Neth. J. Pl. Path. 76 (1970) 79–92

Experiments on the use of latex, bentonite, and water-insoluble antiserum protein polymers in sensitive serological tests

D. Z. MAAT

Instituut voor Plantenziektenkundig Onderzoek (IPO), Wageningen

Accepted 22 December 1969

Abstract

Experiments were performed to detect low concentrations of plant viruses with the latex-agglutination test, the bentonite-flocculation test, and a test using water-insoluble antiserum protein polymers. Special attention was paid to the potato viruses X and S, and to the influence of high concentrations of normal plant constituents, notably from potato plants, on the sensitivity of the methods. It was found that under certain circumstances lower virus concentrations could be detected with these methods than with the conventional micro-agglutination or micro-precipitation test. However, sensitivity was negatively influenced by sap from potato plants, especially in experiments with potato virus S. To really profit from the sensitive tests mentioned, their sensitivity should be greater than in my experiments.

Introduction

The suitability of common serological test methods as the agglutination, precipitation or agar-gel diffusion techniques for diagnostic purposes is limited greatly since they are not sensitive enough to detect those viruses that occur in low concentration in expressed plant sap. Therefore, interest is increasing in serological tests that enable detection of such low virus concentrations. Attention has been paid especially to the latex-agglutination (LA) test, the bentonite-flocculation (BF) test, and the passive haemagglutination (PHA) test (cf. Bercks, 1967a; Abu Salih et al., 1968a, b; Richter, 1969). In these tests, antibodies against plant viruses are first adsorbed to the much bigger particles of latex or bentonite-clay or to red blood cells. The so-called antibody-sensitized particles or cells are then used to react with the viruses. Because of the size of the sensitized particles, much less virus and antibody are needed to result in a visible agglutination or flocculation. To obtain visible reactions, however, in many cases the crude plant extract had to be clarified and/or suitably diluted. Furthermore the results were highly influenced by the individual antisera and their dilution used for sensitizing the particles or cells. With some antisera the detectable virus concentrations in the LA-, BF- or PHA-tests were not lower than in the common serological tests. Nevertheless, with the LA- or BF-tests viruses have been detected in extracts from parts of plants or of potato tubers where the conventional serological test or even the infectivity test failed, or gave positive results only after a more intensive pretreatment of the plant material (Bercks, 1967a, b, c and 1968; Bozicevich et al., 1963; Kahn et al., 1967; Scott et al., 1964).

My experiments concern the LA- and BF-tests, and a test in which biologically active, water-insoluble antiserum protein polymers (Avrameas and Ternynck, 1967) were tried as a possible substitute for sensitized latex or bentonite (APP-test). Attension was paid to the influence of high concentrations of plant constituents on test sensitivity, with the special intention to detect potato viruses. The experiments were mainly for orientation and only a few viruses, antisera and plant saps were tested.

Materials and methods

A. The latex-agglutination test (LA-test)
The LA-test was performed (1) without pre-sensitization of latex with antibodies by successively putting together latex, antiserum and antigen or (2) with sensitization of latex particles with antibodies, prior to addition to the antigen.

(1) Test without pre-sensitization of latex particles with antibodies (LA_1-test)

Performance. The tests were performed in petridishes as drop test under paraffin oil or on standard haemagglutination plates (Visijar Laboratories Ltd, Croydon, England), the latter being placed in plastic bags to avoid evaporation of the liquid. Latex (Bacto, 0.81 μ), diluted 1/15 with 0.85% NaCl, antiserum and antigen were added successively to the petridishes or haemagglutination plates, in equal amounts. The reagents were then mixed by gently shaking by hand or by an electric vibrator (50 Hz; maximum amplitude 2 mm; 5–15 min). After 1½–5 h of incubation reactions were read using a low power binocular microscope. Shortly before reading, dishes or plates were often shaken for 1 min or less.

Treatment of antisera. Besides untreated antisera globulin fractions were used. The latter were usually prepared by mixing 1 ml of antiserum (a.s.) with 11 ml of buffered NaCl pH 7.3 (Bozicevich et al., 1963) and 8 ml of $(NH_4)_2SO_4$ solution saturated at room temperature. After 15 min of storage in a refrigerator the mixture was centrifuged for 10 min at 11,500 g in a refrigerated centrifuge. The precipitate thus obtained was resuspended in 8 ml buffered NaCl pH 7.3 (incidentally 0.85% NaCl or Tris buffer pH 7.2) and dialyzed against this solution overnight, the dialyzation solution being changed after 1–2 h. The resulting globulin suspension was then clarified by centrifuging for 10 min at 11,500 g. A dilution series (dilution factor 4) of a.s. or globulin fraction (gl. fr.) was made using 0.85% NaCl, buffered NaCl, Tris-HCl buffer pH 7.2 (0.05 M Tris) or, in special experiments, diluted normal sera.

