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# Enzyme-linked immunosorbent assay (ELISA) for the detection of potato viruses S and M in potato tubers

J. A. DE BOKX, P. G. M. PIRON and E. COTHER[1]

Research Institute for Plant Protection (IPO), Wageningen, the Netherlands

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**Abstract**

Enzyme-linked immunosorbent assay (ELISA) with alkaline phosphatase successfully detected potato virus S (PVS) and potato virus M (PVM) in secondarily infected tubers of some Dutch potato cultivars. Extinction was higher for PVS than for PVM but values for both declined slightly within 8 weeks of lifting and it is suggested that testing be carried out within this period. Values (A405) of ELISA reactions between healthy and infected tubers were statistically significant and storage at 4° or 20 °C had no effect on detectability of the viruses.

*Additional keywords:* Serology, potato cultivars, storage.

## Introduction

Potato virus S (PVS) and potato virus M (PVM), belonging to the carlavirus group, are widespread in commercial potato stocks all over the world. Both viruses can be detected with test plants like *Chenopodium quinoa* (Kaczmarek and De Bokx, 1977; Kowalska and Waś, 1976), but their detection in certification schemes is mainly done with serological tests (agglutination or precipitin) using foliage of the plants to be tested.

These methods fulfil the major demand in certification schemes for seed potatoes. However, a low virus concentration, the possible presence of virus inactivators in plant extracts or occurrence of spontaneous flocculation may hamper their effectiveness.

To overcome these problems in testing PVS and PVM, the enzyme-linked immunosorbent assay (ELISA) developed by Van Weemen and Schurs (1972) was adapted to potato tubers.

## Material and methods

*ELISA.* A rabbit antiserum to PVS, isolated from cv. Ysselster with a titre of 2048 (micro-precipitin reaction, a titre of 2 to normal plant material) and an antiserum to PVM, isolated from cv. Bintje with a titre of 2048 (micro-precipitin reaction, a titre of 2

[1] Guest worker from April-September 1979 as a fellow of the International Agricultural Centre, Wageningen, the Netherlands, from the Agricultural Research Centre, Yanco, NSW 2703, Australia.

to normal plant material) were purified for γ-globulin as described by Clark and Adams (1977). For sensitizing (coating) plates, a dilution was applied of 1/1000 in coating buffer of the purified γ-globulin. The reagents, equipment and procedure (photometer with filter at wavelength 405 nm) used for ELISA were as described by Clark and Adams (1977) with the modifications given by Maat and De Bokx (1978a, b), by De Bokx and Maat (1979) and by De Bokx et al. (1979).

Alkaline phosphatase (Sigma, St. Louis, USA, type VII) was coupled to the purified γ-globulin with glutaraldehyde (Voller et al., 1976). A dilution of the conjugate of 1/400 was used. The enzyme substrate (*p*-nitrophenyl phosphate, Sigma 104) was added at a concentration of 0.5 mg/ml in 10% diethanolamine adjusted to pH 9.8 with HCl. The reaction at room temperature was stopped after 15 min (PVS) or 30 min (PVM) with NaOH of concentration 3 mol/l.

*Plant material.* Batches of tubers, lifted in July 1978, from field-grown plants either healthy or secondarily infected with PVS, were stored at 4°C. Their crude sap was submitted to ELISA, 49 weeks after storage. In addition, batches of tubers lifted in July 1979, from healthy and secondarily infected (PVS and PVM) field-grown plants were submitted to ELISA after storage for 2, 4, 6 and 8 weeks at 4°C or at room temperature (about 20°C). Crude sap from slices of the heel and rose ends of each of three tubers (healthy and diseased) of each cultivar was prepared with a power-driven crusher and assayed in duplicate. To check whether contamination of healthy tubers had occurred, a top eye of each healthy tuber, was planted in a greenhouse after testing. The plantlets from these eyes were found to be virus free by serological assay.

