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Detection of potato virus M and potato virus S on test plants

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Summary

The influence of environment and virus isolate on PVM detection on eight plant species and PVS detection on six plants species was investigated. *Lycopersicon chilense* was the most reliable test plant for PVM and *Chenopodium quinoa* for PVS. In both cases 12–24 days were required for the symptoms to appear. The most rapid development of PVM symptoms resulted from inoculation on *Phaseolus vulgaris* Red Kidney (4–8 days) and of PVS symptoms on detached leaves of *Solanum demissum* Y (6–8 days). The detectability of the viruses on these test plants was, however, lower than on *L. chilense* and *C. quinoa*.

Introduction

Serological methods are usually used for the detection of potato virus M (PVM) and potato virus S (PVS). They are not always reliable and occasionally test plants must be employed. Many plant species showing distinct symptoms caused by PVM and PVS have been described (Bagnall et al., 1956; Bode, 1966; de Bokx, 1970; Chrzanowska, 1969; Chrzanowska & Waś, 1974; Dziewońska & Ostrowska, 1973; Hiruki, 1970; Horváth, 1971; 1972a; Horváth & de Bokx, 1972; Kaczmarek, 1971; Kowalska, 1973; Ross, 1968; Rozendaal & van Slogteren, 1958; Vulič & Hunnius, 1967).

In this paper the reaction of eight plant species to PVM and of six plant species to PVS is described. The plants were grown in different environmental conditions and inoculated with different isolates of these viruses.

Materials and methods

The following plant species were investigated: *Chenopodium quinoa* Willd., *Cucumis sativus* L., *Datura metel* L., *Gomphrena globosa* L., *Lycopersicon chilense* Dun., *Nicotiana debneyi* Domin., *Phaseolus vulgaris* L. cv. Red Kidney, *Solanum rostratum* Dunal. and *S. stoloniferum* Schlechtd. et Bché EBS 2630 (detached leaves) for PVM, and *C. quinoa*, *G. globosa*, *L. chilense*, *S. rostratum* and *S. demissum* Lindl. Y (SdY) (detached leaves) for PVS.

Different Polish and foreign varieties and breeding lines of potato infected with PVM or PVS were used as a source of virus isolates.

Experiments were conducted in an aphid-free greenhouse or in temperature-controlled chambers. Average temperature in the greenhouse fluctuated between 18 °C (winter) and 23 °C (summer). The highest temperatures were 25 °C and 38 °C, respectively. From October to March there was additional illumination for 6–12 hours per day. In the three temperature-controlled chambers the temperature was constant: 16 °C, 22 °C and 28 °C, respectively. Plants in the chambers were artifically illuminated for 12 hours per day.

Plants of most species were inoculated at the 3 to 7 leaf stage. *P. vulgaris* plants were inoculated at the well expanded primary leaves stage and *C. sativus* plants at the cotyledon stage. Detached leaves of SdY and *S. stoloniferum* plants, grown in the greenhouse, were incubated after inoculation in glass-covered trays, in a room with continuous illumination, at 20–24 °C.

For inoculation, carborundum-dusted leaves of the test plants were rubbed with crushed leaves of infected potato plants (dry inoculation) and rinsed with tap water. At least five plants or detached leaves were inoculated with each virus or virus isolate in each test. Five uninoculated plants or detached leaves served as control. The reaction of the test plants was evaluated up to six weeks, and of the detached leaves up to 10 days after inoculation. In some cases plants were tested serologically six weeks after inoculation for presence of the virus.

Results

Symptoms on test plants

Symptoms which appeared on test plants inoculated with PVM and PVS are described in Table 1. In this table the differences in reaction to different virus isolates are also indicated. In the experiments described below the reaction was evaluated as positive only when distinct symptoms appeared on the test plants.

Effect of temperature on symptom development

The test plants were grown in the greenhouse and after inoculation incubated in the three temperature-controlled chambers. They were inoculated with one PVM isolate (from potato variety Uran) and one PVS isolate (from potato variety Leona). The results of this experiment are presented in Table 2.

