Incidence of Potato Virus X in Foundation and Certified Seed of Seven Cultivars

S. M. TAVANTZIS, Assistant Professor, and S. G. SOUTHARD, Research Assistant, Department of Botany and Plant Pathology, University of Maine, Orono 04469

ABSTRACT

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Differences in levels of infection of potato virus X (PVX) between foundation and commercial seed of seven cultivars were determined by testing 4,315 tubers with enzyme-linked immunosorbent assay. Atlantic, BelRus, Katahdin, Kennebec, Ontario, Russet Burbank, and Superior were the cultivars included in the study. Commercial seed of all cultivars except Kennebec had a significantly ($P = 0.05$) higher percent infection and titer than those of foundation seed. Kennebec was the only cultivar for which virus incidence or titer did not increase when grown commercially. Incidence of PVX in foundation seed of cultivar Ontario, which was freed from the virus by heat treatment and meristem-tip culture, remained as low as 1% for 7 yr after treatment. Some growers were able to maintain percent infection and titer of PVX as low as that of the foundation seed released to them even after 3 yr or more of propagation. In contrast, seed of the same cultivar suffered high levels of PVX contamination when grown by other growers operating at the same location.

Potato virus X (PVX), one of the most frequently found viruses in commercial stocks of potato, *Solanum tuberosum* L. (7,10,17,18), reduces yield (6,8–11,20) and specific gravity of tubers (4). In most cultivars, infection of PVX is latent, without foliage symptoms or apparent effect on plant vigor (10,12,17). Thus, detection of PVX has been based on various serological procedures or on infectivity assay (2,9,21). Enzyme-linked immunosorbent assay (ELISA) is particularly useful because of its high sensitivity, specificity, and suitability for large-scale testing (5,13,15). ELISA has been used successfully for detecting several potato viruses (5,13–15) including PVX (3). Detection of PVX in foundation seed in Maine has been based on the microprecipitin test and infectivity assay using *Gomphrena globosa* as a local-lesion host. ELISA was employed to monitor PVX in nuclear stock for the first time in 1982. Consequently, results presented in this paper were obtained from plant material that had been indexed by the previous two methods.

The objectives of this study were to determine the incidence of plants infected with PVX in seven cultivars of foundation and commercial seed potatoes, the rate of reinfection of foundation seed when grown commercially, and the practicality of maintaining seed free of PVX. The cultivars included in our study, Atlantic, BelRus, Katahdin, Kennebec, Ontario, Russet Burbank, and Superior, represent 90% of the total seed potato acreage in Maine.

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MATERIALS AND METHODS

Plant material. Incidence and titer of PVX in foundation seed were compared with those in commercial seed (ie, generation 1, generation 2, and certified). The term "nuclear" is used to indicate plants during the year they are selected and pathogen-tested. Subsequent increases are designated "elite foundation I, elite foundation II," etc. Nuclear and foundation seed are grown on a farm operated by the Maine Seed Potato Board. Commercial seed is propagated on seed potato farms operated by individual growers. The terms "generation 1, generation 2," and "certified seed" refer to classes of seed potatoes increased for 1, 2, or 3 yr, respectively, beyond the foundation stage. In Maine, certified class seed may be reentered for certification. Nuclear seed of cultivar Ontario that had been freed from PVX by heat treatment and meristem-tip culture was obtained from British Columbia, Canada, in 1975. Attempts to eliminate PVX from nuclear seed of the other six cultivars used in this study were limited to roguing of plants in which PVX was detected by the microprecipitin test or infectivity assay.

Tubers from the cultivars Atlantic, BelRus, Katahdin, Kennebec, Ontario, Russet Burbank, and Superior were obtained immediately after harvest and stored at 6 C until testing. Tubers from cultivar Saco, which is known to be immune to PVX (10), were used as healthy controls in each test. Known PVX-infected tubers of the cultivar Green Mountain were used as diseased controls.

