Managing viruses in Tasmania seed potato stocks

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Final Report for project PT05011

Managing Viruses in Tasmanian Seed Potato Stocks.

December 2006.

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Purpose of project:

This project involved a survey of second generation (G2) crops within the Tasmanian seed potato industry to assess the prevalence and incidence of two viruses – Potato virus S (PVS) and Potato virus X (PVX). Small scale greenhouse experiments were also conducted to examine i) disinfectants applied to the seed tuber following cutting for phytotoxicity and for their ability to reduce virus spread, ii) the ability of PVS to be aphid and mechanically transmitted between plants and iii) the ability of PVS to infect plants at different stages of development. Information gained from these trials will be used to further refine management strategies for the control of these viruses.

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Media summary

This project involved a survey of second generation (G2) crops within the Tasmanian seed potato industry to assess the prevalence and incidence of two viruses – Potato virus S (PVS) and Potato virus X (PVX). Greenhouse experiments were also conducted to examine i) the potential for disinfectants applied to the seed tuber following cutting to reduce virus spread and to assess potential phytotoxicity, ii) the transmission of PVS between plants by aphids or by mechanical transmission and, iii) the ability of PVS to infect plants at different stages of development. Information gained from these trials will be used to further refine management strategies for the control of these viruses.

Technical summary

A survey of 59, G2 seed potato crops (mainly vars. Russet Burbank and Ranger Russet) in Tasmania was undertaken during early 2006. Leaf samples (up to 300) were collected systematically through the crops at flowering. Leaflets were grouped in lots of 20, and tested by enzyme linked immunosorbent assay (ELISA) for potato virus S (PVS) and potato virus X (PVX). PVS was detected in 40/59 (67.8%) of crops tested, with 36 of these crops (61.0%) above the National Standard for Certification of seed potato (<1%). However, 52 crops (88.1%) had low to moderate incidence of PVS (<25%) or no detectable PVS. PVS was detected in the varieties Atlantic, Bintje, Coliban, Desiree, Kipfler Pink Eye Ranger Russet Russet Burbank and Shepody but was not detected in Granola, Nooksack, Pontiac, Tasman or Up-to-date. PVX was detected in 7/59 (11.9%) crops tested, with all seven crops above the National Standard for Certification of Seed Potato (<1%). PVX was detected in varieties Russet Burbank and Pink Eye, but not detected in other varieties (above).

A greenhouse trial was conducted to determine if var. Ranger Russet exhibited mature plant resistance to a local isolate of PVS. Mature plant resistance is manifest as increased resistance to infection as the plant ages, which can effectively reduce the amount of virus spread which occurs within the field. Plants (20) were mechanically inoculated with PVS at 1, 2 and 3 months after planting. Leaflets were tested for PVS by ELISA at 1 week prior to, and 4 weeks following inoculation, and again at 4 months after planting, when plants were nearing senescence. In addition, tubers collected from plants were grown on the following season and leaflets tested at 6 weeks. PVS was not detected in plants prior to, or following inoculation. However, PVS was detected in leaflets of plants grown on from tubers the following season. PVS occurred in 4/20, 0/20 and 0/20 plants from those inoculated at 1, 2 and 3 months respectively. The trial demonstrated i) the difficulty in detecting primary infection of PVS within the season and ii) was suggestive of mature plant resistance in var. Ranger Russet as PVS infection was noted only in tubers from plants which had been inoculated at the first inoculation

time. However, due to the low transmission observed in this trial, further trials with a range of virus isolates and potato varieties would be required to confirm this.

Overseas studies have demonstrated PVS and PVX to be spread readily on cutting knives during seed cutting, and the use of sterilants on knives is recommended to reduce virus spread. Because of the difficulty in sterilising mechanical seed cutters during cutting operations, this project investigated the potential for reducing virus transmission by dipping tubers in different concentrations of antiviral disinfectants (Viraclean and Virkon) immediately following cutting. However in two trials, PVS was not transmitted by hand cutting of infected tubers immediately followed by healthy tubers. Because of a lack of virus transmission, these trials were unable to demonstrate the effectiveness of dipping tubers in disinfectants after cutting as a means of reducing virus spread. However, some concentrations of disinfectants did not exhibit phytotoxicity, suggesting potential for this strategy. Further research would be required to identify non-phytotoxic concentrations and durations of particular disinfectants and to test them for their ability to reduce virus transmission during seed cutting.

A greenhouse trial was conducted to examine the ability of PVS to be transmitted from plant to plant either mechanically or by aphid populations within the greenhouse. Healthy plants (20) were placed in aphid proof cages or were left uncaged, and were either placed in direct contact, or not exposed, to PVS-infected source plants. PVS was not detected in leaflet samples from healthy plants at 10 weeks. However, in plants grown from tubers the following season, PVS was detected in 18/20 and 0/20 plants from caged plants exposed or not exposed to inoculum respectively, and in 16/20 and 0/20 from non caged plants exposed to, and not exposed to inoculum respectively. Results were indicative of mechanical transmission of PVS through plant to plant contact, with no evidence of aphid transmission. The trial also demonstrated the inability to detect primary infections of PVS within the season, which suggested the need for a grow-out test from tubers the following season to more accurately determine virus incidence.

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1. Introduction

Tasmanian seed potato stocks have been considered to be relatively free of virus infection due to geographic isolation afforded by being an Island State and to the Tasmanian seed potato certification scheme, which was instituted in the 1930's. However, a limited survey of viruses in Tasmanian seed potato crops during the 2001/2002 season by the Department of Primary Industries Water and Environment (DPIWE), Simplot Australia Pty. Ltd., McCain Foods (Australia) Pty. Ltd. and Harvest Moon Forth Farm Produce Ltd., detected Potato virus S (PVS), Potato virus X (PVX) and Potato leaf roll virus (PLRV) in 27%, 7% and 7% of crops respectively. A more comprehensive survey of the virus status of Tasmanian seed potato crops was undertaken in 2002/2003 through project PT02037 and in G2 crops during 2003/2004 by project PT03069. Management strategies for reducing virus incidence were subsequently put in place by the Tasmanian Virus Strategy Group, made up of representatives of Simplot Australia Pty. Ltd., McCain Foods (Australia) Ltd., Forth Farm Produce Ltd., fresh and processing potato growers, DPIWE, Tasmanian Institute of Agricultural Research and Tasmanian Farmers and Graziers' Association.

