Management strategy for elimination of viruses from certified seed potato stocks in Tasmania

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Final Report for Horticulture Australia Ltd. Project PT03069 (April 2005)

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Horticulture Australia Ltd. Project PT03069
Management Strategy for elimination of virus from certified seed potato stocks in Tasmania.
April 2005

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Purpose of project: In a previous project (PT02037), *Potato viruses S* and *X* were detected in the Tasmanian seed potato scheme and preliminary management strategies were implemented to control virus levels. Project PT03069 built upon this by carrying out an audit of current seed handling practices and providing recommendations to growers to control these viruses. In addition, a survey of second field generation (G2) crops was undertaken to monitor the success of management strategies. Further research was conducted to underpin the management strategy including assessing the importance of in-field spread compared to mechanised seed cutting, identifying inoculum sources, characterisation of virus strains and identifying the principal means by which they are transmitted. This information was used to refine the management strategy and to develop the most cost-effective means of eliminating virus from Tasmanian seed potato.

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Media summary

Tasmanian seed potato stocks have generally been considered to be relatively free of viruses, although there have been sporadic outbreaks of viruses over the years. Factors contributing to this include the geographic isolation of Tasmania and the Tasmanian seed potato certification scheme. However, a recent survey conducted as part of HAL project PT02037 detected *Potato virus S* (PVS) and *Potato virus X* (PVX) in seed potato stocks. Interim management strategies were put in place to control these viruses. An assessment of these strategies was undertaken by testing second field generation (G2) crops for virus. PVX occurred in 6/108 crops and PVS in 49/112 crops. The low prevalence of PVX suggested that there is a good prospect of eliminating this virus from the seed scheme in the short term. However, the prevalent nature of PVS suggested that the elimination of PVS from the seed scheme will take longer. An audit of seed handling practices was undertaken and further recommendations for management of viruses were adopted by industry. Further research has shown only the ordinary strains of PVS (PVS^O) to be present in Tasmania. Little spread of PVS and PVX occurred over the season at two fields, suggesting that management practices which minimise movement of machinery through the crop are being effective at reducing virus spread in the field. Preliminary monitoring of seed lines before and after cutting suggested some transmission of PVS over the seed cutter. However, the relative contribution of seed cutting to virus levels was not ascertained. In field trials, a significant negative correlation was noted between the incidence of PVS in plots of Russet Burbank and processing yield. On average, plots completely infected with PVS yielded 5.4 t/ha lower than those with no virus. This supported overseas work that has shown PVS to have a relatively mild effect on potato yield in comparison to some other viruses. Virus levels will be monitored in succeeding years to test the effectiveness of the management strategies put in place by the Tasmanian potato industry. Tasmanian seed potato sent interstate is now laboratory tested for PVS and PVX to ensure crops meet the requirements of the National Standard for Certification of Seed Potato and therefore undergoes a higher level of scrutiny than seed in most other states.

Technical summary

Although there have been sporadic outbreaks of viruses such as *Potato leafroll virus* and *Tomato Spotted Wilt virus* over the years, Tasmanian seed potato stocks have generally been considered to be relatively free of viruses. Factors contributing to this include the geographic isolation of Tasmania and the Tasmanian seed potato certification scheme. However recently, *Potato viruses S* (PVS) and *X* (PVX) were detected in the Tasmanian seed potato scheme as part of Horticulture Australia Ltd. project (PT02037). These viruses have been reported before in other states of Australia and in Tasmania. Both are transmitted mechanically, e.g. by plant to plant contact, on machinery and by seed cutting. In addition, some strains of PVS are transmitted inefficiently by some aphid species. These viruses often produce mild symptoms in the field and so are often not detected by visual inspections carried out as part of the National Standard for Certification of Seed Potato.

Preliminary management strategies have been put in place to control virus incidence in Tasmania. Project PT03069 monitored the success of these management strategies by testing second field generation (G2) crops. In addition, an audit of seed handling practices was undertaken to determine how management could be improved and research was conducted to identify how these viruses were being transmitted in Tasmania.

PVX was detected in 6/108 G2 crops, with 5 crops above 1% incidence. PVS occurred in 49/112 crops, with 42 crops above 1% incidence. The low prevalence of PVX suggested that this virus could be eliminated from the seed scheme in the short term. However, the prevalent nature of PVS suggested elimination of this virus would be more difficult to achieve.

Inoculation of 16 PVS isolates to the indicator plant species *Chenopodium quinoa* suggested the presence of both the ordinary strains (PVS^O) and andean strains (PVS^A) of PVS. However, subsequent testing by an RT-PCR technique has indicated that only PVS^O strains are present. Further characterisation of strains will be undertaken during 2005.

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Virus spread over the season was monitored in two fields of var. Russet Burbank by testing 20 plants in each of 49 plots (980 plants) or 51 plants at 1 m intervals along 7 adjacent rows (357 plants) at intervals during the season. In a field with PVS ('PVS field'), the incidence of virus at 58 and 129 days after planting (DAP) was 50.2% and 45.3% respectively in plots and 52.5% and 56.7% respectively in transects. In a field with PVX and trace levels of PVS ('PVX field'), the incidence of PVX at 56 and 132 DAP was 5.4% and 7.7% respectively in plots and 6.1% and 8.3% respectively in transects. The incidence of PVS in plots in this field at 56 and 132 DAP was 0.5% and 0.6% respectively and in transects was 0.3% and 1.1% respectively. Results indicated only limited spread of PVS and PVX occurred in the field over the season, suggesting that modern agronomic practices that minimise movement of machinery through the crop are effective at reducing virus spread in the field. Spatial analysis detected a random pattern PVS and PVX infection, suggestive of planting of infected seed pieces. However, aggregation of PVS along (but not across) rows was detected in transects in the PVS field, which was indicative of some mechanical spread of PVS along rows during the season.

Weed species have been implicated as reservoirs of PVS and PVX in overseas studies. However, neither PVS nor PVX was detected in 165 specimens of weeds, comprising 14 species collected from the PVX field. This included *Chenopodium album* (fat hen) and *Solanum nigrum* (black nightshade). Annual weeds therefore may not be significant as reservoirs of these viruses in Tasmania. However, infected volunteer potato from previous crops may potentially be a source of virus into subsequent crops, where short rotations or poor weed control have been practiced.

Some strains of PVS can be spread by specific aphid species. Aphid trapping was conducted at the PVS and PVX fields. The aphids *Aphis gossypii* and *Macrosiphum euphorbiae* were detected on yellow sticky traps towards the end of the growing season. These species are vectors of some other viruses

of potato, but not PVS. The limited spread of PVS at both fields also suggested that aphids were not transmitting PVS at these fields.

Overseas research has demonstrated that seed cutting can be a significant means of transmission of PVS and PVX. A trial was conducted to determine the contribution of seed-cutting to virus spread. From each of 9 seed lines, a sample of whole tubers was collected prior to passing over a commercial seed-cutting machine and seed pieces collected after cutting. Tubers and seed pieces were grown in the greenhouse and foliage tested for PVS and PVX by ELISA at 8 weeks. The incidence of PVS following seed cutting increased in 5/9 lines, remained similar in 2/9 lines and declined in 2/9 lines following seed cutting. PVX was detected in only 2 crops. While the trial suggested that PVS was spread by seed-cutting, the extent to which seed cutting contributes to virus incidence in Tasmania was unable to be quantified.

The effect of PVS on plant growth and yield was determined. Measurement of plots in the PVS field (above) with a Cropscan radiometer, determined a significant negative correlation between percentage reflectance from the crop canopy at 830 nm (an indicator of green leaf area index) at 134 DAP and the incidence of virus at 129 DAP. Further assessment of the effect of virus on yield was conducted in a replicated plot trial. A significant negative correlation was detected between the incidence of PVS in plots of var. Russet Burbank and processing yield. Regression analysis determined a significant relationship between incidence of PVS and processing yield. On average, plots completely infected with PVS yielded 5.4 t/ha lower than those with no virus. This supported overseas work that has shown PVS to have a relatively mild effect on potato yield in comparison to some other viruses.

Management strategies have been put in place by the Tasmanian industry to eliminate PVX and PVS from the seed scheme. Virus incidence will continue to be monitored in succeeding years to ensure the success of the management strategy. Tasmanian seed potato sent interstate is now laboratory tested for PVS and PVX to ensure crops meet the requirements of

the National Standard for Certification of Seed Potato and therefore undergoes a higher level of scrutiny than seed in most other states.

1. Introduction.

For many years, Tasmanian seed potato stocks have been considered to be relatively free of viruses. This has been attributed to the geographic isolation of Tasmania and to the Tasmanian seed potato certification scheme, which was instituted in the 1930's (Taylor 2003). 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 Forth Farm Produce Ltd., detected *Potato virus S* (PVS) (Brunt *et al*. 1996a), *Potato virus X* (PVX) (Brunt *et al*. 1996b) and *Potato leaf roll virus* (PLRV).

A more comprehensive survey of the virus status of Tasmanian seed potato crops was undertaken through Horticulture Australia Ltd. project PT02037 (Hay *et al*. 2004). PVS was prevalent in a range of varieties of Tasmanian seed potato, occurring in 150/225 (66.7%) of crops with 131/225 (58.2%) of crops above the National Standard for Certification of Seed Potato of 1%. A higher mean incidence of PVS was detected in crops in the North East region of the state, perhaps reflecting earlier introduction of PVS into crops in this region, differences between regions in seed handling practices or potential aphid vector activity or the presence of more transmissible virus strains. PVX was less prevalent in Tasmania seed potato crops, occurring in 30/231 (13.0%) of crops tested, with 19/231 (8.2%) crops above the National Standard of 1%. PVX occurred in a limited range of varieties and at low incidence, with only 2 crops having an incidence above 10%. *Potato virus Y* (PVY) and PLRV occurred infrequently, in 2/26 (7.7%) and 1/28 (3.6%) crops respectively and occurred at low incidence, with no crops recording and incidence over 10%. These viruses have been reported previously in Tasmania. PVX and PLRV were reported in Tasmania in 1929, PVS in 1968 and PVY in 1981 (P. Cross, pers. comm.), but had been considered eradicated from the seed scheme. These viruses have also been reported from other states in Australia.

A comprehensive literature review of PVS and PVX and seed certification schemes was given in a previous report (Hay *et al*. 2004). PVS and PVX are widespread in potato growing regions around the world (Jones 1983; Brunt and Loebenstein 2001). PVS and PVX are generally considered benign viruses, having only a relatively small impact on yield of potato. In other countries, infections with PVS or PVX alone have been associated with yield losses of up to 0-20% and 15-20% respectively (Wright *et al*. 1976; Beukema and van der Zaag 1979; Brunt and Loebenstein 2001; Stevenson *et al*. 2001). However, in some situations, significant yield losses (up to 40%) have been reported in potato crops with co-infections of PVS and PVX (Rozendaal and Brust 1955; Wetter 1971; Wright 1977; Manzer *et al*. 1978; Stevenson *et al*. 2001). The effect of PVS on yield is likely to be dependant upon several factors including virus strain, variety of potato and geographical location.

PVS occurs as two groups of strains, the ordinary (PVS^O) and Andean (PVS^A) strains. Overseas, PVS is considered to be predominately spread by mechanical transmission during seed cutting operations and by plant contact in the field, often as a result of machinery passing over the crop (Franc and Banttari 2001). PVS remains infectious for several hours on various surfaces, and up to 120 hours on burlap (Franc and Banttari 1984). In addition, several strains of PVS are spread inefficiently, and in a non-persistent manner by some species of aphids. *Myzus persicae* Sulzer (Green Peach Aphid) and *Aphis nasturtii* Kaltenbach (Buckthorn aphid) have been shown to be vectors of PVS (Bode and Weidemann 1971, MacKinnon 1974, Kostiw 1975, Santillan 1979, Slack 1983, Weidemann 1986, Wardrop *et al*. 1989, Weidemann and Koenig 1990, Fletcher 1996). Wardrop *et al*. (1989) reported *Aulacorthum solani* Kaltenbach and *Rhopalosiphum padi* Linnaeus were not vectors of PVS. The pea aphid, *Acyrthosiphum pisum* Harris is also reported as a vector of PVS (Stufkens and Teulon 2001). It is likely that similar mechanisms of transmission operate in Tasmania. However, little is currently known about the rate of spread that occurs in the field in Tasmania or the relative contribution of seed cutting, or field transmission e.g. by aphids, on farm equipment or plant to plant contact.

