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Poplar mosaic virus: purification, antiserum preparation, and detection in poplars with the enzyme-linked immunosorbent assay (ELISA) and with infectivity tests on Nicotiana megalosiphon

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Abstract

Poplar mosaic virus (PMV) was purified from *Nicotiana megalosiphon* or *N. clevelandii* and antisera with titres from 256 to 4096 were prepared. One of these was used for the detection of PMV in poplar stools with the enzyme-linked immunosorbent assay (ELISA). Although ELISA was less sensitive than the infectivity test on *N. megalosiphon*, both tests were equally reliable when using lower leaves in July and August, and when applied to tip leaves at the end of the growing season. With both tests more infected trees were detected than with visual inspection. For PMV a particle length of 661 nm was calculated.

Introduction

As recently reviewed by Luisoni et al. (1976), poplar mosaic virus (PMV) is widespread in Europe. In the Netherlands the virus has been studied by Berg (1964). Properties and morphology of its particles (Biddle and Tinsley, 1971a; Boccardo and Milne, 1976) place PMV in the carlavirus group (Fenner, 1976), although aphid transmission is uncertain, and serological relationships with recognized members of the carlavirus group could not be detected (Biddle and Tinsley, 1971c). Electron-microscopical studies of the virus in ultra-thin sections also indicate that PMV belongs to the carlavirus group (Atkinson and Cooper, 1976; Brunt et al., 1976; Boccardo and Milne, 1976).

Little is known about the long term effect of PMV on growth and wood production of poplar. Biddle and Tinsley (1971b), however, detected a strong growth reduction with nursery trees of several cultivars.

In the Netherlands poplars are subject to inspection by the Netherlands General Inspection Service for Woody Nursery Stock (NAKB) (Elzenga, 1974). Nurseries are obliged to use inspected basic material and are only allowed to sell certified planting material derived from the basic material. Basic material is produced on isolated propagation fields which are visually inspected every year. It is essential to remove diseased plants from such fields in the earliest possible stage of infection. With many other viruses and crops, however, detection of infected plants by visual selection is not reliable. We therefore investigated if indexing by sap inoculation on herbaceous indicators, and serological tests with the ELISA method (Clark and Adams, 1977) would

be more reliable than visual inspection for PMV.

Materials and methods

Virus isolates and propagation. For antiserum production an isolate from a mosaic diseased 'Florence Biondi' poplar tree (FB3) was propagated in *Nicotiana clevelandii* or *N. megalosiphon* in an insect-proof, temperature-controlled glasshouse at about 20°C. For purification systemically infected leaves were harvested two to three weeks after inoculation.

For electron microscopy an isolate from 'Robusta' (Robusta LH) in *N. megalosiphon* was used. For other isolates: see Results.

Virus purification mainly was as with *Lonicera* latent virus (Van der Meer et al., 1980). Essential differences are indicated below.

Portions of 85 g of leaf material were homogenized in 300 ml of tris buffer, pH 9, 15 ml of carbon tetrachloride and 15 ml of chloroform. The homogenate was centrifuged at low speed (10 min at 6000 rev/min). While stirring, 20 g of solid $(NH_4)_2SO_4$ was added to each 100 ml of supernatant and stirring was continued for 15 min. The suspension was then centrifuged for 10 min at 10000 rev/min. The sediment obtained was resuspended in 75 ml of tris buffer, pH 9, stirred for 15 min and centrifuged at low speed. The supernatant was centrifuged 1.5 h at 15000 rev/min and the virus sediment obtained resuspended in 5.5 ml of tris buffer, pH 8. After stirring for 1 h the suspension was centrifuged at low speed and the resulting supernatant subjected to CsCl-gradient centrifugation, each of the six tubes of the rotor containing the virus obtained from c. 14 g of leaf material. After isolation and dialysis of virus zones present at about 17–19 mm from the bottom of the tubes, the material was centrifuged on a sucrose gradient. Virus zones obtained were either directly injected intravenously into a rabbit or concentrated to 2 ml for intramuscular injection.