The purpose of this project (PT05011) was to conduct a follow up survey (2005/2006) of G2 crops within the Tasmanian seed potato industry to assess the effectiveness of current strategies for managing PVS and PVX. Greenhouse experiments were also conducted to examine i) the ability of disinfectants applied to the seed tuber following cutting to reduce virus spread and to assess potential phytotoxicity, ii) the mode of transmission of PVS between plants by aphids or by mechanical transmission and, iii) the ability of PVS to infect plants at different stages of development. Information gained from these trials will be used to further refine management strategies for the control of these viruses.

2. Survey of G2 crops for potato viruses S and X

2.1 Introduction

A survey of G2 sown seed potato crops was undertaken in Tasmania during the 2005/2006 season for potato viruses S and X.

2.2 Materials and methods

Samples of between 50-300 leaflets were collected from each of 59, G2 crops by DPIW certification officers between late January and early April 2006. Crops were mainly of the processing varieties Russet Burbank and Ranger Russet, but also encompassed a range of fresh market varieties. Leaflets were obtained in a systematic manner from transects in the crop and were stored at 4^{o}C for no more than 2 days prior to virus testing. Samples of less than 100 leaflets were tested individually, while those of greater than 100 were bulked in lots of 20 for testing. A representative sub-sample was obtained by stacking the 20 leaflets on top of each other. The top half of the leaflets was cut off at right angles to the veins and discarded. A further two cuts were made on each side of the vein at 45^{o} angle to form a blunt arrowhead shape. A strip (2-3 mm wide and 2-3 cm long) was cut from the cut surface running at right angles to the veins. This method ensured that all 20 leaflets comprised part of the sample for extraction and virus testing. Scissors were sterilised in 10% household bleach solution and wiped clean between each group of leaves. Leaflet samples were tested by enzyme linked immunosorbent assay (ELISA) (Clark and Adams 1977), as described (Appendix 1). For bulked samples, virus incidence was estimated by the technique of Gibbs and Gower (1960).

2.3 Results

PVS was detected in 40/59 (67.8%) of crops tested, with 36 of these crops (61.0%) above the National Standard for Certification of seed potato (<1%)

(Table 1). However, 52 crops (88.1%) had low to moderate incidence of PVS (<25%) or no detectable PVS (Table 1). PVS was detected in the varieties Russet Burbank, Ranger Russet, Pink Eye and Bintje, but was not detected in Granola, Nooksack, Pontiac, Tasman or Up-to-date. In addition, PVS was detected in some other less common varieties including Atlantic, Coliban, Desiree and Kipfler.

PVX was detected in 7/59 (11.9%) crops tested (Table 1), with all seven crops above the National Standard for Certification of Seed Potato (<1%). PVX was detected in varieties Russet Burbank and Pink Eye, but not detected in Shepody or Ranger Russet (Table 2) or in other fresh market varieties (above). PVX was not detected in less common varieties.

Table 1. Survey of seed potato crops for Potato carlavirus S (PVS) and Potato potexvirus X (PVX) during the 2005/2006 season.

	Virus	
	PVS	PVX
Number of crops with:		
No detectable virus	19	52
0-1%	4	0
1-10%	25	6
11-25%	4	0
26-99%	0	0
100%	7	1
Total crops surveyed	59	59
Average incidence (%) ± standard deviation	14.8±31.8	2.0±13.0

Table 2. Incidence of Potato carlavirus S (PVS) and Potato potexvirus X (PVX) in common potato varieties during the 2005/2006 season.

	Potato variety:				
	Shepody	Pink Eye	Russet Burbank	Ranger Russet	Other[1]
PVS					
Total crops tested	2	2	27	2	26
Number of crops with:					
No detectable virus	0	0	5	1	13
0-1%	0	0	2	0	2
1-10%	0	1	13	1	10
11-99%	1	0	3	0	0
100%	1	1	4	0	1
Mean incidence (%)	56.3	54.8	18.7	3.2	5.3
Std. deviation (%)	61.8	63.9	34.8	4.5	19.5
PVX					
Total crops tested	2	2	25	4	26
Number of crops with:					
No detectable virus	2	1	22	4	25
0-1%	0	0	0	0	0
1-10%	0	0	5	0	1
11-99%	0	0	0	0	0
100%	0	1	0	0	0
Mean incidence (%)	0	50.0	0.6	0	0.1
Std. deviation (%)	0	70.7	1.5	0	0.3

[1]Other includes other varieties and mixtures of varieties.

In comparison to previous survey results, the incidence of PVS in G2 crops of all varieties in Tasmania increased over three growing seasons (2003/2004, 2004/2005 and 2005/2006 seasons) (Figure 1). The mean incidence of PVS for 2003/2004, 2004/2005 and 2005/2006 seasons was 8.7%, 13.0% and 14.8%, respectively (Figure 1). The mean incidence of PVX in 2003/2004, 2004/2005 and 2005/2006 seasons was 1.0%, 1.9% and 2.0% respectively (Figure 1).

The mean incidence of PVS in var. Russet Burbank G2 seed potato crops increased between the growing seasons of 2003/2004 and 2004/2005, with a mean incidence of 11.6% and 19.9%, respectively (Figure 2). The mean incidence of PVS for the 2005/2006 season (19.6%) remained similar to that of the previous season (Figure 2).

The mean incidence of PVX in seed crops over all varieties remained low (<3%) over the three seasons. The mean incidence of PVX in var. Russet Burbank G2 seed potato crops increased from 0.2% to 5.1% for the growing seasons of 2003/2004 and 2004/2005, respectively (Figure 2). However, in 2005/2006 season the mean incidence of PVX decreased to 0.7% (Figure 2).