Preparation of antigens. Purified virus preparations, or viruses in crude sap or clarified sap were used as antigens. Crude sap was centrifuged at low speed if to be diluted with centrifuged sap from healthy plants. Dilution series (dilution factor 4) were made, using crude sap from healthy plants (diluted or not), clarified sap from healthy plants, 0.85% NaCl, buffered NaCl pH 7 or pH 7.3, or Tris-HCl buffer pH 7.2 (0.05 M Tris). The buffers were sometimes provided with 0.02% polyvinylpyrrolidone (PVP) to avoid non-specific agglutination of latex. Clarification was performed by low-speed centrifugation, preceded or not by heating for some minutes at 55°–60°C. Usually purified preparations were prepared by clarification with ether and carbontetrachlo-

ride, followed by differential centrifugation or ammoniumsulphate precipitation.

Propagation hosts were: *Nicotiana tabacum* 'White Burley' for potato virus X (PVX), alfalfa mosaic virus (AMV), and tobacco mosaic virus (TMV), *Phaseolus vulgaris* 'Bataaf' for cowpea mosaic virus (CPMV isolate Sb, Agrawal and Maat, 1964), and tuber-infected plants of *Solanum tuberosum* 'IJsselster' for potato virus S (PVS). Non-infected greenhouse-grown plants of the same species served as sources for sap from healthy plants, and so did the 'Doré' potatoes and the *Solanum demissum* hybrid 'A6'.

Control reactions. To prove the specificity of the reactions, experiments were made with a heterologous a.s., normal serum and/or the diluent of the a.s. instead of the homologous a.s. Furthermore experiments were made with sap from healthy plants and the diluent instead of the virus-containing sap.

(2) Test with antibody-sensitized latex particles (LA_2-test)

Treatment of antisera, preparation of antigens and control reactions were as with the LA_1-test.

Performance. The tests were according to Bercks (1967a) or in petridishes as drop test under paraffin oil. In petridishes equal amounts of antibody-sensitized latex and antigen were put together and mixed by handshaking. Final readings were made after $1\frac{1}{2}$–5 h with a low power binocular microscope; sometimes the plates or dishes were first shaken shortly.

Sensitization of latex particles. The procedure described by Bercks (1967a) was followed, except that centrifugation was for 10 min at 11,500 g instead of 30 min at 5,000 g, and "Cialit" was replaced by NaN_3. Instead of only one a.s. dilution, three dilutions were used for sensitization, namely the one corresponding with the titre-dilution of the a.s. or gl. fr. in the common serological (drop) test, one higher and one lower dilution (dilution factor 4).

B. The bentonite-flocculation test (BF-test)

The tests were performed as described for the LA_2-test. The bentonite suspension was prepared as recorded in Fig. 1.

Sensitization of bentonite particles. Mainly the procedure of Bercks (1967a) was followed. However, centrifugations were at 7,000 g instead of 700 g, and the buffered NaCl solution used for washing the sensitized bentonite was provided with 0.02% PVP. Here also "Cialit" was replaced by NaN_3. As with the LA_2-test three a.s. dilutions were used.

Information concerning the treatment of antisera, preparation of antigens and control reactions is given on p. 80–81. When 0.85% NaCl or buffered NaCl pH 7.3 was used as antigen diluent, PVP was added.

C. Test with antiserum protein polymers (APP-test)

The tests were performed as described for the LA_2-test. Information concerning the preparation of antigens and the control reactions is given on p. 80–81.

Preparation of antiserum protein polymers. The antiserum protein polymers were prepared with ethyl-chloroformate (Avrameas and Ternynck, 1967) by the procedure of Fig. 2. Of the suspension obtained a dilution series (dilution factor 4) was made with 0.85% NaCl.

Fig. 1. Preparation of the bentonite suspension.

