Detection of potato virus Y in primarily infected mature plants by ELISA, indicator host, and visual indexing

R.P. Singh[1] and J. Santos-Rojas[2]

Potato virus Y was detected in greenhouse- and field-grown seven-week-old potato plants by local lesion host ***Solanum demissum*** P.I. 230579 and by ELISA tests. The virus was detected in 3-4 weeks by mechanical inoculation of local lesion hosts in greenhouse-grownplants and in 4-5 weeks in field-grown plants. However, PVY was detected in these same plants by ELISA one week later. Virus concentration varied in plants inoculated at different leaf positions, reached a peak, then slowly declined. Smaller tubers (less than 30 g, and 30-60 g) from primarily infected field-grown plants gave rise to 'symptomless' plants, which were not diagnosed by visual observation, while they were diagnosed to be PVY infected by ELISA tests. Also, visual observation detected fewer plants as PVY infected from stem end than from bud end pieces of the tubers.

Can. Plant Dis. Surv. 63:2, 39-44, 1983.

Le virus Y de la pomme de terre (PVY) a été détecté chez des plants de pomme de terre semés en serre et au champ et agés de sept semaines, à l'aide de la plante indicatrice ***Solanum demissum*** P.I. 230579 et du test ELISA. L'inoculation mécanique de la plante indicatrice a permis la detection du virus en 3-4 semaines chez les plants de serre et en 4-5 semaines chez les plants de champ toutefois, le test ELISA n'a permis la detection du virus qu'une semaine plus tard chez les même plants. La concentration de virus varie chez les plants inoculbs sur des feuilles diffbrentes, atteint un sommet, puis diminue lentement. Les plus petits tubercules (moins de 30 g et 30 à 60 g.), provenant de plants en champ à infection primaire, ont donne naissance à des plants "sans symptômes" diagnostiqués comme non-infectés par observation visuelle alors que les tests ELISA les ont identifies comme étant porteurs de virus. Lorsque le talon des tubercules est utilisé comme semence la technique d'observation visuelle détecte moins de plants infectés avec PVY que lorsque la couronne des tubercules est utilisée.

Introduction

For large-scale testing of potatoes for virus infection, 'Florida-test' is often used in North America. In this test whole tubers are planted after treatment with dormancy-breaking chemicals. The Florida-test has been useful in detecting mosaics caused by potato virus Y (PVY) in tubers which are even partially infected, because viruses transport within tubers when tubers have been planted and the eyes have developed into stems **(2).** However, the enzyme-linked immunosorbent assay (ELISA) (3) is a laboratory test and has been considered for large-scale testing of potato viruses (4, 5, 7, **8, 10, 12),** in which mostly dormant tubers will be tested, which may not detect partially infected tubers.

The studies dealing with potatoes have used a mixture of primarily and secondary infected (5) or only secondary infected (8) plants. Thus, there is no information available regarding the detection of PVY from mature plants primarily infected, and also whether such late infections result in tubers with partial infection. The aim of the present study was to establish conditions for detecting PVY infection, late in the growing season, compare with indicator host and ELISA, and to determine the extent of partial infection in tubers. For this purpose, the most widely grown North American cultivar, Russet Burbank, was used and inoculations were made to different leaf positions and in late July to mimic the early flights of green peach aphid (*Myzus persicae* (Sulzer)) in New Brunswick.

Materials and methods

Potato (Solanum *tuberosum* L.) cv. 'Russet Burbank', which is widely grown in the Maritimes was obtained as a virus-indexed tuber from the Fredericton Research Station program of Dr. A.R. McKenzie, and propagated under field and greenhouse conditions. Greenhouse and field experiments were similar in most details. In the greenhouse, plants were grown in **18** cm pots and were maintained at **18-22°C** with 14-hour day length. In the field experiments potato plants were selected at random from several rows.