Table 1. Results of ELISA with antiserum to PVS and with PVS-infected or virus-free potato tubers. Both ends of 3 tubers tested after 49 weeks storage at 4°C. Each group includes 3 readings and, in brackets, their mean.

| Cultivar | Ranges of extinction (100E) | | | |
|---|---|---|---|---|
| | heel end | | rose end | |
| | PVS | healthy (H) | PVS | healthy (H) |
| Bintje | 160, 155, 155 (156.7) | 32, 4, 7 (14.3) | 150, 160, 165 (158.3) | 16,11, 9 (12.0) |
| Cardinal | 140, 130, 142 (137.3) | 17, 6, 6 ( 9.6) | 140, 132,162 (144.7) | 10, 6, 11 ( 9.0) |
| Climax | 115, 95, 145 (118.3) | 14, 8, 7 ( 9.6) | 147, 145, 162 (151.3) | 10, 8, 10 ( 9.3) |
| Doré | 140, 122, 117 (126.3) | 8, 7, 5 ( 6.6) | 155, 160, 162 (159.0) | 8, 9, 7 ( 8.0) |
| Eigenheimer | 160, 165, 170 (165.0) | 8, 12, 14 (11.3) | 150, 152, 155 (152.3) | 9,11, 10 (10.0) |
| Mirka | 165, 185, 195 (181.7) | 18, 20, 24 (20.6) | 175, 182, 197 (184.7) | 21, 22, 25 (20.6) |
| Prevalent | 200, 197, 200 (199.0) | 17, 13, 10 (13.3) | 200, 200, 200 (200.0) | 11, 11, 11 (11.0) |
| Sientje | 190, 155, 160 (168.3) | 12, 7, 8 ( 9.0) | 152, 190, 177 (173.0) | 9, 7, 9 ( 8.3) |
| Sirtema | 152, 197, 168 (172.3) | 15, 9, 9 (11.0) | 185, 190, 177 (184.0) | 10, 10, 10 (10.0) |
| Mean | 158.3 | 11.7 | 167.5 | 11.1 |

*Tabel 1. Resultaten van ELISA met een antiserum tegen aardappelvirus S en aardappelknollen, geïnfecteerd met S-virus of virusvrij (H). Iedere groep bestaat uit 3 waarnemingen met, tussen haakjes, het gemiddelde.*

## Results

*PVS.* (Table 1). The mean extinction for ELISA reactions to tubers infected with PVS and stored for 49 weeks at 4°C exceeded 1.5, whereas the healthy samples remained below 0.12. The difference between reactions of infected and healthy tubers could be observed in ELISA plates with the naked eye. The extinction for sap taken from the rose ends of tubers whose dormancy was broken naturally was not significantly higher than that for the heel ends (according to statistical analyses as described by Snedecor and Cochran, 1976).

High extinction was also obtained when early lifted dormant infected potato tubers were submitted to ELISA up to 8 weeks after lifting. Table 2 shows that extinction for infected material was high at all sampling times. At 2, 4 and 6 weeks after lifting, sap taken from the rose end of all cultivars showed extinction higher than 2. Again no differences were observed between the values for sap taken from the rose or heel ends of the tubers. Table 2 shows also that extinction for tubers tended to decrease during storage. Healthy tubers of some cultivars, e.g. cvs Climax, Doré, Eigenheimer and Sirtema showed a high background coloration. As it turned out, this was due to contamination in the sap-extracting device.

There was no effect of storage temperature (4° or 20°C) on the detectability of the virus.