The transmission of PVX occurs mainly by contact between infected and healthy plants by mechanical inoculation of sap (Becks 1970). Sap containing PVX may be carried on skin, clothing, animal fur and farm equipment and be transmitted following contact with healthy potato plants (Beemster and de Bokx 1987). Wright (1974) reported PVX to remain infectious on painted wood, cotton and jute for 6 hours and in soil for 24 hours. Infection may occur during storage as a result of contact between sprouts of infected tubers and non-infected tubers (Brunt and Loebenstein 2001). Several other methods of mechanical transmission have been reported, although their importance is considered to be minimal. Transmission of PVX does not occur through pollen or true seed. PVX is not aphid transmitted, and no specific insect vectors are known. However, chewing insects can transmit PVX including grasshoppers *Tettigonia viridissima* (Brunt and Loebenstein 2001) and *Melanoplus differentialis* (Brunt and Loebenstein 2001). This is believed to occur through retention of virus on mouthparts and mechanical transmission of infected sap to healthy plants (Becks 1970; Beemster and de Bokx 1987). Fungal zoospores of *Synchytrium endobioticum* have also been reported to transmit PVX (Brunt and Loebenstein 2001). In the field, root contact may provide a supplementary means of transmission, although it is considered potentially unimportant (Roberts 1950; Brunt and Loebenstein 2001). PVX has also been transmitted experimentally by the parasitic plant dodder (*Cuscuta campestris*) (Ladeburg *et al*. 1950 cited in Brunt and Loebenstein 2001). Mechanically transmitted viruses such as PVX may also be transmitted in aerosols (Banttari and Venette 1980). Infectious aerosols of PVX were obtained when injured infected tomato and potato plants were subjected to air-blast and water-blast in an enclosed chamber. Although aerosols containing PVX were not detected in a field of highly infected var. Russet Burbank potato, Banttari and Venette (1980) suggested that PVX may be naturally spread in aerosols during more severe weather events including hail, driving rain and wind.

Weed hosts may constitute a potential source of infection for PVS or PVX. The host range of PVS includes *Chenopodium* species such as *C. quinoa* and *C. amaranticolor* (Hinostroza-Orihuela 1973; Brunt and Loebenstein 2001).

PVX has a limited natural host range constrained mainly to solanaceous species (Becks 1970) such as *Solanum nigrum*, *S. tuberosum*, *Nicotiana* spp., *Petunia hybrida*, *Datura stramonium*, *Cyphomandra betacea* and *Lycopersicon esculentum* (Brunt and Loebenstein 2001; Ali and Hassan 2002).

Some viruses of potato such as *Potato virus Y* (PVY), *Potato leafroll virus* (PLRV) and *Tomato Spotted Wilt Virus* (TSWV) produce conspicuous symptoms in infected potato plants in the field. There is therefore opportunity to identify infected crops during the visual inspections undertaken during Seed Certification. There is also opportunity to reduce incidence in the crop by roguing. By contrast, PVS and PVX cause little, if any, visual symptoms (depending on the virus strain) and can only be reliably detected by laboratory techniques. Therefore levels of PVS and PVX can increase within each generation of seed potato without being detected. The Australian National Standard for Certification of Seed Potato currently relies upon visual inspection only, and does not require grow-out test from a sample of tubers as is required by a number of overseas certification schemes.

While PVS is considered to have little impact on potato yield and quality, its presence is of concern to the Tasmanian industry. Management strategies have been implemented to eradicate viruses from Tasmanian seed potato crops. All viruses detected in Tasmania have been recorded previously in other states. Tasmanian seed potato sent interstate is now laboratory tested for PVS and PVX to ensure crops meet the requirements of the National Standard for Certification of Seed Potato and therefore undergoes a higher level of scrutiny than seed in most other states.

The purpose of this project (PT03069) was to build upon previous work by carrying out an audit of current seed handling practices and provide further recommendations to growers to control these viruses. In addition, a survey of second field generation (G2) crops was undertaken in Tasmania to monitor the success of preliminary management strategies. Additional research was conducted to assess the importance of in-field spread compared to

mechanised seed cutting, identify sources of virus into crops, determine the strains of virus present and the principal means by which they are transmitted. This information was used to refine the management strategy and to develop the most cost-effective means of eliminating viruses from Tasmanian seed potato crops.

The project was overseen 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, Department of Primary Industry Water and Environment, Tasmanian Institute of Agricultural Research and Tasmanian Farmers and Graziers' Association.

2. Monitoring of the virus status of G2 crops.

2.1 Introduction and method

A survey of virus incidence in G2 sown seed potato crops was undertaken in Tasmania during the 2003/2004 season. Samples were collected between late January and early April 2004. Leaf samples (between 50-300) were collected from each of the crops by DPIWE certification officers and sent to the laboratory for analysis by enzyme linked immunosorbent assay (ELISA) (Clark and Adams 1977). When over 100 samples were obtained, samples were bulked tested in lots of 10 for ELISA and virus incidence estimated as described by Gibbs and Gower (1960). When less than 100 samples were obtained, samples were tested individually. In total, 112 crops were tested for PVS, and 108 crops for PVX. The ELISA testing procedure is described in Appendix 1. In addition a small number of crops were tested for PVY.

2.2 Results

Potato virus S

PVS was detected in 49/112 (43.8%) of crops tested, with 42 of these crops (37.5%) above the National Standard for Certification of seed potato (Table 1). However, the majority of crops had low to moderate incidence with 105/112 crops (93.8%) having either no detectable PVS or less than 25% incidence (Table 1). PVS was detected in vars. Russet Burbank, Ranger Russet and Pink Eye, with a few crops having complete infection (Table 2). PVS was not detected in var. Bintje, although only a small number of crops were surveyed. In addition, PVS was detected in some other less common varieties (Table 3).

Potato virus X

PVX was detected in 6/108 (5.6%) of crops tested (Table 1). Only 4 crops (3.7%) were above the National Standard for Certification of Seed Potato, and all but one had an incidence of 10% or below. The remaining crop had

Table 1. Survey of seed potato crops for PVS and PVX during 2003/2004.

	Virus	
	PVS	PVX
Number of crops with:		
No detectable virus	63	102
0-1%	7	1
1-10%	21	4
11-25%	14	0
26-50%	1	0
51-99%	0	0
100%	6	1
Total crops surveyed	112	108
Average incidence (%)	8.7	1.0
Std. deviation (%)	22.8	9.7

Table 2. Incidence of PVS and PVX in common potato varieties during 2003/2004.

	Potato variety:				
	Bintje	Pink Eye	Russet Burbank	Ranger Russet	Other[1]
PVS					
Total crops tested	4	6	41	11	42
Number of crops with:					
No detectable virus	4	3	12	5	31
0-1%	0	0	3	2	2
1-10%	0	1	13	0	7
11-99%	0	0	11	2	2
100%	0	2	2	2	0
Mean incidence (%)	0%	33.5%	11.6%	21.4%	1.4%
Std. deviation	0%	51.5%	22.0%	39.5%	3.4%
PVX					
Total crops tested	4	5	39	9	42
No. crops with:					
No detectable virus	4	4	34	9	42
0-1%	0	0	1	0	0
1-10%	0	0	4	0	0
11-99%	0	0	0	0	0
100%	0	1	0	0	0
Mean incidence (%)	0%	20.0%	0.2%	0%	0%
Std. deviation	0%	44.7%	0.6%	0%	0%

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

complete infection (Table 1). PVX was detected in vars. Russet Burbank and Pink Eye, with a few crops having complete infection. PVX was not detected in vars. Russet Ranger or Bintje, although only a small number of crops were sampled (Table 2). PVX was not detected in some other less common varieties (Table 3)

Potato virus Y

A small number of crops with symptomatic plants were tested for PVY. PVY was detected in 3 of 8 crops tested, at incidences of 0.3%, 7.3% and 23.7%. Symptoms of PVY were not noted in any other crops during certification in the 2003/2004 season.

Table 3. Presence of virus in less common potato varieties (+ = detected, - = not detected).

Variety	No. crops tested	PVS	PVX
Bismark	1	+	-
Carrera	1	-	-
Granola	1	-	-
Innovator	1	-	-
King Edward	1	-	-
Mondial	1	+	-
Nicola	1	+	-
Red Rascal	2	-	-
Red Star	1	-	-
Rodeo	1	-	-

2.3 Conclusion

The generally low prevalence (percentage of crops infected) and incidence (mean percentage of plants infected within a crop) of PVX in G2 crops is encouraging and suggests a good prospect of eliminating this virus from the seed scheme in the short term. The prevalent nature of PVS and the moderately high incidence in G2 crops is of concern as there is opportunity for

the virus to increase during seed cutting, mechanical operations or potentially aphid vectors in succeeding generations. This suggests that the elimination of PVS from the seed scheme will take longer and will be more difficult to achieve. Despite this, some 56% of crops had no detectable PVS indicating that current management strategies are capable of maintaining crops free of detectable virus at least during the early field generation stages. Crops with PVY were eradicated from the seed scheme.

3. Audit of seed handling practices.

Dr. Roger Jones of Agriculture Western Australia visited Tasmania to discuss control of viruses in seed potato with the local industry. Dr. Jones had a unique perspective, being involved in the seed potato scheme in Western Australia (Wilson and Jones 1990), which has led to a large reduction in incidence of viruses, including PVS and PVX. Dr Jones visited with fresh and processing seed potato growers, staff of McCain Foods Australia, Simplot Australia Pty. Ltd., Harvest Moon Forth Farm Produce, three of the major storage and seed cutting operations and researchers and DPIWE certification officers. Dr. Jones and Dr. Iain Kirkwood of DPIWE also toured the major seed growing areas in the state (north west coast, north east and south). Dr. Jones gave presentations in Devonport (2), Scottsdale (1) and Hobart (1) on the control of potato viruses as part of his itinerary (Appendix 2). Dr. Frank Hay also gave presentations (2) in Devonport on the findings of the HAL project PT02037 during the 2002/2003 season. Dr. Jones' visit engendered considerable interest and discussion and served to highlight some of the weaknesses in the operation of the current seed scheme that may be contributing to virus increase in seed potato in Tasmania. In addition to his presentations and discussions, Dr. Jones made a series of recommendations that have been circulated to the industry (Appendix 3). In addition, Dr. Jones recently published an article detailing the key strategies that can be used to control viruses on farm (Jones 2004). Many of these recommendations have been adopted by the local industry.

4. Characterisation of PVS strains.

4.1 Introduction

Strains of PVS

Two major groups of strains of PVS are recognised, the ordinary (PVS^O) and the Andean strains (PVS^A) (Brunt 2001). Until the 1980s, it was considered potato cultivars infected with PVS worldwide contained only ordinary strains of PVS (Wetter 1971). These isolates were characterised as causing only local lesions following mechanical inoculation to the indicator host *Chenopodium quinoa*. In the early to mid 1980s novel strains of PVS were reported in Holland (Rose 1983), and the USA (Slack 1981; Jones 1983). These strains and South American sourced strains of PVS were found to cause both local lesions and systemic symptoms when inoculated into *Chenopodium quinoa* (Hinostroza-Orihuela 1973; Brunt and Loebenstein 2001). The term 'Andean strains' (PVS^A) has become generally used to describe those strains of PVS which are systemic in *C. quinoa* and 'ordinary strains' (PVS^O) to describe those which cause only local lesions (Jones 1981, Slack 1983, Dolby and Jones 1987). The introduction and subsequent establishment of PVS^A outside the Andes may have resulted from escape from imported potato germplasm (Jones 1983). Both PVS^A and PVS^O are readily transmitted mechanically by plant to plant contact, however PVS^A has been shown to be more easily transmitted by aphids than PVS^O (Hinostroza-Orihuela 1973; Slack 1981; Brunt and Loebenstein 2001).

In addition to several South American countries (Hinostroza-Orihuela 1973, Santillan 1979), PVS^A has also been reported in the Netherlands (Rose 1983), the U.S.A. (Slack 1981; Jones 1983), the U.K. (Slack 1981), Germany (Slack 1983; Dolby and Jones 1987) and New Zealand (Fletcher 1996, Fletcher *et al*. 1996). In New Zealand, PVS^A was transmitted at low efficiency by aphids, suggesting that mechanical transmission was more important (Fletcher 1996, Fletcher *et al*. 1996). PVS^A has also been shown to infect Pepino (*Solanum muricatum*) and infection of this host has been reported in

the Netherlands and New Zealand (Jones 1983; Brunt and Loebenstein 2001). In addition, PVS^A has occasionally been reported in several weed host species including *Solanum chacoense*, *S. brevidens*, *S. spegazzini* and *S. dulcamara* (Valkonen *et al.* 1992 cited in Brunt and Loebenstein 2001).