Antiserum preparation was by intravenous and intramuscular injection of a rabbit. For the latter virus preparations were emulsified with equal volumes of Freund's incomplete adjuvant. No special injection schedule was followed but material was injected when available. In total the virus from c. 350 g of leaf material was administered, spread over 5 intravenous and 2 intramuscular injections, that were given in a period of 5.5 weeks.

Two antisera to PMV from Italy with titres of 512 (A 52 I) and 2048 (A 56 II) in the slide precipitin test were kindly provided by Dr E. Luisoni, Torino, Italy. An antiserum to a Dutch isolate of PVM with unknown titre (Berg, 1964) was supplied by Ir D. H. M. van Slogteren, Bulb Research Centre, Lisse.

Serological testing with the micro-precipitin test, to determine antiserum titres and for virus identification was as described by Van der Meer et al. (1980). Testing leaves, bark or shoot tips of poplar trees was with the enzyme-linked immunosorbent assay (ELISA). The methods used to prepare γ-globulin fractions and enzyme conjugates differed only in some details from those described by Clark and Adams (1977). The optimum concentrations of these reagents for routine testing in ELISA were determined after testing dilution series of both with dilution series of crude extracts from

virus-free and PMV-infected *N. clevelandii* plants.

Except for one experiment with plants from a glasshouse (Table 1), poplar material was from stools in a propagation field from which all shoots are removed every year to produce cuttings. Growth of such stools starts rather late but shoots are about 2 m long at the end of the season.

Tests were performed as described by Maat and De Bokx (1978). For routine testing poplar extracts were prepared with a power-driven crusher (De Bokx, 1972). After introducing 1–2 g of leaf material or a c. 80 mm long leafless shoot tip into the crusher, c. 1 ml of phosphate-buffered saline, pH 7.4, containing 0.05% Tween 20 and 2% polyvinylpyrrolidon (PBS + Tween + PVP) was applied semi-automatically to the crushed material on the rollers of the crusher and the sap collected for testing. In a few cases some drops of water were applied instead of the PBS + Tween + PVP and the extract was diluted with PBS + Tween + PVP. In one experiment, testing mixtures of virus-free and PMV-infected poplar leaves, 50 g of leaf material was homogenized in a Waring blender with 150 ml of PBS + Tween + PVP and the homogenate squeezed through cheese cloth.

More details about the test and the material tested are given below.

Infectivity tests were by mechanical inoculation of *N. megalosiphon*, using carborundum 500 mesh as an abrasive. Inoculum was prepared by grinding 1 g of poplar leaves in 4 cc of a 1% nicotine solution in tap water.

When ELISA and infectivity on *N. megalosiphon* were compared, pieces of one and the same leaf were used, or two successive leaves of the same shoot were alternately used in each of the tests.

All ELISA experiments and infectivity tests were done in 1978.

Electron microscopy was as with *Lonicera* latent virus, measuring particles from photographic negatives (Van der Meer et al., 1980).

Results

Antiserum titres, serological relationships and γ-globulin concentrations in ELISA. The homologous titres of the FB3 antiserum samples obtained from successive bleedings were from 256–4096, when tested just after harvest. In later tests often other values were recorded probably due to non-specific aggregation in some tests. The data presented below are from tests without evidence of non-specific precipitation (see also Van der Meer et al., 1980). The FB3 antiserum sample used in ELISA had a homologous titre of 1024 in the micro-precipitin test.

In a test performed to identify some of our PMV isolates, the titres of the two Italian PMV antisera against our type isolate FB3 were 256. Against some other, less well defined isolates (a.o. Robusta LH) titres of antiserum A 52 I varied between 64 and 256 and of antiserum A 56 II between 64 and 1024, depending on the isolate. In this test the FB3 antiserum had a homologous titre of only 64, which was much lower than in other tests. In the same test its titre to other Dutch isolates was 64 or 256. In a test performed in 1976 Berg's antiserum (Berg, 1964) had a titre of 256 to isolate Robusta LH. In recent tests the latter proved very closely related to FB3.