Sample preparation and ELISA procedure. ELISA was performed as described by Clark and Adams (1). Test samples consisted of tuber extracts. Wedges of 1 g of tissue were cut from the apical end of each tuber and sap was extracted by pressing the tissue in a mechanical sap extractor. One part sap was mixed with nine parts extraction buffer (0.02 M phosphate, 0.15 M NaCl, pH 7.4, containing 0.05% Tween 20, 0.1% 2-mercaptoethanol, and 2% polyvinyl pyrrolidone, mol wt 10,000) and the mixture was clarified by centrifugation (7,000 *g*, 5 min). Samples were tested in duplicate wells. Buffer, healthy, and known PVX-infected controls were included in each microplate. Absorbance readings ($A_{405\,nm}$) were quantified using a Microelisa Reader (Model 590, Dynatech Laboratories, Inc., Alexandria, VA). Statistical analysis was conducted by analysis of variance and means were compared by Duncan's multiple range test. A reaction was considered positive when the mean absorbance of a sample exceeded the mean absorbance plus two standard deviations of healthy controls.

To determine whether PVX titer could be quantified reliably in tuber extracts, the following experiment was carried out: PVX, purified as described previously (16), was diluted in extraction buffer or clarified healthy potato extracts and absorbance (average values of three wells per dilution) was plotted against known virus concentration (dilution).

To minimize sampling error, the following precautions were taken: PVX titers of samples only within the same potato cultivar were compared. All tubers used were obtained immediately after harvest and stored in a single cold room. The apical end of the tuber was consistently used for ELISA testing. In addition, the PVX antigen present was relatively homogenous because the common latent strain of PVX is known to be prevalent in commercial seed lots in Maine (F. E. Manzer, *personal communication*), whereas most of the mutants of PVX causing visible symptoms are eliminated in field inspections by roguing the virus-infected plants.

RESULTS

Effect of tuber tissue extracts on virus detectability. PVX was detectable in extraction buffer at concentrations as low as 15 ng/ml (Fig. 1). When PVX was mixed with clarified healthy tuber extracts, absorbance values ($A_{405\,nm}$ values) were significantly lower ($P = 0.05$) than those obtained with the corresponding concentrations of virus diluted in extraction buffer. Reactions with clarified extracts containing 31–4,000

ng/ml of virus were significantly positive ($P = 0.05$), however, and absorbance increased as virus concentration increased. Results similar to those of Figure 1 were obtained with tuber extracts from different potato cultivars. The mean $A_{405\,nm}$ value obtained with healthy extracts was 0.03.

Virus incidence in foundation and commercial seed potatoes. In commercial seed samples, which included generations 1 and 2 and certified seed, percent virus infection ranged from 23.1 (Kennebec) to 85.9 (Atlantic). Atlantic, BelRus, Russet Burbank, and Superior were heavily contaminated with PVX, whereas virus incidence was significantly lower in Katahdin and Kennebec (Table 1). In samples of foundation seed, percent virus infection ranged from 5.7 (Katahdin) to 53.8 (Superior). Because foundation seed was obtained from a single source (ie, a single sample), the means (for percent infection) were not analyzed for significant differences between cultivars or between foundation and commercial seed. It is apparent, however, that there was a dramatic increase in incidence of PVX between foundation and commercial seed. Kennebec was the only cultivar for which PVX occurrence did not increase when grown commercially (Table 1). Incidence in cultivar Ontario is presented but not compared with the other cultivars because Ontario commercial seed, in most cases, was obtained from sources outside of Maine.