0.5 g dry bentonite (bentonite powder, technical, The British Drug Houses Ltd., England)
+
100 ml destilled water

Homogenize in Waring blendor (1–2 min)
Leave for 5 min
Homogenize in Waring blendor (1–2 min)
Add destilled water to get 500 ml, and shake
Leave for 2 h

Discard sediment	Centrifuge supernatant 10 min at 350 g
Discard supernatant	Resuspend sediment in 100 ml destilled water; homogenize in Waring blendor; centrifuge 5 min at 200 g
Discard sediment	Keep supernatant + 1 ml 3% NaN_3 solution in refrigerator

Before use the concentration of the bentonite suspension is determined, and, if necessary, brought at 0.4–0.6 mg/ml

Fig. 1. De bereiding van de bentonietsuspensie.

Fig. 2. Preparation of antiserum protein polymers.

1 ml antiserum
+
3 ml 0.2 M Na-acetate buffer pH 4.75
+
0.15 ml ethyl-chloroformate

Stirr for 15 min in a closed bottle
Leave for 1 h
Suspend in 40 ml 0.85% NaCl solution
Homogenize suspension during 10–15 sec by ultrasonic
vibration (e.g. Kerry Vibrason cell disrupter, probe diameter 0.9 cm, output 100 W)
Centrifuge homogenate for 10 min at 12,000 g
Discard supernatant

Rub down sediment and suspend in 40 ml 0.85% NaCl solution
Centrifuge suspension for 10 min at 12,000 g
Discard supernatant
Repeat suspending and centrifuging once using 0.85% NaCl solution and twice using McIlvaine's buffer pH 7

Rub down sediment and suspend in 5 ml 0.85% NaCl solution; add a few drops of a 3% NaN_3 solution and homogenize during 20–30 sec (ultrasonically)

Fig. 2. De bereiding van antiserum eiwitpolymeren.

Experiments and results

A. The latex-agglutination test

(1). Test without pre-sensitization of latex with antibodies

The scheme and results of a characteristic experiment with PVX are recorded in Table 1. In this experiment the gl. fr. of an a.s. with original titre of 1024 was used. The antiserum was diluted with 0.85% NaCl, and the virus with crude sap from healthy tobacco plants. The results of a similar experiment with the same virus with several diluents and an untreated a.s., are summarized in Table 2. Here, non-specific agglutination of latex occurred with sap from potato plants. In other experiments with sap from potato plants, where the non-specific reaction did not occur, the LA_1-test was not more sensitive than the common drop test, even not when using the gl. fr. instead of untreated a.s. Comparing buffered NaCl and Tris buffer as diluents, Tris buffer gave slightly better results than buffered NaCl, when all reagents were diluted with it.

In experiments with CPMV results using an a.s. with a titre of 4096 or the gl. fr. from this a.s. were the same. The lowest detectable virus concentration was only 1/4 of that in the common micro-precipitin test when the virus was diluted with undiluted clarified sap from healthy French bean plants. The sensitivity of the LA_1-test increased when the healthy plant sap used for diluting the virus was diluted first, and was 64 times better than the micro-precipitin test when using sap diluted 1 to 16. Diluting the a.s. with normal serum also negatively influenced the sensitivity of this test. This effect decreased when the normal serum was diluted first and disappeared using normal serum dilutions of 1/256 to 1/1024.

Table 1. Latex-agglutination test without special sensitization of latex particles with antibodies (LA_1-test). Comparison of the LA_1-test with the common micro-agglutination test, the antigen (PVX) being diluted with crude sap from healthy tobacco plants.

Dilutions of PVX	Dilutions of the PVX antiserum globulin fraction											
	LA_1-test							Micro-agglutination test				
	1	4	16	64	256	1024	4096	1	4	16	64	256
1	+	+	+	+	±	−	−	+	+	+	±	−
4	+	+	+	+	+	+	±	+	+	±	−	−
16	±	+	+	+	+	+	±	+	+	±	−	−
64	−	−	±	+	+	+	+	−	−	−	−	−
256	−	−	−	±	±	+	±	−	−	−	−	−
1024	−	−	−	−	−	±	−	−	−	−	−	−
4096	−	−	−	−	−	−	−	−	−	−	−	−

+ positive reaction; ± weak reaction; − no reaction.

Tabel 1. Latex-agglutinatietoets zonder voorafgaande sensibilisering van de latex met antistofdeeltjes (LA_1-test). Vergelijking van de LA_1-toets met de gewone agglutinatietoets, waarbij het virus (PVX) werd verdund met ruw sap van gezonde tabaksplanten.