A common strain of potato virus Y (PVY) in Red Pontiac potatoes was used for inoculation. Five plants were mechanically inoculated (1/20 dilution of potato sap) on the bottom leaf, 5 on the middle leaf (half-way to the plant) and 5 on the top leaf. Similarly, 3 plants were inoculated with buffer alone (one at each leaf position) in the same field and used as healthy controls. In addition, 3 plants infected with PVY were maintained under field conditions to serve as infected controls. The inoculation was made on July **22,** 7 weeks post planting and leaf samples were collected every week for 8 or 9 weeks, up to the topkilling dates in the field. Ten leaflets were taken at random from each plant and two sets of discs

[1] *Research Scientist, Agriculture Canada Research Station, P.O. Box 20280, Fredericton, New Brunswick, E3B 4 2 7, Canada.*

[2] *Visiting Scientist from Instituto de Investigaciones Agropecuarias (I.N.I.A.), Casilla 11 10, Osorno, Chile.*

Accepted for publication January 26, 1983

were punched through the leaflets using a 0.5 cm cork borer. One set of discs was ground in glycine-phosphate buffer (w/v 1:20) (0.05 M glycine + 0.03 M K_2HPO_4, pH 9.2) and used to inoculate S. ***demissum*** plants (13), while the other set of discs was tested by the **ELISA** procedure.

From field-grown plants, the tubers from each plant of each leaf position group, were put in separate bags and divided into different groups according to their weights. The weight groups were, weights of less than 30 g, 30-60 g, 60-90 g, 90-120 g, and 120 g and over. From each tuber both bud and stem end were planted separately in order to determine which of these parts were infected. This was done by visual observation of symptoms in the greenhouse, and by testing of the leaves by **ELISA.**

S. demissum test plants were used when they had **6-8** leaves and inoculations were made on each half-leaf with separate samples. **All** samples were inoculated on 5 right and on 5 left half-leaves. The local lesions were averaged from 10 leaf-halves for each sample for each date.

The **ELISA** procedure was similar to that used in an earlier study (10), except that the enzyme γ globulin conjugate was incubated at 5°C rather than 25°C. The antisera used in this study were a gift from the International Potato Center, Lima, Peru by Dr. C. Fribourg and locally produced against several PVY isolates. The A405 nm values were measured by Titertek Multiskan (Flow Laboratory, Mississauga, Ontario, Canada).

Results

The isolates of PVY, mainly differing in host reactions and virus yields (unpublisheddata) were initially tested by **ELISA. A Saco** PVY was characterized by necrotic local lesions in **Saco** potato and milder leaf-drop symptoms in ***Physalis*** species; while a common PVY was characterized by necrotic local lesions in **Saco** and severe leaf-drop in ***Physalis*** species. **All** these isolates caused similar necrotic local lesions in ***S. demissum.*** The PVY isolates were tested against an antiserum prepared in Peru, and their homologous antisera. There was no indication of strain specificity or interference in **ELISA** tests. **A** typical reaction of Red Pontiac isolate of PVY is shown in Fig. 1**A.** The virus concentration based on A405 nm values was higher in tobacco plants than in potato plants, although both were significantly different than the healthy or buffer controls (Fig. 1A). Similarly, with local lesion host S. ***demissum*** P.I. 230579, tobacco had higher virus concentration than potato (1B) and this bioassay was able to detect PVY up to a sap dilution of 320 fold (1B). In addition, 75 mosaic samples collected from different parts of New Brunswick were tested against antisera from Peru; these all reacted positively. Thus it appears that the main strain of PVY in New Brunswick is a common strain and one antiserum can detect most of the infections from the field.

Detection of PVY from greenhouse- and field-grown plants

The results (Table 1) from greenhouse-grown plants show that PVY was detected in 4 out of 15 plants within 3 weeks of inoculation by ***S. demissum*** but not by **ELISA.** However, by the fourth week virus was detected in all the plants by ***S. demissum*** test as compared to 13 of the 15 by **ELISA.** The plants with the bottom leaves inoculated had a low detection rate throughout the experiment, while those inoculated at top leaves had high virus detection rate.