The concentrations of γ-globulin chosen for routine testing of poplar material were

1 µg/ml for coating and c. 1.3 µg/ml in the enzyme conjugate. ELISA was considered positive when extinction values at 405 nm were 0.15 or higher.

Symptoms on test plants. Symptoms on *N. megalosiphon* were similar to those mentioned by Schmelzer (1966), i.e. chlorotic or necrotic lesions, followed by a systemic vein necrosis. There was a close correlation between numbers of local lesions on the test plant and the extinction values in ELISA. In several comparative tests *N. megalosiphon* did not produce local lesions and showed only systemic symptoms when inoculated with material inducing extinction values below 0.15 in ELISA.

Electron microscopy. The average length of 142 particles measured was 661 nm.

Comparison of ELISA with infectivity tests on N. megalosiphon and presence of symptoms on poplar. A first experiment was done on 12 April with leaves from shoots that had been forced in a glasshouse at 20 °C. Shoots were from stools on a propagation field and known to be diseased or virus free by frequent testing and observation during 1977. No symptoms were present on leaves of these forced shoots and results with ELISA and infectivity tests were poor. Extinction values for the virus-free trees were from 0.05–0.08. Material from 11 out of 18 mosaic-diseased trees also gave extinction values of 0.08 or less. Of the seven others two were 0.10, one 0.14, and four 0.16 or

Table 1. Results of ELISA (extinction values × 100) and results of infectivity tests on *N. megalosiphon*, compared with presence of symptoms on leaves of cv. Zeeland on 9 June 1978. Leaves are numbered from base to tip (see text).

Isolate Robusta LH				Isolate FB3			
leaf	symptoms[1]	ELISA	inf. test[2]	leaf	symptoms[1]	ELISA	inf. test[2]
1	–	34	+	1–4	× × ×	79–120	+
2	?	34	+	5	× ×	92	+
3	–	14	–	6–10	×	48–97	+
4	–	11	+	11	–	1	–
5	×	66	+	12	–	6	+
6	× ×	72	+	13	–	15	–
7	× ×	74	+	14	–	2	+
8–10	× × ×	77–78	+	15	–	6	+
11	× ×	76	+	16	–	15	+
12	× × ×	87	+	17–19	–	7–9	+
13–16	×	34–66	+	20	–	2	+
17	–	2	+	21	–	1	+
18–22	–	0–2	–	22	–	2	–

[1] No symptoms present (–); mild (×), moderate (× ×), and severe (× × ×) symptoms.
[2] Infectivity test positive (+), infectivity test negative (–).

Tabel 1. Resultaten van ELISA en resultaten van toetsingen met N. megalosiphon vergeleken met aanwezigheid van symptomen op bladeren van 'Zeeland' op 9 juni 1978. Bladeren genummerd van basis naar top.

higher. With sap inoculation on *N. megalosiphon* out of these 18 infected trees 10 were detected.

Table 1 shows the results of an experiment on 9 June with leaf halves of two poplars 'Zeeland' from a glasshouse with a constant temperature of 21 °C. One contained the type isolate FB3, whereas the other contained the isolate Robusta LH. Both were infected by sap inoculation from infected *N. megalosiphon* in April 1977. After hibernating in the open, they were pruned back to soil level and put back in the glasshouse in the middle of April. Each plant consisted of one single shoot from which each leaf was tested separately. Except for the two basal leaves of the plant infected with the Robusta LH isolate with extinction values of 0.34, the symptomless leaves gave extinction values in ELISA of only 0.15 or less. From these, however, 11 reacted positively in the infectivity tests. In the tip leaves virus could not be detected at all.

Results of an experiment on 29 June with leaves from 0.7 m long shoots of two infected trees each of 'Loenen' and 'Serotina' and of two apparently healthy trees of 'Spijk' (Table 2), also indicated a much lower concentration, or possibly absence, of the virus in the tip leaves. The infected 'Loenen' and 'Serotina' trees showed faint symptoms in only a few basal leaves, and were overlooked during routine inspections in August. In this experiment too the infectivity test was more sensitive than ELISA.