With AMV the LA_1-test had a greater sensitivity only when the gl. fr. of the a.s. (titre 64) was used. Even then the lowest virus concentration detectable was only 1/4 of that in the test without latex; this was true when the virus was diluted with buffered NaCl as well as with clarified sap from healthy tobacco plants. The increase in sensitivity disappeared when the gl. fr. was diluted with normal serum diluted 1/20 or 1/200 instead of with buffered NaCl.

The LA_1-test was not more sensitive than the micro-precipitin test with purified TMV using a three-months old a.s. (titre 1024) or the gl. fr. out of this a.s. Both a.s. and virus were diluted with Tris buffer.

Dilution series of PVS were made with Tris buffer (+ PVP), clarified sap, undiluted crude sap and crude sap from healthy potato plants diluted 1/4 and 1/16. Antiserum samples of different ages and originating from two rabbits, or the gl. fr. out of the sera, were used in dilutions made with Tris buffer. The LA_1-test only incidentally was more sensitive than the test without latex, this greater sensitivity being found only when the virus was diluted with buffer. With the other diluents the test without latex sometimes was better. As with PVX, the sensitivity of the test without latex was clearly influenced by the virus diluent (see Table 2).

(2) Test with antibody-sensitized latex particles

The results of two experiments with PVX, carried out with latex sensitized with two different antisera and/or the gl. fr. from these sera are summarized in Table 3. Antiserum 444 had a titre of 4096 and a.s. 416 a titre of 1024 in the micro-precipitin test.

The results of two experiments with PVS are summarized in Table 4. Antiserum samples used in these experiments were at least 5 months old. Earlier experiments performed with fresh antisera or their globulin fractions gave no reaction in the LA_2-test, not even when purified virus preparations were used. Antiserum samples mentioned in Table 4 originate from one and the same rabbit, but are from different bleedings or are stored in different ways. Antiserum 1 had a titre of 1024 and the antisera 2 and 3 had titres of 4096.

Table 2. Comparison of the LA_1-test with the normal drop test. Results of an experiment with PVX, using several diluents for the virus.

Diluent used for the virus	Virus dilution end points	
	LA_1-test	Normal drop test
0.85% NaCl + PVP	4096	256
Undiluted crude sap from tobacco plants	256	16
Undiluted centrifuged sap from tobacco plants	1024	64
Undiluted crude sap from potato plants	non-specific	16
Crude sap from potato plants diluted 1/4	non-specific	64
Undiluted centrifuged sap from potato plants	non-specific	64

Tabel 2. Vergelijking tussen de LA_1-toets en de gewone druppeltoets. Overzicht van de resultaten van een proef met het PVX, waarbij het virus met verschillende middelen werd verdund.

CPMV could be detected with the LA_2-test at concentrations 16 times lower than in the micro-precipitin test, using buffered NaCl or clarified sap from healthy bean plants as diluents. When diluted with crude sap, no visible reaction was obtained. The a.s. was untreated and had a titre of 4096.

Purified TMV, tested with five a.s. samples of about 3 months old and originating from two rabbits, did not show agglutination of latex when the antisera were untreated. In an experiment with latex sensitized with the gl. fr. of one of these a.s. samples, latex

Table 3. Comparison of the LA_2-test with the common drop test. Results of experiments with PVX, using several diluents for the virus.

Diluent used for the virus	Virus dilution end points in:					
	LA_2-test, latex sensitized with					Common drop test[1]
	a.s. 444			a.s. 416		
	untreated	gl.fr.	gl.fr.	untreated	gl.fr.	
Tris-HCl buffer + PVP	4096	16384	$\geqslant$ 4096	1024	1024	64
Centrifuged sap from tobacco plants	16384	16384		4096	4096	256
Crude sap from potato plants	0	256	0	0	0	16
Centrifuged sap from potato plants	1024	4096	1024	256[2]	4096	64

[1] The results of the common drop test were the same in the different experiments.
[2] Reactions questionable. 0: no visible reaction.

Tabel 3. Vergelijking van de LA_2-toets met de gewone druppeltoets. Overzicht van de resultaten van proeven met het PVX, waarbij het virus werd verdund met verschillende middelen.

Table 4. Comparison of the LA_2-test with the common drop test. Results of experiments with PVS, using several diluents for the virus.

