Improving management of Potato Virus S through a better understanding of mechanisms of virus transmission.

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Project Number: PT06044

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This report is published by Horticulture Australia Ltd to pass on information concerning horticultural research and development undertaken for the potato industry.

The research contained in this report was funded by Horticulture Australia Ltd with the financial support of TAS Department of Primary Industries, Water and Environment.

ISBN 0 7341 2055 9

Published and distributed by:
Horticulture Australia Ltd
Level 7
179 Elizabeth Street
Sydney NSW 2000
Telephone: (02) 8295 2300
Fax: (02) 8295 2399

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FINAL REPORT

Improving management of Potato Virus S through better understanding of mechanisms of virus transmission

Final report for Horticulture Australia Limited

Project PT 06044 (July 2009)

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July 2009

Horticulture Australia Ltd.

Project PT06044

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Purpose of project: In previous projects funded through Horticulture Australia Ltd. (PT02037, PT03069 and PT05011), *Potato virus S* (PVS) and *Potato virus X* (PVX) were detected in the Tasmanian seed potato scheme. These projects focused on monitoring virus incidence, auditing seed handling practices and providing recommendations to growers and industry. As a result of this research, preliminary management strategies were implemented to control virus levels. As a result, PVX has practically been eliminated from the Tasmanian seed potato scheme, with only one or two crops infected at low levels in recent years. However, PVS has proven more difficult to manage. This project (PT06044) aimed to gain a better understanding of the means by which PVS enters early generation seed crops. The project involved monitoring the incidence of PVS in five generation one (G1) crops, and the subsequent G2 crops in the following season, and in an attempt to relate the incidence of PVS to agronomic practices. In addition, greenhouse experiments were conducted to determine if strains of PVS present in Tasmania are transmissible by the aphid, *Myzus persicae*. Seed cutting experiments were conducted to determine if the antiviral chemical (Virkon® S) can be applied to seed pieces immediately following cutting to reduce virus transmission without phytotoxicity or compromising yield and quality.

Funding: This project was facilitated by Horticulture Australia Limited., and funded by voluntary contributions from Simplot Australia Pty. Ltd., McCains Foods (Australia) Ltd., Harvest Moon Forth Farm Produce Ltd. and Simplot and McCain growers, with matched funding from the Australian Government.

Media summary

Management strategies for control of Potato Virus S (PVS) in seed potato crops in Tasmania have been effective at reducing the incidence of this virus in early generation seed crops. However, some growers continue to have difficulty in eradicating PVS from their farms. This project involved monitoring virus levels and recording agronomic practices of five early generation seed potato crops within the Tasmanian seed potato industry, in an to attempt to identify agronomic practices which allowed PVS to enter seed crops. Isolation of early generation seed crops from later generation seed crops and from ware crops was identified as the most effective strategy for preventing PVS infection. Other means by which PVS may enter crops is through aphid transmission, which has been reported for some strains of the virus in overseas studies. However, small scale greenhouse experiments undertaken as part of this study indicated that PVS isolates in Tasmania were not transmissible by the aphid, *Myzus persicae*. Therefore, aphid transmission may not be an important factor in spread of the virus. As PVS can be transmitted during seed cutting, experiments were conducted to determine if treating seed pieces with an antiviral chemical after cutting could reduce virus transmission without phytotoxicity. Poor transmission of PVS in the trial prevented an assessment of the ability of the antiviral chemical to reduce virus transmission. However, yield and quality of tubers was unaffected by treatment, suggesting this strategy would be worthy of further development.

Technical summary

A virus survey was undertaken of five generation one (G1) crops (2007/2008 season) and following crops in the proceeding season (G2 crops) (2008/2009 season) within the Tasmanian seed potato industry. Agronomic practices used by the growers were documented, in an attempt to relate incidence of *Potato virus S* (PVS) to agronomic practices. PVS was not detected in any of the five G1 crops at flowering. However, the following season (2008/2009), PVS was detected in one G2 crop located at Riana, with an incidence of 9.6%. During the 2007/2008 season, the G1 crop from which this G2 crop had originated had been grown amongst an unrelated G2 crop. This crop was shown to have >50% incidence of PVS during routine testing as part of the seed certification process. This suggested the unrelated G2 crop provided a source of PVS infection for the G1 crop, which became evident when grown on the following season (2008/2009) as a G2 crop. By contrast, PVS was not detected in the remaining four G2 crops which originated from G1 crops that had been grown in isolation from other potato. PVS was not detected in a limited sample of the perennial weed *Solanum laciniatum* collected from Tasmanian farms, although previous research has shown this to be an experimental host of PVS.

Some PVS isolates have been reported overseas to be transmissible in a non-persistent manner by some aphid species. The ability to be aphid transmitted has implications for the management of this virus. Greenhouse experiments were conducted to determine if five isolates of *Potato virus S* (PVS) sourced from different potato growing regions in Tasmania were transmissible by the aphid, *Myzus persicae*. No evidence of aphid transmission was obtained, suggesting that the principle means of spread in Tasmania is via mechanical transmission.

One of the main means by which PVS may be increased is through mechanical transmission during mechanical seed cutting operations. Hygiene practices, such as washing down and disinfecting machinery, are commonly employed between seed lines. However, this is time consuming and expensive for large centralized seed cutting facilities. Similarly, disinfestation of equipment between seed lines does not

prevent spread of virus on cutting knives within a seed line. Seed cutting experiments were conducted to determine if treating seed pieces with the antiviral chemical Virkon® S immediately after cutting could reduce virus transmission without phyto-toxicity in the subsequent crop. Two field trials demonstrated no reduction in yield from applying concentrations of Virkon® S at less than 1%, a concentration at which the chemical should be efficacious. However, the low transmission of PVS in the trials prevented an assessment of the ability of Virkon ® S to reduce PVS transmission. Results suggest that this treatment may be worthy of further study as a means of reducing virus transmission during mechanized seed cutting. Information gained from these trials will be used to further improve management strategies for the control of PVS in Tasmanian seed potato crops.

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1. Introduction

Potato (*Solanum tuberosum* L.) is an important crop in Tasmania, with annual production of 301,747 metric tonnes grown over 6,618 hectares, with an average yield of 46 t/ha (Anon.2008a). Potato production in Tasmania equates to 25% of the total potato grown in Australia, with a gross value of $AUS82.5 M in 2007 (Anon. 2008b). Any disease capable of limiting the productivity of potato in Tasmania is treated very seriously.

The Tasmanian Institute of Agricultural Research (TIAR) is responsible for the certification of seed potato crops in Tasmania. The current seed certification process follows the guidelines set out in the National Standard for Certification of Seed Potato. This standard encompasses all potato virus considered to be of economic importance, including *Potato Virus S* (PVS). A Tasmanian Virus Strategy Group was established in 2002/2003 to address issues of potato virus in the Tasmanian seed potato industry. Members of the Tasmanian Virus Strategy Group include representatives of McCains Food Australia, Simplot Australia Pty. Ltd., Harvest Moon Forth Farm Produce Ltd., TIAR and potato grower representatives. Commonwealth funded projects through Horticulture Australia Limited (HAL) and involving voluntary contributions from members of the Tasmanian Virus Strategy Group have enabled research to be undertaken, mainly on potato virus S (PVS) and potato virus X (PVX). Comprehensive state-wide potato virus surveys for PVS and PVX in Tasmanian seed potato crops were undertaken in 2002/2003 as part of HAL project PT02037 (Hay *et al*. 2004) and in 2003/2004 as part of project PT03069 (Hay *et al*. 2005). PVS was prevalent in Tasmanian seed potato crops and occurred at greater incidence in the north-east of Tasmania. The finding of PVS at high incidence in some crops can be explained in that until recently the seed certification process relied on identifying visual symptoms of the various diseases in the field. However, PVS is generally symptomless in the field and has therefore not been picked up during routine field inspections and allowed to increase. PVX was less prevalent and infected crops were restricted to the north-west region of Tasmania.

PVS is considered a relatively benign virus in potato production. Under Tasmanian conditions, field trials with variety Russet Burbank have demonstrated up to a 10% reduction in yield in plots containing 100% PVS infected plants in comparison to those with no detectable virus (Hay *et al*. 2005). Other studies conducted in Tasmania include characterising the strain(s) of PVS present, assessing spatial patterns of PVS in seed potato and determining mechanisms of spread of PVS (Hay *et al*. 2004, 2005 & 2006). As a result of this research, various management strategies have been put in place by the Tasmanian Virus Strategy Group to reduce the incidence of virus in Tasmanian seed potato crops.