The detection of PVY by **ELISA** procedure in the ninth week after inoculation was unreliable because only 5 of 15 plants were found to be positive, although by the ***S. demissum*** test

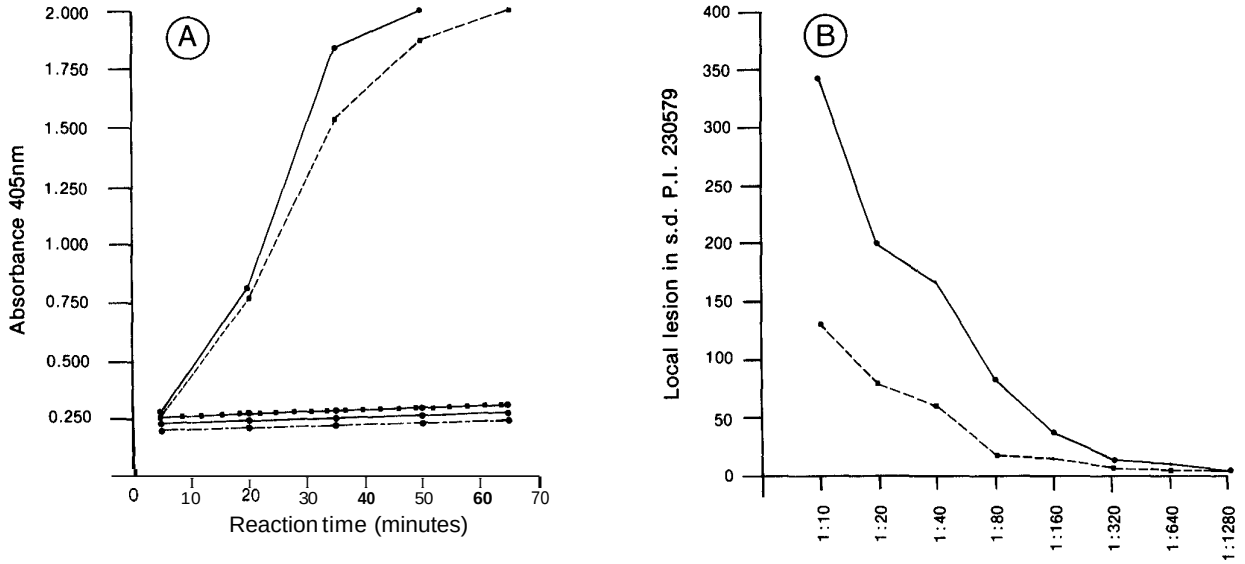

Figure 1A Absorbance values (A405 nm) for potato virus Y from tobacco (●—●—●), and from potato (-■- -■-) using γ globulins from Peru; Lower part, healthy tobacco (—■—■—), healthy potato (—●—●—) and buffer (-●— — —●-)

Figure 1B Bioassay of PVY using *Solanum* demissum P.I. 230579 from tobacco (—●—●—) and from potato (-■- -■-) at various sap dilutions.

Table 1. Detection of PVY in Russet Burbank potatoes grown in the greenhouse and inoculated seven weeks post planting.

Treatment	Plant No.	Time in weeks: Local lesion assay 3	4	5	6	7	8	9	ELISA* 3	4	5	6	7	8	9
PVY top leaf	1	+	+	+	+	+	+	+	–	+	+	+	+	+	–
	2	–	+	+	+	+	+	+	–	+	+	+	+	+	+
	3	–	+	+	+	+	+	+	–	+	+	+	+	+	–
	4	–	+	+	+	+	+	+	–	+	+	+	+	+	+
	5	–	+	+	+	+	+	+	–	+	+	+	+	+	+
PVY middle leaf	1	+	+	+	+	+	+	+	–	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	–	+	+	+	+	+	–
	3	–	+	+	+	+	+	+	–	+	+	+	+	+	+
	4	–	+	+	+	+	+	+	–	+	+	+	+	+	–
	5	–	+	+	+	+	+	+	–	+	+	+	+	–	–
PVY bottom leaf	1	–	+	+	+	+	+	–	–	+	–	+	+	–	–
	2	–	+	+	–		+	+	+	–	–	+	+	+	+	–
	3	–	+	+	+	+	+	+	–	+	+	+	+	+	–
	4	–	+	+	–	+	+	+	–	–	+	+	+	+	–
	5	+	+	+	+	+	+	+	–	+	+	+	+	–	–
Healthy control	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	4	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	5	–	–	–	–	–	–	–	–	–	–	–	–	–	–
PVY tobacco		+	+	+	+	+	+	+	+	+	+	+	+	+	+
Healthy tobacco		–	–	–	–	–	–	–	–	–	–	–	–	–	–