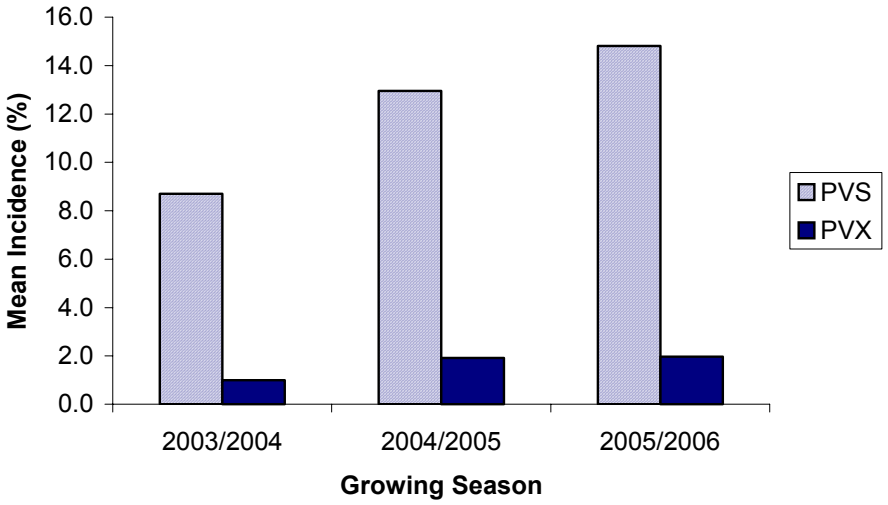

Figure 1. Mean incidence of Potato virus S (PVS) and Potato virus X (PVX) in generation two seed potato crops during the growing seasons of 2003/2004, 2004/2005 and 2005/2006 in Tasmania.

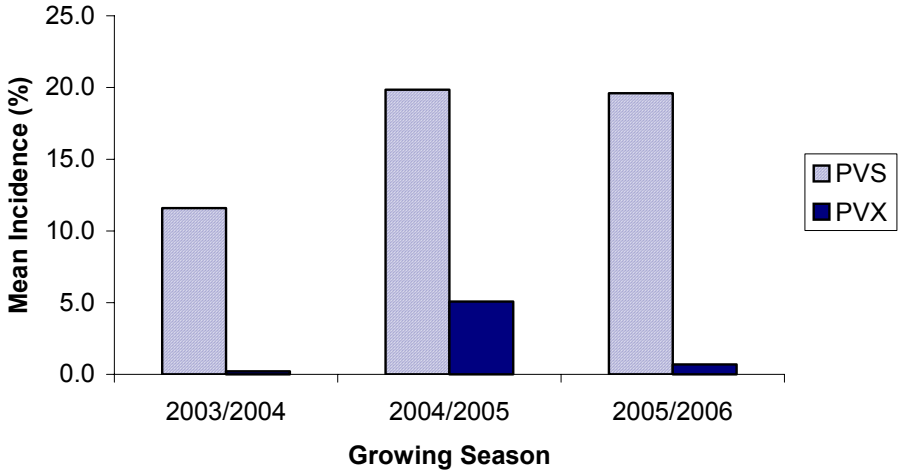

Figure 2. Mean incidence of Potato virus S (PVS) and Potato virus X (PVX) in var. Russet Burbank generation two seed potato crops during the growing seasons of 2003/2004, 2004/2005 and 2005/2006 in Tasmania.

2.4 Discussion

There have been few comprehensive surveys for PVS and PVX in Australia, other than in Western Australia (Wilson and Jones 1990). PVS and PVX were once prevalent in the seed scheme in Western Australia and occurred at high incidence, with up to 100% incidence in some cases. Within the old scheme, production of seed potato historically involved generally one cultivar (var. Delaware) summer planted in coastal swamplands of wind-exposed areas. Crop rotation was not utilised in this scheme, and elimination of unharvested tubers was assumed to occur through sheep foraging (grazing) and natural winter flooding. Management of potential virus spread involved: i) the planting of large selected tubers, ii) visual inspections during the growing season, iii) rogueing of symptomatic plants, and iv) the application of aphicide to minimise aphid populations.

The Western Australian scheme has changed considerably and now involves a 'flush through', limited generation scheme based on the National Standard (RAC Jones pers. comm.). The key components are the release of virus free minitubers and the isolation of seed crops from ware crops. The Western Australian scheme also involves a three-row gap between plantings of different generations to ensure no plant contact. Two inspections are conducted during the growing season, at flowering and pre-senescence. For G2 (sown) crops, 500 leaves are collected per generation, per site at the second inspection and bulk tested in lots of 10 leaves. Samples are tested for PVS, PVX, and TSWV by ELISA and for PLRV by an petiole immunoblotting technique. Tuber testing is also conducted with an eye from the rose end of tubers excised and grown on in the greenhouse prior to testing. The virus tolerances at first and second inspection for G1 (sown) are 0.10 and 0.01 respectively, for G2 (sown) are 0.25 and 0.10 respectively and for G3 (sown) are 1.0 and 1.0 respectively. Later generation crops are also tested if they are to be exported to Sri Lanka and Mauritius.

The new scheme has been successful at reducing the prevalence and incidence of viruses. A survey of potato tubers harvested from seed crops

grown in the Albany swamp region of Western Australia was conducted during 1987/1988 (Wilson and Jones 1990). PVX was detected in 22/23 crops, with 13/23 crops having incidences above 90%. PVS was detected in 20/23 crops, with 9/23 having 80% incidence or greater and PLRV was detected in 4/23 crops at incidences of 2% or less (Wilson and Jones 1990). During the monitoring of G2 (sown) crops conducted during 2003, the viruses PVS, PVX, PLRV, TSWV and PVY occurred in only 0.04%, 0.04%, 0.07%, 0.6% and 0% of 10,450 samples respectively (RAC Jones, DAWA, pers. comm.).

Surveys for PVS and PVX in other states of Australia have been limited. PVS was detected in 2 of 9 Foundation crops at an estimated incidence of 0.105% and 100% during a survey of fresh market certified seed potato varieties in Victoria during 1979-1980 (Moran et al. 1983). PVX was not detected. PVS was detected in 7 of 20 certified seed crops with an estimated incidence of infected crops ranging from 0.105% to 100%. PVX was detected in 5 of 20 certified seed crops with an estimated incidence within infected crops ranging from 0.105% to 1.597%. During 1980-1981, PVS and PVX were detected in 1 of 7 Foundation crops with an estimated incidence of 0.211% (Moran et al. 1983). PVS was detected in 4 of 21 certified seed crops with an estimated incidence ranging from 0.325% to 100%, while PVX was detected in 8 of 21 certified seed crops with an estimated incidence ranging from 0.103% to 1.189% (Moran et al. 1983). A survey of commercial potato crops in the Lockyer Valley, Queensland detected PVS and PVX in 57% and 15% of crops respectively with PVY occurring sporadically (Holmes and Teakle 1980). In a later survey of Queensland potato, PVS was detected in tuber samples taken from 1 of 3 crops of certified seed potato from NSW, but not in 6 crops from Victoria, while PVX was not detected (Jafarpour et al. 1988). In tests of tubers from commercial crops, PVS was present in 7 and PVX in 8 out of 11 crops, with an average incidence of 3.3% and 3.5% respectively.