Only two plant species, *L. chilense* and *S. rostratum*, developed distinct symptoms at all the three temperatures when inoculated with PVM. A further four species, *C. quinoa*, *C. sativus*, *D. metel* and *P. vulgaris* cv. Red Kidney, produced no symptoms at 28 °C, and on *G. globosa* symptoms appeared only at 22 °C. Thus in most cases higher temperatures proved to be unsuitable for PVM detection. A different reaction was obtained with *S. rostratum* inoculated with PVM. Local and systemic symptoms were produced in a shorter time, and systemic symptoms were more distinct (Fig. 1), on this plant, at 22 °C and 28 °C than at 16 °C.

PVS produced symptoms at all three temperatures on three plant species: *C. quinoa*, *L. chilense* and *S. rostratum*. On *C. quinoa* symptoms caused by PVS developed in the shortest time and they were the most pronounced at 28 °C.

Table 1. Reactions of test plants inoculated with PVM and PVS.

Plant species[1]	Local symptoms[2]		Systemic symptoms[3]	
	type of symptoms[4]	number of days after inoculation[5]	type of symptoms[4]	number of days after inoculation[5]
PVM				
Chenopodium quinoa	GrSp or YSp*	11–24	0	
Cucumis sativus	CSp	13–28	0	
Datura metel	CSp, NSp**	7–18	CSp, NSp, BrMy, Stu, Dis**, ***	19–40
Gomphrena globosa	RSp, CSp, NSp**	14–27	0	
Lycopersicon chilense	0		Ep, Dis, Stu, LeAb***	12–22
Nicotiana debneyi	NSp**	14	0	
Phaseolus vulgaris cv. Red Kidney	NSp or NSp, NRi*	4– 8	0	
Solanum rostratum	NSp	11–19	NSp, Vn, Dis, NSt, LeAb, Stu***	11–23
S. stoloniferum EBS 2630[x]	NSp**	4	–	
PVS				
Chenopodium quinoa	CSp, NSp	12–24	0	
Gomphrena globosa	RSp, CSp, NSp**	16–27	0	
Lycopersicon chilense	0		Ep, LeAb**, ***	15–34
Nicotiana debneyi	0		Vc, M, NSp***	22–40
Solanum demissum Y[x]	NRi	6– 8	–	
S. rostratum	0		NSp, LeAb***	19–37

Ab = Abscission – *Blattfall – Abscission;* Br = Brown – *Braun – Brun;* C = Chlorotic – *Chlorotisch – Chlorotique;* Dis = Distortion – *Verdrehung – Déformation;* Ep = Epinasty – *Epinastie – Epinastie;* Gr = Green – *Grün – Vert;* Le = Leaves – *Blätter – Feuilles;* M = Mosaic – *Mosaik – Mosaïque;* My = Midvein – *Mittelrippe – Nervure centrale;* N = Necrotic – *Nekrotisch – Nécrotique;* R = Red – *Rot – Rouge;* Ri = Rings – *Ringe – Anneaux;* Sp = Spots – *Flecken – Taches;* St = Stem – *Stengel – Tige;* Stu = Stunt – *Stauche – Arrêt de croissance;* Vc = Vein clearing – *Nervenaufhellung – Eclaircissement des nervures;* Vn = Vein necrosis – *Nervennekrosen – Nécrose des nervures;* Y = Yellow – *Gelb – Jaune;* 0 = No symptoms – *Keine Symptome – Pas de symptôme;* [x] = Detached leaves – *abgeschnittene Blätter – Feuilles détachées*

* Type of symptoms varied with virus isolate – *Die Art der Symptome änderte sich in Abhängigkeit vom Virusisolat – Le type de symptômes variait avec le virus isolé.*

** Symptoms were produced only by some virus isolates – *Die Symptome wurden nur von einigen Virusisolaten hervorgerufen – Les symptômes étaient seulement occasionnés par quelques virus isolés.*

*** Intensity of symptoms varied with virus isolate – *Die Intensität der Symptome änderte sich in Abhängigkeit vom Virusisolat – L'intensité des symptômes variait avec le virus isolé.*

[1] *Pflanzenart – Espèces de plante;* [2] *Lokale Symptome – Symptômes localisés;* [3] *Systemische Symptome – Symptomes généralisés;* [4] *Art der Symptome – Type de symptômes;* [5] *Zahl der Tage nach der Inokulation – Nombre de jours suivant l'inoculation*

Tabelle 1. Reaktionen der Testpflanzen nach Inokulation mit PVM und PVS.
Tableau 1. Réactions des plantes-tests inoculées avec les virus M et S.

