TSWV denatured with 6.5 M guanidine-HCl fused with the precipitin line obtained between denatured TSWV antiserum (Fig. 8). Both reactions could be abolished by absorption of the corresponding sera with polysaccharides. Fourthly, proteins 2+3, 4 and 5 stain clearly with Schiff's reagent indicating that they contain covalently bound carbohydrate (Tas et al., 1977). Host antigens in the form of glycoproteins have been reported with influenza virus (Laver and Webster, 1966).

The results obtained indicate that host derived antigens in the form of carbohydrate are covalently bound to proteins 2, 3 and 4. No serological reaction was found between proteins 5, 6 and 7 and the antisera used. This may be due to the low concentrations of these proteins in the virus and hence in the immunizing mixture.

Whether the carbohydrate moiety of the glycolipids extracted from purified virus (Tas et al., 1977) are also host derived, was not investigated. It is not likely that the genome of TSWV contains information for the order and structure of carbohydrate chains. This requires that the virus codes for a number of glycosyltransferases, one for each monosaccharide (Burge and Strauss, 1970). Thus, the carbohydrate moiety of the glycolipids of TSWV is probably also host derived. Host antigen in the form of glycolipid has been demonstrated with parainfluenza virus SV 5 (Klenk et al., 1970) and vesicular stomatitis virus (Klenk and Choppin, 1971; Cartwright and Brown, 1972).

Samenvatting

Zuivering en serologische analyse van het tomatebronsvlekkenvirus

Het bronsvlekkenvirus van de tomaat (TSWV) werd gezuiverd met een procedure die bestond uit een cyclus van differentiële centrifugering, behandeling van de virussuspensie met antiserum tegen bestanddelen van gezonde planten *(N. rustica)* en dichtheidsgradiëntcentrifugering (Fig. 1 en Fig. 3). De zone rotor kon worden gebruikt wanneer grote hoeveelheden bladmateriaal werden gebruikt (Fig. 2).

Twee antisera, die elk een verschillende activiteit tegen TSWV (Tabel 1, Fig. 4) hadden, werden bereid. Deze sera reageerden ook met sap en polysacchariden uit *N. rustica* (Fig. 6). Door verzadiging met polysacchariden werd de reactie tegen TSWV aanmerkelijk verzwakt. Analyse van de eiwitbandjes die na polyacrylamidegel-elektroforese waren gevormd, toonde aan dat de activiteit van de sera tegen de eiwitcomponenten van TSWV was gericht (Fig. 5) en dat deze activiteit na verzadiging met polysacchariden zwakker was. Uit deze reacties kan geconcludeerd worden dat polysacchariden van de waardplant aan de structurele eiwitten van TSWV zijn gebonden. Niet geabsorbeerd antiserum geeft met TSWV drie precipitatielijnen (Fig. 4), maar na absorptie met compleet virus trad nog slechts één precipitatielijntje op (Fig. 7). Mogelijk wordt dit lijntje gevormd door het nucleocapside-eiwit dat door de dissociatie van het virus vrij komt. Sera die tegen normale bestanddelen van *N. rustica* zijn bereid, vormen geen zichtbare reactie met compleet virus; wel wordt er een lijntje gevormd indien het virus met guanidine-HCl wordt gedissocieerd (Fig. 8). Deze waarneming kan verklaren dat deze antisera kunnen worden gebruikt bij de zuivering van TSWV ondanks het feit dat dit virus polysacchariden uit de waardplant draagt.

References

Ackers, G. K. & Steere, R. L., 1962. Restricted diffusion of macromolecules through agar-gel membranes. Biochim. biophys. Acta 59: 137–149.

Best, R. J., 1968. Tomato spotted wilt virus. Adv. Virus Res. 13: 65–146.

Best, R. J. & Hariharasubramanian, V., 1967. Serological studies on tomato spotted wilt virus (strains E and R_1). Enzymologia 32: 128–134.

Best, R. J. & Katekar, G. F., 1964. Lipid in a purified preparation of tomato spotted wilt virus. Nature, Lond. 203: 671–672.

Best, R. J. & Palk, B. A., 1964. Electron microscopy of strain E of tomato spotted wilt virus and comments on its probable biosynthesis. Virology 23: 445–460.

Black, L. M., Brakke, M. K. & Vatter, A. E., 1963. Purification and electron microscopy of tomato spotted wilt virus. Virology 20: 120–130.

Burge, B. W. & Strauss Jr, J. H., 1970. Glycopeptides of the membrane glycoprotein of Sindbis virus. J. molec. Biol. 47: 449–466.

Cartwright, B. & Brown, F. 1972. Glycolipid nature of the complement fixing host cell antigen of vesicular stomatitis virus. J. gen. Virol. 15: 243–245.

Gumpf, D. J. & Weathers, L. G., 1972. Identification and purification of tomato spotted wilt virus isolated from ageratum. Pl. Dis. Reptr 56: 869–872.

Ie, T. S., 1964. An electron microscope study of tomato spotted wilt virus in the plant cell. Neth. J. Pl. Path. 70: 114–115.

Ie, T. S., 1970. Tomato spotted wilt virus. C.M.I. Descriptions of Plant Viruses No. 39, Commonwealth Mycological Institute, Kew.

Joubert, J. J., Hahn, J. S., Wechmar, M. B. von & Regenmortel, M. H. V. van, 1974, Purification and properties of tomato spotted wilt virus. Virology 57: 11–19.

Kammen, A. van, Henstra, S. & Ie, T. S., 1966. Morphology of tomato spotted wilt virus. Virology 30: 574–577.

Klenk, H. D. & Choppin, P. W., 1971. Glycolipid content of vesicular stomatitis virus grown in baby hamster kidney cells. J. Virol. 7: 416–417.

Klenk, H. D., Caliguiri, L. A. & Choppin, P. W., 1970. The proteins of parainfluenza virus SV 5. II. The carbohydrate content and glycoproteins of the virion. Virology 42: 473–481.

Laver, W. G. & Webster, R. G., 1966. The structure of influenza viruses. Chemical studies on the host antigen. Virology 30: 104–115.

Lin, M. T. & Campbell, R. N., 1972. Characterization of broccoli necrotic yellows virus. Virology 48: 30–40.

MacLean, G. D., Wolanski, B. S. & Francki, R. I. B., 1971. Serological analysis of lettuce necrotic yellows preparation by immuno-diffusion. Virology 43: 480–487.

Martin, M. M., 1964. Purification and electron microscope studies of tomato spotted wilt virus (TSWV) from tomato roots. Virology 22: 645–649.

Paliwal, Y. C., 1974. Some properties and thrip transmission of tomato spotted wilt virus in Canada. Can. J. Bot. 52: 1177–1182.

Peters, D. & Kitajima, E. W., 1970. Purification and electron microscopy of sowthistle yellow vein virus. Virology 41: 135–150.

Tas, P. W. L., 1975. Chemische en structurele eigenschappen van het bronsvlekkenvirus van de tomaat. Diss. Wageningen.

Tas, P. W. L., Boerjan, M. L. & Peters, D., 1977. The structural proteins of tomato spotted wilt virus. Submitted to J. gen. Virol.

Tsakiridis, J. P. & Gooding, G. V., 1972. Tomato spotted wilt virus in Greece. Phytopath. mediterr. 11: 42–47.

Willers, J. M. M., Michel, M. F., Sysma, M. J. & Winkler, K. C., 1964. Chemical analysis and inhibition reactions of the group and type antigens of group F. streptococci. J. gen. Microbiol. 36: 95–105.

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