In comparative tests with 167 'Gelrica' trees on 10 October ELISA showed 34 trees to be infected whereas with sap inoculation on *N. megalosiphon* 49 infected trees were found, including the 34 trees detected with ELISA. Of these 167 trees only 25 showed symptoms in July. When the tests were performed most leaves of the 2 m long shoots had already been shed. At the time of testing only four trees still had symptoms on the

Table 2. Results obtained 29 June 1978 with successive leaves from base to tip that were alternately used in ELISA and in infectivity tests. Figures represent range of extinction values (× 100) and number of infections obtained with six comparable leaves from six different shoots (see text).

Leaf pairs	'Loenen'		'Serotina'		'Spijk'	
	ELISA[1]	inf. test[2]	ELISA[1]	inf. test[2]	ELISA	inf. test[2]
1	45–145 (107)	6	56–∞ (151)	6	5	0
2	61–190 (109)	6	⩾200 (∞)	6	5	0
3	13–51 (32)	6	∞ (∞)	6	5–6	0
4	7–55 (28)	6	∞ (∞)	6	5–6	0
5	5–84 (25)	6	26–∞ (101)	6	5–6	0
6	7–16 (13)	6	8–85 (39)	6	5–8	0
7	5–8 (6)	5	7–14 (10)	6	5	0
8	5–18 (8)	2	8–24 (12)	6	4–5	0

[1] Mean value between brackets; ∞ = > 200.
[2] Number of leaves positive.

Tabel 2. Resultaten verkregen op 29 juni 1978 met opeenvolgende bladeren van de basis naar de top, afwisselend gebruikt in ELISA en in de infectietoets. Weergegeven zijn de reeksen extinctiewaarden (× 100) en de aantallen infecties die verkregen werden met zes vergelijkbare bladeren van zes verschillende scheuten.

Table 3. Extinction values in ELISA (× 100) at 405 nm of leaf samples of four poplar cultivars taken from base (B), middle (M) and tip (T) of shoots of 16 different trees at five different data in 1978.

Cultivar	29/5	20/7		10/8			7/9			10/10		
	B	B	M	B	M	T	B	M	T	B	M	T
Florence	5	5	5	40	20	6	∞	140	49	140	81	37
Biondi	–	18	140	15	26	5	∞	200	12	51	∞	60
	5	5	140	110	150	5	95	∞	8	∞	15	40
	–	9	190	∞	23	5	∞	22	8	150	11	200
	6	6	5	6	7	5	7	7	6	7	7	6
Barn	5	∞	∞	∞	∞	24	∞	∞	78	28	∞	∞
	–	–	–	∞	∞	40	200	63	∞	26	∞	∞
	6	12	43	6	5	7	7	6	7	6	–	6
	5	10	9	6	6	7	7	7	7	8	8	7
Serotina	23	170	∞	∞	26	7	∞	50	80	8	∞	∞
	35	∞	∞	∞	140	6	∞	175	8	∞	∞	110
	8	∞	∞	∞	200	11	200	∞	24	140	∞	140
	7	5	6	8	8	7	7	7	6	7	7	6
Loenen	6	∞	44	100	52	6	20	6	13	7	∞	74
	5	∞	25	∞	100	6	∞	8	6	6	7	∞
	5	6	5	6	6	5	6	6	6	6	7	7

∞ = > 200; –: not tested.

Tabel 3. Extinctiewaarden in ELISA (× 100) van bladmonsters van vier populiercultivars, op verschillende data in 1978 genomen van de basis (B), het midden (M) en de top (T) van de scheuten van 16 verschillende bomen.

leaves, 0.3 m below the tips. These were also used in the tests.

On 14 September 169 'Florence Biondi' trees were tested with ELISA. From the 65 trees that showed symptoms 61 gave a positive reaction whereas from the remaining 104 symptomless trees six gave a positive reaction. No infectivity tests were done with these 169 trees.