Hall and Wicks (2005) reported the incidence of virus in samples from ware crops received by the Horticulture Pathology Diagnostic Service in South Australia since 1999. PLRV, TSWV, PVX and PVY were all detected. PVX

was detected in foliar samples from 6.5% of 200 crops sampled and in 31.3% of 48 tuber lots sampled. No samples were tested for PVS.

3. Mature plant resistance

3.1 Introduction

This experiment was conducted to determine if var. Ranger Russet becomes more resistant to infection with a local isolate of PVS as the plant ages. This is potentially important as it has implications for how rapidly PVS may spread in the field. For example in Europe, potato varieties have been shown to develop greater resistance to infection by local strains of PVS as the plant ages (De Bokx 1968). However, in studies in Minnesota U.S.A, varieties were susceptible at all stages (Franc and Banttari 1996). Spread of European isolates of PVS to tubers was therefore limited to the early part of the season, thereby reducing the potential for re-infection in seed-lots in European production areas even when inoculum was present. By contrast, the lack of mature plant resistance in the U.S.A., would allow spread from infected plants to tubers throughout the season, especially late in the growing season when contact between foliage and stems became more pronounced and the likelihood of mechanical transmission from plant to plant was increased.

3.2 Materials and methods

Ranger Russet minitubers obtained from DPIW were planted in potting mix in 200 mm diameter pots on 11/11/2005. Twenty plants were mechanically inoculated with PVS at each of the following times (22/12/2005, 18/1/2006 and 13/2/2006). Inoculum was 0.25 g dried, infected leaf material which had been stored at 10^{o}C over calcium chloride. Inoculum was prepared by pulverising leaf material with a mortar and pestle along with 0.25 g celite abrasive powder and 20 ml phosphate buffer. Four leaves of each plant were inoculated by rubbing inoculum over the surface of the leaf.

For virus testing, one leaflet per plant was collected immediately prior to inoculation and two non-inoculated leaflets per plant were collected at approximately 4 weeks after inoculation and again when plants were nearing

senescence on 2/3/2006. To avoid potential virus transmission during collection of samples, a plastic bag was placed over each leaflet and the leaflet pinched off in the bag. Leaflets were tested for PVS by ELISA as described (Appendix 1). Tubers were collected from senesced plants on 1/5/2005, stored at 4^{o}C and one tuber from each treatment was replanted in potting mix on 2/10/2005. Plants were grown for 6 weeks and one leaflet collected from each stolon and a subsample comprising part of each leaflet tested for PVS by ELISA.

3.3 Results

PVS was not detected by ELISA in any leaflet samples collected prior to mechanical inoculation, at 4 weeks following inoculation or nearing senescence on 2/3/2006. However, PVS was detected in 4/20, 0/20 and 0/20 leaf samples from tubers which had been harvested and grown on in the greenhouse the following season, from plants inoculated on the 22/12/2005, 18/1/2006 and 13/2/2006 respectively.

3.4 Discussion

The number of positive samples detected in this trial was too low to statistically analyse the data or to make any firm conclusions regarding the presence or absence of mature plant resistance in var. Ranger Russet. However, PVS infection was noted only in tubers from plants which had been inoculated at the first inoculation time which might suggest that mature plant resistance was occurring. Further trials with a range of virus isolates and other potato varieties would be necessary to confirm this.

4. Disinfestation of tubers after cutting

4.1 Introduction

Whole seed tubers are usually cut into smaller seed pieces to increase the amount of material available for planting. Overseas studies have demonstrated that PVS and PVX can be transmitted between infected and healthy seed pieces on the cutting knife (e.g. Larson 1950; Franc and Banttari 1984). In Tasmania, much of the crop is cut on a few large, centralised seed cutters. Some precautions are taken to ensure that pathogens are not transmitted during seed cutting. Seed cutting equipment is washed down at the end of the day and disinfected periodically during cutting. Furthermore, early generation seed crops are often cut separately to those of later generations and earlier generations scheduled for cutting earlier in the day than later generations. Some seed cutters have indicated a difficulty in disinfecting machinery more frequently during the day due to the downtime associated with sterilising, washing and allowing machinery to dry. An alternative strategy may be to disinfect tubers with antiviral chemicals soon after cutting. The purpose of this experiment was to test the ability of the antiviral disinfectant Viraclean (Hospital Grade 4.255 g/L benzalkonium chloride, (distributed by Whiteley Medical, Tomago New South Wales) and Virkon (50% potassium peroxomonosulfate, 15% sodium alkyl benzene sulphonate and 5% sulphamic acid (distributed by United Biosciences P/L Carindale, Queensland) applied to tubers directly after cutting to reduce PVS transmission during seed cutting operations. Viraclean is a broad range disinfectant which kills a range of bacteria, with activity against human viral pathogens. Virkon is a predominately broad-spectrum antiviral chemical (Lister 2004).

4.2 Experiment 1

Materials and methods

Tubers were obtained from a highly infected seed lot of var. Ranger Russet identified as part of the survey. Tubers were sprouted, and sprouts tested for PVS infection by ELISA (Appendix 1). Twenty PVS-infected tubers were selected as virus sources. For each of the following treatments (except treatment 7) individual knives were sliced through each of the 20 infected tubers and immediately sliced through 20 virus-free minitubers. Treatments were as follows:

1) Inoculated and cut minitubers dipped in 1% Virkon solution (w/v).
2) Inoculated and cut minitubers dipped in 5% Virkon solution (w/v).
3) Inoculated and cut minitubers dipped in undiluted Viraclean solution.
4) Inoculated and cut minitubers rolled in cement containing 1% (w/w) Virkon powder.
5) Inoculated and cut mintubers rolled in cement only after cutting.
6) Inoculated and cut minituber with no further treatment (positive control)
7) Non-inoculated and cut minituber with no further treatment (negative control).