Molecular differentiation of PVS strains

Foster *et al*. (1990) used cDNA solution hybridisation to compare sequence homologies between RNAs of 5 isolates of PVS^O and 4 isolates of PVS^A. They found a high degree of sequence homology (90-100%) between the majority of PVS^O and PVS^A isolates. MacKenzie *et al*. (1989) sequenced the 3' terminal region of PVS^A. Weidemann and Koenig (1990) compared isolates of PVS^A from the Andean region and from Germany that were able to infect *C. quinoa* systemically. Isolates were unable to be differentiated by the serological technique, ELISA, indicating that they were serologically closely related. However, differences were noted between the isolates using quantitative cDNA hybridisation. Weidemann and Koenig (1990) also demonstrated biological differences between strains, with PVS^A from the Andes successfully aphid-transmitted by *Myzus persicae* (Sulz.) to 36/426 (8.5%) of *Solanum demissum* plants, while the strain from Germany was transmitted to only 5/210 (2.4%) of plants in aphid transmission studies. Dolby and Jones (1987) also showed different symptomatology of isolates in test plants and potatoes. Weidemann and Koenig (1990) concluded that the use of the term 'Andean strain' was somewhat misleading as it consisted of a number of heterogeneous strains.

Matoušek *et al*. (2000) used more sensitive molecular techniques to investigate the sequence variability of Central European isolates of PVS^O, which were unable to develop systemic infection in *C. quinoa*. A phylogenetic tree based on the coat protein amino acid sequences demonstrated greater than 96.7% homology between isolates. The 3' terminal portion of one of these isolates was sequenced and was shown to have 81.4% homology with PVS^A, along with some shifts in open reading frame patterns. The most significant differences were at the N-terminal regions of the 7-kDA protein, coat protein and 11-kDA protein encoded by the 3' terminal region of the

genome, suggesting these could be responsible for differences in virus movement and symptom development between PVS^O and PVS^A .

Host plants

Although the natural host range of PVS is very limited, experimental transmission can be attained by mechanical inoculation to many species including in excess of 56 additional solanaceous species and 33 species from 12 different families (Brunt and Loebenstein 2001). Symptoms induced by PVS infection on particular indicator species are outlined (Table 4).

Table 4. Indicator species of potato virus S (PVS) (adapted from Brunt and Loebenstein 2001; Fletcher 1996).

Experimental Host	Symptoms
Chenopodium album,	Chlorotic local lesions, often with green halo on older leaves.
C. amaranticolor	Chlorotic local lesions and PVS^A inoculation result in systemic necrotic lesions and yellow vein banding
C. quinoa	PVS^O induces chlorotic local lesions, often with green halo on older leaves. PVS^A inoculations result in systemic infections and characteristically induce chlorotic spotting.
Cyamopsis tetragonoloba	Small brown necrotic lesions in inoculated cotyledons, but no subsequent systemic infection.
Lycopersicon esculentum	Symptomless systemic infection by PVS^A but immune to PVS^O
Nicotiana clevelandii	Conspicuous chlorosis of systemically infected leaves. Use host for maintenance and propagation of virus cultures.
Nicotiana debneyi	Symptomless local infection but vein-clearing, mottling and necrosis of systemically infected leaves. Useful in the separation of PVS and PVM (immune to PVM).
Solanum rostratum	Numerous small necrotic lesions in inoculated and systemically infected leaves.
Nicotiana benthamiana	Symptomless infection

A study was undertaken to determine what strains of PVS were present in Tasmania.

4.2 Materials and methods

Indicator plants

Chenopodium quinoa seedlings at the 8-10 leaf stage were placed in the dark for 24 hours prior to inoculation. PVS isolates were obtained from fresh leaf samples taken from potato plants that tested positive to PVS by ELISA (Appendix 1). Leaf samples were macerated in 10 ml phosphate buffer with a mortar and pestle. Phosphate buffer contained 13 ml of 0.2 M solution (31.2g $NaH_2PO_4.2H_2O$, made up to 1 L with distilled water) and 87.0 ml of a 0.2 M solution (28.39g Na_2HPO_4, made up to 1 L with distilled water), diluted to a total of 200 ml to give pH 7.6. Leaves of *C. quinoa* were dusted with celite abrasive. Ground PVS sample in phosphate buffer was rubbed liberally onto three leaves of five separate *C. quinoa* plants for each PVS isolate. Plants were placed in the dark for 24 hours then maintained in the greenhouse for 24 days. Plants were visually scored at four-day intervals for lesion development on inoculated leaves and symptoms on upper leaves. Control plants were inoculated with phosphate buffer only.

Leaf samples from three inoculated leaves and three top leaves were collected from each inoculated *C. quinoa.* The top half of each leaf 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 1-3 cm long) was cut from the cut surface running at right angles to the veins. Scissors were sterilised in 4.2 g/l sodium hypochlorite and wiped clean between each individual leaf. Each leaf sample was crushed between two rollers in a motorised leaf press and tested for virus by ELISA (Appendix 1).

Molecular characterisation of strains

A molecular method of differentiating strains of PVS was developed as part of a University of Tasmania Institutional Research Grants Scheme project. This was based on a reverse transcriptase polymerase chain reaction (RT-PCR) technique similar to that developed by Heldak (2001). RNA extraction was conducted using the Qiagen Plant Mini Kit according to manufacturer protocol. PCR primers were designed from published sequences of PVS on Genebank to produce an 863 bp amplicon from the coat protein region of the PVS genome. RT-PCR was conducted using a Qiagen One-Step RT-PCR kit. Computer analysis of restriction enzyme sites in the amplicon was conducted which identified a set of restriction enzymes that would differentiate between the ordinary strains (PVS^O) and Andean strains (PVS^A) of the virus. Incubation of PVS c-DNA with these restriction enzymes and subsequent gel electrophoresis (restriction fragment length polymorphism – RFLP) was predicted to differentiate strains on the basis of banding patterns.

4.3 Results and conclusions

Sixteen strains of PVS were inoculated onto *C. quinoa* in a series of experiments. Eight strains showed no systemic symptoms in upper leaves and leaves above inoculated leaves tested negative to PVS by ELISA. These would be considered ordinary strains (PVS^O). A further eight strains produced a few systemic symptoms and upper (non-inoculated) leaves tested positive to PVS by ELISA. These would be considered Andean strain (PVS^A). However, all 10 isolates tested by the RT-PCR RFLP technique were identified as PVS^O. This included some isolates that were systemic in *C. quinoa*. This suggested that the inoculation of *C. quinoa* with PVS might not be a robust method of differentiating strains. Further strain characterisation will be undertaken during 2005 to better determine the strain variants of PVS in Tasmania. However pending further investigation, results suggest that only PVS^O strains are present in Tasmania.

5. Determining the importance of in-field spread of virus.

5.1 Introduction

Eliminating PVS and PVX from potato seed lines and maintaining virus-free seed as part of a Certification Scheme is a costly and difficult process. This study aimed to assess the rates of spread of PVS and PVX in the field and to determine the main mechanisms of spread. This information is important to assist the Tasmanian potato industry in developing the most cost-effective strategy for control of these viruses.

5.2 Materials and methods

Trials were established within two certified seed potato fields (G4) containing the potato variety Russet Burbank. One field had moderate incidence of PVS and trace PVX ('PVS field') and the other had low incidence of PVX and trace PVS ('PVX field'). Fields were planted on 10/11/2003 (PVS field) and 20/11/2003 ('PVX field'). The PVS field was located at Riana and the PVX field at South Riana, located on the north west coast of Tasmania. Commercial recommendations were followed for planting, fertiliser application and weed control at each field.

To identify suitable locations within the fields for trials, an initial 20 leaf samples were collected from each of 20 random locations within each field at an early growth stage of the crop and tested by ELISA (Appendix 1).

Trial layout

Two different trials were conducted within each field to examine spread of virus. The first trial consisted of a section of the field divided and marked into 49 plots arranged in a 7 x 7 lattice. At the PVS field, each plot consisted of 10 rows wide X 10 m long (8 m wide X 10 m long), with approximately 500 plants/plot. At the PVX field, each plot consisted of 9 rows wide X 10m long (8.3 m wide X 10 m long), with approximately 450 plants/plot. Twenty leaf samples from each of the 49 plots were tested for PVS and PVX by ELISA, at two (PVS field) and three (PVX field) intervals during the season (below).

The second trial within each field consisted of a primary transect marked along a row at 1 m intervals over a total distance of 50 m. Three rows either side of the primary transect were marked in a similar manner and one leaf sample was collected at each 1 m interval along all seven rows (starting at position 0). Samples were collected at two times (PVS field) and three times (PVX field) during the season and tested for viruses by ELISA (below).

Virus testing

In the plots in each field, a single leaf was taken from 20 arbitrarily chosen plants within each of the 49 plots at each sampling period and leaves were virus tested by ELISA (Appendix 1). Leaf samples were collected from plots at the PVS site on 06/01/04 at 58 DAP when plants were approximately 25-35 cm tall and on 17/3/04 (129 DAP) when plants were starting to yellow prior to senescence. Leaf samples from the PVX site were collected on 14/01/04 (56 DAP) when plants were 10-15 cm high, on 03/02/04 (76 DAP) just prior to row closure, and on 30/03/04 (132 DAP) prior to senescence. Leaves were either stored at 5^{o}C for no more than 5 days prior to virus testing or frozen at -20^{o}C for no more than three weeks before virus testing.

In the transects in each field, single leaf samples were collected from 1 m intervals along transects at the PVS field on 58 DAP (06/01/04) and 130 DAP (18/03/04). Transects were sampled at the PVX field at three intervals; 58 DAP (16/01/04), 77 DAP (04/02/04) and 133 DAP (31/03/04).

Examination of stunted plants

At the PVX site, leaves were collected from an additional 20 plants exhibiting symptoms of stunting and from 10 plants considered to be healthy. Samples were collected on 03/02/04 (76 DAP). Leaves were tested by ELISA for both PVS and PVX (Appendix 1).

Weed species

A total of 165 potential weed hosts of 14 different weed species were collected from within and along irrigation runs and from the edge of the field and along the headlands at the PVX field at 77 and 133 DAP. Leaves were tested by ELISA as potential sources of virus inoculum.

Spatial analysis

Spatial analysis was conducted to determine the spatial pattern of infected plants and therefore develop hypotheses on how PVS and PVX are being spread in Tasmania. The incidence of viruses in the plots in each field at each sampling time was statistically analysed by the method of Spatial Analysis by Distance IndicEs (SADIE) (Perry 1985). Briefly, through a series of simulations, SADIE calculates the minimum distance that infected plants would need to be moved to achieve a regular pattern across the sample area and from this determines the Index of aggregation (I_a). Significant I_a values greater than unity indicate spatial aggregation or a clumped distribution of infected plants. Spatial distribution of virus infected plants in transects was analysed by Ordinary Runs Analysis (ORA) (Gibbons 1976). A run was calculated as 'a succession of one or more plants of like status (healthy or infected) followed and preceded by plants of opposite status', in which a calculated Z statistic of less than –1.64 (P=0.05) indicates non-random (aggregation) of infected plants. Spatial analysis can provide insights into whether the pattern of infected plants is aggregated, suggesting short-range mechanical transmission, or random suggesting planting of infected seed pieces or perhaps aphid transmission. Due to constraints of the statistical technique, spatial analysis was only conducted when virus incidence was greater than 5% and less than 95%.

Effect of virus on crop growth

To objectively assess whether virus was having an effect on the growth of potato during the season, each plot in both fields was assessed with a hand-held multi-spectral radiometer (Cropscan® MSR5; Rochester, Minnesota). The radiometer works on the principle that every substance transmits, emits, absorbs or reflects electromagnetic radiation in its own characteristic manner. Reflectance from the crop canopy in the near infrared spectrum (esp. 750-900 nm) has been found to be closely correlated with the green leaf area index of a crop and useful for assessing the effect of various stresses, such as that caused by disease. Reflectance was measured in the plots in each field. Measurements were taken at the PVS field on 22/03/04 (134 DAP) when the crop was nearing senescence, leaves were beginning to yellow and plants had reduced in height to approximately 40 cm. At the PVX field, measurements were taken on 05/03/04 (117 DAP), when plants were approximately 50 cm in height and were approaching row closure. Percentage reflectance was recorded between 12-2 pm at wavelengths of 485, 560, 660, 830, 1650 nm. Measurements were taken from a height of 2 m above the soil at five arbitrarily chosen locations in each plot. At this height, canopy reflectance was measured from 1 m diameter areas ($0.8m^2$). Plots had minimal weed growth at these times. Percentage reflectance values were expressed as a percentage of the voltage value for the reflected radiation divided by the voltage values for the incident radiation. The reflectance at certain wavelengths was used to calculate the Weighted Difference Vegetation Index or WDVI (see Table 14), which has been demonstrated to be a good indicator of foliar growth of potato (Bouman *et. al*. 1992). The correlation coefficient (r) was calculated between the reflectance values at each wavelength in each plot and virus incidence in each plot, to determine whether virus was having an effect on plant growth. Where the correlation coefficient was statistically significant, regression analysis was conducted.