ELISA at successive data during 1978 with leaves of different age. Table 3 shows the results of ELISA with 16 trees from which leaves from the base, the middle and the tip of a shoot were tested at different data. Eight other trees are not mentioned because they showed extinction values between 0.05 and 0.10 during the whole season as did the last-mentioned tree of each cultivar given in this table. Except for one 'Barn' tree, all trees with extinction values higher than 0.10 reacted positively in infectivity tests. No virus was isolated from trees with extinction values below 0.10 and from the already mentioned 'Barn' tree (Table 3, line 8), which, probably due to an experimental error, showed a high extinction value only on 20 July (0.43). This tree did not show symptoms and 48 different leaves from this tree tested by ELISA on 7 September gave extinction

values between 0.06 and 0.08. The other two infected 'Barn' trees had severe symptoms during the whole summer. Two of the four infected 'Florence Biondi' trees showed mild symptoms in some leaves in the second half of the summer whereas the other two had no symptoms. The infected 'Serotina' and 'Loenen' trees showed mild symptoms in only a few basal leaves. Table 3 indicates that on 29 May with the exception of those of 'Serotina', basal leaves did not contain much virus (extinction values below 0.08). On 20 July and 10 August, however, the virus concentration in basal leaves was much higher, indicating that the virus had invaded and (or) multiplied very strongly in full-grown leaves.

Apparently because of the dense planting of the poplar stools, the first yellow basal leaves began to drop shortly after 10 August. Such yellow leaves of infected trees of ('Zeeland', 'Barn' and 'Serotina' induced extinction values between 0.34 and ∞ (> 2.00) in ELISA on 7 September. Comparable leaves from 'Brandaris', 'Florence Biondi' and 'Loenen', however, induced values between 0.06 and 0.33 only, with a mean value of 0.095. Therefore, yellow leaves were not used in any other test, and leaves mentioned under B in Table 3, taken later than 10 August were then in fact the then lowest available green leaves on the shoots. Thus at 10 October 'basal leaves' were taken from about 50 cm below the tips of the more than 2 m long shoots. When starting the tests on 29 May (Table 3) the shoots were about 15 cm long.

From Table 3 it appears also that, with exception of 'Barn', ELISA with tip leaves was only reliable on the last testing date. Tests on 10 August with base, middle and tip leaves of three infected trees each of 'Zeeland', 'Brandaris', 'Gelrica', 'Dorskamp', 'Flevo', 'Heidemij', and 'Robusta', were in agreement with this. Tip leaves of these cultivars showed very low extinction values in ELISA, whereas with base and middle leaves reactions were clearly positive. In similar tests with 'Donk' and 'Rap' on the same date, tip leaves showed extinction values between 0.37 and 1.85. However, also with these cultivars extinction values obtained with middle and basal leaves were much higher.

Detection of PMV in bark and in shoot tips. Table 4 shows the results of an ELISA experiment on 24 July with leaves from four different sites of 1 m long shoots of two infected 'Zeeland' trees, with pieces of bark from different sites and with the 10 cm long succulent tips, all of the same shoots. In all cases tip leaves gave lower extinction values than did shoot tips from which all leaves were removed. These shoot tips, however, induced much lower extinction values than did base and middle leaves. Bark tissue from 10 cm below the tips (Table 4, Bark, tip) gave higher extinction values than older bark tissue, but both induced lower values than base and middle leaves. In experiments with 'Zeeland' and several other cultivars on 1 August and 10 August, and in tests performed on 7 September and 19 October with the cultivars mentioned in Table 4, very low extinction values were obtained with shoot tips. On the last two data shoot tips were more woody and difficult to crush, which possibly influenced the results of the tests.

Erratic distribution of PMV in poplar trees. Two infected trees of 'Zeeland' had some symptomless shoots. With one tree (Table 4, tree A), only one tip leaf sample of one of the symptomless shoots 1–5 gave an extinction value of 0.20, whereas all of the other 39 samples taken from the five symptomless shoots of this tree, induced values below 0.15 with a mean value of 0.07. With two symptomless shoots of the other tree (B1 and 2)

Table 4. Results of ELISA on 24 July 1978 (extinction value × 100), with leaves from four different sites of shoots and with leafless tips and bark from the same shoots. Shoots were from two different 'Zeeland' trees (A and B). Shoots A1–5 and shoots B1 and 2 did not show symptoms.