Table 2. The effects of temperature on the time of appearance of PVM and PVS symptoms on different test plants (in days).

Plant species[1]	Local symptoms[2]			Systemic symptoms[3]		
	16 °C	22 °C	28 °C	16 °C	22 °C	28 °C
PVM						
C. quinoa	14	12	0	0	0	0
C. sativus	19	19	0	0	0	0
D. metel	16	16	0	25	40	0
G. globosa	0	18	0	0	0	0
L. chilense	0	0	0	18	14	19
P. vulgaris cv. Red Kidney	5	5	0	0	0	0
S. rostratum	19	11	11	23	14	14
PVS						
C. quinoa	23	16	12	0	0	0
L. chilense	0	0	0	34	25	34
N. debneyi	0	0	0	30	30	0
S. rostratum	0	0	0	37	25	37

[1–3] *Siehe Tabelle 1 – Voir tableau 1*

Tabelle 2. Einfluss der Temperatur auf die Zeit des Auftretens von Symptomen von PVM und PVS bei verschiedenen Testpflanzen (in Tagen).
Tableau 2. Effet de la température sur le temps d'apparition des symptômes des virus M et S sur différentes plantes-tests (en jours).

Effect of virus isolate and time of testing on symptom development

The reaction of different plant species inoculated with twelve PVM isolates and eight PVS isolates was investigated. The experiments were conducted in the greenhouse in the summer and in the winter.

The efficiency of PVM detection on most plant species was higher during winter than during summer tests (Table 3). Only two species, *L. chilense* and *S. rostratum*, showed distinct symptoms in both summer and winter independently of the virus isolate used. On *C. quinoa*, *C. sativus* and *P. vulgaris* cv. Red Kidney all PVM isolates produced symptoms, but only in winter tests. In summer conditions two PVM isolates did not produce symptoms on *C. quinoa* and none produced symptoms on *C. sativus* and *P. vulgaris* cv. Red Kidney. On *D. metel* and on the detached leaves of *S. stoloniferum* EBS 2630 symptoms were produced only by some PVM isolates. Both these plants reacted better in winter than in summer tests. Seven PVM isolates produced symptoms on *G. globosa* and one produced symptoms on *N. debneyi* independently of time of testing.

Experiments conducted with PVS isolates revealed that there was no clear influence of the time of testing on the virus detectability. On *C. quinoa* all PVS isolates produced symptoms in summer as well as in winter. All PVS isolates were detected on *S.*

Fig. 1. *Solanum rostratum* 23 days after inoculation with PVM incubated at 16 °C (left) and at 28 °C (right).

Fig. 1. Solanum rostratum 23 Tage nach der Inokulation mit PVM, inokuliert bei 16 °C (links) und 28 °C (rechts).
Fig. 1. Solanum rostratum 23 jours après l'inoculation de virus M, à 16 °C (à gauche) et à 28 °C (à droite).

rostratum only in summer tests and on *N. debneyi* only in winter tests. *L. chilense* was infected with all PVS isolates independently of the time of year (as revealed in serological tests) but distinct symptoms were produced on this test plant only by some isolates. In each period of testing PVS isolates occasionally produced necrotic rings on the detached leaves of SdY plants. The presence of symptoms on SdY leaves was not connected with the properties of a particular virus isolate.

Effectivness of PVM and PVS detection on some most suitable test plants
Some plant species which appeared, from the foregoing experiments, to be most suitable for PVM or PVS detection, were used for testing potato plants in which the presence of PVM or PVS was expected. Two hundred tests were conducted at different times of the year. The results showed that the greatest number of positive reactions was obtained on *L. chilense* and *C. quinoa* for PVM and on *C. quinoa* for PVS (Table 4).

