Evaluation of polyacrylamide gel electrophoresis, bioassay and dot-blot methods for the survey of potato spindle tuber viroid

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Three methods (e.g. polyacrylamide gel electrophoresis (PAGE), bioassay using ***Solanum berthaultiiand*** dot-blot) were compared for the detection of potato spindle tuber viroid (PSTV). Composite samples ranging in dilution from 1:3 to 1:500 (infected discs:healthy discs) were used. Extracted nucleic acid was used for all the tests. PAGE could not detect PSTV beyond 1:10 dilution whereas both bioassay and dot-blot detected PSTV reliably up to 1:300 dilution. Using bioassy and dot-blot, PSTV was surveyed in 103 tablestock fields of potato. Tablestock fields of potato cultivars Kennebec, Russet Burbank, Shepody and Superior planted with seed grade Elite II to Certified were surveyed. No PSTV was found in any of the 618 composite samples tested, which represented 51,500 leaves. Freedom of the seed potato crop from PSTV was thus confirmed by laboratory tests.

Can. Plant Dis. Surv. 65:2, 61-63, 1985.

On a comparé la capacité de detection du viroide de la filosité de la pomme de terre (PSTV) à l'aide de trois techniques, l'électrophorèse au gel de polyacrylamide (PAGE), l'essai biologique avec ***Solanum berthaultii*** et la tache-point. en utilisant des Bchantillons composes dont la dilution varie de 1:3 à l:500 (disques infectés: disques sains). Tous les tests ont utilisé des extraits d'acide nucléique. PAGE n'a pas permis la detection du PSTV dilué au-deld de 1:10, tandis que l'essai biologique et la tache-point l'ont détecté sans erreur jusqu'à une dilution de 1:300. La presence du PSTV fut inventorié dans 103 champs de pommes de terre de table en utilisant les techniques de l'essai biologique et de la tache-point. Cet inventaire couvre des champs de pommes de terre de table des cultivars Kennebec, Russet Burbank, Shepody et Superior plantés avec de la semence de catégorie Elite II d Certifiée. Aucun PSTV ne fut détecté dans les 618 échantillons composés représentant 51,500 feuilles. Les tests en laboratoire ont ainsi permis de confirmer l'absence du PSTV des pommes de terre de semence.

Introduction

Although potato spindle tuber viroid (PSTV) was first observed on the North American continent in the early 1920s (1), it is not an economic problem now (6,8). However, in order to ensure that the potato seed crop is free of PSTV, it is necessary to monitor the crop during the growing season. Visual indexing has been used for a long time and has been successful in reducing the PSTV incidence in the seed crop (8). However, availability of additional methods of PSTV detection make it necessary to determine their applicability for monitoring of large acreages of potato fields for PSTV. Therefore, the method of polyacrylamide gel electrophoresis (PAGE) (2,4,5), bioassay using *Solanum berthaultii* (7), and nucleic acid hybridization (3) were compared for large-scale testing. The latter two methods were also used to survey the potato crop for PSTV in New Brunswick.

Materials and Methods

Viroids and Detection Procedures — In order to obtain field-grown potato plants with current-year PSTV symptoms, as an aid for visual indexing, the following was done. Virus and viroid-free tubers of several cultivars were obtained from the Potato Breeding Program of the Fredicton Resarch Station. Single-eyed tuber pieces were planted in peatmoss and when the plants were 10-12 cm in height, they were inoculated with PSTV containing nucleic acid extract (5,7). One day after the inoculation, plants were transplanted into the field (May 20, 1984). Plants were observed for symptoms every two weeks. As a comparison, viroid-free plants grown from the same tubers were planted in the adjoining plot.

For the comparison of test methods, nucleic acid was extracted as described previously (4,7). One PSTV-infected leaf disc (7 mm) was mixed with 2,4,9,49,99,249,299,399 and 499 leaf discs of viroid-free potato. For nucleic acid extraction, leaf to extracting buffer ratio of 1:3 (w/v) was always used. The extracted nucleic acid was dissolved in distilled water. Three μl of each nucleic acid sample was applied to the dot-blot membrane (Agdia, Inc., Mishawake, Indiana, U.S.A.); 250 μl of the nucleic acid was diluted equally with glycine phosphate buffer and used to inoculate S. *berthaultii* seedlings (7); and another 100 μl of the nucleic acid extract was used for the PAGE test (4,5).

Survey of Potato Fields for Potato Spindle Tuber Viroid — One hundred and three potato fields, selected at random were used for the survey of PSTV. All the fields were planted for processing or tablestock purposes. Because processing or tablestock fields are the farthest removed potatoes from nucleus virus-free stocks, under the Seed Potato Certification Program, it was expected that if there is any PSTV present, it should be more easily found in tablestock fields than in seed fields, which are inspected thoroughly every year. Once fields were selected, the planting date, cultivar, source and grade of seed used for planting were recorded. The survey field was

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Accepted for publication April 16, 1985

Fig 1. The field symptoms of potato spindle tuber viroid in cultivars. Kennebec, Russet Burbank, and Shepody from experimental plot. Upright growth, reduced upper leaves, and bushy appearance of plant is evident in all cultivars.

Table 1. Comparison of potato spindle tuber viroid detection in composite leaf samples of potato by various methods.