The purpose of this project (PT06044) was to build upon previous research briefly outlined above. The aims were to: i) monitor G1 and subsequent G2 crops for the presence of PVS and attempt to relate incidence of PVS to agronomic practices, ii) determine if strains of PVS present in Tasmania are transmitted by *Myzus persicae* (green peach aphid), and iii) determine if treating seed pieces with an antiviral chemical (Virkon®S) after cutting can reduce virus transmission without phytotoxicity. Information gained from these trials will be used to further improve management strategies for the control of PVS in Tasmanian seed potato.

2. Monitoring G1 and G2 crops for PVS

2.1 Introduction

Prior to 2001, virus incidence in Tasmanian seed potato was considered to be very low, with the exception of sporadic and isolated reports of *Potato leaf roll virus* (PLRV) and *Tomato spotted wilt virus* (TSWV). This had been attributed to isolation afforded by being an Island State and to the Tasmanian seed potato certification scheme. However, a limited study conducted by Department of Primary Industries, Parks, Water and Environment (DPIPWE), Simplot Australia Pty. Ltd., McCain Foods (Australia) Pty. Ltd. and Harvest Moon Forth Farm Ltd. to assess the presence of viruses in Tasmanian seed potato crops during the 2001/2002 season detected PVS, PVX and PLRV in 27%, 7% and 7% of crops respectively (Kirkwood 2003). This study was based on low numbers of fields, and therefore indicated only the presence or absence of virus. Furthermore, due to the low numbers of samples within fields, the survey would probably have detected virus only in those fields with high incidence.

As a result of findings of 2001 virus survey, a comprehensive state-wide potato virus survey for PVS and PVX in Tasmanian seed potato crops was undertaken in 2002/2003 which included mixed generations (Hay *et al.* 2004) and 2003/2004 which included G2 crops only (Hay *et al.* 2005). PVS was prevalent in Tasmanian seed potato crops, occurring in 66.7% and 42% of crops in 2002/2003 and 2003/2004 respectively. It occurred in a range of cultivars and seed producing regions. PVS also occurred at high incidence, with 58.2% and 36.5% of crops above the National standard for Certification of Seed Potato of 1% incidence in the 2002/2003 and 2003/2004 seasons, respectively.

Despite putting in place management strategies for PVS, some growers continue to experience problems with PVS entering early generations of their seed crops. The purpose of this part of the project was to monitor virus incidence in the G1 crop and subsequent G2 crop in the following season and to link any ingress of PVS with agronomic practice. In addition samples of a potential perennial weed host of PVS (*Solanum laciniatum*) were collected from farms to assess the potential of this weed as a source of inoculum into early generation seed crops. *S. laciniatum* is sometimes observed growing as a weed in close proximity to fields in Northern Tasmania. Lambert *et al*. (2007) found experimentally a total of 13/15 (86.6%) Tasmanian PVS isolates tested infected *S. laciniatum* by mechanical transmission. Thus *S. laciniatum* may provide a host for PVS in Tasmania

2.2 Materials and methods

During 2007/2008 potato growing season five G1 crops were selected for virus testing for PVS to investigate incidence of PVS-infection with agronomic practices. Information was collected on various practices including potato seed storage, planting, irrigation, machinery movement in the field, seed cutting, volunteer potatoes, incidence of weeds, length of rotation between potato crops and distance of nearest potato crop.

In addition seven samples of *Solanum laciniatum* were collected within and around potato fields and roadsides and were virus tested for PVS as described below (section 2.2.2). Two samples of *S. laciniatum* were collected on 26 March 2008 from the roadside near a garden at Forth, Tasmania. Five samples were collected from the edges of potato fields located at Riana and Wilmot on 15 February 2008.

2.2.1 Sampling

Five G1 crops were surveyed during the 2007/2008 growing season for the presence of PVS (Table 1). In 2008/2009 season, G2 crops resulting from the G1 crops were sampled and virus tested for PVS. Sampling for virus testing on G1 and G2 crops coincided with first field inspections conducted by seed certification officers in mid

January/mid February of each season. From each field a single leaf was collected from between 50-150 (G1) and 150 plants (G2) prior to row closure. Plants were at arbitrarily chosen locations along transects at field edges and along irrigator runs within the crop. This sampling strategy was chosen to minimise movement through a crop that may contribute to mechanical transmission of PVS in the field. Further precautions to prevent virus transmission included wearing protective boot covers and coveralls which were discarded between fields. In addition boots were cleaned and disinfected between fields. Sample bags were placed over leaflets and the leaflet broken off in the bag to prevent sap coming into contact with fingers and therefore providing a means of mechanical transmission.

The five G1 crops were located at Mt. Seymour, Woodstock, Riana, South Riana, and Moina and were sampled on 17 January 2008, 17 January 2008, 29 January 2008, 30 January 2008 and 22 February 2008 respectively. All crops were of Russet Burbank, except for the Woodstock crop (Field 5) which was a mixture of Russet Burbank and Umatilla (Table 1). All G1 crops were isolated from other potato fields except at Riana (Field 1) where the G1 were planted amongst the G2 (Table 1).

2.2.2 Virus testing

Leaflets (50-150) collected from each G1 crop were individually virus tested for PVS. For G2 crops virus incidence was estimated from grouped samples using the Gibbs and Gower technique (1960) (section 2.2.3). For grouped samples, leaflets were grouped in lots of 10 and a subsample (*ca.* 1.0g) was homogenized in a rotary leaf press in 1.0 ml of 0.01 M phosphate-buffered saline (pH 7.4) containing polyvinylpyrrolidone (MW 40,000; 20 g/L), bovine serum albumin (2.0 g/L) and Tween 20 (20 ml/L). Leaflet samples were stored at 4ºC for no more than 4 days prior virus testing. The presence of PVS in sap extracts (100 µl) was tested by DAS-ELISA (Clark and Adams 1977) using polyclonal antisera (Agdia Inc. Elkart, Indiana, USA) in polystyrene microtitre plates (Nunc). A Titertek photometer (Flow Laboratories, Helsinki, Finland) was used to record the Absorbance (A_{405}) of each well. Where absorbance was greater than the mean absorbance of the negative controls plus three times the standard deviation of the buffer only, samples were considered positive (Sutula *et al.* 1986).

2.2.3 Estimation of virus incidence

One hundred and fifty leaflets were collected from each G2 field and grouped in lots of 10 for virus testing. The formula of Gibbs and Gower (1960) was used to provide an estimate of the true incidence of infection from each crop. In this technique:

N is the number of grouped samples (15)
i is the number of leaflets in each grouped sample (10)
R is the number of grouped samples that give a positive virus test
p is the proportion of infected plants in the crop being tested
*p** is the maximum likelihood estimate of *p*
p = 1-p is the proportion of uninfected plants in the population and *q** = 1-*p** is the estimate of *q*.

The probability that none of *i* leaflets is infected is q^i and the probability of a positive virus test when *i* leaflets is tested is 1 - q^i. The estimate *q** is given by:

$R/N = 1 - q^{*i}$ Therefore $p^* = 1 - q^* = 1-(1-R/N)^{1/i}$

2.3 Results

PVS was not detected in any of the five G1 seed potato crops virus tested during the 2007/2008 growing season. PVS was detected in one of the five G2 crops at Riana during the 2008/2009 season, with an incidence of 9.6%. PVS was not detected in the remaining four G2 seed potato crops (Table 1). Commercial agronomic practices for each seed potato field are outlined (Tables 2-6).

Table 1. Incidence of PVS in G1 and G2 Tasmanian seed potato crops.

Field	Location	Variety	Isolated*	Incidence of PVS (%)	
				G1	G2
Field 1	Riana	RB[a]	No	0[c]	9.6
Field 2	South Riana	RB	Yes	0[c]	0
Field 3	Moina	RB	Yes	0[c]	0
Field 4	Mount Seymour	RB	Yes	0[d]	0
Field 5	Woodstock	Mixed[b]	Yes	0[c]	0

*Isolated = no other potato crop in the G1 field
[a] RB = Russet Burbank
[b] Mixed = Russet Burbank and Umatilla
[c] 50 individual leaflets virus tested
[d] 100 individual leaflets virus tested
[e] 150 individual leaflets collected and bulked in groups of 10 for virus testing.