Diluent used for the virus	Virus dilution end points in:				
	LA_2-test, latex sensitized with				Common drop test[1]
	a.s. 1 gl.fr.	a.s. 2 gl.fr.	a.s. 2 gl.fr.	a.s. 3 gl.fr.	
Tris-HCl buffer + PVP	1024	1024	1024	1024	64
Crude sap from potato plants			0	0	4
Centrifuged sap from potato plants	4	16	64	16	16

[1] Results of the common drop test were the same in the different experiments. 0: no visible reaction.

Tabel 4. Vergelijking van de LA_2-toets met de gewone druppeltoets. Overzicht van de resultaten van proeven met het PVS, waarbij het virus werd verdund met verschillende middelen.

agglutination did occur and the LA_2-test was found to be 4–16 times more sensitive than the micro-precipitin test. The antisera had titres of 1024. Dilutions of the virus were made with Tris buffer (+ PVP).

B. The bentonite-flocculation test

The results of two experiments with PVX and PVS are recorded in Table 5 and 6, respectively. The antisera used were the same as in the LA_2-test.

Table 5. Comparison of the BF-test with the common drop test. Results of experiments with PVX, using several diluents for the virus.

Diluent used for the virus	Virus dilution end points in:						
	Exp. I					Exp. II	
	BF-test using				Common drop test	BF-test using a.s. 444 gl.fr.	Common drop test
	a.s. 444		a.s. 416				
	untreated	gl.fr.	untreated	gl.fr.			
Buffered NaCl + PVP	1024	4096	1024	1024	256	4096	64
Centrifuged sap from tobacco plants	1024[1]	4096	1024	4096	256		
Crude sap from potato plants	256	1024	0	256	16	0	16
Centrifuged sap from potato plants	1024	4096	256	1024	256	256	64

[1] Reactions questionable. 0: no visible reaction.

Tabel 5. Vergelijking tussen de BF-toets en de gewone druppeltoets. Overzicht van de resultaten van proeven met PVX, waarbij het virus met verschillende middelen werd verdund.

Table 6. Comparison of the BF-test with the common drop test. Results of experiments with PVS, using several diluents for the virus.

Diluent used for the virus	Virus dilution end points in:					
	Exp. I			Exp. II		
	BF-test using		Common drop test	BF-test using		Common drop test
	a.s. 1 gl.fr.	a.s. 2 gl.fr.		a.s. 2 gl.fr.	a.s. 3 gl.fr.	
Buffered NaCl + PVP	256	256	64	64	256	16
Crude sap from potato plants				0	0	4
Centrifuged sap from potato plants	16	4	16	64	64	16

0: no visible reaction.

Tabel 6. Vergelijking tussen de BF-toets en de gewone druppeltoets. Overzicht van de resultaten van proeven met PVS, waarbij het virus werd verdund met verschillende middelen.

Table 7. Comparison of the APP-test with the common drop test. Results of experiments with PVX, using several diluents for the virus.

Diluent used for the virus	Virus dilution end points in:									
	Exp. I			Exp. II			Exp. III		Exp. IV	
	APP-test using a.s.		Common drop test	APP-test using a.s.		Common drop test	APP-test using a.s.	Common drop test	APP-test using a.s.	Common drop test
	444	416		444	416		444		444	
Tris-HCl buffer				4096	1024	64				
NaCl solution	4096	4096	256				1024	64	256	256
Centrifuged sap from tobacco plants	4096	4096	256	1024	256	256			256	64
Undiluted crude sap from potato plants	?	4096	16	0	0	16		16	0	16
Crude sap from potato plants diluted 1/4									16	64
Undiluted centifuged sap from potato plants	4096	4096	256	4096	4096	64	256	64	1024	64

0: no visible reaction; ?: reactions doubtful.

Tabel 7. Vergelijking tussen de APP-toets en de gewone druppeltoets. Overzicht van de resultaten van proeven met het PVX, waarbij het virus met verschillende middelen werd verdund.

Table 8. Comparison of the APP-test with the common drop test. Results of experiments with PVS, using several diluents for the virus.

Diluent used for the virus	Virus dilution end points in:								
	Exp. I			Exp. II			Exp. III		
	APP-test using		Common drop test	APP-test using		Common drop test	APP-test using		Common drop test
	a.s. 1	a.s. 3		a.s. 2	a.s. 3		a.s. 2	a.s. 4	
NaCl solution	256	256	64	64	64	16	64	64	64
Centrifuged sap from tobacco plants							64	0	64
Undiluted crude sap from potato plants				0	0	1	0	0	> 4
Crude sap from potato plants diluted 1/4				4	4	16	0	0	4
Undiluted centrifuged sap from potato plants	4	16	16	64	64	16	64	?	16

0: no visible reaction; ?: reactions doubtful.