Aphid monitoring

Two yellow sticky aphid traps (9.5 x 23.0 cm) were placed at the edge of each field facing outward into the prevailing wind to monitor flights of alatae (winged) aphids. Aphid traps were placed on the 28/01/04 (80 DAP for the

PVS field and 70 DAP for the PVX field) and mounted on a peg with the aphid trap approximately 1m above ground level. Traps were retrieved and replaced on an approximately weekly basis until 07/04/04. Traps were wrapped in cling film and stored in a cool store (10^{o}C) until processed for aphid identification. Traps were processed by Cathy Young (Department of Primary Industries Water and Environment, New Town Laboratories, Hobart). Sticky traps were initially scanned under a dissecting microscope to locate any aphids present on the trap surface. Aphids were removed by soaking sections of the trap containing aphids in a dipentine-based solvent (DeSolvit™). Aphids were identified using keys from Blackman and Eastop (2000) and by comparison with specimens held in the insect collection at the DPIWE, New Town, Tasmania. Voucher aphid specimens were preserved in ethanol-lactic acid and stored at DPIWE.

5.3. Results

Virus incidence (PVS field)

Virus incidence in the plots at the PVS field was high and showed a slight decrease (4.9%) between 58 and 129 DAP (Table 5). PVX was detected in only one plant of the 980 tested at 129 DAP (Table 5). The incidence of PVS in the transects was high, and increased slightly (4.2%) between 58 and 129 DAP (Table 6). PVX was not detected in the transects at either time interval (Table 6).

Table 5. Incidence of PVS and PVX in var. Russet Burbank seed potato from 20 leaf samples collected from each of the 49 plots at the PVS field at 58 and 129 days after planting (DAP).

Sample Time	Total Number of PVS Infected Plants	Total Number of PVX Infected Plants
58 DAP	492[1] (50.2%)	0[1]
129 DAP	444[1] (45.3%)	1[1]

[1] 980 leaves tested at each time interval

Table 6. Incidence of PVS and PVX in var. Russet Burbank seed potato in transects at the PVS field recorded at 58 and 130 days after planting (DAP).

Sample Time	Total Number of PVS Infected Plants	Total Number of PVX Infected Plants
58 DAP	189[1] (52.9%)	0[1]
129 DAP	204[1] (57.1%)	0[1]

[1] 357 leaves tested at each time interval

Virus incidence (PVX field)

The incidence of PVX in plots declined slightly between 56 and 76 DAP, then increased slightly to 7.7% at 132 DAP (Table 7). The incidence of PVS remained low at all sample times, with minimal increase in incidence (Table 7).

The incidence of PVX infected plants in the transects increased slightly (by 1.1%) between 58 and 77 DAP and by a further 1.1% between 77 and 133 DAP (Table 8). PVS occurred at low incidence in the transects and increased from 0.3% to 1.1% between 58 and 133 DAP (Table 8).

Table 7. Incidence of PVS and PVX in var. Russet Burbank seed potato from 20 leaf samples collected from each of the 49 plots at the PVX field at 56, 76 and 132 days after planting (DAP).

Sample Time	Total Number of PVS Infected Plants	Total Number of PVX Infected Plants
56 DAP (Time 1)	5[1] (0.5%)	53[1] (5.4%)
76 DAP (Time 2)	0[1] (0.0%)	42[1] (4.3%)
132 DAP (Time 3)	6[1] (0.6%)	75[1] (7.7%)

[1] 980 leaves tested at each time interval

Table 8. Incidence of PVS and PVX in var. Russet Burbank seed potato collected from transects at the PVX field at 58, 77 and 133 days after planting (DAP).

Sample Time	Total Number of PVS Infected Plants	Total Number of PVX Infected Plants
58 DAP (Time 1) [1]	1 (0.3%)	22 (6.2%)
77 DAP (Time 2)	1 (0.3%)	26 (7.3%)
133 DAP (Time 3)	4 (1.1%)	30 (8.4%)

[1] 357 leaves tested at each time interval

Examination of stunted plants

PVS was not detected in either stunted or healthy plants collected from the PVX field. PVX was detected in 2/20 stunted and 1/20 healthy plants. This indicated no apparent relationship between these viruses and stunting at this site.

Virus status of weed species

Neither PVS nor PVX were detected in any weed leaf samples (Table 9). *C. album* was the most common weed species at the PVX field at 77 DAP. Weed samples were also collected at the PVX field at 133 DAP. Of the five different weed species tested, none were positive for either PVS or PVX (Table 10).

Table 9. Virus status of weed species collected from the PVX field within and near plots and irrigation runs at 77 days after planting.

Species	Common name	No. samples	PVS	PVX
Chenopodium album	Fat Hen	73	-	-
Solanum nigrum L.	Black Nightshade	11	-	-
Polygonum persicaria L.	Redshank	28	-	-
Rumex acetosella L.	Sorrel	8	-	-
Sisymbrium officinale (L.) Scop.	Hedge Mustard	31	-	-
Fagopyron esculentum	Buckwheat	2	-	-
Trifolium repens L.	Clover	4	-	-
Geranium dissectum L.	Cut-leaf Crainsbill	1	-	-
Carduus teniuflorus Curt. or *Carduus pycnoceohalus* L.	Slender Thistle	1	-	-
Coronopus didymus (L.) Sm.	Lesser Swincress	1	-	-
Senecio vulgaris L.	Groundsel	2	-	-
Sonchus asper (L.) Hill	Prickly Sow Thistle	2	-	-
Rumex crispus L.	Curled Dock	1	-	-

Table 10. Virus status of various weed species collected from the PVX field within and near plots and irrigation runs at 133 days after planting.

Species	Common nane	No. samples	PVS	PVX
Chenopodium album	Fat Hen	13	-	-
Solanum nigrum L.	Black Nightshade		-	-
Capsella bursa-pastoris (L.) Medic	Shepherd's Purse	3	-	-
Rumex acetosella L.	Sorrel	1	-	-
Trifolium repens L.	Clover	1	-	-

Spatial Analysis

In the PVS field, spatial analysis by SADIE indicated that the pattern of plants infected with PVS in the plots was random at 59 and 129 DAP (Table 11). In

the transects, the pattern of infected plants was random across rows at both time intervals, however significant aggregation occurred along rows (Table 12).

In the PVX field, spatial analysis by SADIE indicated that the pattern of plants infected with PVX in the plots was random at 56, 76 and 132 DAP (Table 13). Similarly, ordinary runs analysis demonstrated that PVX infected plants were distributed in a random pattern along and across rows in the transects (Table 12).

Table 11. Spatial analyses of incidence of PVS in var. Russet Burbank seed potato (PVS field) at intervals during the 2003/2004 season, using spatial analysis by distance indices (SADIE).

Sample Time	I_a	Probability
59 DAP (Time 1)	0.80	0.81[a]
129 DAP (Time 2)	1.18	0.17[a]

[a] The index of aggregation (*Ia*) was not significantly different from a value of 1, thus the null hypothesis of spatial randomness is accepted.

Table 12. Spatial analysis of PVS and PVX infected plants by Ordinary Runs Analysis (Z-statistic) along and across rows in transects in var. Russet Burbank seed potato at intervals during the 2003/2004 season.

	Days after planting	
PVS field	**56**	**129**
Across rows	1.02	-1.13
Along rows	-1.96[a]	-2.96[a]
PVX field	**56**	**132**
Across rows	1.49	-0.51
Along rows	0.56	0.51

[a] The calculated Z-statistic < -1.65, indicating non-random aggregation of plants (*P*=0.05).

Table 13. Spatial analyses of incidence of PVX in Russet Burbank seed potato (PVX field) at intervals during the 2003/2004 season, using spatial analysis by distance indices (SADIE).

Sample Time	I_a	Probability
56 DAP (Time 1)	1.22	0.1031[a]
76 DAP (Time 2)	1.02	0.3363[a]
132 DAP (Time 3)	1.21	0.1165[a]

[a] The index of aggregation (*Ia*) was not significantly different from a value of 1, thus the null hypothesis of spatial randomness is accepted.

Assessment of effects of virus on crop growth

At the PVX field, there was no significant correlation between percentage reflectance from the crop canopy at any wavelength at 117 DAP and the virus incidence within plots at 56, 76 or 132 DAP (Table 14).

At the PVS field, there was also no significant correlation between percentage reflectance at 134 DAP and incidence of PVS at 58 DAP. However at 134 DAP, there was a significant negative correlation between the percentage reflectance from the crop canopy at 560, 830, 1650 nm and the incidence of PVS in plots at 129 DAP (Table 14). The percentage reflectance from the crop canopy at these wavelengths was higher in plots with low virus incidence than those with high virus incidence. Regression analysis also showed a relationship between reflectance at 560, 830 and 1650 nm and incidence of PVS at 129 DAP (Figures 1 to 3). Although the R^2 values were low, there was a significant inverse relationship between reflectance and virus incidence. The WDVI at 134 DAP was also significantly correlated with incidence of PVS at 129 DAP (Table 14).

Aphid monitoring

Aphids were not detected on the yellow sticky traps until late in the development of the potato crops (Table 15). Some aphids were trapped between 3/3/2004 and 17/3/2004, with the majority occurring between

Table 14. Correlation coefficient (r) between percentage reflectance at different wavelengths from the crop canopy of 49 potato plots at 117 DAP (PVX field) or 134 DAP (PVS field) and incidence of PVX at three times (PVX field) or incidence of PVS at two times (PVS field).

Field	Days after planting	Wavelength (nm) 485	560	660	830	1650	WDVI[a] calculated with wavelengths: (830, 560)	(830, 660)
PVX field	56	0.18	0.09	0.13	0.03	0.07	0.01	0.02
	76	-0.09	-0.07	-0.09	-0.03	-0.03	-0.02	-0.04
	132	-0.17	0.12	-0.16	0.15	0.11	0.16	0.15
PVS field	58	n/a	-0.10	-0.23	0.07	-0.03	0.10	0.10
	129	n/a	-0.40 **	-0.24	-0.36 *	-0.43 **	-0.27	-0.32**

* P=0.05, ** P=0.01

[a]Weighted difference vegetation index (WDVI) calculated as NIRc – (NIRs/VIs) * VIc, where NIRc and NIRs are reflectance of crop and bare soil respectively at 830 nm, and VIc and VIs are reflectance of crop and bare soil respectively at either 560 nm or 660 nm

Figure 1. Regression between percentage reflectance at 560 nm at 134 DAP and percentage of plants with PVS in each of 49 plots at 129 DAP. P<0.001, R^2=0.16, adjusted R^2=0.14. Y=2.35 x 10^2 + -1.74 x 10^1 X

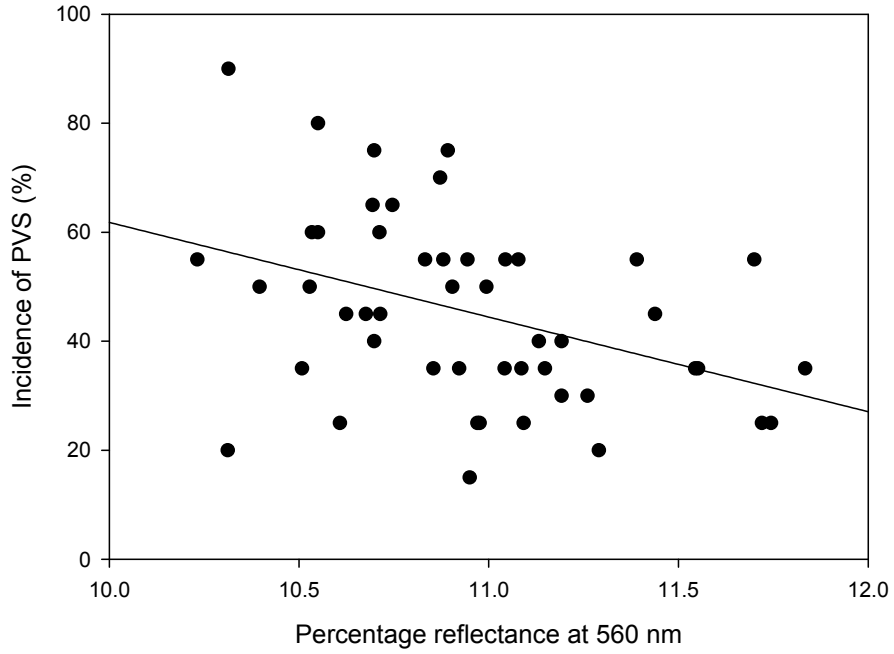


Figure 2. Regression between percentage reflectance at 830 nm at 134 DAP and percentage of plants with PVS in each of 49 plots at 129 DAP. P<0.001, R^2=0.13, adjusted R^2=0.11. Y=1.69 x 102 + -1.80 X