The main cultural difference between Field 1 where PVS was detected in the subsequent G2 crop, and other fields where PVS was not detected, was the proximity of the G1 crop to other potato. In field 1, the G1 crop was planted in the middle of another G2 crop, with a fallow area (1.5- 2.0 m) between the G1 and G2 crops. Virus testing of the G2 crop surrounding the G1 crop was undertaken as part of seed certification during the 2007/2008 season and PVS was found at high incidence (>50%) (Lamberts *et al.* unpublished). This suggested that the G2 crop had acted as a source of PVS into the G1 crop in 2007/2008 and that this was not detected until the G1 was planted, and the resulting G2 crop was sampled in 2008/2009. Comments were also provided by the grower at Field 1 highlighting the high number of grasshoppers in Field 1 during 2007/2008 season and the presence of several *S. lacinatum* plants located along the field edge of G2 crops in 2007/2008 season. *S. lacinatum* and other weeds were removed by hand early in the season which prevented virus testing of samples.

At Field 2, 3, 4 and 5 wash down procedures were performed at the end of each season on wooden bins used for storage of seed potato. All fields prior to planting of

G1 potato crop were free of potato crops for more than 7 years or had never been planted with potato crops. The majority of fields were irrigated with a solid set irrigation thus providing reduced machinery movement through the crop, with the exception of Field 2, which was irrigated with a travelling gun irrigator. However, in the latter case the grower had ensured wide irrigation runs which prevented contact between potato plants and irrigation equipment. For G1 crops a cup planter was used to plant tubers in 2007/2008 season at Field 2, 4 and 5, with wash down procedures implemented on equipment between seasons in Field 4 and 5. A mintituber planter was used to plant minitubers at Field 3, and wash down procedures included high pressure hose wash down followed by application of sterilant (Virkon® S) directly sprayed onto machinery. Field 1 was planted by hand. Few or no weeds were reported in the fields. Volunteer potatoes were not present in fields assessed due to the long rotation periods employed. Additional comments provided by growers regarding their opinions to why on farm reduction of PVS or elimination of PVS in seed stocks is occurring on some farms included long potato rotations, isolation, scheduling operations in order of earlier generations to later generations, and locating crops at high altitude to avoid potential aphid transmission.

PVS was not detected in samples of *S. laciniatum* collected from field edges and road sides from potato fields, although Lambert *et al*. (2007) was able to experimentally infect *S. laciniatum*. Thompson (1976) suggested that commercial crops of *S. laciniatum* should not be planted adjacent to potato crops to reduce the possibility of infection of PVX. Thus it seems plausible that wild *S. laciniatum* growing adjacent to potato crops in Tasmania might pose a potential threat as an inoculum source of PVS (and PVX). Although not common, the perennial nature of this weed may allow long term survival of PVS sources. Further work is required to assess whether natural infection of *S. laciniatum* by PVS occurs in Tasmania, to assess whether this weed host may act as a source of infection into potato crops.

Table 2. Agronomic practices of Field 1 located at Riana during production of Tasmanian seed potato crops generation one (G1) and generation two (G2) in 2007/2008 and 2008/2009 growing season, respectively.

Generation (G)	Seed cutting	Seed storage	Planting	Harvest	Irrigation	Potato rotation and closest crop	In-field machinery movement	Weeds & volunteers
G1 (2007/2008 season)	N/A*	On farm in wooden bins (no wash down of bins)	Hand planted (sprouts on tubers at planting, no aphids observed)	Dug by hand with forks	Solid set	50 years free of potato, with the closest potato crop within the same field (G2 crop, with PVS infection of >50%)	No movement through the crop	Few weeds – *Chenopodium album* (fat hen) *S. lacinatum* (kangaroo apple) *Solanum nigrum* (black night shade), no volunteers
G2 (2008/2009 season)	Hand cut (no sterilant)	Same as for G1 storage	Cup planter	Commercial harvester	Solid set	7 years since previous potato crop, 2 km to nearest commercial crop	Moulding operations through the field and 4-5 applications of chemicals using a boom sprayer	No weeds and no volunteers

* All G1 crop planted using whole minituber, no cutting.

Table 3. Agronomic practices of Field 2 located at South Riana during production of Tasmanian seed potato crops generation one (G1) and generation two (G2) in 2007/2008 and 2008/2009 growing season, respectively.

Generation (G)	Seed cutting	Seed storage	Planting	Harvest	Irrigation	Potato rotation and closest crop	In-field machinery movement	Weeds & volunteers
G1 (2007/2008 season)	N/A*	On farm in wooden bins. All bins washed down at the end of the season	Cup planter (sprouts on tubers at planting, no aphids observed)	Twin row harvester and pick up by hand	Travelling gun irrigator (wide irrigation runs, no contact with plants)	18 years since last potato crop planted in the field and closest potato crop was a G3 crop approximately 200 m distant.	Wide runs left for tractor to access field without contact with crop, with 4-5 applications of chemicals using a boom sprayer	No weeds and no volunteers
G2 (2008/2009 season)	Hand cut	Same as for G1 storage	Cup planter	Commercial harvester	Travelling gun irrigator	No previous potato crops, 2 km to nearest commercial crop	Moulding operations through the field and 4-5 applications of chemicals using a boom sprayer	No weeds and no volunteers

* All G1 crop planted using whole minituber, no cutting.

Table 4. Agronomic practices of Field 3 located at Moina during production of Tasmanian seed potato crops generation one (G1) and generation two (G2) in 2007/2008 and 2008/2009 growing season, respectively.

Generation (G)	Seed cutting	Seed storage	Planting	Harvest	Irrigation	Potato rotation and closest crop	In-field machinery movement	Weeds & volunteers
G1 (2007/2008 season)	N/A*	Seed stored at coolstore in wooden bins, all bins washed down each season[1]	Simplot minituber planter, washed down prior to use[1]	Twin row harvester and pick up by hand.	Solid set irrigation	>40 years since last potato crop planted in the field and closest potato crop approximately 2 km	Wide areas left fallow for tractor to access field for chemical spray with boom. No moulding operations through the field.	No weeds and no volunteers
G2 (2008/2009 season)	Pre-cut with machine dedicated cutter for G1 seed only[1]	Same as for G1 storage[1]	Clamp planter, wash down procedure as for G1[1]	Commercial harvester[1]	Travelling gun with wide irrigation runs	Same as for G1	No moulding operations through the field, and herbicide for weed control using a boom sprayer	No weeds and no volunteers

* All G1 crop planted using whole minituber, no cutting.
[1] Wash down procedure with high pressure hose and then sprayed with sterilant (Virkon® S).

Table 5. Agronomic practices of Field 4 located at Mount Seymour during production of Tasmanian seed potato crops generation one (G1) and generation two (G2) in 2007/2008 and 2008/2009 growing season, respectively.

Generation (G)	Seed cutting	Seed storage	Planting	Harvest	Irrigation	Potato rotation and closest crop	In-field machinery movement	Weeds & volunteers
G1 (2007/2008 season)	N/A*	On farm in wooden bins. All bins washed down at the end of the season	Cup planter, no wash down. A few sprouts on tubers	Harvester	Solid set irrigation	7 years since last potato crop planted in the field and nearest potato crop (commercial) approx. 700 m distant.	Moulding operations through the field	Very few weeds and no volunteers
G2 (2008/2009 season)	Machine cut (on farm) wash down between each potato line	same as for G1 storage	Cup planter	Harvester	Solid set irrigation	7 years since last potato crop planted, nearest potato crop was G4 crop in the same field	Moulding operations through the field, and herbicide for weed control using a boom sprayer	No weeds due to chemical control (spray) and no volunteers

* All G1 crop planted using whole minituber, no cutting.

Table 6. Agronomic practices of Field 5 located at Woodstock during production of Tasmanian seed potato crops generation one (G1) and generation two (G2) in 2007/2008 and 2008/2009 growing season, respectively.