Discussion

Our experiments confirmed observations of other workers (Bagnall et al., 1959; Chrzanowska, 1969; Ross, 1968; Rozendaal & van Slogteren, 1958) that on *G.*

Table 3. Number of PVM and PVS isolates detected on different plant species in tests conducted in summer and winter.

Plant species[1]	Number of isolates causing symptoms[2]			
	PVM (out of 12 tested)[3]		PVS (out of 8 tested)[4]	
	winter tests[5]	summer tests[6]	winter tests[5]	summer tests[6]
C. quinoa	12	10	8	8
C. sativus	12	0	–	–
D. metel	8	2	–	–
G. globosa	7	7	1	1
L. chilense	12	12	3	5
N. debneyi	1	1	8	6
P. vulgaris cv. Red Kidney	12	0	–	–
*S. demissum Y**	–	–	6	5
S. rostratum	12	12	6	8
S. stoloniferum EBS 2630*	10	2	–	–

* Detached leaves – *Abgeschnittene Blätter – Feuilles détachées.*
– Not tested – *Nicht geprüft – Non testées.*

[1] *Pflanzenart – Espèces de plante;* [2] *Zahl der Isolate, die Symptome hervorrufen – Nombre d'isolats occasionnant des symptômes;* [3] *PVM (von 12 getesteten)-Virus M (sur 12 tests);* [4] *PVS (von 8 getesteten) – Virus S (sur 8 tests);* [5] *Wintertest – Tests d'hiver;* [6] *Sommertest – Tests d'été*

Tabelle 3. Zahl von PVM – und PVS-Isolaten, die auf verschiedenen Pflanzenarten bei Testungen im Winter und im Sommer gefunden wurden.
Tableau 3. Nombre d'isolats de virus M et de virus S détectés sur différentes espèces de plante dans des tests réalisés en été et en hiver.

Table 4. Percentage of potato plants which were found to be infected with PVM or PVS using the most efficient test plants.

Plant species[1]	PVM	PVS
C. quinoa	84	97
L. chilense	88	–
P. vulgaris cv. Red Kidney	70	–
S. rostratum	75	81
S. demissum Y*	–	31

* Detached leaves – *Abgeschnittene Blätter – Feuilles détachées.*
– Not tested – *Nicht geprüft – Non testées*

[1] *Pflanzenart – Espèce de plante*

Tabelle 4. Prozentsatz von Kartoffelpflanzen, die bei Verwendung der am besten geeigneten Testpflanzen mit PVM oder PVS infiziert waren.
Tableau 4. Pourcentage de plantes de pomme de terre infectées par les virus M et S en utilisant les plantes-tests les plus efficientes.

globosa, *D. metel*, *N. debneyi* and *S. stoloniferum* EBS 2630 symptoms are caused only by some PVM isolates. These plant species are therefore not suitable for PVM detection in potato plants infected with unknown virus strains. The most reliable test plant for PVM detection was *L. chilense*. High reliability of tests performed on this test plant was also shown by Lodochkin et al. (1974). Unfortunately rather a long time is required for PVM symptoms to appear. The test plant which produced symptoms in the shortest time after inoculation with PVM, independently of the virus isolate used, was *P. vulgaris* cv. Red Kidney, but in our experiments local lesions appeared on this test plant only in some environmental conditions (Table 2 and 3). Hiruki (1973) and Hiruki et al. (1974) proved that the appearance and number of local lesions caused by PVM on *P. vulgaris* cv. Red Kidney depended on many factors such as age of test plants and the use of an appropriate buffer. It therefore may be possible to increase the reliability of PVM detection even in less favourable environmental conditions.