* The A405 nm value twice or more than the healthy control was used to classify + PVY; each sample was replicated 4 times in the plate.

14 of the 15 were found to be positive (Table 1). In the field-grown plants (Table 2), the virus detection was delayed by 2 weeks. The earliest PVY detected by the S. demissum test was in the fifth week, while by the ELISA procedure was in the sixth week. Contrary to greenhouse-grown plants, in field-grown plants the virus infection from leaves inoculated at various positions did not differ significantly. There was more variation in virus detection from the field-grown plants than those from the greenhouse. All of the plants were found to be positive by ELISA in the eighth week whereas only 12 of 15 were detected by the S. *demissum* test (Table 2).

In the greenhouse- and field-grown plants the inoculated leaves from those plants which were inoculated at bottom and mid-points became chlorotic then necrotic and dropped off, while in those plants inoculated on the top leaves, no leaf-drop occurred. The symptoms, mottle and various degrees of mosaic in the new growth, were obvious in greenhouse-grown plants, but were seldom observed under field conditions.

The concentration of virus based on A405 nm value increased sharply in both greenhouse- and field-grown plants and then declined (Fig. 2A,B). The highest values were obtained in 4 weeks and 6-7 weeks in greenhouse- and field-grown plants, respectively. The local lesion assay followed closely the same pattern (Fig. 2C) in the greenhouse-grown plants, but the number of lesions fluctuated with inoculum obtained from the field-grown plants (2D).

Detection of PVY infection by visual-indexing and ELISA

The infections of tubers in the groups classified according to the weights of the tubers showed that smaller tubers (less than 30 g, and 30-60 g) either showed partial infection or

Table 2. Detection of PVY in Russet Burbank pototoes grown in the field and inoculated seven weeks post planting.

Treatment	Plant No.	Time in weeks: Local lesion assay 3	4	5	6	7	8	ELISA* 3	4	5	6	7	8
PVY top leaf	1	–	–	–	+	+	+	–	–	–	+	+	+
	2	–	–	–	+	+	+	–	–	–	–	+	+
	3	–	–	–	+	+	+	–	–	–	+	+	+
	4	–	–	+	+	+	+	–	–	–	–	+	+
	5	–	–	+	+	+	+	–	–	–	–	+	+
PVY middle leaf	1			+	+	+	+	–	–	+	+	+	+
	2	–	–	–	+	+	+	–	–	–	+	+	+
	3	–	–	+	+	+	+	–	–	–	+	+	+
	4	–	–	–	+	+	+	–	–	–	+	+	+
	5	–	–	+	+	+	–	–	+	–	+	–	+
PVY bottom leaf	1	–	–	+	+	–	–	–	–	–	–	–	+
	2	–	–	+	+	+	+	–	–	–	+	+	+
	3	–	–	+	+	+	+	–	–	–	+	+	+
	4	–	–	+	+	+	+	–	+	–	+	+	+
	5	–	–	+	+	+	+	–	–	–	+	–	+
PVY potato control	1	+	+	+	+	+	+	+	+	+	+	–	+
	2	+	+	+	+	+	+	+	+	+	+	–	+
	3	+	+	+	+	+	+	+	+	+	+	+	+
Healthy potato control	1	–	–	–	–	–	–	–	–	–	–	–	–
	2	–	–	–	–	–	–	–	–	–	–	–	–
	3	–	–	–	–	–	–	–	–	–	–	–	–
PVY tobacco		+	+	+	+	+	+	+	+	+	+	+	+
Healthy tobacco		–	–	–	–	–	–	–	–	–	–	–	–