Generation (G)	Seed cutting	Seed storage	Planting	Harvest	Irrigation	Potato rotation and closest crop	In-field machinery movement	Weeds & volunteers
G1 (2007/2008 season)	N/A*	Seed stored at coolstore in wooden bins, all bins washed down at the end of the season	Minituber planter, washed down prior to use with high pressure hose	Harvester	Solid set irrigation	No previous potato crop in the field, no commercial potato crops in the district	Moulding operations through the field	No weeds and no volunteers
G2 (2008/2009 season)	Hand cut	Same as for G1 storage	Cup planter, wash down procedure as for G1	Harvester	Solid set irrigation	No previous potato crop in the field, no commercial potato crops in the district	Moulding operations through the field, and Herbicide for weed control using a boom sprayer	No weeds due to chemical control (spray) and no volunteers

* All G1 crop planted using whole minituber, no cutting.

2.4 Discussion

The recent introduction of nursery farms for the production of G1 crops within the Tasmanian seed potato industry has resulted in reduced prevalence and incidence of PVS in G2 crops. In the 2005/2006 season and 2008/2009 season, PVS was detected in 40/59 (67.8%) and 13/56 (23.2%) of G2 crops tested, respectively, with 36 and 12 of these crops above the National Standard for Certification of seed potato (1%), respectively (Hay *et al.* 2006, Lamberts *et al.* unpublished). Interviews conducted with seed potato growers during this study suggest that several growers in isolated areas with no previous history of PVS in seed potato crops had not changed practices considerably. However, growers in areas with PVS infection in G2 crops had altered on-farm hygiene practices in recent years, with many growers adopting isolated nursery farm production of G1. As a result elimination of PVS from early generations (G1 and G2) has been achieved on farms adopting such agronomic practice changes.

During 2003, a virus survey of three different generations (G1, G2 and G3) within the same season was conducted by Lambert (2007). There was a general trend for increase in PVS in succeeding generations. PVS was detected at low incidence in three early generation crops (G1) at 0.3-3.5%. The finding of virus in such early generation material is of major concern given the potential for increase over generations (G1-G4). Results of this study showed in most cases PVS incidence increased in each subsequent generation (G1-G3) for each field. In some fields a levelling off or decrease of PVS incidence was detected between G3 and G4 (Lambert 2007). The study conducted by Lambert (2007) highlights the importance of maintaining early generation seed potato free of PVS.

Routine testing of G2 crops conducted by the Tasmanian Seed Potato Certification Scheme showed PVS incidence for Field 1 (Riana) var. Russet Burbank G2 in 2007/2008 was greater than 50% PVS infection. This suggests the G2 crop may have provided a source of PVS infection for the G1 crop, which was planted in the middle of the G2 field. However, no movement was recorded within the G1 plot and PVS was not detected at flowering within the G1 crop. This indicates PVS infection of G2 in

2008/2009 season may have occurred either, i) later in the G1 season, ii) at G1 harvest, iii) during G1 storage, iv) during G2 seed cutting, v) at G2 planting, vi) within the field during the G2 crop, or vii) a combination of the above.

In Field 1 (Riana) the grower reported high numbers of grasshoppers during the 2007/2008 season. Grasshoppers and planthoppers have been reported to facilitate transmission of PVX between PVX-infected plants and healthy plants (Brunt and Loebenstein 2001), probably through mechanical transmission. Transmission of PVX can be facilitated by the grasshopper, *Tettigonia viridisima* (Schmutter 1961 cited in Brunt and Loebenstein 2001) and *Melanoplus differentialis* (Schmutter 1961 cited in Brunt and Loebenstein 2001). However, the contribution of insects to the spread of PVX in the field is not known. Transmission of PVS by grasshoppers has not been reported but further research may be beneficial to test whether this is possible.

Other studies have found seed cutting can attribute to the transmission of PVS from infected seed pieces to healthy seed pieces (e.g. Franc and Banttari 1984). In field 3 the G2 crop was pre-cut with a dedicated G1 cutter following strict wash down hygiene protocol of high pressure hose washing followed by application of sterilant (Virkon® S). Pre-cutting seed pieces reduced the risk of virus transmission due to the lack of sprouts on tubers. The importance of adopting hygiene protocols for storage bins is highlighted by the retention of PVS in the absence of a host plant. Franc and Banttari (1984) found PVS to remain infectious for 120 hr and 204 hr, where sap from PVS infected plants was maintained in beakers with buffer absent and buffer present, respectively. PVS particles can remain viable in sap for 72-96 hours (Brunt *et al.* 1996). PVS particles were not viable on unpainted wood after a period of 180 hr at 4°C and 100% relative humidity (Franc and Banttari 1984), however PVS particles have been shown to remain viable for over 120 hours on some surfaces (Table 7.) (Banttari *et al.* 1993).

Table 7. Retention of infectivity (in hours) by *Potato virus S* (PVS) on various materials (from Banttari *et al.* 1993).

Material	Retention of infectivity of PVS (hours)
Iron or aluminum foil	7
Unpainted wood	0
Painted wood	-
Burlap	120
Cotton	-
Soil	25
Rubber	25
Human skin	-
Expressed sap from potato foliage	120

Weeds were considered to be at low incidence or not present in the fields, and volunteers potatoes were not reported in any of the fields surveyed. Given the majority of fields assessed had rotations between potato of seven years to greater than 50 years, or fields that had never been planted to potato, indicates it is unlikely that potential weed hosts have contributed to PVS infection. The lack of PVS infection in fields 2, 3, 4, and 5 where G1 crops were isolated from other potato, indicates isolation is effective for maintenance of low virus incidence in seed potato.

3. Aphid transmission studies of Tasmanian PVS isolates

3.1 Introduction

All isolates of PVS are mechanically transmissible. However, some are transmitted in a non-persistent fashion by aphids. Aphid species reported as vectors of PVS include *Aphis nasturtii* (buckthorn aphid), *Aphis fabae*, *Rhopalosiphum padi* L. (bird cherry/oat aphid) and *Myzus persicae* Silz. (green peach aphid) (Brunt and Loebenstein 2001). Of these *R. padi* and *M. persicae* are present in Tasmania (L. Hill, Department of Primary Industries, Parks, Water and Environment (DPIPWE), *personal communication*). The ability of PVS to be transmitted by aphids has implications for how the virus would need to be managed in seed potato crops. This study was undertaken to determine whether isolates of PVS in Tasmania can be transmitted by a commonly occurring aphid vector (*Myzus persicae*).

3.2. Materials and methods

3.2.1 Isolates used in aphid transmission studies

Five PVS isolates collected from Tasmanian seed potato were assessed for aphid transmissibility by *M. persicae*. PVS isolates were collected from tubers on 6 August 2007 from different potato seed lines within the Simplot Australia Pty. Ltd. Grower's Line Trial conducted in the 2006/2007 season. Tubers from each seed line were stored in hessian bags at 4°C for 4 months. Tuber dormancy was broken by placing tubers in individual containers at room temperature (15-20°C) for 4 days prior to planting of tubers. Tubers were planted in 200 mm pots in commercial potting mix and maintained at ~20°C under greenhouse conditions. One leaflet from each plant was collected and tested for PVS by DAS-ELISA (section 2.2.2). A total of five PVS isolates (from different seed lines) were selected - TAS07-10.1 (Riana), TAS07-5.4 (Ridgley), TAS07-11.6 (Scottsdale), TAS07-3.8 (Ridgley) and TAS07-22.6 (Mt Seymour).

3.2.2 Aphid experiment

An aphid colony of *Myzus persicae* (green peach aphid) was established at the greenhouse and maintained on radish plantlets in an aphid proof enclosure. Aphid were collected by gently dislodging them from leaves with a paintbrush and collecting them in clean plastic containers. All handling of aphids was done with care to prevent breaking of the stylets (mouthparts) of aphids whilst feeding. Aphids were starved for 1 hour prior to transmission experiments.

Four leaves from each of the five PVS infected source potato plants were added to individual containers of starved aphids (one isolate per plastic container). Aphids were observed for probing activity and allowed to probe infected leaf material for 15 minutes. Ten aphids were carefully transferred to a 2 cm diameter aphid cage mounted on a hair clip. One aphid cage containing 10 aphids was placed onto a leaf on each of 20 healthy potato plants, var. Russet Burbank. Healthy potato plants were grown from minitubers sourced from the TIAR minituber production facility and were grown in the greenhouse in an aphid proof enclosure. Plants were tested by DAS-ELISA approximately 1 week prior to use (section 2.2.2). Aphids were allowed to feed on healthy potato plants for 4-14 hours. Aphids were destroyed at the end of the feeding period and plants were sprayed with an insecticide to ensure any remaining aphids had been killed . Plants were placed in an aphid proof enclosure in the greenhouse and maintained at 20°C for 4-8 weeks. A total of 20 control potato plants, not inoculated with aphids, were also maintained under the same conditions. Three leaflets from each of the control and aphid compromised potato plants were collected 4 weeks and 8 weeks after the aphid inoculation experiments. Leaflets were individually virus tested for PVS by DAS-ELISA (section 2.2.2).