Minitubers were cut and treated on 18/11/2005. Cut minitubers were dipped in sterilants no longer than 2 minutes after cutting and for no more than 10 seconds duration. Minitubers were dried on the bench and half of each was planted in potting mix in 200 mm diameter pot on 25/11/2005 and grown on. Leaflets were collected on 23/1/2006 and tested by ELISA for PVS. To avoid potential virus transmission during collection of samples, a plastic bag was placed over each leaflet and the leaflet pinched off in the bag. At senescence on 1/5/2006, tubers were collected and placed in cool store (4^{o}C). Two tubers from each treatment were replanted into single pots (1 L) on 5/9/2006 and grown on for 7 weeks. One leaflet from each stolon was collected on

23/10/2006 and a subsample comprising part of each leaflet tested for PVS by ELISA (Appendix 1).

Results

No virus was detected by ELISA in any leaf samples collected prior to senescence or from leaflets obtained from tubers grown on in the following season. Some bleaching and retardation of sprout growth was noted with 5% Virkon solution and with undiluted Viraclean solution. Other treatments appeared unaffected. Treatment with undiluted Viraclean appeared to reduce emergence, with only 14/20 tubers developing into plants. In all other treatments more than 17/20 tubers planted developed into plants. However, there was no observable difference in plant growth between treatments at 6 weeks after treatment.

4.3 Experiment 2

Materials and methods

Tubers of var. Ranger Russet were obtained from a seed lot which had been tested as part of the survey and found to have no detectable PVS. Tubers were removed from cool storage (4^{o}C) on 10/10/2005 and held at 10^{o}C On 18/11/2005, tubers were cut and treated as follows, with 20 tubers per treatment. Note there was no virus treatment in this trial.

1) Cut tubers dipped in Virkon 1%.
2) Cut tubers dipped in Virkon 5%
3) Cut tubers dipped in undiluted Viraclean
4) Tubers cut and not dipped.

Dipping of tubers in disinfectants was for up to 10 seconds duration and was conducted less than 2 minutes after cutting. Tubers were dried on the bench overnight and half of each was planted into pots (200 mm) in potting mix on

the day following cutting. Plants were grown to maturity in the greenhouse. Plants were harvested on 1/5/2005, and yield and size range of tubers measured.

Results

Disinfectants had no observable effect on emergence, and no statistically significant effect on the subsequent number of tubers, average tuber weight or total weight of tubers produced (Table 3).

Table 3. The effect of dipping cut tubers in disinfectants for up to 10 seconds on subsequent average number of tubers, average tuber weight and total weight of tubers per plant.

Treatment	Number of tubers	Average tuber weight (g)	Total weight of tubers (g)
Non treated	6.5	44.5	269.2
Viraclean	6.9	39.4	261.2
Virkon (1%)	5.1	51.3	241.0
Virkon (5%)	5.4	54.5	248.4
P=	0.11 (ns)	0.31 (ns)	0.78 (ns)

4.4 Experiment 3

Materials and methods

Tubers were obtained from a highly infected seed lot of var. Ranger Russet identified as part of the survey. Tubers were sprouted, and sprouts tested for

PVS infection by ELISA (Appendix 1). Fifteen PVS-infected tubers were selected as virus sources.

Healthy tubers (var. Russet Burbank) were obtained from a seed lot harvested from a field in which virus had not been detected during the survey (above). Tubers were sprouted and tested for PVS infection by ELISA prior to the experiment. Healthy tubers were treated on 24/11/2005 as follows, with 15 replicates per treatment.

Negative control:	Cut healthy tubers only.
Positive control:	Cut infected tuber followed by healthy tuber.
Virkon 0.1% (w/v):	Cut infected tuber, followed by healthy tubers then dipped in Virkon.
Viraclean 10% (v/v):	Cut infected tuber followed by healthy tuber then, dipped in Viraclean.

Half of each cut tuber was discarded. For disinfectant treatments, the remaining half was dipped in disinfectants for up to 10 seconds duration, no longer than 2 minutes after cutting. Tubers were dried on the bench overnight prior to planting in potting mix in 200 mm pots on 25/11/2005. Plants were maintained in the greenhouse for 3 months. Leaflets (1 per plant) were collected on 2/3/2006 and tested for PVS by ELISA. To avoid potential virus transmission during collection of samples, a plastic bag was placed over each leaflet and the leaflet pinched off in the bag. Tubers were collected from senescing plants on 1/5/2006 and stored (4^{o}C). Two tubers from each treatment were planted in single pots (1 L) in potting mix on 2/10/2006, grown on for approximately 6 weeks and 1 leaflet collected from each stolon on 21/11/2006. A subsample comprising part of all leaflets was tested for PVS by ELISA (Appendix 1).

Results

There was no observable difference between treatments in emergence with 11, 14, 11 and 13/15 tubers producing plants for the negative control, positive

control, Viraclean and Virkon treatments respectively. Similarly there were no significant differences between treatments in the number of tubers, average tuber weight or total weight of tubers (Table 4). However, the latter bordered on significance, with Viraclean having the lowest total tuber weight (Table 4).

Table 4. The effect of various treatments on average number of tubers, average tuber weight and total weight of tubers per plant.

Treatment	Number of tubers	Average tuber weight (g)	Total weight of tubers (g)
Control (-ve)	5.4	47.6	248.1
Control (+ve)	5.3	55.0	277.6
Viraclean (10%)	5.6	48.6	239.7
Virkon (0.1%)	6.1	50.5	283.2
P=	0.68 (ns)	0.65 (ns)	0.11 (ns)

Discussion

Experiments 1 and 3 were unable to demonstrate any transmission of PVS on knives by cutting infected tubers followed by healthy tubers. The lack of transmission of PVS by seed cutting in these experiments is at odds with the results of similar experiments overseas. Franc and Banttari (1984) demonstrated that transmission of PVS from infected to uninfected tubers by hand cutting in var. Russet Burbank, increased significantly if the cutting knife passed through a sprout of a tuber (45.2%) compared to knife contact with non-sprout tuber tissue (24.5%). Franc and Banttari (1984) also demonstrated that transmission of PVS by seed cutting differed between cultivars. Cutting infected tubers followed by healthy tubers led to 76.7% and 62.6% infected plants for vars. Russet Burbank and Kennebec respectively, significantly higher (P=0.01) than for var. Norland (25% infection). Spread of PVS on a

contaminated seed-cutting machine was demonstrated for the var. Norgold Russet (Wright 1987). A seed line with 0.0-1.5% incidence of PVS was passed through a seed cutter after cleaning or after cutting a line with 20% PVS on the same day. Seed cut on the cleaned machine remained at between 0.0-1.5% incidence, while in two lots cut on the contaminated machine the incidence of PVS increased to 1.9-7.2% and 15% respectively. PVX has been shown to be transmitted readily during seed-cutting. Larson (1950) showed a ringspot strain of PVX to be transmitted with greater frequency by the cutting knife when virus infected source tubers were cut through the eyes (52%) compared with cuts through source tubers that avoided eyes (24%).