Shoot	Leaves from				Bark			Tips
	base	mid. 1	mid. 2	tip	base	middle	tip	leafless
A1–5[1]	6	7	7	8	8	7	6	8
A6	6	∞[2]	∞	180	7	11	49	190
A7	200	∞	∞	75	9	12	17	120
B1	60	120	∞	52	11	48	∞	88
B2	120	180	∞	36	17	31	80	46
B3	∞	∞	∞	150	9	15	200	170
B4	∞	∞	∞	18	8	17	60	44
B5	∞	∞	∞	10	14	120	130	27
B6	44	∞	∞	48	34	200	64	88
B7	∞	∞	∞	33	8	180	69	130

[1] Mean values are given for these five symptomless shoots.
[2] ∞ = > 200.

Tabel 4. Resultaten van ELISA op 24 juli 1978 (extinctiewaarden × 100) met bladeren van vier verschillende plaatsen van de scheuten en met bast en 10 cm lange ontbladerde toppen van dezelfde scheuten. De scheuten waren afkomstig van twee 'Zeeland' bomen (A + B). De scheuten A1 tot 5 en B1 en 2 vertoonden geen symptomen.

Table 5. Influence on extinction values in ELISA of homogenizing diseased leaf material in different proportions mixed with healthy leaf material.

Cultivar	Extinction values at 405 nm with						
	healthy material	mixed material[1]					diseased material
		1/1000	1/250	1/100	1/50	1/10	
Florence Biondi	0.07	0.11	0.14	0.40	0.58	1.75	>2.00
	0.06	0.12	0.28	0.39	0.49	1.4	>2.00
Zeeland	0.07	0.14	0.26	0.53	0.85	>2.00	>2.00
	0.08	0.11	0.19	0.39	0.69	1.5	>2.00
Brandaris	0.06	0.07	0.45	0.93	0.60	2.00	>2.00

[1] Numerator: denominator = diseased material: healthy material.

Tabel 5. Effect van het vermalen van ziek bladmateriaal in verschillende verhoudingen gemengd met gezond materiaal, op de extinctiewaarden in ELISA.

extinction values in ELISA were as high as with symptom bearing shoots (Table 4).

Because symptoms on infected 'Florence Biondi' were mild, and often present in only some leaves, symptomless leaves situated near leaves with symptoms were tested with ELISA on 14 September. From 49 leaves 13 gave extinction values between 0.07 and 0.12, those of the remaining 36 between 0.21 and 2.00.

When using more leaves per sample, however, the erratic distribution of PMV in poplar apparently has little influence on the reliability of ELISA. Results of experiments on 14 September, in which leaves with symptoms from infected trees of three cultivars were homogenized together with leaves from healthy trees, showed that even mixtures of one part of infected leaf tissue and 99 parts of healthy leaf tissue induced sufficiently high extinction values (Table 5).

Non-specific reactions. With some of the microtitre plates non-specific reactions up to 0.20 were obtained in the outer rows of wells, especially around plate corners. For reasons of efficiency, however, and because extinction values with PMV were usually rather high, outer wells were always used and in case of doubtful results samples were retested.

When wells induced higher than maximum extinction values on the photometer (> 2.00), sometimes the next well, incubated with sap from healthy material, induced also rather high values. Retesting of such healthy material, and experiments in which intensely coloured wells were tested alternately with uncoloured wells, indicated that extinction values up to 0.40 can be caused by contamination of pipettes and the photometer cuvette, in spite of subsequent rinsing with distilled water and with part of the sample to be tested.

Discussion

Although it is not known if our PMV-isolates are identical with those of other authors, our tests show that isolates FB3 and Robusta LH are serologically closely related to those of Berg (1964) and Luisoni et al. (1976).