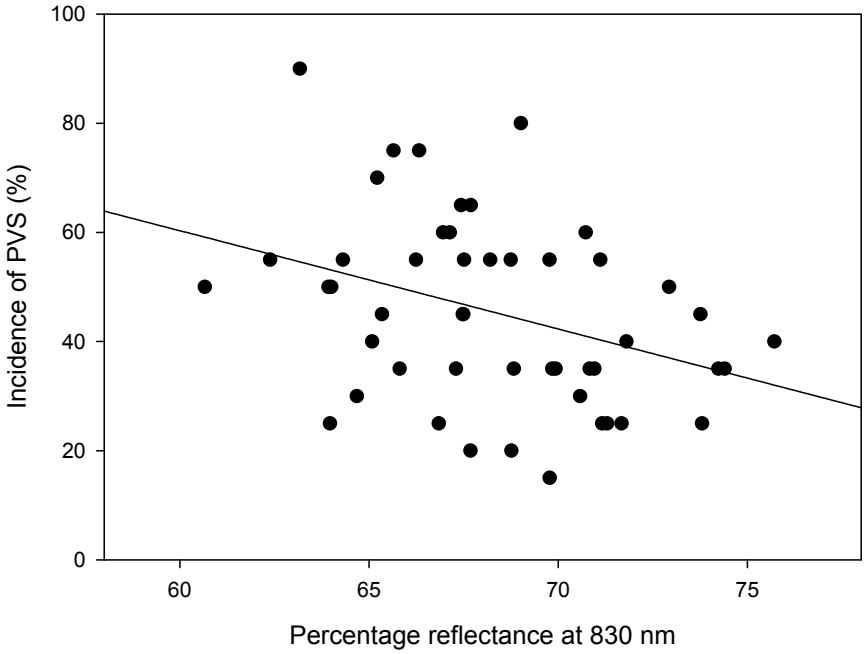


Figure 3. Regression between percentage reflectance at 1650 nm at 134 DAP and percentage of plants with PVS in each of 49 plots at 129 DAP. P<0.001, R^2=0.19, adjusted R^2=0.17. Y=2.56x10^2 + -1.08 x 10^1 X

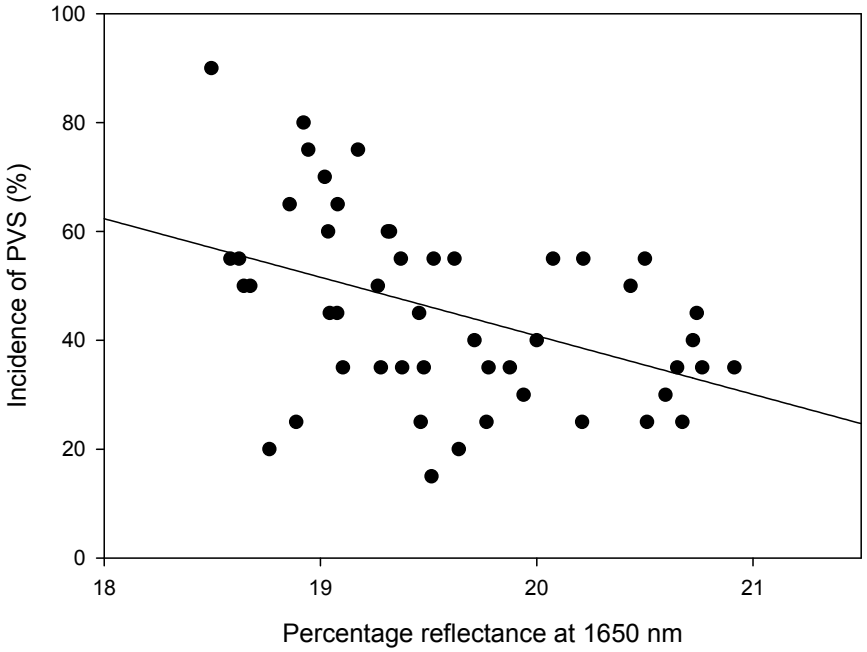


Table 15. Number of aphids trapped on two yellow sticky traps at the PVX and PVS fields during 2004.

		No. alatae aphids/trap	
Date placed	**Date retrieved**	**PVX field**	**PVS field**
3 February	10 February	0	0
10 February	17 February	0	0
17 February	25 February	0	0
25 February	3 March	0	0
3 March	10 March	1*	0
10 March	17 March	0	2*
17 March	23 March	0	0
23 March	1 April	1*	-
1 April	7 April	3**	28*

* = *Aphis gossypii*, **=2 *A. gossypii* + 1 *Macrosiphum euphorbiae*

1/4/2004 and 7/4/2004. The most common aphid trapped was the melon/cotton aphid *Aphis gossypii*, along with an individual *Macrosiphum euphorbiae*. These species are not known as vectors of PVS, but are known vectors of some other potato viruses. In addition, traps were retrieved from a third potato crop in the North East of Tasmania between 27/1/2004 – 2/2/2004, 2/2/2004 – 11/2/2004 and 11/2/2004 and 23/2/2004. No aphids were recovered from these traps.

5.4 Discussion

Virus spread

The results of trials in two fields in Tasmania during the 2003/2004 season indicated that there was only limited transmission of PVS and PVX in the field. Spatial analysis suggested that the pattern of infection was random and mostly remained random during the season. This is indicative of the planting of infected tubers, with no evidence of further mechanical spread during the season. In addition, there was no suggestion of PVS spread by aphids at either field. In the PVS field, ordinary runs analysis showed significant aggregation of infection along, but not across, rows. This suggested that

there was some spread of PVS between adjacent plants along rows, suggestive of mechanical spread. The difference across and between rows perhaps may be related to plants touching within rows at an earlier stage than between rows, leading to more opportunity for spread. PVS and PVX were not detected in any weed species within the PVX field, suggesting that weeds were also not virus sources at this site.

Overseas studies on spread of PVS and PVX

There have been several studies into the rate of reinfection of PVS and PVX into virus free potato crops and into the increase in virus incidence between field generations (Franc and Banttari 2001). Most studies have involved assessment of inter-generational re-infection rates, which makes it difficult to separate spread that occurred in the field with that which may have occurred during storage, seed cutting or planting. The findings of several studies have been summarised (Table 16).

For PVX, which is transmitted only by mechanical means, physical separation of virus free potato stocks from background sources of virus is very effective in reducing reinfection. This is illustrated by the studies of Cockerham (1958) and MacKinnon *et al*. (1972) (Table 16). Cockerham (1958) also showed that where plants with an incidence of approximately 1% PVX were grown in close proximity to infected plants, high rates of reinfection (82.9%) occurred in 3 generations (Table 16). However, where plants were grown in isolation, the incidence of PVX increased to only 4.8% in 3 generations. Cockerham (1958) suggested that the main means of transmission of PVX in his study was from plant to plant contact between infected plants surrounding the plots and plants within plots. However, implements used in cultural operations were also implicated in virus transmission.

Many studies have shown very high reinfection rates with PVS (Table 16). In general, reinfection rates of virus-free material with PVS are more rapid than for PVX, e.g. Bantarri *et al*. (1978), Manzer *et al*. (1978), Wardrop *et al*. (1989). Similarly, Omer and El-Hassan (1992) reported that the local var. Zalinge grown for many years in Sudan had a mean incidence of 83.1% PVS

in comparison to 15.2% PVX. The faster rate of spread for PVS could be due to the presence of more background sources of PVS inoculum in the surrounding environment in these studies. Alternatively it may indicate that PVS is more efficiently mechanically transmitted than PVX or that alternative means of transmission of PVS (e.g. aphid) are important in introducing and increasing this virus in potato crops. However, studies indicate that both PVX and PVS are spread readily by mechanical means, suggesting the importance of aphids in PVS transmission.

Table16. Summary of overseas research into field spread of PVS and PVX.

Authors	Location	No. generations	Varieties	PVS incidence (%)	PVX incidence (%)
Cockerham (1958)	U.K.	3	Var. Majestic with ca. 1% PVX planted in isolated plots	N/A	4.8%
			Var. Majestic with ca. 1% PVX planted in plots surrounded by PVX infected plants	N/A	82.9%
Bantarri et al. (1978).	USA	3	Various vars.	Norland (24%), Norchip (16%), Kennebec (15%), Norgold Russet (31%), Russet Burbank (32%)	Norland (1%), Norchip (trace), Kennebec (1%), Norgold Russet (Trace), Russet Burbank (6%)
Hahm et al. (1981)	USA	1 and 3	Norgold Russet and Ontario	59% (year 1) and 70% (year 3), low in vars. Monana and LaChipper	N/A
		1 and 3	LaChipper, Norchip & Norgold Russet	N/A	19% (year 1) and 55% (year 3), low in Kennebec, Monona, Norland and Superior
De Bokx (1972)	Netherlands	1	Eersteling with ca. 10% infection at planting	56-76	N/A
			Alpha with ca. 10% infection at planting	2-28%	N/A
Fletcher	New	1	Various varieties[1]	56%	19%

(1984)	Zealand				
Omer and El-Hassan (1992)	Sudan	Various generations	Alpha[1] from Holland	G0 (1.3%), G1 (1.9%), G2 (4.2%)	G0 (4.5%), G1 (7.2%), G2 (9.3%)
MacKinnon et al. (1972)	Prince Edward Island	3 years	5 varieties[1] at an Elite seed farm where virus infected potato also grown	ca. 10% for each variety	ca. 10% for each variety.
		2 years	8 varieties[1] at a farm in the absence of virus infected potato	4 vars. with no detectable virus, and 4 vars. with PVS (<5%)	0 %
MacKinnon (1974)	U.S.A	1 year	Netted Gem[1]	(12%)	N/A
		1 year	Various vars.	Green Mountain (57%), Kennebec (19%), Sebago (9%)	N/a
		1 year	Various vars.	Green Mountain[1] (14%), Kennebec (0%), Sebago (0%)	N/A
Sip (1974)	Czech Republic	1 year	Variety grown under insect-proof cage[2]	Trace	N/A
			Variety grown in open area but fenced to prevent human and animal activity	82.1%	N/A
Kamenikova (1978)	Czech Republic		Var. Jiskra[1] where only mechanical transmission possible	3.1% (year 1) and 34.4% (year 2)	N/A

			Var. Jiskra[1] where accessible to aphid vectors but not humans or animals	51.5% (year 1) and 90% (year 2)	N/A
			Var. Jiskra[1] where accessible to vectors only	32.7% (year 1) and 56.3% (year 2)	N/a
			Var. Jiskra[1] grown under 'ordinary' conditions in the vicinity of PVS infected plants	67-100% (year 1)	
Kostiw (2002)	Poland	1 (over 4 year period)	Var. Baszta	19% (mean of 4 years)	N/A
Fletcher et al. (1996)	New Zealand	3	Various vars. surveyed_pathogen tested (PT) and non-pathogen tested (non-PT)	Year 1: 0/16 PT crops, 0/4 non-PT crops, Year 2: 2/17 PT crops (1%-5%), 3/11 non-PT crops (7-80%), Year 3: 0/19 PT crops, 2/6 non-PT (15%-100%),	N/A
Franc and Banttari (1996)	U.S.A.	2	Various vars.	Norland[1] (71.8%), Russet Burbank (73.0%), Kennebec (29.5%)	N/A
Weidemann (1986)	Germany	1 (measured each year for 3 years)	Unknown variety[1] planted alongside infected	27% (year 1), 21% (year 2), 31% (year 3)	N/A

Wardrop et al. (1989)	U.S.A.	1	Var. Red Pontiac caged with PVS infected source plants and M. persicae	4% leaf infection (9 weeks), 100% of plants with 1 or more infected tubers, 87.3% tuber infection.	N/A
			Var. Red Pontiac (uncaged but with PVS infected source plants)	2% leaf infection (9 weeks), 14.6% of plants with 1 or more infected tuber, 46.2% tuber infection	
			Var. Red Pontiac caged with PVS infected source plants but no M. persicae	0% leaf and tuber infection.	

[1] Initially virus free or from pathogen tested schemes.

[2] Possibility for spread by leaf/root contact and on contaminated clothing and implements.

Table 17. Summary of studies into aphid transmission of *potato virus S*.

Author	Virus strain	Aphid species	Source of virus	Assay host	No. with virus/no. plants inoculated
Slack (1983)	PVS^{A}	*M. persicae*	Potato	Potato	7/69 (10.1%)
				Potato	0/30
			C. quinoa	*C. quinoa*	3/23 (13.0%)
			Purified	Potato	7/26 26.9%)
				C. quinoa	0/25
	PVS^{O}	*M. persicae*	Potato	Potato	0/103
				Potato	0/30
			Purified virus	Potato	0/29
Fletcher (1996)	PVS^{A}	*M. persicae*	Potato	*C. quinoa*	0/10
				Potato	0/10
	PVS^{A}	*A. solani*		Potato	1/10 (10%)
	PVS^{O}			Potato	0/10
	PVS^{A}	*M. persicae*		Potato	0/10
	PVS^{O}			Potato	0/10
	PVS^{A}	*M. persicae*	Purified	Potato	1/9^{1} and 2/9^{2} (11.1 and 22.2% respectively)
				C. quinoa	2/5^{1} and 2/5^{2} (40% each)
Weidemann and Koenig (1990) Weidemann (1986)	PVS^{A}				36/426 (8.5%)
	PVS^{O}				5/210 (2.4%) (2.9%)
Santillan (1979)	PVS^{A}	*M. persicae*		*C. quinoa*	Up to 40-50%

MacKinnon (1974)		*M. persicae*		*S. demissum*	3.4%
Wardrop et al. (1989)	PVS	*M. persicae* (alatae)	Potato	Potato	[3]RP=1/172 (5.9%), RB=0/17, Shep=1/16 (6.3%), Seb=1/15 (6.7%)
		M. persicae (apterae)			[3]RP=2/17 (11.8%), RB=0/17, Shep=2/16 (12.5%), Seb 2/14 (14.3%)
		A. nasturtii (alatae)			[3]RP=1/17 (5.9%), RB=0/17, Shep=0/16, Seb=0/15
		A. nasturtii (apterae)			[3]RP=1/17 (5.9%), RB=1/17 (5.9%), Shep= 1/16 (6.3%), Seb=0/14.