However, previous unpublished experiments in Tasmania have also failed to demonstrate transmission of Tasmanian strains of PVS from infected to uninfected tubers by hand-cutting (Susan Lambert TIAR pers. comm.). This suggests that strains of PVS within Tasmania may not be as readily mechanically transmissible by seed cutting as those overseas or that transmission of PVS requires transmission of significant amounts of infected sap, e.g. on a mechanical seed cutter.

The lack of virus transmission in these experiments prevented an assessment of the ability of disinfectants to reduce virus transmission during seed cutting. However, Fletcher et al. (2004) demonstrated that exposure of blades treated with PVX to concentrations of Virkon (0.1 and 1.0%), similar to that used in this experiment for a period of 30 seconds was able to significantly reduce subsequent transmission of PVX to indicator plants. Presumably disinfectants applied to tubers would be active on the cut surface of the tuber for somewhat longer than this, and would permeate into the surface layers, which would further indicate potential for eradication of virus from the cut surface.

Some damage to sprouts of dipped seed tubers was evident following treatment with concentrated Viraclean and Virkon 5% w/v, but not Virkon 1% w/v. However, there were no obvious differences in plant growth or in

subsequent tuber numbers per plant, total weight of tubers per plant or average tuber weight in these experiments. While further larger scale field trials are required, this would suggest that treatment of tubers with disinfectants following cutting may be a viable strategy to reduce virus transmission. Other disinfectants should be examined for their ability to reduce virus transmission and for reduced phytotoxicity and for their potential to reduce transmission of PVS and PVX during seed cutting. Fletcher et al. (2004) studied the ability of several disinfectants to reduce transmission of PVX and Tobacco mosaic virus on knives (Table 5).

Table 5. Effect of dipping blades contaminated with Potato potexvirus X (PVX) or Tobacco tobamovirus (TMV) in disinfectants for 30 seconds on subsequent ability to inoculate indicator plants by cutting (Fletcher et al. 2004)

Disinfectant	Concentration (% a.i.)	% virus transmission to indicator plants	
		PVX	TMV
Sodium hypochlorite[1]	0.24%	10	0
	2.4%	0	10
Benzoic acid[2]	1%	0	100
	5%	0	90
Hydrogen peroxide[3]	1%	40	90
	10%	0	80
Chitosan	0.01%	11.1	100
	0.1%	0	90
Potassium peroxomonosulfate[4]	0.1%	20	100
	1.0%	10	40
Didecylmethyl ammonium chloride	1%	30	100
	10%	20	80
Water control	-	40	100

1 Dynawhite (4.8% sodium hypochlorite)

2 Culticlean (9% benzoic acid)

3 Geosil (25% hydrogen peroxide and silver)

4 Virkon (50% potassium peroxomonosulfate and 15% sodium alkyl benzene)

5 Sporekill (12% didecylmethyl ammonium chloride)

Some of these disinfectants have low phytotoxicity at rates which are virucidal. Benzoic acid has been used at low concentrations within recirculating hydroponic systems and sprayed onto plants without phytotoxicity. Similarly, concentrations of Virkon below 1% are generally considered to be not phytotoxic to a variety of plants. Chitosan has been used as a postharvest treatment of carrot at 2 or 4% for control of Sclerotinia rot without evidence of phytotoxicity (Cheah et al. 1997) and for control of powdery mildew when sprayed onto plants. Therefore, there would appear to be opportunities for developing protocols for treating tubers with disinfectants to reduce transmission of virus and other pathogens during seed cutting.

5. Transmission of PVS between potato plants

5.1 Introduction

PVS is transmitted readily by mechanical transmission via wounds, e.g. through movement of animals or equipment through the crop or from plants rubbing against each other (Stevenson et al. 2001). In addition, some strains of PVS are spread in a non-persistent manner and at varying levels of efficiency by aphids, including *Myzus persicae* (green peach aphid) and *Aphis nasturtii* (buckthorn aphid) (Stevenson et al. 2001). The ability of strains of PVS to be aphid transmitted has major consequences for the management of the virus. This trial was conducted to test whether local isolates of PVS are aphid transmissible or transmissible through plant to plant contact.

5.2 Materials and methods

Ranger Russet minitubers obtained from DPIW were planted on 4/11/2005 and grown under aphid proof conditions. Tubers from a crop with a high incidence of infection were removed from cool storage and sprouted on the laboratory bench. Sprouts were tested for PVS by ELISA (Appendix 1). Infected tubers were planted and used as virus source plants in the subsequent trial. The trial was established on 22/12/2005. There were 4 treatments (Table 6), with healthy plants either caged or non caged and with or without virus source plants. For each treatment, 20 healthy plants were arranged in two rows of 10 plants, approximately 1 pot width apart. Where required, 5 virus source plants were placed between the two rows, so that each source plant was in contact with 4 adjacent healthy plants.

Table 6. Conclusions that could be drawn if there had been transmission (+) or no transmission (-) in particular treatment combinations are given below:

	Infected plants	No infected plants
Caged	+=mechanical	+=contamination
(aphids excluded)	-=no mechanical	-= no contamination
Not caged	+=mechanical/aphid	+=aphid transmission
(aphids not excluded)	-=no mechanical/aphid	-=no aphid transmission

The four treatments were located in the same greenhouse and separated by a distance of approximately 6 metres. Uncaged plants were exposed to naturally occurring populations of aphids in the greenhouse. Leaflets were collected from plants nearing senescence on 2/3/2006 and tested for PVS by ELISA (Appendix 1). To avoid potential virus transmission during collection of samples, a plastic bag was placed over each leaflet and the leaflet pinched off in the bag. Tubers were collected from each plant on 1/5/2006, placed in paper bags and maintained in the cool store (4^{o}C). Two tubers from each plant were replanted into single pots in commercial potting mix on 2/10/2006, and grown on. Leaflets were collected following 6 weeks growth on 21/11/06, and tested for PVS by ELISA.