Tabel 8. Vergelijking tussen de APP-toets en de gewone druppeltoets. Overzicht van de resultaten van proeven met het PVS, waarbij het virus met verschillende middelen werd verdund.

C. Test with antiserum protein polymers

The results of the APP-tests with PVX and PVS are recorded in Table 7 and Table 8, respectively. Antiserum 4 against PVS had a titre of 1024. The other antisera used were those mentioned with the LA_2-test. Some more experiments were performed with purified or highly clarified preparations of TMV, CPMV, and TRV (tobacco rattle virus). The viruses were diluted with 0.85% NaCl. The lowest virus concentrations detectable were 1/8, 1/64 and 1/4 of those in the micro-precipitin test for TMV, CPMV, and TRV, respectively. These results were obtained only when antisera with titres of 1024 or higher were used.

Discussion and conclusions

My experiments were carried out during a period of about 3 years and often with materials that happened to be available. They were mainly meant as an orientation. The results show that under certain circumstances with the LA-, BF-, and APP-tests, lower virus concentrations can be detected than with the conventional micro-precipitation or micro-agglutination methods. This was earlier demonstrated for the LA-, BF-, and PHA-tests (a.o. Bercks, 1967a; Abu Salih et al., 1968a, b; Richter, 1969); the last test was not included in our experiments.

Several factors influence the sensitivity of the tests mentioned, but not all are completely understood. So for the LA-, BF-, as well as for the PHA-tests it has been reported that not all antisera gave satisfactory results. It was often found that complete antisera gave poor results in the LA- and BF-tests, especially when they were fresh (Bercks, 1967a). The results were sometimes better when these sera were heated or when the gl. fr. was used for sensitization. The dilution of the a.s. or gl. fr. used for sensitization

also influenced test sensitivity. The antibody-globulin concentration required for optimum results may vary between antisera; antisera with titres less than 1024 seemed unsatisfactory for BF- and PHA-tests because of the high concentrations of serum globulins necessary for optimal sensitization (Abu Salih et al., 1968a). Furthermore, the virus source and even individual source plants were found to influence sensitivity of the LA- and BF-tests (Bercks, 1967a).

Likewise, in our LA-, BF- and APP-tests often no reactions were obtained, or no or hardly any increase in sensitivity was observed as compared with the common micro-agglutination, or micro-precipitation reaction.

The negative influence of high concentrations of normal plant constituents, mainly from potato and especially with PVS on sensitivity of the methods tested can easily be read from Table 2–8. When diluting the virus with undiluted crude sap, often no reaction was observed, whereas sometimes it was in the common micro-agglutination test. Dilution of the crude sap was sometimes profitable, as can be read for the APP-test from Table 7 and 8. Supplementary experiments not mentioned here were done with the LA-, and BF-tests, in which the virus was diluted with crude sap from healthy potato plants diluted 1/4. Here sensitivity was also impeded. The frequent absence of visible reactions in undiluted crude plant extracts may, at least partly, be due to the intransparency of this sap. However, with the LA_2- and BF-tests for PVS (Table 4 and 6) and clear, centrifuged sap from potato plants, the tests were much less sensitive than when Tris buffer or NaCl solution was used as diluent. More or less the same holds for the LA_1-test with CPMV diluted with clarified sap from healthy French bean plants. When this sap is diluted 1/16, sensitivity is much better than with undiluted sap or sap diluted 1/4. Abu Salih et al. (1968a) mention crude saps should be diluted at least 1/5 for getting a visible reaction with PHA- and BF-tests. Richter (1969), using the PHA-test for strawberry latent ringspot virus in peach trees, reports that centrifugation and dilution of sap both are necessary to get positive reactions.

In my experiments, the LA-, BF-, and APP-tests were compared with the common drop test under paraffin oil. With the latter normal plant constituents also influenced sensitivity. The data can be read from Table 2–8; a review of three tests is given in Table 9. In these experiments, the dilution end point of the virus in general is four times higher when the virus was diluted with crude sap from healthy potato plants diluted 1/4, than when the virus was diluted with undiluted crude sap. However, in individual cases the difference was greater. Thus testing potato plants for virus by using crude sap diluted 1/4 is not, or only slightly, better than using undiluted crude sap. In diluting the sap the virus is diluted also. Low-speed centrifugation of the sap may be a real improvement, however.