[1] Aphid feeding for 1 hour or overnight[2] on assay host

[3] RP=var. Red Pontiac, RB= var. Russet Burbank, Shep= var. Shepody, Seb= var. Sebago.

Reinfection rates with PVS have been shown to differ with season. For example, MacKinnon (1974) reported more PVS infection in three initially virus-free varieties in one year in comparison to the following year (Table 16). This perhaps reflects seasonal differences in aphid vector activity or in the amount of PVS inoculum.

There are also differences in reinfection rates of different potato varieties with PVS. This has been observed over a single season (e.g. MacKinnon 1974), suggesting that some varieties are more resistant to infection than others. Differences in reinfection rates between potato varieties has also been observed over a number of field generations (e.g. Bantarri *et al*. 1978, Franc and Bantarri 1996) (Table 16). Franc and Banttari (1984) noted differences between potato varieties in the efficiency of transmission of PVS during seed cutting that may explain some of the observed differences in reinfection rates between varieties over a number of generations. In addition some varieties may exhibit a greater level of adult plant resistance to PVS than others, leading to less rapid increase in PVS incidence between generations (Hahm *et al*. 1981).

The type of planting material can also influence the rate of reinfection with PVS. McDonald (1987) reported that PVS reinfection of initially virus-free material was significantly faster when plantlets were transplanted into the field, compared to plants propagated from tubers. Plantlets flowered 1-2 weeks later than plants from tubers and McDonald (1987) suggested that the faster onset of mature plant resistance in plants from tubers may have accounted for the difference in virus reinfection. Boiteau *et al*. (2000) also noted greater sensitivity of potato plants originating from plantlets or mini-tubers compared to plants from field grown tubers to aphid vectored viruses. They were unable to detect differences in aphid behaviour, activity or numbers between different types of planting material. Alatae of *Myzus persicae* were found to alight at greater numbers on older plants compared to younger plants regardless of origin. Boiteau *et al*. (2000) considered that where aphid vectors were present early in the season, the greater numbers of

aphids alighting on relatively older and larger transplants might contribute to higher virus infection, but that later in the season, plant physiological factors that affect virus multiplication and translocation might be important.

The importance of aphid transmission of PVS will depend upon the virus strain present and whether vector species are prevalent. Some ordinary strains of PVS are not aphid transmissible (Table 17), while some strains of PVS^O and all PVS^A strains are. The majority of laboratory studies have shown PVS to be inefficiently transmitted by aphids. However, in one study (Santillan 1979), some strains were transmitted at high efficiency (Table 17). Both alatae and apterae of *M. persicae* and *Aphis nasturtii* were demonstrated to transmit PVS, while *R. padi* and *Aulocorthum solani* were not (Wardrop *et al*. 1989). In field studies, the importance of aphid transmission has been variable, probably reflecting the presence of different strains. De Bokx (1972) stated that the pattern of infected plants indicated foliar contact was the main means of spread of PVS. Similarly, Fletcher (1984) suggested that mechanical transmission of PVS was more important than aphid transmission in New Zealand. However, in the Czech Republic, Sip (1974) and Kamenikova (1978) demonstrated that aphid transmission of PVS was more important than mechanical transmission (Table 17). Weidemann (1986) also demonstrated more transmission of PVS to widely spaced plants (60 x 100 cm) where plants barely touched than more narrowly spaced plants (37 x 62 cm) and suggested that aphid transmission may have played a greater role in transmission than contact between adjacent plants. Similarly, Wardrop *et al*. (1989) demonstrated very high levels of PVS infection in tubers from field grown plants when caged with *M. persicae* and PVS source plants in comparison to nil infection in plants exposed to a PVS source but caged to prevent aphid entry (Table 17). This suggested the importance of aphids in PVS transmission in comparison to plant to plant contact. Kostiw (2002) monitored virus incidence in crops over a four-year period in Poland and showed the greatest spread of PVS (and PVY and PVM) occurred between mid-July to early August. This perhaps reflected a time of aphid activity or of greatest plant susceptibility. Kostiw (1980) considered *A. nasturtii* to be a more effective vector of PVS (and PVM) than *M. persicae* in Poland. These

studies therefore indicate that aphid transmission may play a major role in reinfection and increase of PVS in healthy potato stocks in some situations.

Potential reasons for the slow in field spread of PVS and PVX in Tasmania.

Although many overseas studies have indicated that PVS and PVX can spread rapidly within and between seasons, the two field trials conducted in 2003/2004 in Tasmania suggest that there was limited spread of PVS and PVX within a season. A comparison with overseas studies, many of which are dated, is difficult. Methods of detecting viruses have improved greatly in recent years. In earlier trials, the lack of serological testing methods may have made it difficult to accurately assess the virus status of apparently 'healthy' material used in trials or to assess the subsequent spread. In addition, it is not apparent from many older studies what strains of virus were present and (in the case of PVS) whether mechanical transmission alone or in combination with aphid transmission was contributing to the measured rate of spread. Similarly, methods of growing seed potato have changed considerably and production methods differ between countries. In older trials, there may have been considerable mechanical operations through the crop for weed control and moulding of rows and ground spraying for weeds, insect pests and fungal pathogens. Such operations would have led to rapid spread of mechanically transmitted viruses through the crop. By contrast, in the two fields in our study in Tasmania there was minimal traffic, with no moulding of rows and the use of aerial spraying where required. At the PVS field, solid set irrigation was used, which further minimised possibilities for virus spread. At the PVX field, irrigation was by travelling irrigator. However, the laneways were unplanted and were sufficiently wide that contact between the irrigator and crop plants was minimised. The plots that were virus tested in this field lay between two irrigator laneways. However, there was no suggestion of increased virus spread in plots next to laneways, again suggesting that the travelling irrigator was not contributing to virus spread. The situation in Tasmania where there is minimal traffic (if any) through the crop during the season would be expected to contribute to only a slow rate of spread. This is also suggested by the study of Cockerham (1958) in Scotland, who

demonstrated that PVX spread in seed potato of cv. Majestic planted in isolated plots to prevent introduction of virus from outside sources, increased only slightly, from approximately 1% to 4.8% over a 3-year period. The PVX field in Tasmania was in a similarly isolated situation and had a similarly low rate of spread. By contrast, Cockerham (1958) showed that PVX incidence in plots of the same stock planted in plots surrounded by PVX infected potatoes increased from approximately 1% to 82.9% over the same 3-year period.

The slow rate of spread detected in the field in Tasmania during 2003/2004 is not consistent with the high incidence of PVS and PVX in some Tasmanian seed crops. This might suggest that other methods of transmission such as seed cutting are a more important contributor to virus increase between seasons. However, it should be noted that an initial trial during 2002/2003 failed to demonstrate transmission over a mechanical seed cutter. Results of further trials to investigate seed cutting are given in Chapter 6 of this report.

The lack of significant spread of PVS and PVX noted in the field trials in Tasmania could also be due to spread being confined to the latter part of the season. These late season infections may not have been detected by the ELISA test. Khalil and Shalla (1982) reported higher ELISA readings from leaves of PVS infected plants at two months after planting (before flowering) than at one or three months after planting. At two months after planting, leaves sampled from the middle of the plant rather than the top of the plant showed the highest ELISA readings (Khalil and Shalla 1982), although virus was detectable in all material. Several studies have reported difficulty in detecting late season infections. In each of two treatments, Wardrop *et. al*. (1989) detected 4% and 2% incidence of PVS respectively in leaves collected at 9 weeks, however subsequent testing of foliage from tubers collected and grown on the following season demonstrated that 100% and 14.6% of plants respectively had one or more infected tuber, and 87.3% and 46.2% of all tubers respectively had PVS. This indicated that virus testing of leaves within the season could substantially underestimate the amount of PVS infected plants and tubers. MacKinnon *et. al*. (1972) also found recent infections were more reliably detected from post harvest tuber testing on *Nicotiana debneyi*

than serological or indicator testing of foliage sampled during the growing season. 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.

However, there is evidence that we would have detected at least some in season infections in our trials for the following reasons:

i) Tuber samples were collected from the PVS and PVX fields at harvest, cool stored and grown on in the greenhouse the following season. Testing of leaves by ELISA indicated that the incidence of virus was similar to that detected in the fields the previous season. This suggested that inability to detect within season infections was not an issue in these trials.

ii) There was no reduction in the strength of ELISA reactions during the season at either trial site. Reactions were either strongly positive or negative, with no weak reactions suggestive of in-season infections occurring at low concentrations in the plant.

iii) Khalil and Shalla (1982) showed transmission of a non aphid-transmissible strain of PVS to occur in an insect free green-house to 5/30 previously uninfected plants when in physical contact with infected plants for a period of two months. Similarly, in field plots, 11/108 plants became infected during the season. This demonstrated that at least some new infections can be detected during the season.

iv) 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. PVS was translocated from inoculated leaves to daughter tubers within 13 days for vars. Russet Burbank and Norland, and 20 days for var. Kennebec and within 14 days for European varieties (De Bokx 1968, De Bokx and

Waterreus 1967). These results suggested rapid spread of PVS throughout the plant following mechanical inoculation and that all but very late season infections would have been detected in our trials.

v) Beemster (1958) reported that PVX infected the tubers of several European potato cultivars within 2-4 days after inoculation of leaflets, suggesting rapid spread of this virus through the plant following inoculation.

The difference in detection between some of these studies may be the nature of transmission. For PVS, aphids potentially inoculate relatively small amounts of virus in comparison to mechanical inoculation and the former may take longer to attain detectable concentrations within the plant.

A further complicating factor is the potential for mature plant resistance in potato, which can affect the transmission rate of virus between plants and tubers. Therefore late season infections may not actually contribute to an increase in virus in the next generation. De Bokx (1968) reported mature plant resistance to PVS in European cultivars at four weeks after planting, which became more pronounced at six weeks. The degree of resistance varied with cultivar and with PVS isolate. However, Franc and Banttari (1996) were unable to detect mature plant resistance to a Minnesota isolate of PVS and postulated that this at least partially explained the rapid reinfection of healthy potatoes in Minnesota. Spread of European isolates of PVS to tubers would be limited to the early part of the season and become more limited with the onset of mature plant resistance, thus reducing reinfection in seed-lots in European production areas even when inoculum was present. By contrast, the lack of mature plant resistance to Minnesota isolates of PVS 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. Weidemann (1986) also reported that not all tubers from an infected plant were infected after harvest and that the proportion of tubers infected was higher for plants infected early than those in which infections

were detected later. Dedic (1978) noted that 12-14 days following artificial inoculation of the top leaves of potato with PVS, the percentage of tuber infection was 18.5-25.0% in var. Jara and only 6.2% in var. Krasava after 21 days. Tuber infection was not detected in var. Krasava following inoculation of plants more than 6 weeks old. It is not currently known whether strains of PVS in Tasmania are subject to mature plant resistance in local varieties.