5.3 Results

PVS was not detected by ELISA in leaflets collected from plants grown from minitubers just prior to senescence (Table 7). However, virus was detected in leaflet samples from virus source plants (results not shown).

In plants collected from tubers grown the following season, PVS was not detected in plants subjected to either caged or non caged treatments in which there were no infected source plants (Table 7). However, there was a high

incidence of PVS in plants which had been exposed to infected source plants in both caged and non caged treatments (Table 7). This indicated mechanical transmission occurred between plants but gave no evidence of aphid transmission (Table 6).

Table 7. The incidence of PVS in foliar samples collected from potato plants maintained in aphid proof cages or non caged and exposed to contact with infected source plants (+) or not exposed (-).

	Number of PVS infected/number tested on:	
Treatment	2/3/2006[a]	21/11/2006[b]
Caged (–)	0/20	0/20
Caged (+)	0/20	18/20
Non caged (–)	0/20	0/20
Non caged (+)	0/20	16/20

Leaflets were collected and tested from plants prior to senescence[a] and from tubers collected from plants and grown on the following season[b].

5.4 Discussion

In this trial, primary infections of PVS could not be detected by ELISA within the season. This may have been due to virus transmission occurring late in the season, which did not allow virus sufficient time to increase to levels detectable by ELISA.

The finding that plants grown from healthy minitubers became infected in both caged and non caged treatments and only in those treatments exposed to infected source plants was suggestive of mechanical transmission between plants. Conversely the lack of transmission to non caged plants without infected source plants provided no evidence of virus transmission by aphids in

this trial. However, caution is required as it is not known if vector species were present. The high amount of transmission to healthy plants grown in close contact with PVS-source plants is interesting given that plants in this trial would have been exposed to considerably less wind-rub and damage than field-grown plants.

6. Communication/extension activities.

A meeting was held with the Tasmanian Virus Strategy Group on 14th August 2006 and results were presented at the 9th Annual Potato and Vegetable Agricultural Research and Advisory Committees, Research Development and Extension Day on 25th July 2006.

7. General conclusions

Management of viruses

The survey of G2 crops for PVS and PVX indicated that management strategies have been effective at maintaining low levels of PVX in Tasmanian seed potato. However, PVS remains at a higher prevalence and incidence. The difficulty in managing PVS may, in part, be due to:

i) The higher initial prevalence and incidence of PVS in comparison to PVX in Tasmanian seed potato crops provides greater sources of inoculum for spread of PVS.
ii) While PVX produces mild, but distinguishable, symptoms of infection in the field, PVS does not produce observable symptoms. This makes the identification of PVS infected fields by DPIW certification staff or growers difficult.
iii) While PVS and PVX are spread by mechanical transmission, some strains of PVS are also spread by some species of aphid. While it is possible that aphid transmission may be a means by which PVS enters and increases rapidly in seed crops, results of this and previous projects have so far provided no evidence that aphid transmission occurs or is a major contributor to virus transmission in Tasmania.

General methods to manage viruses are given in appendix 2. One of the cornerstones of virus management is adherence to the National Standard and isolation from virus sources. It is noteworthy that some Tasmanian growers have implemented geographic separation of G1 and G2 crops from later generation and commercial crops and this has been successful at maintaining nil or low levels of virus in their G2 crops. In general, infection of G2 material is more likely to occur where it is grown in proximity to later generation or commercial crops which are potentially infected.

Virus testing

Monitoring and accurate determination of virus incidence is an important component of a management strategy. However, this project has indicated that testing leaflets by ELISA may not detect primary infections with PVS that have occurred within the season. Therefore virus testing of leaflet samples taken at flowering may underestimate virus incidence for the subsequent crop. This may explain why in some instances leaflets gathered at flowering from G1 crops have had no detectable PVS, while in the following year leaflets gathered from the subsequent G2 crop have had high incidence. This is similar to findings overseas. For example, Weidemann (1986) reported that the incidence of PVS increased to 27% (year 1) and 31% (year 3) within initially healthy plants planted and grown adjacent to infected plants. By comparison eye plug testing of harvested tubers showed a PVS incidence of 64% and 79.1% at the end of years 1 and 3 respectively. Conversely, Franc and Banttari (1996) reported that PVS moved out of mechanically inoculated potato leaves within 24 hours, with virus detectable by ELISA in foliage above and below the inoculated leaf within 13 and 20 days respectively. The results of Franc and Banttari (1996) would suggest that there would be little difficulty in detecting primary infections in the field. The ability to detect PVS needs to be confirmed in the field, perhaps by testing both leaflets collected at different times within the season (early, mid and late) and sprouted tubers (between seasons) as particular crops progress through generations. While more labour intensive, additional testing of sprouted tubers or eye plug testing between seasons may be a more accurate determinant of the incidence of virus within a seed crop than testing leaflet samples within season. Some form of tuber testing or grow out testing is adopted by many other seed potato certification schemes around the world.

Contribution of seed cutting to virus spread

Results from this project would suggest that hand seed cutting may not be a major contributor of spread of PVS in Tasmanian seed potato. This is at odds with overseas studies which have shown PVS to be readily transmitted on

cutting knives. Therefore the authors recommend that growers maintain the practice of disinfesting knives when hand cutting for PVS and especially for PVX. Conversely, it is likely that there would be greater opportunity for spread of PVS in mechanical seed cutters in which large volumes of material are processed and there is opportunity for build up of infected sap on surfaces and for wounds on tubers. Therefore, strict adherence to hygiene practices on mechanical cutters is of paramount importance. Where possible, non-sprouted tubers should be cut and planted, as virus concentrations tend to be lower in non sprouted than sprouted tubers. In addition, sprouts may be easily damaged during cutting, handling and planting operations and thus contribute to transfer of infected sap between tubers.

Due to the poor transmission of PVS during hand-cutting experiments, this project was unable to demonstrate reduced virus transmission from disinfectants applied to cut tubers shortly after cutting. However, some concentrations of disinfectants which are known to be virucidal under other conditions, were not phytotoxic when applied to the cut tuber and grown on in greenhouse trials. This would suggest that this strategy may have potential for reducing virus transmission during cutting. Further work is required to assess a range of disinfectants against a range of potato varieties, with field evaluation of the effect on agronomic performance and eventual registration for use.