Table 2. Results of ELISA with antiserum to PVS on potato tubers infected (PVS) or virus free (H) after lifting and stored for 2, 4, 6 and 8 weeks at 20°C. Rose ends of 3 tubers tested.

| Cultivar | Average extinction (100E) after lifting and storing (weeks) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 2 | | 4 | | 6 | | 8 | |
| | PVS | H | PVS | H | PVS | H | PVS | H |
| Bintje | >200 | 6.3 | >200 | 4.7 | >200 | 12.0 | 177.3 | 3.7 |
| Cardinal | >200 | 6.3 | >200 | 19.0 | >200 | 7.3 | 175.7 | 4.3 |
| Climax | >200 | 10.0 | >200 | 19.7 | >200 | 12.0 | 176.3 | 3.7 |
| Doré | >200 | 5.0 | >200 | 25.0 | >200 | 7.3 | 171.3 | 3.7 |
| Eersteling | >200 | 6.7 | >200 | 12.7 | >200 | 10.7 | 171 | 4.0 |
| Eigenheimer | >200 | 10.3 | >200 | 19.3 | >200 | 11.3 | 173.3 | 4.0 |
| Mirka | >200 | 4.7 | >200 | 5.3 | >200 | 7.0 | 158.7 | 4.7 |
| Prevalent | >200 | 7.3 | >200 | 6.3 | >200 | 22.3 | 158 | 4.0 |
| Sirtema | >200 | 4.3 | >200 | 19.0 | >200 | 12.7 | 159.7 | 6.3 |
| Range | >200 | 3–14 | >200 | 3–50 | >200 | 4–23 | 148–188 | 3–8 |
| Mean | >200 | 6.8 | >200 | 14.6 | >200 | 11.4 | 169 | 4.3 |

*Tabel 2. Resultaten van ELISA met een antiserum tegen aardappelvirus S en aardappelknollen, geïnfecteerd met S-virus of virusvrij (H), 2, 4, 6 en 8 weken na rooien en bewaard bij 20°C. Apicale einden van 3 knollen werden getoetst.*

Table 3. Results of ELISA with antiserum to PVM on potato tubers infected (PVM) or virus free (H) after lifting and stored for 2, 4, 6 and 8 weeks at 20 °C. Rose ends of 3 tubers tested.

| Cultivar | Average extinction (100E) after lifting and storing (weeks) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 2 | | 4 | | 6 | | 8 | |
| | PVM | H | PVM | H | PVM | H | PVM | H |
| Bintje | 41.0 | 4.7 | 36.7 | 4.7 | 32.7 | 8.7 | 15.0 | 5.7 |
| Cardinal | 25.7 | 5.0 | 37.7 | 4.7 | 30.0 | 8.3 | 17.0 | 5.7 |
| Climax | 26.7 | 5.7 | 36.3 | 5.0 | 25.7 | 10.3 | 16.7 | 5.3 |
| Désirée | 27.0 | 6.3 | 35.0 | 5.7 | 26.0 | 7.3 | 20.3 | 4.0 |
| Doré | 33.3 | 6.0 | 36.0 | 4.0 | 24.0 | 7.0 | 18,3 | 4.3 |
| Eersteling | 38.3 | 6.0 | 40.0 | 4.3 | 27.0 | 6.7 | 21.3 | 6.3 |
| Element | 18.7 | 7.3 | 15.3 | 6.0 | 29.3 | 7.7 | 16.0 | 4.3 |
| Marijke | 36.7 | 5.7 | 39.7 | 4.3 | 27.7 | 7.3 | 15.3 | 4.0 |
| Mirka | 37.7 | 13.3[1] | 39.3 | 3.7 | 25.7 | 5.7 | 13.3 | 5.7 |
| Prevalent | 33.3 | 6.3 | 32.3 | 4.3 | 25.7 | 6.7 | 19.3 | 5.0 |
| Range | 16–43 | 4–18 | 15–44 | 3–7 | 23–35 | 5–13 | 11–23 | 3–7 |
| Mean | 31.8 | 6.6 | 34.8 | 4.7 | 27.4 | 7.6 | 17.3 | 5.0 |

[1] Separate values for healthy tubers of cv. Mirka after 2 weeks are 12, 10, 18.