In our experiments *C. quinoa* was the most reliable test plant for PVS detection. In the studies by Hinostroza-Orihuela (1973) only some PVS isolates produced symptoms on *C. quinoa*, and de Bokx (1970) obtained plants showing symptoms, independently of virus isolate, only from one out of four *C. quinoa* seed smaples tested. On the *C. quinoa* plants used in our experiments all PVS isolates tested produced distinct symptoms, and, as found by Gooth & Webb (1972) these symptoms developed in the shortest time after inoculation at a high temperature (28 °C). Nevertheless even in these conditions a rather long period of time was required for symptoms to develop. The most rapid development of symptoms after inoculation was given by using detached leaves of SdY. Until now no PVS isolate has been found which would not produce symptoms on SdY (Chrzanowska & Waś, 1974; Kowalska, unpublished). The fact that some PVS isolates were not detected in the experiments described in this paper (Table 3) was not connected with their properties but rather with a relatively low frequency of SdY leaves producing symptoms when inoculated with PVS. It is likely that the efficiency of SdY as a test plant for PVS could be improved by finding more suitable test conditions.

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Zusammenfassung

Nachweis von Kartoffel-M- und -S-Virus mit Testpflanzen

An 8 bzw. 6 Pflanzenarten wurde die Reaktion auf eine Inokulation mit dem Kartoffel-M-virus (PVM) bzw. dem Kartoffel-S-virus (PVS) untersucht. Die Symptome sind in Tabelle 1 beschrieben.

Untersuchungen, die in 3 temperaturkonstanten Räumen (16, 22 und 28 °C) durchgeführt wurden, ergaben, dass unabhängig von der Temperatur nach Inokulation mit PVM auf *Lycopersicon chilense* und *Solanum rostratum* Symptome auf-

traten, nach Inokulation mit PVS auf *Chenopodium quinoa*, *L. chilense* und *S. rostratum* (Tabelle 2).

Tests, die im Winter und im Sommer mit 12 Isolaten des PVM und 8 Isolaten des PVS durchgeführt wurden, ergaben, dass sich auf *L. chilense* und *S. rostratum* für PVM und auf *C. quinoa* für PVS unabhängig von der Jahreszeit und vom Virusisolat Symptome bildeten (Tabelle 3). Die am besten geeigneten Testpflanzen waren *L. chilense* für PVM und *C. quinoa* für PVS. Insgesamt wurden 200 Versuche zu verschiedenen Zeiten des Jahres durchgeführt (Tabelle 4). Jedoch, die vielleicht am besten geeignete Testpflanze für PVM ist *Phaseolus vulgaris* Red Kidney und für PVS sind es abgeschnittene Blätter von *Solanum demissum* Y, da die Symptome bereits wenige Tage nach der Inokulation mit allen getesteten Virusisolaten auftreten. Die Schwierigkeit bei der Verwendung dieser Testpflanzen ergibt sich aus der sehr starken Abhängigkeit der Symptomausbildung von den Versuchsbedingungen.

Résumé

Détection du virus M et du virus S de la pomme de terre sur des plantes-tests

La réaction au virus M et au virus S, respectivement de 8 et 6 espèces de plante, a été étudiée. Les symptômes qui sont apparus sur les plantes-tests, inoculées avec le virus M et avec le virus S, sont décrits dans le tableau 1.

Des expériences conduites dans trois chambres à température contrôlée (16, 22 et 28 °C) ont montré, qu'indépendamment de la température, le virus M produisait des symptômes sur *Lycopersicon chilense* et *Solanum rostratum*, et le virus S sur *Chenopodium quinoa*, *L. chilense* et *S. rostratum* (tableau 2).

Des tests réalisés en hiver et en été avec 12 isolats de virus M et 8 isolats de virus S ont montré que les plantes-tests qui manifestaient chaque fois des symptômes, indépendamment de l'isolat utilisé, étaient *L. chilense* et *S. rostratum* pour le virus M, et *C. quinoa* pour le virus S (tableau 3).

Deux cents tests réalisés sur la pomme de terre à différentes époques de l'année ont montré que *L. chilense* pour le virus M et *C. quinoa* pour le virus S étaient les plantes-tests les plus sûres (tableau 4).

Toutefois, la plante-test la plus prometteuse est peut-être pour la détection du virus M, *Phaseolus vulgaris* Red Kidney; et pour la détection du virus S, les feuilles de *Solanum demissum* Y, car les symptômes étaient apparus quelques jours seulement après l'inoculation, quel que soit l'isolat testé. La difficulté d'utiliser ces plantes-tests réside dans le fait que le développement des symptômes est hautement dépendant des condition d'environnement.

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