8. Future work

1) **Virus detection**: The effectiveness of testing leaflets within season for assessment of PVS incidence needs to be further ascertained by comparison to grow-out tests from tubers.

2) **Virus transmission**: The difficulty in preventing entry of PVS into early generation crops in Tasmania could be suggestive of a vector. Isolates of PVS should be tested for their ability to be aphid transmitted by the most common vector (*Myzus persicae*) in dedicated transmission tests.

3) **Seed tuber treatments:** Preliminary work in this project has indicated that treatment of seed tubers with antiviral disinfectants shortly after cutting may be conducted without phytotoxicity. A range of disinfectants applied in this manner should be further tested for their ability to reduce transmission of viruses and potentially other pathogens and, if successful, progressed towards registration.

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Appendix 1. Virus testing protocol.

For virus testing, sap was expressed from leaflets using a motorised roller press. Extraction buffer (100 ml Phosphate Buffered Saline (PBS) 10X, 1.3 g anhydrous sodium sulfite, 20.0g polyvinylpyrrolidine (MW 24-40,000), 2.0 g Bovine Serum Albumin, 20 ml Tween 20, made up to 1 L and adjusted to pH 7.4) was run down the rollers and sap and extraction buffer collected in a 1 ml sample tube. Rollers were washed with tap water between samples to prevent cross-contamination of samples.

Antisera for PVS and PVX was obtained from Agdia Inc. Elkart, IN, USA. A double antibody sandwich ELISA technique was used (Clark and Adams 1977). Antiserum was pipetted into 96 well polystyrene microtitre plates (Nunc) at 1/400 dilution in carbonate coating buffer (1.59 g Na_2CO_3 and 2.93 g $NaHCO_3$, made up to 1 L with distilled water and adjusted to pH 9.6). A total of 100 µl was added to each well. Microtitre plates were incubated at 37^{o}C for 4 hours or at 4^{o}C overnight and washed (below). Sap samples prepared as above were pipetted into wells (100 µl/well) and incubated overnight at 4^{o}C. Plates also contained wells with 4 known negative samples, buffer only and a positive sample. Antiserum conjugated with alkaline phosphatase was prepared for each virus at the same dilution used to coat the plate. Conjugated antiserum was diluted in conjugation buffer (100 ml PBS 10x, 2.0 g Bovine serum albumin, 20.0 g Polyvinylpyrrolidine (MW 24-40,000), made up to 1 L with distilled water and adjusted to pH 7.4). To each well, 100 µl dilute conjugated antiserum was added and microtitre plates incubated at 37^{o}C for 4 hours. Plates were washed (below). Substrate tablets, each containing 5 mg p-nitrophenyl phosphate were added at a rate of 1 tablet per 10 ml of substrate buffer, (48.5 ml Diethanolamine and 400 ml of distilled water adjusted to pH 9.8). Substrate (100 µl) was added to each well. Plates were incubated for 30-60 minutes at room temperature to allow colour development and absorbance of each well was read at 405 nm using a Titertek photometer (Flow Laboratories, Helsinki, Finland). Samples were considered positive if they were greater than the mean absorbance of the

negative controls plus three times the standard deviation of the negative controls (Sutula *et al*. 1986).

Microtitre plates were washed between each step with 3 changes of wash buffer (100 ml PBS 10x stock, 900 ml distilled water, 0.5 ml Tween 20 and 1.0g milk powder, made up to 1 L and adjusted to pH 7.4). Plates were immersed in wash buffer to fill wells and soaked for at least 3 minutes between each change. After the final soak, plates were emptied of wash buffer, patted on paper towels and allowed to drain upside down over paper towels for approximately 5 minutes before the next step.

Appendix 2. Methods for management of viruses in potato.

The basis of the control of viruses is strict adherence to the National Standard for Certification of Seed Potato. This is based on a flush through, limited generation mode of seed potato production that has been successful in reducing virus problems in potato worldwide. Further steps to limit the spread of viruses, which could be implemented with industry agreement, include:

Isolation:

- Isolation of G1 and G2 generation crops from later generation crops.
- Increase the physical separation between i) generations in the field (currently 1 blank row) to 2-3 blank rows (Western Australia use three blank rows and Victoria use two blank rows) and ii) between seed potato crops and ware crops.

Hygiene:

- Ensure that low generation seed is not graded or cut at the same facilities as seed of high generation or ware crops. For example, hand-cutting of early generation material (G1/G2) with strict disinfectant protocols for knives. Alternatively, the implementation of strict hygiene standards at cutting and grading and storage facilities and cutting of early generation material prior to later generation material.
- Disinfestation of equipment during seed cutting operations, especially between lines. If hand cutting, then regular disinfestation of cutting equipment between and also within lines.
- Improved bin hygiene and introduce a bin cleanliness certificate (as is required under the National Standard). Use plastic bins that are easier to clean.
- Improve field officer hygiene for company and seed certification staff between each crop and enforce hygiene plan.
- Improved farm and machinery hygiene – develop in-field wash down procedures and disinfest machinery between generations or different crops.

Cultural:

- Conduct machinery operations in order of generation, i.e. from G1 to G5. Later generations will have been exposed to potential infection for a longer period and have a greater risk of harbouring virus infection than earlier generations.
- Handle seed carefully and cut and plant seed before sprout formation to reduce transmission of mechanically transmitted viruses at planting.
- Restrict movement of machinery through the crop to avoid mechanical transmission of viruses, e.g. employ aerial spraying and do not plant traveller irrigator runs with potato crop. Ensure that irrigator runs are sufficiently wide that wheels or hose are not in contact with the crop. For pivot irrigators, wheel tracks should be cleared of potato foliage to prevent transmission of mechanically transmitted viruses. Solid set irrigation would be preferable to minimise movement throughout the crop.
- Inspection and rogueing of symptomatic plants at an early stage of crop growth.
- Control of weeds (especially nightshade and fathen for PVS and PVX) and volunteer potato that may be reservoirs of vectors and/or viruses.
- Control of vectors of vector transmitted viruses through e.g. isolation of crops, chemical control, removal of alternative hosts and adjustment of planting date to avoid vector flights.

Quarantine:

- Remove seed crops with a greater than 1% level of virus from the seed scheme, e.g. by processing tubers. This requires routine testing to identify infected crops.
- Restrict the movement of seed that has not been virus tested.

Note that guidelines for control of viruses in potato are also given by Fletcher (2000) and Jones (2004).