Antiserum 444 against PVX had a higher titre in the micro-precipitin test than a.s. 416. The results in the LA_2-, and BF-tests were slightly better with a.s. 444 (Table 3 and 5). Reactions obtained with latex or bentonite sensitized with the gl. fr. from these antisera were also somewhat better than those with latex or bentonite sensitized with the complete antisera; also the higher virus concentrations were better detectable. The LA_1-test with AMV was only more sensitive than the conventional drop test when the gl. fr. was taken. The titre of the a.s. used was low (64). Both with AMV and CPMV dilution of antisera with normal serum decreased sensitivity of the LA_1-test. This may suggest that the titres of the antisera influence the sensitivity of these tests. There are other explanations, however (see below). In the APP-test, only antisera with titres of

Table 9. Influence of normal plant constituents on the sensitivity of the common drop test. Results of experiments with PVX and PVS, derived from Table 2 and 8.

Diluent used for the virus	Dilution end points of:		
	PVX	PVS	
NaCl solution	256	64	16
Undiluted crude sap from tobacco plants	16		
Undiluted centrifuged sap from tobacco plants	64	64	
Undiluted crude sap from potato plants	16	> 4	1
Crude sap from potato plants diluted 1/4	64	4	16
Undiluted centrifuged sap from potato plants	64	16	16

Tabel 9. Invloed van normale plantebestanddelen op de gevoeligheid van de gewone druppeltoets. Overzicht van resultaten van proeven met het PVX en het PVS, afgeleid van de tabellen 2 en 8.

1024 or higher gave reasonable results. These results were not better when the globulin fractions were used instead of the complete antisera.

For the sensitization of the latex and bentonite particles, three antibody concentrations were used. In the BF-test the highest concentration usually gave the best results. This especially was true when the complete antisera were used. In the LA_2-test with PVX the best results were usually obtained with the middle concentration, but with PVS differences were observed even within one and the same experiment and this depended on the diluent used for the virus. Concerning the LA_1-test, the example given in Table 1 is not representative because the lowest virus concentration is detected with a serum dilution that is 16 times higher than the titre in the test without latex. In general this optimum antiserum dilution approached the titre in the test without latex. The protein polymers in the APP-test usually were at optimum concentration at a dilution of 1/16 to 1/64. Here the fineness of the polymerization product, which was not always reproducible was another influencing factor.

Since my experiments were mainly for orientation, only a few viruses, antisera, and plant saps were tested. Thus, generalization should be avoided, but it seems clear, that when the LA-, BF-, or APP-tests are used to detect viruses in sap from potato plants, this sap has to be clarified and/or diluted. The difference I found between the LA-, BF- and APP-tests on one side, and the common micro-agglutination and micro-precipitation tests on the other side, often was small. To really profit from the LA-, BF-, and APP-tests this difference should be greater as it will be neutralized for the greater part by diluting the sap, and thus the virus. That for the LA-, and BF-tests in some cases the optimum globulin concentration was not always used for sensitization might have been the reason for this difference being so small. However, several of our sera more likely contain inhibiting components, which were not removed by isolating the gl. fr. by means of ammoniumsulphate precipitation. Recently Bercks and Querfurth (1969) reported that several of their antisera needed a further purification. They successfully separated antisera by electrophoresis and in a number of cases were thus able to increase the sensitivity of their LA-test.

Samenvatting

Enkele ervaringen met de toepassing van latex, bentoniet en gepolymeriseerde serumeiwitten in gevoelige serologische toetsmethodieken

Veel virussen komen in een dusdanig lage concentratie in plantemateriaal voor, dat ze daarin met behulp van de gebruikelijke agglutinatie-, precipitatie- of agar-geldiffusietoets niet zijn aan te tonen. Er bestaat derhalve veel interesse voor serologische methodieken, waarmee lage virusconcentraties kunnen worden aangetoond. Een aantal orienterende proeven werd uitgevoerd met de latex-agglutinatietoets (in twee uitvoeringen, aangeduid als LA_1- en LA_2-toets), de bentoniet-uitvlokkingstoets (BF-toets) en een toets met behulp van gepolymeriseerde serumeiwitten (APP-toets). De gevoeligheid van deze toetsen werd vergeleken met die van de gewone micro-agglutinatie of -precipitatietoets. De meeste aandacht werd besteed aan de aardappelvirussen X (PVX) en S (PVS).