3.3. Results

For each of the five isolates of PVS, virus was not detected in leaflets from the 20 plants inoculated with aphids, or in the 20 control (non inoculated plants), at 4 or 8 weeks after aphid feeding occurred.

3.4 Discussion

Various overseas reports have shown that isolates of PVS vary with respect to their ability to be aphid transmitted (Table 8). Currently PVS is believed to occur as two strains – PVS^O (ordinary) and PVS^A (Andean). The latter strain is generally more aphid transmissible than the former (Table 8). Lambert *et al.* (2007) found no molecular evidence to suggest the presence of PVS^A amongst 23 isolates of PVS suggesting that isolates in Tasmania were predominately PVS^O. This suggested that aphid transmission of PVS in Tasmania might be limited. Results from this current study indicated five PVS isolates were not aphid transmissible by *M. persicae*. While the number of isolates tested for aphid transmissibility in this study was limited, they were sourced from different potato growing regions in Tasmania and would have been expected to be representative of the spectrum of isolates in Tasmania. This preliminary evidence suggests that aphid transmission by *M. persicae* is not a factor in transmission of PVS in Tasmania. However, this does not preclude transmission by other known aphid vectors present in Tasmania (*R. padi*), or perhaps some as yet unknown aphid vector, given that the range of aphid species tested for their ability to transmit PVS is limited (Table 8).

Table 8. Summary of studies into aphid transmission of *Potato virus S* (PVS).

Author	Virus Strain	Aphid species	Source of virus	Assay host	No. with virus/no. plants inoculated
Slack (1983)	PVS^A	*Myzus 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	Potato	0/29
Fletcher (1996)	PVS^A	*M. persicae*	Potato	*C. quinoa*	0/10
				Potato	0/10
	PVS^A	*Aulacorthum 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%, respectively)
				C. quinoa	$2/5^1$ and $2/5^2$ (40% each)
Weidemann and Koenig (1990)	PVS^A				36/426 (8.5%)
	PVS^O				5/210 (2.4%)

Table 8. Summary of studies into aphid transmission of *Potato virus S* (PVS) cont.

Author	Virus Strain	Aphid species	Source of virus	Assay host	No. with virus/no. plants inoculated
Weidemann (1986)					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 (6.6%)
		M. persicae (apterae)			[3]RP=2/17 (11.8%), RB=0/17, Shep=2/16 (12.5%), Seb=2/14 (14.3%)
		Aphis 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 overnigh

t[2] on assay host

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

4. Seed cutting experiments

4.1. Introduction

Seed cutting operations have been shown to be a means by which PVS is transmitted and increased within seed lines (Fanc and Banttari 1984). The Tasmanian seed potato industry is dependent on centralised seed cutters for a large proportion of potato cutting operations in Tasmania. Disinfestation of cutting blades with sterilant during the seed cutting process is recommended to reduce the possibility of virus transmission between seed lots and within seed lines. The efficacy of various sterilants was reported by (Lister 2004). The purpose of this part of the project was to determine if the antiviral chemical Virkon® S could be applied to cut seed pieces following cutting without inducing phytotoxicity in the subsequent crop. This would be beneficial in terms of reducing down time associated with cleaning seed cutting machinery between lines and in terms of reducing virus transmission within a seed line.

4.2 Materials and methods

4.2.1 Effect of Virkon® S on plant growth of Russet Burbank (2007/2008)

An initial field experiment was conducted to assess what concentrations of Virkon® S could be applied to cut seed tubers without inducing any phytotoxicity or negative effects on yield or quality. Results from this trial were used to determine Virkon® S concentrations suitable for virus transmission experiments in the 2008/2009 season. Russet Burbank tubers sourced from a crop grown at Sisters Creek were stored at 4ºC for four months. On 18 August 2007 tubers were removed from cold storage to break dormancy. Tubers were cut and treated on 8 October 2007. For each of treatments C to F (Table 9), 30 tubers were cut in two and dipped in sterilant (Virkon® S) for approximately 1 minute, no longer than 5 minutes after being cut. For treatment B, cut seed pieces were rolled in cement, while for the control (treatment A), tubers were cut with no further treatment. Cut tubers were dried in onion bags at room temperature.

The field trial was located at the TIAR Vegetable Research Facility at Forth, Tasmania on a Krasnozem soil type. The trial was established a completely randomized design with six replications of each treatment. Each plot consisted of 10 seed pieces planted in 1 row, 3.2 m long. Seed pieces were planted on 17 October 2007 with a Faun Planter at a spacing of 320 mm, with a buffer of 6 Red Norland tubers planted at the end of each plot. Fertiliser (11:13:19) was pre-drilled in bands with the Faun planter, at a rate of 1812 kg/ha.

The trial was harvested on 27 February 2008. Potato tubers were lifted with a Myers twin row potato harvester and placed into hessian sacks. Tubers were graded into different size categories 0-80 g, 80-250 g, 250-650 g, 650-850 g and >850 g. In commercial terms tubers less than 80 g would be considered too small for processing, while tubers in the 80-850 g range are processed, although 80-250 g is the preferred size range. Tubers greater than 850 g are also processed, but are undesirable due to the propensity for hollow heart in large tubers. Tubers were also graded as 'misshapen' if there were knobs or growth abnormalities, 'rots', and for 'cracks'. Hollow heart and brown centre was assessed by cutting in half 10 tubers from the largest available size grade for that sample and data presented as a percentage of the number of tubers affected. Flesh colour was also assessed visually on tubers used for hollow heart assessment, using a scale of 1 (white), 2 (off/creamy white), 3 (cream), 4 (dark cream), and 5 (yellow).

A subsample was assessed for specific gravity according to the equation (weight in air/(weight in air - weight in water)). Percentage dry matter was calculated as ((specific gravity – 0.983214)/0.004813). A sample of 5 tubers was assessed for bruising at the stem and rose ends and for shatter according to a scale of 0=nil, 1=slight, 2=moderate and 3=severe. A chip was excised from each of 10 tubers from each replicate and fried for 150 seconds at a temperature of 190^{o}C in cottonseed oil. Overall colour of each fry was visually scored by comparison to the USDA 1988 French fry colour chart, on a scale of C000, C00, C0, C1, C2, C3 and C4, with C000 being a desirable white colour and C4 being blackened. Commercially acceptable ratings for fry colour range from C000 to C1. Data was subjected to analysis of variance using Genstat V 9.1. Where the F test was significant (P<0.05), means were separated by least significant difference.

Table 9. Rates used to assess potential phytotoxicity of Virkon® S when applied to the cut surface of seed potato.

Treatment No.	Treatment Description
A	Seed piece cut and no further treatment
B	Seed piece cut and rolled in cement dust only after cutting
C	Seed piece cut and dipped in 0.1% Virkon®S solution
D	Seed piece cut and dipped in 0.5% Virkon®S solution
E	Seed piece cut and dipped in 1.0% Virkon®S solution
F	Seed piece cut and dipped in 2.5% Virkon®S solution

4.2.2 Assessment of PVS-transmission protocol

Prior to the commencement of the 2008/2009 seed cutting experiment it was necessary to assess whether any reduction in virus transmission by applying Virkon® S to the cut surface of tubers arose from the antiviral nature of the sterilant, or through the mere act of dilution following application of a liquid. On 18 August 2008 one sprout from two different eyes (approximately 5-10cm in length) was taken from each of five PVS-infected tubers (var. Red Norland) and virus tested for PVS by DAS-ELISA (section 2.2.2). The same tubers were used as PVS-source material for assessment of PVS-transmission protocol trial and cutting trial conducted in 2008/2009 season.