*Tabel 3. Resultaten van ELISA met een antiserum tegen aardappelvirus M en aardappelknollen, geïnfecteerd met M-virus of virusvrij (H), 2, 4, 6 en 8 weken na rooien en bewaard bij 20 °C. Apicale einden van 3 knollen werden getoetst.*

*PVM*. The extinction for PVM-infected tubers was much lower than for PVS-infected ones (Table 3).

The concentration of PVM in infected tubers decreased rapidly during storage (Table 3). The mean extinction of the rose ends of infected tubers dropped from 0.32 to 0.17 in 6 weeks.

The differences in mean extinction at the various sampling times were statistically significant.

The values for uninfected tubers remained constantly low during storage. An exception were the values for tubers of cv. Mirka 2 weeks after lifting. These values, higher than 0.10, overlapped with the 'diseased' range of some other cultivars. Since extinction was much lower at later dates, it is not clear whether the high values observed 2 weeks after lifting are due to a varietal effect.

As with PVS-infected tubers, the differences in extinction for heel and rose ends of PVM-infected tubers were not statistically significant. A difference in storage temperature (4 ° versus 20 °C) did not affect virus detectability (data not shown).

## Discussion

Checking secondarily infected potatoes for PVS with ELISA gives reliable results.

Extinction for infected tubers were extremely high, apparently for the whole storage period. High values were observed in tubers 49 weeks after storage as well as in early lifted potatoes that were stored for up to 8 weeks at temperatures of 4° or 20°C.

The extinction of sap samples of PVM-infected tubers was much lower than for PVS-infected material. It never exceeded 2. However the extinction for healthy tubers was very low and overlap between healthy and infected material was rare.

The difference between mean values of healthy and diseased tubers was not great at all sampling times, but diseased tubers could be detected reliably. Table 3 shows that the virus concentration in tubers decreased during storage. It is not yet clear how far the virus concentration would fall and whether it would be detectable with ELISA.

The results of experiments with potato virus $Y^N$ ($PVY^N$) and the test plant 'A6' (De Bokx, 1964) showed that the concentration of $PVY^N$ in early lifted tubers would fall below the detection limit until the tubers start to germinate. This may also come to light for other viruses in potato tubers, whatever the method of detection.

However we have shown that ELISA is a reliable method for detecting PVS and PVM in early lifted secondarily infected potato tubers for at least 8 weeks after lifting.

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**Samenvatting**

*'Enzyme-linked immunosorbent assay' (ELISA) voor het aantonen van de aardappelvirussen S en M in secundair geïnfecteerde aardappelknollen*

Aardappelvirus S kon zowel in knollen van oogst 1978, die gedurende 49 weken bij 4°C waren bewaard en waarvan de kiemrust reeds was verbroken, als in knollen van oogst 1979, die bij 4° of 20°C waren bewaard en nog in de kiemrust verkeerden, tot 8 weken na rooien betrouwbaar met ELISA worden aangetoond. Aardappelvirus M kon eveneens met ELISA betrouwbaar worden aangetoond in knollen van oogst 1979, bewaard bij 4° of 20°C, tot 8 weken na het rooien.

De extinctiewaarden voor aardappelvirus S waren hoger dan die voor aardappelvirus M. De waarden voor beide virussen vertoonden een daling gedurende de onderzoekperiode (tot 8 weken na rooien). Er kon geen effect van de bewaartemperatuur (4° en 20°C) op de aantoonbaarheid van de virussen worden aangetoond. Geen verschillen werden waargenomen tussen de extinctiewaarden van het sap uit navel- en krooneinden van de knollen, die nog in de kiemrust verkeerden.

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## Adresses

J. A. de Bokx and P. G. M. Piron: Instituut voor Plantenziektenkundig Onderzoek, Postbus 42, 6700 AA Wageningen, the Netherlands.
E. Cother: Agricultural Research Centre, Yanco, NSW 2703, Australia.