Like other authors (Luisoni et al., 1976; Biddle and Tinsley, 1971c) we also came across the problem of virus aggregation in purification experiments and also in serological testing. However, with the purification method described, yields were good and aggregation was negligible.

The particle length calculated for PMV-Robusta LH (661 nm) is very close to that of *Lonicera* latent virus (c. 656 nm; Van der Meer et al., 1980) and only somewhat shorter than most of the other values given in literature (626–736 nm; see Biddle and Tinsley, 1971a, 1971c; Boccardo and Milne, 1976).

Under the circumstances prevailing in a propagation field in the Netherlands in 1978, it appeared that the detectability of PMV in poplar stools with ELISA and with infectivity tests varied with the time of the year and with the age of the leaves used in the tests. Optimum conditions were similar for both tests and with each of them more infected plants were detected than with visual selection. Infectivity tests on *N. megalosiphon* were more sensitive than ELISA, apparently because with the former lower virus concentrations could be detected than with the latter (Tables 1 and 2). As to the detection of infected trees, however, both tests seem equally reliable when using the lower leaves (Tables 1 and 2). Results in Tables 3 and 4 and results on 10 August with

eight additional varieties indeed show that no diseased trees were missed when ELISA was done with basal leaves, provided that tests were not performed too early in summer (Table 3). Results obtained with 167 'Gelrica' trees on 10 October, when most leaves had dropped, show that with the infectivity test using the lowest available leaves, more infected trees were detected than with ELISA. Tip leaves of those 'Gelrica' trees might have given better results, because with two out of the four cultivars mentioned in Table 3 results on 10 October were more reliable with tip leaves than with the lowest leaves and with tip leaves all infected trees were detected with ELISA. The poor results with tip leaves on 10 August and 7 September, as compared with the much better results on 10 October, indicate a translocation of the zone with the highest virus concentration from older to younger parts. Factors which may influence rate and speed of this movement, such as initial virus concentration and multiplication in older parts, and shoot extension velocity, may vary with weather conditions. Tests in later years may elucidate this point.

Missing infected trees because of erratic virus distribution, as was observed with single leaf samples of 'Florence Biondi' and with symptomless shoots of infected 'Zeeland' (Table 4), can probably be avoided by using more leaves, or possibly parts from more leaves, per sample (Table 5).

Although bark tissue apparently contained much virus (Table 4), as was earlier demonstrated by Berg (1964), leaves were superior in this respect and are much easier to handle. Results with leafless shoot tips were unreliable. With the great majority of tests there were few unspecific reactions caused by irregularities of the microtitre plates. Experimental errors caused by contamination of pipettes and photometer cuvette, in case of extremely high extinction values, can easily be avoided in routine tests by examining such wells only visually. Also contamination via the power-driven crusher may cause experimental errors. The apparatus must be cleaned thoroughly between samples. There is no indication that such contamination occurred in our experiments.

Based on the experince of one season we conclude that both ELISA and infectivity tests on *N. megalosiphon* are very helpful in selecting PMV-free poplar trees and in detecting PMV-infected trees in propagation fields. For reasons of efficiency ELISA should be preferred, provided that tests are done in July and August with leaves of the lower part of the shoots, or with tip leaves at the end of the growing season. Considering extinction values over 0.15 positive, we could reliably distinguish between infected and healthy trees.

Samenvatting

Populieremozaïekvirus: zuivering, antiserumbereiding en detectie in populier met ELISA en met een infectietoets op Nicotiana megalosiphon