In all cultivars except Kennebec, commercial seed had PVX titers significantly ($P = 0.05$) higher than those of foundation seed (Table 1). Again, Kennebec was the only cultivar that showed no increase in virus content after release of foundation seed to commercial seed growers. The mean $A_{405\,nm}$ values presented in Table 1 reflect the mean of all tubers tested. The PVX level appeared to be very low in foundation seed, the highest mean $A_{405\,nm}$ value being 0.22, whereas the range was much broader in commercial seed (0.16–0.89). The ability of PVX to reach high concentrations within individual tubers of a given cultivar was directly proportional to the amount of recontamination of the foundation seed when grown commercially. For example, BelRus seed, which had the highest degree of reinfection, contained numerous individual tubers with relatively large amounts of PVX ($A_{405\,nm}$ 1.20–3.23). In contrast, Kennebec seed had no individual tubers in the above category.

There was also considerable variation with regard to percent infection and virus titer within cultivars, ie, among individual seed potato growers. Results obtained with certified seed of cultivar Katahdin are shown in Table 2 as a typical example. The range for percent infection varied from 1.4 to 78.0. Mean $A_{405\,nm}$ values ranged from 0.10 to 0.78. These differences in the increase in percent infection and virus content took place during the 3 yr or more of seed propagation after release of foundation seed. The vast majority of commercial seed grown in Maine is of the certified class, with the generation 1 and 2 classes representing a small percentage of the total acreage. This, in conjunction with the fact that the samples we used were obtained randomly, resulted in the collection of a relatively small number of generation 1 and 2 samples. Thus, statistical comparisons of PVX incidence and titer among the different classes of certified seed were not made. The "grower" factor, however, was far more important than the number of years since the seed had been removed from the foundation stock.

DISCUSSION

Incidence and titer of PVX varied in foundation seed of the seven cultivars. Katahdin contained the lowest percent infection (Table 1). Although percent infection appeared high in some cultivars, virus titer was low (Table 1). According to a gross estimation of virus titer obtained from the graph of Figure 1, the highest mean $A_{405\,nm}$ value (0.22), observed in foundation seed of Russet Burbank and Superior, corresponds to a mean concentration of PVX of about 250 ng/ml. A large percentage of individual tubers contained significantly lower PVX levels than the above mean value.

Because the sensitivity of ELISA is higher than that of indexing methods used in the past, it is believed that using ELISA to monitor PVX or other latent potato viruses will improve the quality of foundation seed in Maine and elsewhere.

When Atlantic was released in 1976, it was reported immune to PVX (19). Commercial seed of Atlantic, however, had a PVX incidence of 85.9%, the highest among the seven cultivars tested (Table 1). There was a considerable increase in percent infection and virus titer when foundation seed was released

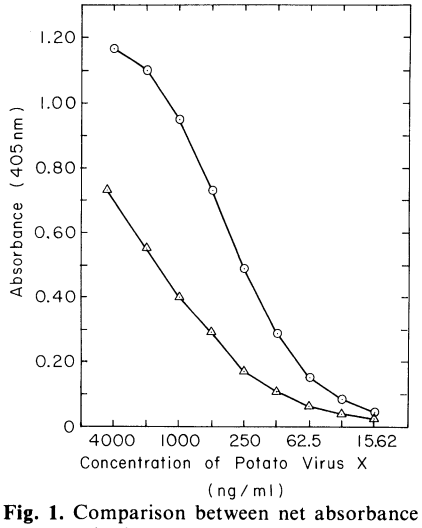

Fig. 1. Comparison between net absorbance ($A_{405\,nm}$ units) in enzyme-linked immunosorbent assay (ELISA) and concentration of potato virus X. Purified virus was diluted in PBS-T-PVP buffer containing 0.1% mercaptoethanol (o—o) or clarified tuber extract of cultivar Saco (△—△). Three wells were used for each sample and the mean of their net $A_{405\,nm}$ values was calculated after substracting the mean buffer or healthy control absorbance values.