De belangrijkste met PVX en PVS bereikte resultaten zijn weergegeven in Tabel 1–9. Geconcludeerd kan worden dat met de LA-, BF- en APP-toets onder bepaalde omstandigheden inderdaad lagere virusconcentraties kunnen worden aangetoond. Hoge concentraties normale bestanddelen, met name uit aardappelplanten, hadden echter dikwijls een negatieve invloed op de gevoeligheid, vooral met het PVS. Ook was in onverdund ruw sap dikwijls geen reactie zichtbaar, terwijl dit met de gewone agglutinatietoets wel het geval was.

De gevonden verschillen in gevoeligheid tussen de LA-, BF- en APP-toetsen en de agglutinatie- en precipitatietoetsen, waren soms zo klein, dat ze door verdunning van het sap met buffer of fysiologische zoutoplossing (om de remmende invloed van normale plantebestanddelen te beperken) weer grotendeels zouden worden teniet gedaan. Werk van andere onderzoekers doet echter veronderstellen, dat verdere verhoging van de gevoeligheid van de LA_2-toets mogelijk is door zeer bepaalde fracties uit de antisera te gebruiken.

Met de gewone agglutinatietoets kon in ruw sap bij een verdunning van 1 op 4 virus in lagere concentraties worden aangetoond dan in onverdund ruw sap. Ook hier wordt de grotere gevoeligheid weer grotendeels tenietgedaan door het verdunnen. Een duidelijke verbetering werd echter verkregen wanneer het ruwe sap werd gecentrifugeerd, d.w.z. wanneer in plaats van de agglutinatie- de precipitatietoets werd toegepast (zie Tabel 9).

References

Abu Salih, H. S., Murant, A. F. & Daft, M. J., 1968a. Comparison of the passive haemagglutination and bentonite flocculation tests for serological work with plant viruses. J. gen. Virol. 2: 155–166.

Abu Salih, H. S., Murant, A. F. & Daft, M. J., 1968b. The use of antibody-sensitized latex particles to detect plant viruses. J. gen. Virol. 3: 299–302.

Agrawal, H. & Maat, D. Z., 1964. Serological relationships among polyhedral plant viruses and production of high-titred antisera. Nature, Lond. 202: 674–675.

Avrameas, S. & Ternynck, T., 1967. Biologically active water-insoluble protein polymers. I. Their use for isolation of antigens and antibodies. J. biol. Chem. 242: 1651–1659.

Bercks, R., 1967a. Methodische Untersuchungen über den serologischen Nachweis pflanzenpatho-

gener Viren mit dem Bentonit-Flockungstest, dem Latex-Test und dem Bariumsulfat-Test. Phytopath. Z. 58: 1–17.
Bercks, R., 1967b. Über den Nachweis des Tomatenzwergbusch-Virus (tomato bushy stunt virus) in Reben. Phytopath. Z. 60: 273–277.
Bercks, R., 1967c. Über den Nachweis des Tabakmosaik-Virus in Reben. Z. PflKrankh. PflPath. PflSchutz. 74: 346–349.
Bercks, R., 1968. Über den Nachweis des Himbeerringflecken-Virus (raspberry ringspot virus) in Reben. Phytopath. Z. 62: 169–173.
Bercks, R. & Querfurth, G., 1969. Weitere methodische Untersuchungen über den Latextest zum serologischen Nachweis pflanzenpathogener Viren. Phytopath. Z. 65: 243–256.
Bozicevich, J., Scott, H. A. & Vincent, M. M., 1963. The bentonite flocculation test for detection of plant viruses and titration of antibody. Proc. Soc. exp. Biol. Med. 114: 750–754.
Kahn, R. P., Scott, H. A., Bozicevich, J. & Vincent, M. M., 1967. Detection of potato viruses X, M, and S in dormant potato tubers by the bentonite flocculation test. Phytopathology 57: 61–65.
Richter, J., 1969. Die Verwendung Glutaraldehyd-fixierter Erythrozyten für den serologischen Nachweis des latenten Erdbeerringfleckenvirus (strawberry latent ringspot virus) in Pfirsich. Acta phytopath. hung. 4: 13–18.
Scott, H. A., Kahn, R. P., Bozicevich, J. & Vincent, M. M., 1964. Detection of potato virus X in tubers by the bentonite flocculation test. Phytopathology 54: 1292–1293.