PVS-infected sprouted tubers (var. Red Norland) were cut into pieces and macerated in a motorised roller press in a phosphate buffer solution. 0.1M phosphate buffer, pH 7.6 containing 65 ml of 0.2 M solution (31.2g $NaH_2PO_4.2H_2O$, made up to 1 L with distilled water) and 435 ml of 0.2 M solution (28.39g Na_2HPO_4, made up to 1 L with distilled water), diluted to a total of 1 L with distilled water to give pH 7.6.

On 22 August 2008 an experiment was conducted to assess potential influence on PVS transmission with rinsing of cut tubers pieces with water. Twenty healthy minitubers were cut in half using a sterilised knife then dipped in the PVS-infected phosphate solution, containing PVS-infected tubers and sprout pieces, for 1 minute. Phosphate buffer was used to improve the transmission of PVS. For Treatment A, twenty of the total 40 seed pieces were removed from the PVS-infected phosphate solution and placed on a clean metal tray and allowed to cure for two days. Seed pieces were then planted individually in 200 mm pots with commercial potting mix, and maintained under glasshouse conditions for 8 weeks. For Treatment B, the remaining twenty seed pieces were removed and dipped in distilled water (for 5 seconds) to resemble a rinsing action that tubers would be subjected to under a Virkon® S trial conducted in the previous growing season in 2007/2008. Seed pieces from Treatment B were planted out as previously mentioned for Treatment A. One leaflet from each potato plant from Treatment A and Treatment B was collected on 16 October 2008 and virus tested for PVS by DAS-ELISA (section 2.2.2).

4.2.3 Effect of Virkon® S on PVS transmission and crop yield and quality (2008/2009)

Healthy tubers were sourced from tissue culture plantlets (var. Russet Burbank) from TIAR Seed Potato Certification minituber production centre (Devonport). Tissue culture plantlets were planted into 400 mm pots on 23 July 2007 and grown under glasshouse conditions for six months. Minitubers were harvested on 16 January 2008 and stored at 4ºC until required for cutting experiments in 2008.

A trial was conducted on 20 October 2008 to determine the effect of treating seed pieces after the cutting process with Virkon® S (antiviral chemical), cement and water and the ability of each treatment to reduce virus transmission of PVS. Healthy minitubers of var. Russet Burbank sourced from the TIAR mintuber production facility were used to assess a range of treatments for disinfecting the tuber surface during hand seed cutting operations. A total of 450 healthy minitubers were cut in half (a total of 900 seed pieces) with a sterilised knife. Cut healthy seed pieces were placed in phosphate solution, containing a macerate of PVS infected tubers and sprout pieces, for 1 minute as previously described (Section 4.2.2). Seed pieces were exposed to

different treatments (Table 10.), with the exception of Treatment A (negative control), where seed pieces were cut with a sterilised knife and no further treatment was applied. Treatments included 0.1% and 1% Virkon® S, an antiviral agent, cement dust and water (Table 10). Cement dust was included as it has been historically used as a means of curing the cut surface of seed potato. A total of three replicates (50 tubers per treatment per replicate) were conducted for each treatment. Fresh PVS-infected phosphate buffer was used for each replicate.

Table 10. Different treatments used for hand cutting experiment to assess transmission of *Potato virus S* (PVS).

Treatment	Description
A	Negative healthy control - cut and no treatment
B	Positive control - cut and dipped in PVS macerate in phosphate buffer and no treatment
C	Tuber cut, placed in PVS macerate in phosphate buffer and dipped in cement dust
D	Tuber cut, placed in PVS macerate in phosphate buffer and sprayed with distilled water (500 ml spray bottle)
E	Tuber cut, placed in PVS macerate in phosphate buffer and sprayed with 0.01% Virkon® S solution (500 ml spray bottle)
F	Tuber cut, placed in PVS macerate in phosphate buffer and sprayed with 1% Virkon® S solution (500 ml spray bottle)

Prior to planting cut seed pieces were placed in onion bags and maintained at room temperature for 4 days to cure seed pieces. The trial was established at the TIAR Vegetable Research Facility, Forth, Tasmania. Fertiliser (11:13:19) at 1840 kg/ha was band placed by predrilling into moulds with a Faun planter on 17 October 2008. Seed pieces were planted at a spacing of 300 mm with a Faun planter on 24 October 2008. For each plot there were 2 rows of 25 seed pieces, with plots 7.5 m long. The experiment was established as a completely randomised design with 3 replicate plots per treatment. Applications of the herbicides Sprayseed (2 L/ha), Lexone (350 g/ha) and activator (80 ml/100l) were applied on the 11 November 2008. The

insecticide chlorpyrifos was applied at 15 ml/15 L and 35 ml/10 L on 21 November (2008) and 4 December 2008) with a backpack sprayer to control cutworm damage.

Foliage from potato plants was virus tested for PVS by DAS-ELISA (section 2.2.2) at emergence, prior to row closure and before senescence. To minimise potential in-field PVS virus transmission, no movement of machinery occurred in the field during the growing season.

The trial was harvested on 26 March 2009 as described in section 4.2.1, and tubers size graded. Data was subjected to analysis of variance using Genstat V 9.1. Where the F test was significant ($P<0.05$), means were separated by least significant difference. Due to cutworm damage in some plots the yield and size grading was analysed both on a total plot area (Table 13), and on an adjusted area based on the number of plants per plot (Table 14).

4.3. Results

4.3.1. Effect of Virkon® S on plant growth of Russet Burbank (2007/2008)

Treatment C (Virkon® S 0.1%) had significantly higher total yield ($P<0.05$) than non-treated (A), 0.5% Virkon® S (D), 1.0% Virkon® S (E) and 2.4 % Virkon® S (F), with cement (B) intermediate between and statistically indistinguishable from these groups (Table 11). There was no significant difference between treatments in terms of bruise rating, specific gravity, dry matter, hollow heart assessment or flesh colour (Table 12). All fry assessments between 000 and 0 are commercially acceptable so any significantly higher or lower ratings within this range were not considered to be of practical significance.

Table 11: Effect of different concentrations of Virkon® S on yield of Russet Burbank.

	No treatment	Cement	0.01% Virkon® S	0.5% Virkon® S	1.0% Virkon® S	2.4% Virkon® S	LSD *P*=0.05
No. of tubers in different size and quality categories (per m^2):							
0-80 g	7.5	9.1	7.7	7.3	7.2	6.2	ns
80-250 g	27.6	26.9	28.1	24.0	19.7	23.9	ns
250-650 g	4.3	3.9	4.3	4.5	5.7	5.0	ns
650-850 g	0	0	0	0	0	0	na
>850 g	0	0	0	0	0	0	na
Yield (t/ha) in different size and quality categories:							
0-80 g	3.3	4.2	3.4	3.3	3.3	2.9	ns
80-250 g	41.8	42.4	43.1	26.9	30.5	36.5	ns
250-650 g	12.6	11.7	12.8	15.2	18.6	16.0	ns
650-850 g	0	0	0	0	0	0	na
>850 g	0	0	0	0	0	0	na
Fry grade >80 g	54.3	54.1	56.0	52.1	49.1	52.4	ns
Total yield	58.3 ab	59.8 ab	63.2 a	57.5b	54.9b	57.5b	5.0
80-650g Yield	54.3	54.1	56.0	52.1	49.1	52.4	ns
% of Fry grade Wt. >250g	23.6	21.6	23.3	29.4	38.5	30.9	ns
Waste Yield	0.7	1.4	3.9	2.1	2.5	2.2	ns

ns = not significant, na = not analysed

Table 12: Effect of different concentrations of Virkon® S on potato processing quality of Russet Burbank.