Weeds as reservoirs of virus

PVS and PVX were not detected in weed samples collected from one potato field in Tasmania, suggesting weed hosts were not playing a significant role in virus transmission at this field. During a study conducted by Khalil and Shalla (1982), PVS was not detected in thirteen native plants and weeds obtained from potato fields and the nearby surroundings. Similarly, Thomas and Richards (2003) were unable to inoculate PVS to 15 common weeds in the Columbia Basin, USA including groundsel (*Senecio vulgaris*), Shepherd's purse (*Capsella bursa-pastoris*), chickweed (*Stellaria media*) or Mallow (*Malva neglecta*). Similarly PVS was not detected in 10 weeds in the field, including Shepherd's purse and Mallow. Thomas and Richards (2003) concluded that PVS could not survive endemically in winter weeds in the Columbia basin. Overseas work has shown weeds to be significant sources of PVX. Allen and Davis (1982) reported that PVX occurred in 11 of 28 weed species studied. Hairy nightshade (*Solanum sarachoides*) was found in 6 of seven fields examined and plants infected with PVX were found in 5 fields. PVX occurred in leaves, roots, fruits and seeds. Redroot pigweed (*Amaranthus retroflexus*) and Lambsquarter (*Chenopodium album*) were found in 7/7 and 6/7 fields respectively and were shown to harbour PVX in 5 and 3 fields respectively. It was suggested that infected potato plants acted as an initial source of inoculum for weeds (Allen and Davis 1982). Such weeds could then act as a 'green bridge' for subsequent potato crops. Locatelli *et al*. (1978) detected PVX infection in 4/18 *Amaranthus powellii* and 7/14 hairy nightshade plants located in areas adjacent to potato fields in Oregon. Beemster (1977) reported that Bromegrass (*Bromus commutatus*) could be inoculated with PVX. Bromegrass developed symptoms of infection and PVX could be

recovered. However, only a small percentage of plants became infected and it is not known if bromegrass can be infected naturally and act as a reservoir of inoculum for potato crops. Volunteer potatoes may serve as a source of inoculum in climates with mild winters or where snow cover protects tubers from freezing (Franc and Banttari 2001). Wright and Bishop (1981) described a field with an estimated 30,300 volunteer potato stems per hectare, with 65% infection with PVX. Inadequate control of volunteer potato prior to planting a potato crop would allow transmission of virus from volunteers to the crop during the growing season.

Results from our limited survey of a field in Tasmania show that although the removal or reduction of weeds from potato fields is desirable from many reasons, this cultural practice may not have an impact on the control of PVS and PVX in Tasmania. Presumably the relatively long crop rotations between seed potato crops (at least 4 years), the lack of other Solanaceous crops in the rotation, the degree of weed control in other crops grown in those fields between potato crops and the low transmission rate of virus (if any) to weed seeds, results in little carry over of virus in weeds between potato crops in this region.

Aphids

There was no suggestion of aphid spread of PVS in either field. However, aphid traps were not placed in the fields until between 70 and 80 DAP, therefore any early flights of alatae aphids would have been missed. Aphids were not trapped until late in the life of the crop at both fields. Only two aphid species were detected (*Aphis gossypii* and *Macrosiphum euphorbiae*). While neither species is known as a vector of PVS, work on aphid transmission of PVS is limited and many species of aphid have not been tested for their ability to transmit this virus. *Aphis gossypii* has become an important pest of potato in Europe, where it has developed insecticide resistance. Potato crops in New Zealand have required insecticide treatment to control this aphid, and it has been recommended that its pest status in New Zealand potato be reviewed regularly (Stufkens and Teulon 2001).

Effect of virus on crop growth.

The green leaf area index (GLAI) is the leaf area per unit ground area and is closely related to the yield of crops. Reflectance from crop canopies at wavelengths of around 830 nm (near infra-red) is a good indicator of the green leaf area index of various crops. Bouman *et. al*. (1992) reported a high correlation coefficient ($r>0.90$) between ground cover of potato crops measured by visual assessment and WDVI, calculated from the reflectance from the crop canopy at different wavelengths. The negative correlation between incidence of PVS in plots at 129 DAP in the PVS field, and percentage reflectance from the crop canopy in plots at 560, 830 and 1650 nm indicated that PVS was adversely affecting plant growth. The statistically significant negative correlation between WDVI at 134 DAP and PVS incidence as 129 DAP also indicated that PVS was reducing foliar growth, as WDVI has been shown to be an accurate measure of groundcover in potato crops (Bouman *et al*. 1992). An alternative explanation may be that PVS infected plots senesced earlier and that this affected the reflectance characteristics of the crop canopy. However, yellow leaves have a higher reflectance in the green (560 nm) or red (660 nm) part of the spectrum (Bouman *et al*. 1992). Therefore had plots with higher incidence of PVS senesced earlier, it may have been expected that there would have been a positive correlation between PVS incidence and percent reflectance at 560 or 660 nm, rather than a negative correlation. There was no correlation between reflectance and virus status of plots in the PVX field. However, incidence of PVX may not have been sufficiently high to observe any differences at this site. In addition, the stage of crop growth at which measurements were taken was less in the PVX field than the PVS field.

6. Determining the importance of seed cutting in spread of virus.

6.1 Introduction.

Whole seed tubers are usually cut into 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. This study was instituted to determine the extent to which seed cutting contributes to the prevalence and incidence of PVS and PVX in Tasmania.

6.2 Materials and methods.

Whole seed pieces were collected from two commercial seed cutters, one located on the North West Coast of Tasmania and another in the North East of Tasmania. A total of 15 seed lines were collected. However, due to high virus levels in whole tubers, only 9 lines were subsequently assessed for virus spread. For each seed line, a sample of whole tubers was collected from a half-tonne bin prior to passing over the cutter and a sample of seed pieces collected after cutting. Seed was stored at room temperature for one to two weeks, then planted into pots in potting mix and grown in an aphid proof cage. At 8 weeks after planting, young leaflets were collected and tested by ELISA for PVS and PVX (Appendix 1).

6.3 Results

Results from this trial were ambiguous. In 5 seed lines, moderate to large increases in PVS infection occurred between pre-cutting and post-cutting (lines 1, 2, 4, 5 and 6) (Table 18). However in other seed lines, PVS incidence was similar (lines 3, 8) or declined (lines 7, 9) between pre-cutting and post cutting. Most crops were free of detectable PVX (Table 18). In seed line 4, PVX was detected only after cutting. Conversely, PVX was detected in seed line 5 prior to cutting, but not post cutting.

Table 18. Virus incidence in seed lines before and after seed cutting.

Seed line	Location[a]	Total tubers tested (Pre/Post cut)	PVS incidence (%)		PVX incidence (%)	
			Pre cut	Post cut	Pre cut	Post cut
1	NWC	42/28	16.7	64.3	0	0
2	NWC	38/25	26.3	36.0	0	0
3	NWC	46/82	19.6	14.6	0	0
4	NWC	40/76	30.0	44.7	0	6.6
5	NWC	47/58	27.7	63.8	10.6	0
6	NWC	50/72	14.0	25.0	0	0
7	NE	37/36	89.2	77.8	0	0
8	NE	34/39	88.2	82.1	0	0
9	NE	39/56	97.4	66.1	0	0

[a] NWC = North West Coast, NE= North East

6.4 Discussion and conclusions

Although the majority of crops showed an increase in PVS after cutting, the decline in incidence in some crops calls into question the veracity of this result. While seed-cutting is known to spread PVS and PVX, this trial failed to demonstrate conclusively that seed cutting is a source of virus spread in Tasmania. Furthermore the low sample numbers tested are likely to have contributed to a large sampling error in this trial.

Other studies have shown seed cutting to be a source of PVS transfer (Franc and Banttari 1984). In var. Russet Burbank, transmission of PVS from infected to uninfected tubers via hand seed cutting 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. PVS replication within the tuber is induced when potato tubers are mechanically wounded (Morelli and Vayda 1996). Poor handling of tubers prior to cutting might therefore stimulate PVS replication and increase virus transmission between tubers during the cutting procedure.

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%).

Therefore while several studies have implicated seed cutting in spread of PVS, the contribution of seed-cutting to overall virus levels in Tasmania is yet to be determined.

7. Effects of potato viruses S and X on yield of Russet Burbank

7.1 Introduction

The recent finding of PVS and PVX in Tasmanian seed potato crops is of concern to growers due to potential problems of achieving certification and sending seed interstate and the potential effects of these viruses on yield. Overseas studies have shown yield reductions of up to 10-20% when these viruses occur singly, or up to 40% when they occur together or with other viruses (Stevenson *et al*. 2001). Differences in the magnitude of yield reduction between studies have been attributed to different strains of virus, geographic locality of the study and the variety of potato. Generally it is considered that PVS and most strains of PVX have a relatively mild effect on potato production. Limited work has been conducted on the effects of PVS and PVX on yield in Australia. Mulcahy (2000) carried out a preliminary investigation of the effect of generation and PVS on yield of potato in Tasmania. He reported that there was no significant difference in processing yield in var. Russet Burbank between G2 (with no detectable PVS) and G15 with complete infection, with yields of 49.0-51.6 t/ha and 49.5-53.5 t/ha respectively. However for var. Kennebec there was a significant difference (17.2%) in processing yield between G2 (69.5 t/ha) and G17 completely infected with PVS (57.6 t/ha).

This study utilised the Growers' Lines Trial conducted annually by Simplot Australia Pty. Ltd. in which seed is sourced from different growers and planted in replicated plots at one location to assess the relative performance of lines. As seed may also have different incidence of virus, this trial afforded the opportunity to investigate the effects of local strains of PVS and PVX on yield and quality of the processing potato variety Russet Burbank under Tasmanian conditions.

7.2 Materials and methods.

Trial site

The trial was located at Riana, on the north west coast of Tasmania during the 2002/2003 season. The trial was planted, grown, harvested and assessed by staff of Simplot Australia Pty. Ltd. The following methods and yield and quality data were kindly provided by Simplot Australia (Mark Heap pers. comm.). For planting the trial, a sample of seed from each of 60 seed lines of var. Russet Burbank was sourced from tubers harvested from a single field of each grower. A 50 kg composite sample of seed tubers was obtained from each seed line, consisting of 5 kg from each of 8 half-tonne bins in the cool-store after harvest. Seed was chosen without regard to size or appearance.

The trial was arranged as a randomised block design with three replicate plots of each of the 60 seed lines arranged in three blocks. Blocks were separated by four unplanted rows. Each plot consisted of 2, 10 m long rows of potato. Seed was hand cut and knives were sterilised with methylated spirits or bleach between lines. Seed was planted at 30 cm spacing using a Faun planter. Fertiliser was band applied with the seed at a rate of 175 kg N/ha , 240 kg P/ha and 220 kg K/ha plus trace elements. Other aspects of crop agronomy (irrigation, pesticides etc.) were carried out as per commercial practice.

Observations of plant health and growth were made on the central 5 m of row during the season. Tubers from the central 5 m of the rows were harvested with a 2 row lifter and weighed and sorted into size classes (0-74 g, 75-249 g, 250-849 g and >850 g). Tubers were assessed for shape and visually scored for scab severity. The 10 largest tubers from each plot were cut open and visually scored for hollow heart. An 8 kg sample was taken from each plot and assessed for specific gravity and cooking quality.

Correlation analysis was used to determine relationships between virus incidence within plots and yield and quality. Regression analysis was used to investigate the relationships between particular variables in more detail.

Virus testing

For virus testing, 20 leaflets were collected from individual plants within each of the 3 replicate plots of 40 seed lines. Leaflets were collected on 5/3/2003, just prior to senescence of the crop and stored at 5^{o}C for no more than 5 days prior to virus testing. ELISA testing was carried out as described (Appendix 1)

7.3 Results

Potato virus S

PVS was prevalent in the growers' lines, occurring in 38/40 lines and 93/120 plots, with an average plot incidence of 41.8% (Table 19). PVX was less prevalent, occurring in 13/40 lines and 22/120 plots with an average plot incidence of 6.7% (Table 19).

Table 19. Summary of incidence of PVS and PVX within plots.

	No. plots with virus (n=120)	Average	Minimum	Maximum	Standard deviation
PVS	93	41.8%	0%	100%	41.2%
PVX	22	6.7%	0%	85%	19.2%

Significant negative correlations (P<0.05) were noted between the incidence of PVS and the weight of tubers (kg/plot) in the larger size ranges and with calculated processing yield (t/ha) (Table 20). Regression analysis of the relationship between PVS incidence and processing yield (t/ha) demonstrated a predicted yield at 0 and 100% PVS incidence of 63.8 t/ha and 58.4 t/ha

respectively, a difference of 5.4 t/ha (Figure 4). While the regression equation was highly statistically significant, it should be noted that there was considerable scatter of data points around the line leading to low R^2 values and therefore low predictive power of the analysis (Figure 4).

The incidence of PVS was negatively correlated with the scab visual score on tubers (Table 20), but graphical examination of the data suggested that the relationship (if any) was poor. PVS incidence was not significantly correlated with other factors, including specific gravity of tubers (Table 20).