Table 1. Incidence of potato virus X in foundation seed stock and commercial seed of seven cultivars

	Foundation seed			Commercial seed		
Cultivar	No. tubers tested	Percent infection[x]	Absorbance (405-nm units)	No. tubers tested	Percent infection	Absorbance[y] (405-nm units)
Atlantic	151	37.1	0.18	127	85.9 b	0.43*
BelRus	207	13.5	0.12	363	79.4 b	0.89*
Katahdin	247	5.7	0.08	959	36.6 a	0.27*
Kennebec	153	25.5	0.15	305	23.1 a	0.16
Ontario[z]	243	1.2	0.04	166	61.2	0.26
Russet Burbank	152	48.0	0.22	378	80.1 b	0.70*
Superior	212	53.8	0.22	652	63.9 b	0.38*

[x] A sample was considered positive when mean absorbance (405-nm units) was higher than the mean of the healthy control plus two standard deviations. Means followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test. Calculations for mean percent infection were based on percent infection for individual growers. Because foundation seed was obtained from a single source, the means (for percent infection) of the cultivars were not analyzed for significant differences between cultivars.

[y] Within a cultivar, means followed by an asterisk (*) are significantly different ($P = 0.05$).

[z] Cultivar Ontario was not compared with the other cultivars because Ontario commercial seed in most cases was obtained from sources outside of Maine.

Table 2. Comparison of virus content or percent infection of PVX in certified seed potatoes of cultivar Katahdin grown on nine commercial farms

Grower	No. tubers tested	Percent infection	Absorbance (405-nm units)
1	36	78.0	0.78
2	103	66.9	0.52
3	41	61.0	0.58
4	41	43.9	0.22
5	40	40.0	0.19
6	68	7.7	0.16
7	59	7.1	0.13
8	116	6.9	0.13
9	91	1.4	0.10

for propagation on commercial farms (Table 1). BelRus, Atlantic, and Russet Burbank were the least resistant to PVX reinfection. In contrast, Kennebec appeared to be highly resistant to reinfection (Table 1). Kennebec was the only cultivar for which percent infection and virus content between foundation and commercial seed were not significantly different. This is in agreement with Hahm et al (6), who reported that Kennebec, Monona, Norland, and Superior were resistant to PVX reinfection. In our study, Superior appeared somewhat less resistant to reinfection than Kennebec (Table 1). Commercial seed of cultivar Ontario, most of which was originally obtained from sources outside of Maine, had percent infection as high as 61.2, whereas that of foundation seed was 1.2 (Table 1). Interestingly, this foundation seed was freed from PVX in 1975 by heat treatment and meristem-tip culture. Thus, 7 yr of propagation on the Maine Seed Potato Board farm resulted in a very low level or absence of recontamination. This information, in conjunction with data concerning the high level of resistance to reinfection characterizing Kennebec, indicates that PVX, and possibly other potato viruses, can be eliminated or kept at very low levels if careful cultural practices are used in combination with cultivars resistant to virus reinfection.

The importance of careful cultural practices becomes apparent from the data of Table 2, which was similar to results obtained from tests with the other six cultivars. After 3 yr or more of propagation, some growers were able to keep PVX incidence and titer as low as that of foundation seed released to them (Tables 1 and 2). In other cases, however, the seed contained high levels of PVX contamination when grown by other growers operating at the same location as the "good" growers. When multiple samples, representing different fields, were collected from individual growers, they were consistently clean or consistently contaminated, depending on the grower.

Another interesting aspect of the data shown in Table 2 is the nearly perfect correlation existing between percent infection and virus titer; an increase in PVX incidence was associated with an increase in mean virus titer, apparently because the number of infected plants influences the mean absorbance ($A_{405\ nm}$) more than the virus titer per plant. This correlation is expected when virus titer varies within a rather narrow range, as was the case with Katahdin (Table 1).

Based on the data of this study, we believe that despite the concerns of the potato industry, it is practical to maintain seed potatoes free of certain viruses. Use of PVX-free plant material obtained through heat treatment and meristem-tip culture in combination with reliable indexing, resistance to reinfection, adoption of the "flush-out" system, and field inspection will pay dividends to the potato industry. Finally, the importance of the appropriate cultural practices in preventing reinfection of seed should be emphasized to commercial seed growers.

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