	No treatment	**Cement**	**0.01% Virkon® S**	**0.5% Virkon® S**	**1.0% Virkon® S**	**2.4% Virkon® S**	**LSD *P*=0.05**
	1.0	1.0	1.0	1.0	1.0	1.0	na
Flesh colour							
Stems per plant	2.6	2.6	2.5	2.5	2.3	2.4	ns
Bruise ratings:							
Stem end	5.1	5.8	6.0	5.2	5.7	5.5	ns
Rose end	3.5	4.7	4.0	4.1	3.5	4.0	ns
Shatter	0.1	0	0	0.2	0.2	0.2	ns
Specific Gravity	1.089	1.089	1.09	1.092	1.091	1.091	ns
Dry matter (%)	22.1	22.0	22.1	22.5	22.3	22.4	ns
Fry colour: percentage of tubers in following categories:							
C000	0	96.7	95.0	94.8	78.3	86.7	13.9
C00	96.5	3.3	5.0	5.2	13.3	13.3	11.4
C0	0	0	0	0	8.3	0	na
C1	3.5	0	0	0	0	0	na
Internal disorders (% of tubers)							
Hollow heart	7	3	3	10	5	5	ns
Brown centre	15	20	15	22	13	12	ns
Total disorders	7	20	7	10	12	12	ns

ns = not significant, na = not analysed

4.3.2. Assessment of PVS-transmission protocol

When seed pieces were dipped in PVS macerate in phosphate buffer and allowed to cure (treatment A) prior to planting, PVS was detected in leaflets of 3/17 (17.7%) of the plants which subsequently grew. By comparison, when seed pieces were dipped in PVS macerate in phosphate buffer and then dipped in distilled water prior to curing and planting, PVS was not detected in leaflets from any of the resulting plants. This suggested that any trial to determine if liquid sterilant is capable of reducing virus transmission, should incorporate a control of water only to distinguish between the activity of the sterilant and the dilution effect.

4.3.3 Effect of Virkon® S on PVS transmission during seed cutting and crop yield and quality (2008/2009)

PVS was detected in 4/37 (10.8%) leaflets at emergence from plants grown from seed pieces treated with cement (Treatment C), in one of the three replicate plots. However, PVS was not subsequently detected in this plot of at flowering or senescence. The trial was affected by cutworm which led to plant death in some plots which may have explained the inability to detect PVS in this plot subsequently. All other treatments were free of PVS at all times of virus testing, including emergence, flowering and before senescence.

In the data unadjusted for differences in plant numbers arising from cutworm infestation, the negative control had significantly higher total numbers of tubers in size range of 0-80 g ($P<0.05$) than the positive control, water only, and 0.1% Virkon® S. The 1.0% Virkon® S treatment was intermediate between and statistically indistinguishable from these two groups (Table 13). The negative control had a slightly higher yield ($P<0.001$) of cracked tubers than other treatments (Table 13). Cement treatment had significantly ($P=0.04$) higher percentage of yield as fry grade greater than 250 g in size than all other treatments, except 1.0% Virkon® S (Table 13). Treatment had no significant effect on other size gradings, tubers per plant or internal disorders (hollow heart and brown centre). There were significant differences in plants per plot between treatments (Table 13), however this was a reflection of cutworm damage rather than any treatment effect.

When data was adjusted for plant deaths due to cutworm, the negative control again had a significantly (P<0.001) higher yield of cracked tubers than all other treatments (Table 14). Similarly the cement treatment had a significantly higher percentage of fry grade tubers greater than 250 g in size than all other treatments except the Virkon 1% treatment (Table 14).

Table 13: Effect of potential antiviral (Virkon® S) treatments on the mean yield and quality of potato var. Russet Burbank (harvest data based on plot size at planting and not adjusted for differences in plant numbers at harvest).

	Negative control	Positive control	Cement	Spray with water	Spray with 0.01% Virkon® S	Spray with 1% Virkon® S	F test P=
No. of tubers in different size and quality categories (per m^2):							
0-80 g	16.23 a	9.13 b	8.26 b	8.92 b	15.18 a	12.01 ab	0.03 (5.51)[1]
80-250 g	17.37	14.40	9.50	13.69	15.88	11.84	0.158 ns[2]
250-650 g	10.22	8.06	6.70	8.29	8.83	7.86	0.282 ns
650-850 g	0.19	0.43	0.63	0.27	0.46	0.35	0.468 ns
>850 g	0.03	0.09	0.25	0.08	0.05	0.08	0.306 ns
Cracked	0.14	0.0	0.03	0.03	0.03	0.05	0.11 ns
Misshapen	4.07	3.98	3.33	2.79	3.50	4.50	0.717 ns
Rots	0.03	0.03	0.00	0.05	0.08	0.03	0.674 ns
Yield (t/ha) in different size and quality categories:							
0-80 g	5.54	4.15	2.90	4.80	5.75	4.44	0.118 ns
80-250 g	25.10	20.40	13.30	20.50	24.20	17.80	0.08
250-650 g	36.10	29.10	27.10	29.30	33.70	30.00	0.502 ns
650-850 g	1.35	3.60	4.47	2.47	3.23	2.65	0.587 ns
80-650 g	61.20	49.50	40.30	49.80	57.90	47.80	0.207 ns
>850 g	0.25	0.82	2.29	0.81	0.53	0.84	0.340 ns
Fry grade >80 g	62.8	53.9	47.1	53.1	61.6	51.3	0.326 ns
Total yield	81.6	72.4	62.9	71.1	78.5	70.5	0.217 ns
Cracked	0.36 a	0.00 b	0.04 b	0.03 b	0.04 b	0.09 b	<0.001 (0.125)
Misshapen	12.87	14.26	12.85	13.02	10.98	14.70	0.785 ns
Rots	0.07	0.12	0.0	0.17	0.08	0.05	0.854 ns
Waste	13.31	14.38	12.89	13.22	11.10	14.84	0.773 ns
Percentage of fry grade yield >250 g	59.9 b	62.1 b	72.1 a	62.2 b	60.9 b	65.4 ab	0.043 (7.64)
Tubers per plant	13.09	11.67	11.20	10.64	12.95	11.99	0.263 ns
Brown centre (% of tubers)	13.3	13.3	20.0	13.3	13.3	13.3	0.993 ns
Hollow centre (% of tubers)	6.7	3.3	20.0	6.7	6.7	16.7	0.499 ns
Total internal disorders (% of tubers)	20.0	16.7	40.0	20.0	20.0	30.0	0.532 ns
Plants per plot	45.3 a	35.7 bc	31.3 c	39.3 a	41.7 ab	38.0 bc	0.012 (6.8)

[1] Least significant difference in parentheses, [2] ns = not significant

Table 14: Effect of potential antiviral (Virkon® S) treatments on the mean yield and quality of potato var. Russet Burbank (harvest data adjusted for differences in plant numbers at harvest).

	Negative control	Positive control	Cement	Spray with water	Spray with 0.01% Virkon® S	Spray with 1% Virkon® S	F test P=
No. of tubers in different size and quality categories (per m^2):							
0-80 g	17.85	12.00	13.05	11.63	18.18	15.98	0.208 ns[1]
80-250 g	19.15	18.93	15.21	16.99	18.98	15.73	0.713 ns
250-650 g	11.32	10.57	10.46	10.37	10.57	10.33	0.980 ns
650-850 g	0.22	0.57	1.06	0.34	0.56	0.48	0.313 ns
>850 g	0.03	0.12	0.42	0.11	0.07	0.10	0.203 ns
Cracked	0.15	0.00	0.04	0.04	0.03	0.07	0.193 ns
Misshapen	4.46	5.20	5.29	3.72	4.18	6.02	0.646 ns
Rots	0.03	0.04	0.00	0.07	0.10	0.03	0.675 ns
Yield (t/ha) in different size and quality categories:							
0-80 g	6.11	5.44	4.54	6.03	6.88	5.87	0.428 ns
80-250 g	27.70	26.80	20.90	25.50	29.00	23.30	0.382 ns
250-650 g	40.00	38.20	42.30	36.70	40.40	39.30	0.911 ns
650-850 g	1.53	4.78	7.47	3.04	3.89	3.63	0.372 ns
80-650 g	67.7	65.0	63.1	62.2	69.3	62.7	0.918 ns
>850 g	0.29	1.08	3.87	1.12	0.66	1.05	0.229 ns
Fry grade >80 g	69.5	70.9	74.5	66.4	73.9	67.4	0.810 ns
Total yield	90.2	95.1	99.5	89.6	94.1	92.8	0.486 ns
Cracked	0.40 a	0.00 b	0.06 b	0.04 b	0.05 b	0.11 b	<0.001 (0.159)[2]
Misshapen	14.1	18.7	20.5	17.0	13.2	19.4	0.290 ns
Rots	0.08	0.15	0.00	0.20	0.10	0.07	0.856 ns
Waste	14.61	18.83	20.52	17.26	13.31	19.58	0.298 ns
Percentage of fry grade yield >250 g	59.9 b	62.1 b	72.1 a	62.2 b	60.9 b	65.4 ab	0.043 (7.64)