Dilution Ratio	Test	Methods: PAGE* 1 2 3	Bioassay** 1 2 3	Dot-blot† 1 2 3
1:3		+ + +	+ + +	+ + +
1:5		+ + +	+ + +	+ + +
1:10		+ + +	+ + +	+ + +
1:50		+ – –	+ + +	+ + +
1:100		NT	+ + +	+ + +
1:250		NT	+ + +	+ + +
1:300		NT	+ – +	+ + ?
1:400		NT	– – +	+ – –
1:500		NT	– – –	– – –

*Polyacrylamide gel electrophoresis.
**Bioassay on *Solanum* berthaultii plants.
†Nucleic acid hybridization, using Agdia Inc. kit.
NT = Not tested.

observed by two to four persons, each covering a large cross-section of the field and collecting leaflets at random. A total of 500 leaflets (in lots of 100) were collected. In case of suspicious looking plants, additional samples were collected. All leaflet samples were kept cold in a Styrofoam box containing ice. On arrival at the laboratory, discs were cut (7 mm) and nucleic acid was extracted as described (7). For each field, one PSTV control was used. A PSTV control consisted of one PSTV-infected disc mixed with 99 viroid-free discs of same potato cultivar. Extracted nucleic acid was used for bioassy and dot-blot tests.

Results

Field Symptoms of Current Year Infection by PSTV — Because PSTV is very rare in potato-growing areas, the procedure of growing plants in the greenhouse and their transplanting into the field soon after inoculation was the nearest thing to a natural PSTV infection in the field. This procedure provided close to 100% infection in 29 potato cultivars inoculated in this way (10 plants in each cultivar). All inoculated plants developed moderate to severe symptoms within six weeks of transplanting. The symptoms of three cultivars used in the survey are shown in Fig. 1. Availability of PSTV symptomatic cultivars, particularly the newly released Shepody (9), was of assistance in visual inspection of fields to be surveyed.

Comparison of Detection Procedures — Three experiments were done with field-grown leaf material using PAGE, bioassay and dot-blot tests as shown in Table 1. PSTV was detected from potato leaves by PAGE up to a dilution of 1:5 to 1:10 but not beyond. Both bioassay on S. berthaultii and dot-blot nucleic acid hybridization detected PSTV up to a dilution of 1:300. Although PSTV was detected at higher dilutions with both bioassay and dot-blot (Table 1) the detection was not consistent. Therefore, for survey purposes, a dilution of 1:100 was selected.

Table 2. Survey and detection of potato spindle tuber viroid in potato fields planted for tablestock use.

Potato Cultivar	Seed Grade Planted	No. of Fields	Detection of PSTV	
			Bioassay*	Dot-blot**
Kennebec	E II	1	0/5+	0/5
Kennebec	E III	1	015	0/5
Kennebec	F	4	0120	0120
Kennebec	C	2	0110	0/10
PSTV controls++	–	8	8/8	818
Russet Burbank	E III	20	0/100	0/100
Russet Burbank	F	27	01135	0/135
Russet Burbank	C	6	0130	0/30
PSTV controls	–	53	53/53	50153
Shepody	E III	6	0130	0/30
Shepody	F	9	0/45	0/45
Shepody	C	8	0140	0/40
PSTV controls	–	23	23/23	21/23
Superior	E II	1	015	0/5
Superior	E III	3	0/15	0115
Superior	F	8	0/40	0/40
Superior	C	7	0135	0135
PSTV controls	–	19	18/19	19119

*Bioassay on Solanum *berthaultii* plants.

**Nucleic acid hybridization, using Agdia Inc. kits.

+From each field 5 samples consisting of 100 leaflets each were used to extract nucleic acid.

++For each field there was one PSTV control included, consisting of 1 PSTV infected leaflet combined with 99 healthy leaflets of the same cultivar.

Survey of PSTV in Potato Fields – A total of 103 potato fields were surveyed. The four major cultivars and their respective number of fields surveyed were: Russet Burbank 53, Shepody 23, Superior 19, and Kennebec 8. A total of 51,500 leaflets were collected and 618 nucleic acid extractions were made. The seed grades planted ranged form Elite 112 fields; Elite III, 30 fields; Foundation, 48 fields; and Certified, 23 fields. No PSTV was found in any field either by bioassay or by dot-blot test (Table 2) irrespective of cultivar or seed grade used for planting.

Discussion

In an earlier study (8) we showed that visual inspection data indicated that PSTV was not present in seed potato fields since 1980. This study further extends that observation to the tablestock fields, as well as by the sensitive detection procedures of bioassay and dot-blot (Table 2). The probable reasons for the absence of PSTV in seed potato fields have been discussed before (8) and may apply to tablestock fields as well.

The procedures of dot-blot and bioassay both are more sensitive than PAGE. However, the bioassay requires large greenhouse space for testing, while the dot-blot as performed by a commercial agency (Agdia Inc.) is quite inexpensive. The problem of membrane dispatching and minimizing delays by mail could further improve the dot-blot's usefulness.

Acknowledgements

The authors thank seed potato inspectors, Food Production and Inspection Branch, Agriculture Canada, for their assistance in location and selection of fields; Miss G.F. Pittoello, and Miss K. Jaswal for their excellent technical assistance.

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