* The A405 nm value twice or more than the healthy control was used to classify + PVY; each sample was replicated 4 times in the plate.

failed to show clear mosaic symptoms in the greenhouse (Table 3). Of the 99 tubers tested, only 78 and 85 were diagnosed as mosaic showing by visual indexing from stem and bud ends, respectively. However, when tested by the ELISA method, all plants, irrespective of weights or tuber ends, were found positive for PVY. The potato plants which failed to show clear mosaic symptoms were rechecked by 'ELISA KIT' (Boehringer-Mannheim) and by local lesion assay. All samples caused local lesion in S. *demissum* but they were very low (average from 10 leaves, 5 to 25), but reacted positively in 'ELISA KIT' (A405 values 0.299 to 1.987). There was no clearcut correlation with lesion number and ELISA values.

Discussion

From this study it is clear that diagnosis of infected plant either in the field, from late infection or by planting smaller tubers for visual indexing in the greenhouse, can be misleading. In earlier studies in Europe (1) small tubers were found to have lower percentage of infection; however, in the present study, they were infected, but failed to show clear symptoms. This may be due to different cultivar used and also longer period of time between inoculation and harvest of the tubers. Since both stem and bud ends of each tuber, irrespective of weight or position of leaf inoculated on the plant, yielded tubers which were positive for PVY by ELISA, this procedure can be relied upon to detect most of the infection in routine laboratory testing.

Since there is a marked preference in the field for top and bottom leaves of potato by M. *persicae* (6) and our mechanical inoculation tests from the field experiments show almost similar infection rate on bottom and top inoculated