Plantgoed van populier mag in Nederland alleen verhandeld worden als het goedgekeurd is door de NAKB en afkomstig is van door de NAKB gekeurde vermeerderingsvelden. Vooral in vermeerderingsvelden is het van belang dat besmette bomen in een zo vroeg mogelijk stadium verwijderd worden. De beoordeling van populieren op aanwezigheid van populieremozaïekvirus (PMV) geschiedde tot nu toe vrijwel uitsluitend visueel. Van veel virussen is echter bekend dat besmette planten vaak geen

symptomen vertonen. Daarom werden in 1978 twee toetsmethoden vergeleken, zowel onderling als met de resultaten van visuele selectie. Daarbij bleek een infectietoets op *Nicotiana megalosiphon* gevoeliger dan een recent door Clark en Adams (1977) beschreven serologische methode (enzyme-linked immunosorbent assay: ELISA). Beide toetsmethoden zijn echter even betrouwbaar als de toetsingen worden uitgevoerd in juli en augustus met de onderste en middelste bladeren van jonge eenjarige scheuten, of in begin oktober met de topbladeren. Met beide toetsen werden belangrijk meer besmette planten gevonden dan met visuele beoordeling. Daar men niet, zoals bij de uitvoering van de infectietoets, over kasruimte hoeft te beschikken, kunnen met ELISA grote aantallen monsters per seizoen worden getoetst. De ELISA methode verdient daarom de voorkeur boven de infectietoets. Het voor ELISA benodigde antiserum werd verkregen door een konijn te injecteren met virus gezuiverd uit *N. megalosiphon* en *N. clevelandii*.

De deeltjeslengte van PMV (Robusta LH) werd berekend op 661 nm.

References

Atkinson, M. A. & Cooper, J. I., 1976. Ultrastructural changes in leaf cells of poplar naturally infected with poplar mosaic virus. Ann. appl. Biol. 83: 395–398.

Berg, Th. M., 1964. Studies on poplar mosaic virus and its relation to the host. Meded. LandbHogesch. Wageningen 64: 59 pp.

Biddle, P. G. & Tinsley, T. W., 1971a. Poplar mosaic virus in Great Britain. New Phytol. 70: 61–66.

Biddle, P. G. & Tinsley, T. W., 1971b. Some effects of poplar mosaic virus on the growth of poplar trees. New Phytol. 70: 67–75.

Biddle, P. G. & Tinsley, T. W., 1971c. Poplar mosaic virus. C.M.I./A.A.B. Descr. Pl. Viruses No. 75: 3 pp.

Boccardo, G. & Milne, R. G., 1976. Poplar mosaic virus: Electron microscopy and polyacrylamide gel analysis. Phytopath. Z. 37: 120–131.

Bokx, J. A. de, 1972. In: J. A. de Bokx (Ed.), Viruses of potatoes and seed-potato production. Pudoc, Wageningen, p. 102–110.

Brunt, A. A., Stace-Smith, R. & Leung, E., 1976. Cytological evidence supporting the inclusion of poplar mosaic virus in the carlavirus group of plant viruses. Intervirology 7: 303–308.

Clark, M. F. & Adams, A. N., 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. gen. Virol. 34: 475–483.

Elzenga, G., 1974. Reproduction and distribution of healthy plant material in the Netherlands and the tasks of the inspection services NAK-S and NAK-B in the field. Proc. 19th. int. Hort. Congr., Warszawa Vol. 1-A: 21.

Fenner, F., 1976. Classification and nomenclature of viruses. Second report of the international committee on taxonomy of viruses. Intervirology 7: 1–115.

Luisoni, E., Boccardo, G. & Milne, R. G., 1976. Purification and some properties of an Italian isolate of poplar mosaic virus. Phytopath. Z. 85: 65–73.

Maat, D. Z. & Bokx, J. A. de, 1978. Potato leafroll virus: antiserum preparation and detection in potato leaves and sprouts with the enzyme-linked immunosorbent assay (ELISA). Neth J. Pl. Path. 84: 149–156.

Meer, F. A. van der, Maat, D. Z. & Vink, J., 1980. Lonicera latent virus, a new carlavirus, serologically related to poplar mosaic virus: host range, purification, serology and inactivation in vivo by heat treatment. Neth. J. Pl. Path. 86: 69–78.

Schmelzer, K., 1966. Untersuchungen an Viren der Zier- und Wildgehölze, 5. Mitteilung: Virosen an Populus und Sambucus. Phytopath. Z. 55: 317–351.

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