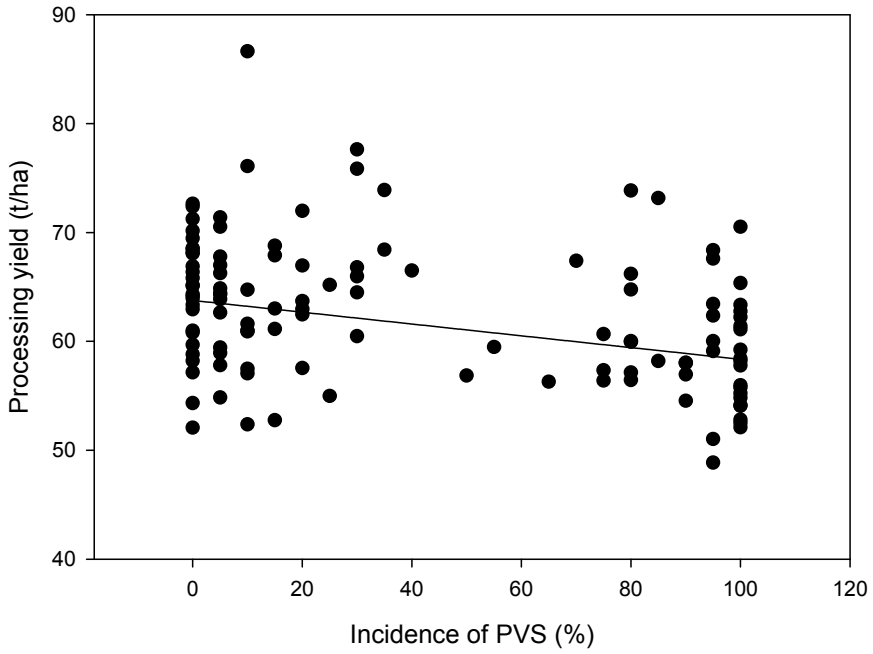
Table 20. Correlation coefficients (r) between yield and quality of potato and incidence of PVS and PVX in each plot.

	PVS	PVX
Weight (kg/plot) in size range:		
0-74 g	-0.09	0.27 ***
75-249 g	-0.08	0.15
250-849 g	-0.28 ***[a]	-0.16
> 850 g	-0.21 **	0.12
Processing yield (t/ha)	-0.36 ****	-0.02
Size (%)[b]	-0.12	-0.20 **
No. plants/10 m	0.01	-0.17
No. stems/10m	0.12	-0.09
Mean stems/plant	0.13	-0.04
Scab visual score	-0.22 **	-0.03
Hollow heart visual score	-0.13	-0.05
Specific gravity	-0.05	0.01

[a] Statistical significance of correlation coefficients: ** $P<0.05$, *** $P<0.01$, **** $P<0.001$

[b] Weight of tubers in 250-849 g category as a percent of processing weight.

Figure 4. Regression between incidence of PVS (%) and processing yield of potato (t/ha). Y= 63.786 + -0.054 X (*P*<0.0001, R^2=0.14, adjusted R^2=0.13)[a]



[a] Note that a regression omitting the 86 t/ha data point in the top left hand side of the graph had little effect on the regression equation, with *P*<0.0001, R^2 = 0.13, adjusted R^2=0.13, Y=63.570 + -0.052 X. The predicted yield at 0 and 100% PVS incidence was 63.6 t/ha and 58.4 t/ha respectively, a difference of 5.2 t/ha.

Potato virus X

A significant positive correlation was noted between the incidence of PVX and the yield of tubers in the smaller size category (Table 20). There was a negative correlation between the incidence of PVX and the percentage by weight of processing yield in the size range 250-949 g (Table 20). There were no significant correlations between PVX incidence and other factors including processing yield and specific gravity (Table 20). As there were a large number of plots with no PVX, and PVX occurred in combination with PVS, it was difficult to draw conclusions on the effect of PVX alone from this data. However, the average yield of three plots with high incidence of PVX was comparable to plots without virus, suggesting that the strain of PVX present in this trial was having a minimal effect on yield and quality (Table 21).

Table 21. Processing yield (t/ha) of selected plots containing different combinations of virus.

	No. of plots	Yield (t/ha) Average	Minimum	Maximum	Standard deviation
No virus	21	64.3	52.1	72.6	5.1
High PVS (>75%)	32	59.9	48.9	73.8	6.2
High PVX (>80%)	3	63.1	54.3	68.5	7.6
High PVS (>95%) & high PVX (45-75%)	4	60.6	58.4	63.4	2.3

Mixed infections of PVS and PVX

Mixed infections of PVS and PVX have been reported to cause larger yield reductions than single infections with either virus alone (Stevenson *et al*. 2001). The data set did not have sufficient combinations over a range of virus incidence to assess this hypothesis. However, four plots with high levels of PVS and moderately high levels of PVX had average yield only slightly below those with no virus and similar yield to those that had high levels of PVS alone (Table 21). This suggested that combinations of PVS and PVX might not reduce yield any more than single infections of PVS alone.

7.4 Conclusions

The moderate effect of PVS on yield in this trial is in agreement with overseas studies. Correlation analysis showed a highly significant negative relationship between the incidence of PVS and processing yield (t/ha) of var. Russet Burbank. Regression analysis demonstrated that, on average, plots completely infected with PVS yielded 91.6% that of plots with no PVS, a difference of some 5.4 t/ha. Negative relationships were also shown between the incidence of PVS and yield of tubers in the larger size ranges.

The limited data available for PVX suggested that the strain of PVX present in the trial had minimal impact on yield of var. Russet Burbank when it occurred

as single infections, and that combinations of PVS and PVX had no more impact than PVS alone. However, this situation could change if more severe strains of PVX entered Tasmania.

Results of a survey in Tasmania during 2002/2003 demonstrated that PVS was present in 69% of 235 seed crops. The majority of crops (63%) had no PVS or incidences below 10%. Therefore on the basis of this trial, PVS is currently unlikely to be having a major impact on yield of Tasmanian crops. However, some 15% of crops had incidences of PVS above 50%, and these crops might be expected to be experiencing significant yield penalties. The widespread nature of PVS in seed crops is of concern as there is potential for rapid increase to levels where yield losses become apparent. It is therefore important that steps be taken to reduce and/or eradicate PVS (and PVX) from Tasmanian seed potato.

8. General conclusions

The generally low prevalence (percentage of crops infected) and incidence (percentage of plants infected within crops) of PVX in G2 crops in Tasmania is encouraging and suggests that there is a good prospect of eliminating this virus from the seed scheme in the short term. The prevalent nature of PVS and the moderately high incidence in G2 crops is of concern as there is opportunity for the virus to increase during seed cutting or by mechanical operations or potentially aphids in succeeding generations. This suggests that the elimination of PVS from the seed scheme will take longer and will be more difficult to achieve. Preliminary analysis of PVS strains suggests that only PVS^{O} strains are present in Tasmania. However, further strain characterisation will be undertaken during 2005.

A very low rate of field spread of PVS and PVX was detected in trials in Tasmania, with no evidence of aphid transmission of PVS. Further trials will be conducted during 2005 to confirm this finding. Results suggest that practices used by seed growers to minimise traffic through the crop are effective at reducing virus spread in the field (e.g. solid set irrigation or sufficiently wide laneways for travelling irrigators, aerial spraying of pesticides, restricted entry to crops).

Transmission of PVS and PVX by seed cutting has been demonstrated in overseas trials and was suggested by the trial in this project. However results were variable, and this project was unable to ascertain the importance of seed-cutting in virus transmission in Tasmania.

The recommendations of Dr. Jones have been circulated to industry and many aspects have been incorporated into a management plan by the Tasmanian potato industry.

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- Dr. Roger Jones, Agriculture Western Australia for conducting an audit of seed handling practices and providing further recommendations for management of viruses by the Tasmanian Seed Potato Industry.

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.

For PVY, a compound direct ELISA was used involving coating polystyrene microtitre plates with virus specific polyclonal antiserum (1/400 dilution), adding plant sap, adding virus specific monoclonal antiserum together with an anti-animal antiserum conjugated to alkaline phosphatase enzyme, followed by the addition of the substrate. Washing procedure between each step was as described above.

Appendix 2. Itinerary for Dr. Roger Jones 14- 20th March 2004

Sunday 14th March

Arrive Devonport

Monday 15th March

Visit McCains early generation seed farms in the North coast – Phillip Richard, Keeton Miles, Chris Murfet, Larry Arstal
Visit Cherryhill and Langworthy Coolstore and seed cutting operations.
Visit and discussion with McCains staff.
Evening presentation DPIWE conference room 7:00 pm: Limiting the impact of potato viruses - industry perspective (Technical presentation)

Tuesday 16th March

Visit Simplot Factory
Visit Simplot Seed farms on the north coast – Lloyd Langham, Chris Fielding, Andrew Hamilton, Shane Ranson.
Evening Presentation DPIWE conference room 7:00 pm: Controlling the Spread of Potato Viruses in Tasmania - from the growers' perspective

Wednesday 17th March

Visit Kinburn Farm, Harvest Moon - Fraser Mearns
Travel to Scottsdale visit Simplot seed growers – Kevin Hall, Clint Lette, Michael Coote
Visit Jondi Coolstore – John Lette
Evening presentation Scottsdale RSL 7:00 pm: Controlling the Spread of Potato Viruses in Tasmania - from the growers' perspective.

Thursday 18th March

Travel to Hobart Visit DPIWE TASAG ELISA Diagnostic Labs - Calum Wilson and Peter Cross

Friday 19th March

Visit Colebrook, Huonville Ouse processing and fresh market growers. Graham Coombs, Andrew Denholm, Peter Wilson, Stewart Wilson.
Evening Presentation Pontville Football Grounds 7:00 pm: Controlling the Spread of Potato Viruses in Tasmania - from the growers perspective

Saturday 20th March

Return Devonport and depart for Perth.

Appendix 3. Recommendations for the Tasmanian Seed Potato Industry for the Control of Potato Viruses

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Recommendations to seed growers

- Leave three row spaces between generations
- Leave three row spaces between lines within generations
- Control weed rigorously, especially nightshade and volunteers
- Avoid strips of G1 (or G2). Plant as rectangular blocks to minimise edges adjacent to other generations
- Place G1 and G2 at the edge of the paddock if possible. If there is a high risk of grazing by wildlife (eg wallaby, grasshoppers etc) it may be necessary to reconsider and plant within the paddock
- When planning machinery movements (eg sprayers, spreaders, irrigators etc) always work from the earliest to the latest generations.
- Always clean and sterilise machinery between paddocks
- Do not allow uncertified seed on to seed farms. Minimise the introduction of anything but minitubers on to the farm or use only virus tested G2 from specialised seed growers
- Consider the supply of certified seed (eg Nicola) to Dutch Cream / backyard growers
- Restrict access to early generation plots (no unauthorised personnel)
- Clean down boots and leggings frequently using disinfectant / detergent.

Recommendations to seed cutters

- Clean and disinfect seed cutters frequently – where possible between lines using pressure wash and a suitable disinfectant (eg Virkon or farmclense)
- Trial Virkon on cut tuber surfaces to determine the impact on germination and growth
- Spray the cutting knives after each line
- Clean the cutter down using a pressure wash at the end of each day
- Sequence the cutting operation so as the earliest generations are cut first and later generations last
- If the seed line is known to contain virus ensure the cutter is cleaned down immediately after the line is cut
- Early generations should all be hand cut on farm. Several knives should be used to allow a longer period of disinfection (eg 10 minutes) between use.
- Remove discard piles/bins promptly. Regularly monitor for aphids on sprouting tubers
- Minimise surface damage during grading (skinning)

Recommendations to the potato industry

- Seed potato movements should be restricted to within designated seed areas. Only virus tested seed should be moved between seed growers.
- Wherever possible multiply minitubers and G2 on specialised farms in low aphid population areas. This seed can then be passed on to other seed growers for further multiplication.
- Alternatively, isolate clean material from current lines to provide a flush out of any infected material. Separate clean seed pipeline as far as practical from infected lines (never in the same paddock)
- Contract machinery – Ensure rigid hygiene protocols before transfer between farms.
- Promote the awareness of the importance of virus free seed stocks to commercial growers
- Set high standards of cleanliness when personnel visit growers' properties.

Recommendations to DPIWE

- Virus test G3 seed crops. Sample at second inspection. Test for X and S, and in high risk areas Y, PLRV, TSWV.
- No other potato material to be brought near any minituber facility
- Ensure strict hygiene protocols for officers entering growers properties, promote high standards and set an example
- Use virus test kits to raise awareness in the industry of the virus issue and to give instant Ids
- The certification tolerances should be graduated so that stringency decreases with generation
- Enforce planting distances by introducing a 3 row spacing between generations and lines and increasing the distance between certified seed and ware and no uncertified seed to be sown on a seed farm.
- Improve the minimum requirements in the National Standards / or adopt higher standards in Tasmania as has been done in WA
- Benchmark National Standards against other national schemes.
- Institute a computerised seed tracking service
- Set a temporary special tolerance for PVS

Recommended Research

- Test the spread of PVS and PVX through the seed cutters
- Determine the strains of PVS and PVX
- Survey backyards, volunteer potatoes, nightshade and fat hen for virus reservoirs
- Trial disinfectant efficacy and toxicity on cut tuber surfaces
- Test the survival of PVX and PVS on different surfaces - current information is inadequate
- Examine the transmission of PVS and PVX by grasshoppers, sandblasting and grazing
- Test the breeding lines from Toolangi for PVY on DPIWE farm and elsewhere in Tasmania

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