[1] ns = not significant, [2] Least significant difference in parentheses

4.4 Discussion and conclusions

Franc and Banttari (1984) demonstrated PVS to be readily transmitted from PVS-infected tubers to healthy tubers of var. Russet Burbank during hand cutting, especially if the cutting knife was in contact with a sprout of a tuber (45.2% transmission) compared to the knife being passed through non-sprout tuber tissue (24.5% transmission). Seed cutting is considered an important means by which PVS can be spread. However, several experiments conducted previously by the project investigators (Hay *et al*. 2006, Lambert 2007) have been unable to unequivocally demonstrate transmission of Tasmanian isolates of PVS by seed cutting. To increase the likelihood of transmission in this study, we used PVS infected tuber sprout tissue and tuber tissue macerated in phosphate buffer as an inoculum source for cutting experiments. This resulted in 17.7% infection in plants grown from seed pieces treated in this manner (section 4.3.2). However, in a subsequent field trial (section 4.3.3) this technique resulted in a low level of PVS transmission which prevented a full assessment of the ability of Virkon®S to reduce virus transmission when applied to the surface seed pieces following cutting. The two field trials did however did suggest no phytotoxic effect of Virkon®S at the rates used, suggesting that if it could be shown to reduce virus transmission, it might be employed in this regard. Such a treatment would have considerable advantages for the large mechanized central seed cutting facilities, in terms of reducing the need to wash down machinery between seed lines and affording a means of reducing virus transmission within seed lines.

5. Acknowledgements

The authors would like to gratefully acknowledge the financial support from the Commonwealth Government through Horticulture Australia Ltd. and voluntary contributions from Simplot Australia Ltd., McCains Foods (Australia) Pty. Ltd., Harvest Moon Forth Farm Produce, and Tasmanian seed potato growers. The authors would also like to thank TIAR Seed Potato Certification Officers including Leonie White, Phillip Beveridge, Ann Marie Donaghue for collecting samples as part of the survey, and Leon Hingston and Lyndon Butler (TIAR) for technical assistance and maintenance of field trials. The authors would also like to thank members of the Tasmanian Virus Strategy Group for advice and guidance.

6. References

Anonymous (2008a) Agricultural Commodities, Australia Cat. No. 7121.0 (year ending 30 June 2007) Australian Bureau of Statistics (online). 28/07/2008. http://www.ausstats.abs.gov.au/ausstats/subscriber.nsf/0/6F17DC7080D215F8CA2574560014ADB0/$File/71210do003_200607.xls#TopOfTable_Table_2 [Accessed 05/08/2008].

Anonymous (2008b) Value of agricultural commodities produced, Australia Cat. No. 7503.0 (year ending 30 June 2007) Australian Bureau of Statistics (online). 28/07/2008. http://www.abs.gov.au/AUSSTATS/abs@.nsf/DetailsPage/7503.02006-07?OpenDocument [Accessed 05/08/2008].

Banttari, E.E., Ellis, P.J., and Khurana, S.M.P. (1993). Management of disease caused by viruses and virus like pathogens. Pp. 127-133. *In:* R.C. Rowe (Ed.) Potato Health Management. American Phytopathological Society Press, St. Paul, Minnesota, USA.

Brunt, A.A., Crabtree, K., Dallwitz, M.J., Gibbs, A.J., and Watson, L. (1996) Viruses of plants. CAB International, Wallingford, U.K.

Brunt, A.A, and Loebenstein, G. (2001) The main viruses infecting potato crops. Pp. 65-133. *In*: G. Loebenstein, P.H. Berger, A.A. Brunt and R.H. Lawson (Eds.) Viruses and virus-like

disease of potato and production of seed-potatoes. Kluwer Academic Publishers. Dordrecht. The Netherlands.

Clark, M.F., Adams, A.N. (1977) Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *Journal of General Virology* **34,** 475-483.

Fletcher, J.D. (1996) Potato virus S^A - characteristics of an isolate from New Zealand. *New Zealand Journal of Crop and Horticultural Science* **24,** 335-339.

Franc, G.D. and Banttari, E.E. (1984) Translocation and mechanical spread of a Minnesota isolate of potato virus S in potatoes. *American Potato Journal* **73,** 123-133.

Gibbs, A.J., Gower, J.C. (1960) The use of multiple-transfer method in plant virus transmission studies - some statistical points arising in the analysis of results. *Annals of Applied Biology* **48,** 75-83.

Hay, F., Kirkwood, I., Lambert, S., Cross, P., Wilson, C., Pethybridge, S. (2004) A survey to determine the prevalence and incidence of common viruses in potato seed stocks in Tasmania. Final Report for Project PT02037 (March 2004). Horticulture Australia Ltd.

Hay, F., Lambert, S., Kirkwood, I., Cross, P., Wilson, C., Pethybridge, S. (2005) Management strategy for elimination of virus from certified seed potato stocks in Tasmania. Final Report for Project PT03069 (April 2005). Horticulture Australia Ltd.

Hay, F., Lambert, S., Cross, Kirkwood, I., P., Wilson, C., Pethybridge, S. (2006) Managing viruses in Tasmania seed potato stocks. Final Report for Project PT05011 (December 2006). Horticulture Australia Ltd.

Kirkwood, I. (2003) Potato virus testing. Department of Primary Industries and Water (online) http://www.dpiw.tas.gov.au/inter.nsf/WebPages/TTAR-5CL4EH?open [Accessed 30/06/09].

Lambert, S.J. (2007) Epidemiology of Potato virus S and Potato virus X in seed potato in Tasmania, Australia. Thesis. University of Tasmania, Hobart, Tasmania, Australia.

Lambert, S.J., Hay, F.S., Pethybridge, S.J., Kirkwood, I.A. and Wilson, C.R. (2007) Strain characterisation of Potato Virus S from seed potato in Tasmania, Australia. Proceedings of the 16th Biennial Australasian Plant Pathology Society Conference, 24-27 September 2007, Adelaide, South Australia. Pp. 135.

Lister, R. (2004) Literature survey of plant virus/viroid decontaminating agents. Crop and Food Research Report No. 1023. New Zealand Institute for Crop and Food Research Ltd., Christchurch, New Zealand. 23 pp.

MacKinnon, J.P. (1974) Detection, spread, and aphid transmission of potato virus S. *Canadian Journal of Botany* **52,** 461-465.

Santillan, F.W. (1979) Estudio comparativo de once aislamientos de virus S de la region Andina. MSc. Thesis, Universidad Nacional Agraria, Lima.

Slack, S. (1983) Identification of an isolate of the Andean strain of potato virus S in North America. *Plant Disease* **67**, 786-789.

Sutula, C.L., Gillet, J.M., Morrissey, S.M., Ramsdel, D.C. (1986) Interpreting ELISA data and establishing the positive-negative threshold. *Plant Disease* **70**, 722-726.

Thomas, A.D. (1976) Virus diseases of *Solanum laciniatum* Ait. in New Zaeland. *New Zealand Journal of Agricultural Research* **19,** 521-527.

Wardrop, E.A., Gray, A.B., Singh, R.P., and Peterson, J.F. (1989) Aphid transmission of potato virus S. *American Potato Journal* **66,** 449-459.

Weidemann, H.-L. (1986) The spread of potato viruses S and M under field conditions. *Potato Research* **29**, 109-118.

Weidemann, H.-L., and Koenig, R. (1990) Differentiation of isolates of potato virus S which infect *Chenopodium quinoa* systemically by means of quantitative cDNA hybridization tests. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz* **97**, 323-327.

Appendix 1. Technology transfer activities undertaken as part of project PT06044.

The main technology transfer has been through annual meetings with members of the Tasmanian Virus Strategy Group through the life of the project. Results from this project were also communicated to potato industry representatives at a 'Post Harvest Wrap Up' held at TIAR Vegetable Research Facility, Forth (Tasmania) on 24th September 2008. Two articles on *Potato virus S* research were published:

Hay, FS and Lambert, SJ, 'Managing the hidden threat of PVS and PVX in seed potato crops', *Potatoes Australia*, October 07, pp 20-21 (2007) [Magazine Article]).

Lambert, SJ, 'Update on viruses in Tasmanian seed potatoes', Tas Regions - TIAR Vegie, Department of Primary Industries & Water, Hobart, Tasmania, March, Autumn (2009).