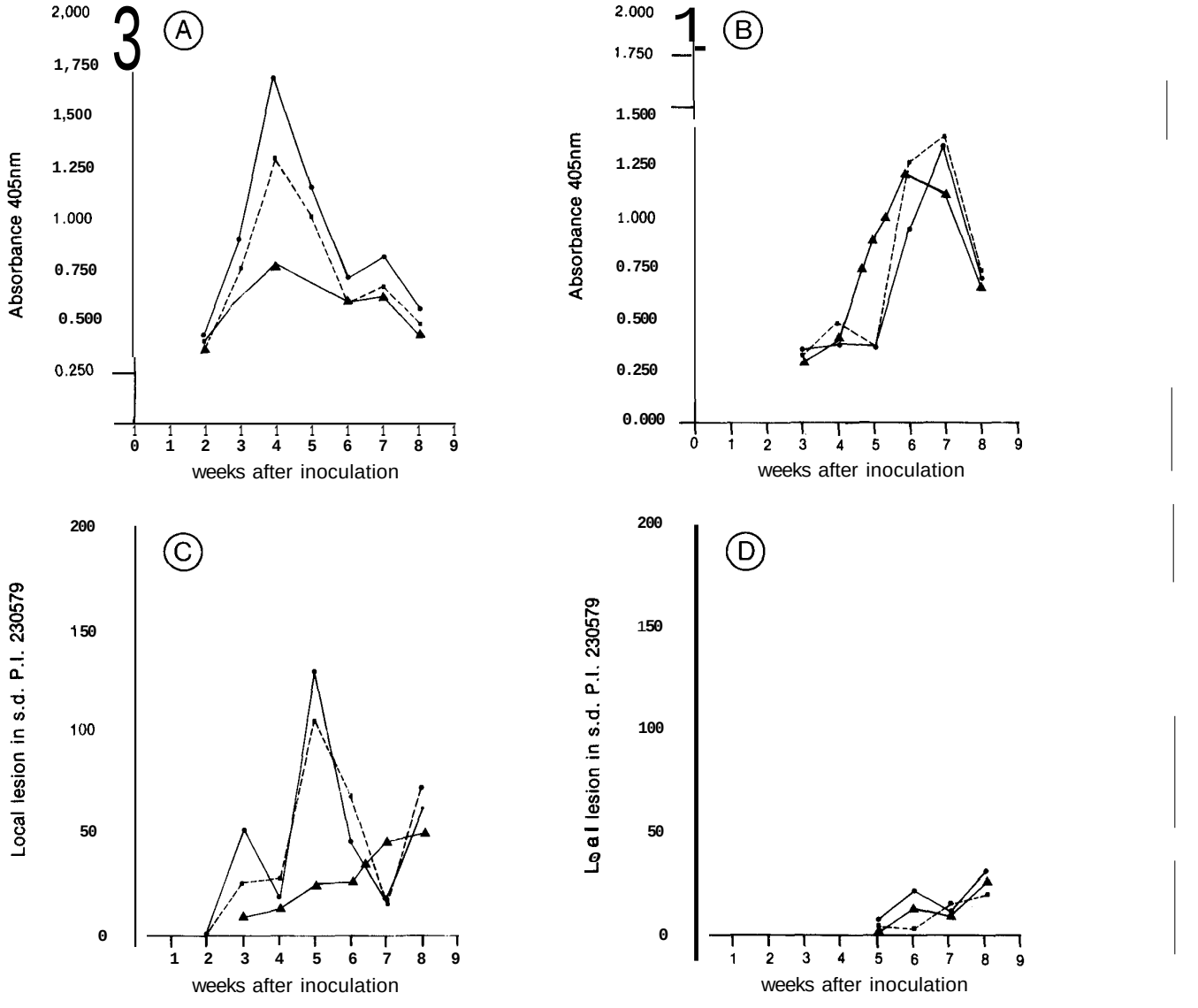

Figure 2A Absorbance values (A405) from greenhouse-grown PVY infected plants, inoculated at various leaf positions, top leaf (-●-●-), middle leaf (- ■ - - ■ -), and bottom leaf (-▲-▲-).

Figure 2B Similar to 2A, except field-grown plants.

Figure 2C Bioassay of PVY using *Solanum demissum* P.I. 230579 from greenhouse-grown PVY infected plants, inoculated at various leaf positions, to leaf (-●-●-), middle leaf (- ■ - - ■ -), and bottom leaf (-▲-▲-).

Figure 2D Similar to 2C, except field-grown plants.

plants, the finding that such plants infected at mature stage can be detected by ELISA is of significance.

The observation that PVY was detected sooner by bioassay using *S. demissum* P.I. 230579 than ELISA indicates that infectivity assay in this host is more sensitive than ELISA. This is not surprising because the ELISA test has been shown to be less sensitive than bioassay with certain other host-virus combinations (9,11). However, ELISA was reported to be superior than A6 test for PVY in another study (12).

The greater ease of PVY detection and higher concentration of the virus in greenhouse-grown plants may be the reflection of the optimum environmental conditions, favoring the virus multiplication rather than any intrinsic properties of plants or the methods used. It is similar to those of German potato variety Leo, studied in the greenhouse (8), where a peak virus concentration was reached, then it declined slowly.

Table 3. Detection of PVY infection from stem and bud end of Russet Burbank pototo tubers after primary infection under field conditions.

Treatment	Weight grams	Number of tubers tested	Visual-Symptoms positive		ELISA positives	
			Stem end	Bud end	Stem end	Bud end
PVY top leaf	less than 30	3	1	2	3	3
	30-60	5	3	3	5	5
	60-90	5	4	4	5	5
	90-120	1	1	1	1	1
	over 120	18	16	16	18	18
PVY middle leaf	less than 30	2	0	2	2	2
	30-60	2	2	2	2	2
	60-90	8	7	6	8	8
	90-120	3	3	3	3	3
	over 120	18	18	18	18	18
PVY bottom leaf	less than 30	2	0	2	2	2
	30-60	3	0	0	3	3
	60-90	2	2	2	2	2
	90-120	8	5	6	8	8
	over 120	19	16	18	19	19
Total		99	78	85	99	99

Acknowledgement

J. Santos-Rojas would like to acknowledge the award of associateship given by the International Development Research Center (I.D.R.C.), Canada.

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