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Improving virus control in seed schemes by combining aphid monitoring and virus testing

Mark Holland
Department of Agriculture
Western Australia

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IMPROVING VIRUS CONTROL IN SEED SCHEMES BY COMBINING APHID MONITORING AND VIRUS TESTING

FINAL REPORT

Mark Holland *et al.*
Department of Agriculture
Western Australia

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IMPROVING VIRUS CONTROL IN SEED SCHEMES BY COMBINING APHID MONITORING AND VIRUS TESTING

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Purpose:

This is the final report for Project PT03061 'Improving virus control in seed schemes by combining aphid monitoring and virus testing'.

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1. MEDIA SUMMARY

The WA potato industry is keen to ensure the quality of its seed is of the highest standard. In the past serious outbreaks of virus have occurred which concern the domestic industry and pose a threat to emerging export markets. This project introduced an improved method of virus detection to determine more objectively than field inspection alone, the level of virus in generation 3 seed. It also introduced a preventative strategy by operating a pest monitoring service to detect the presence of vectors of viruses in crops. Collation of the data from this and preceding projects confirmed the high health status of Western Australian seed potatoes. Results also illuminated areas of improvement at the grower, seed scheme and state levels and has implications for the improvement of the current National Standard for the Certification of Seed Potatoes.

The project found:

- In 2003/04 the incidence of the following viruses in 6,892 generation two sown plants, sampled close to senescence was: potato leafroll virus 0.14 per cent, tomato spotted wilt virus 0.03 per cent, potato virus Y 0.03 per cent, potato virus S 0.12 per cent and potato virus X 0.52 per cent. The results showed that both seed schemes were meeting their respective objectives. Overall, this laboratory determined level compares favourably with the visually determined national standard for virus in generation three seed, which is 0.1 per cent for seed for further multiplication and 1.0 per cent for certified seed for sale. The PVY find resulted in steps being taken to eradicate the disease in WA.
- Australian seed certification schemes rely on visual identification of viruses. As many viruses are difficult to detect by symptoms alone, virus testing significantly enhances the effectiveness of the seed certification system by identifying and quantifying infection levels. Appropriate virus management strategies (Eyes on Potatoes, March 2004) can then be employed.
- Results of the monitoring showed that pest pressure and the need for action varied between regions, within regions and on individual farms.
- Collation of the results from this and preceding projects show that some seed growers had recurring problems with the same virus.

Recommendations stemming from the project are:

- Virus testing become a permanent component of the seed schemes of Western Australia and be deployed to later generations in addition to generation two sown crops.
- Virus testing be considered for adoption by the National Standard for the Certification of Seed Potatoes to improve the effectiveness of Australian seed certification schemes and to demonstrate the viral health of certified seed.
- Future research to develop a cost effective, rapid, quantitative, PCR test and sampling procedure to measure virus level in dormant tubers. This contrasts with the current choice of testing leaf material to estimate virus level in tubers or waiting several weeks for tuber dormancy to break before testing can be conducted.

2. TECHNICAL SUMMARY

The WA potato industry is keen to ensure the quality of its seed is of the highest standard. In the past serious outbreaks of virus have occurred which concern the domestic industry and pose a threat to emerging export markets. This project introduced an improved method of virus detection to determine more objectively than field inspection alone, the level of virus in generation 3 seed. Collation of the data from this and preceding projects confirmed the high health status of Western Australian seed potatoes. Results also illuminated areas of improvement at the grower, seed scheme and state levels and has implications for the improvement of the current National Standard for the Certification of Seed Potatoes.

In 1997 two new seed schemes, a Certified Seed Scheme and a Registered Seed Scheme were introduced by the Western Australian Department of Agriculture in consultation with the seed potato industry. The Certified Seed Scheme was created primarily to facilitate exports and the Registered Seed Scheme upgraded the pre existing Approved Seed Scheme.

This project used a combination of insect monitoring and virus testing to supplement the procedures of the National Standard for the Certification of Seed Potatoes in Australia. Insect monitoring was combined with virus testing to demonstrate the effectiveness of a monitoring program. It is expected to encourage seed growers to adopt their own monitoring program rather than rely on calendar spraying to protect crops from insect transmitted viruses.

Virus testing was conducted to estimate the incidence of five major tuber-borne viruses in generation three seed. Samples from 6,892 plants from fourteen seed growers were tested for potato leafroll virus (PLRV), tomato spotted wilt virus (TSWV), potato virus Y (PVY), potato virus S (PVS) and potato virus X (PVX). Approximately five hundred leaf samples were taken across each of fourteen seed grower's generation two sown crops when they were close to senescence.

Plant samples were tested in the Department of Agriculture's virology laboratory under the supervision of Dr Roger Jones. PLRV was detected by Tissue blotting immunoassay (TBIA) (Hsu and Lawson, 1991; Lin *et al.* 1990). Samples were tested for the other viruses by Enzyme-linked immunosorbent assay (ELISA) in groupings of 10 leaves per test. The number of infected samples identified by the ELISA method was then estimated using the formula of Gibbs and Gower (1960).

This project found:

- In 2003/04 the incidence of the following viruses in samples from 6,892 generation two sown plants, sampled close to senescence was: PLRV 0.14 per cent, TSWV 0.03 per cent, PVY 0.03 per cent, PVS 0.12 per cent and PVX 0.52 per cent. The results showed that both seed schemes were meeting their respective objectives. Overall, this laboratory determined level compares favourably with the visually determined national standard for virus in generation three seed, which is 0.1 per cent for seed for further multiplication and 1.0 per cent for certified seed for sale. The PVY find resulted in steps being taken to eradicate the disease in WA.

- Australian seed certification schemes rely on visual identification of viruses. As many viruses are difficult to detect by symptoms alone, virus testing significantly enhances the effectiveness of the seed certification system by identifying and quantifying infection levels. Appropriate virus management strategies (Eyes on Potatoes, March 2004) can then be employed.
- PLRV, the most important virus affecting seed potato crops in Western Australia, was found to be under control in both seed schemes. However, PLRV was still detected at five of the fourteen seed growers crops tested. This underlines the need for growers and the seed certification service to maintain vigilance and an effective management strategy.
- When aphid monitoring and virus testing are applied in addition to the National Certified Seed Scheme procedures all major viruses found in Western Australia were successfully controlled.
- Results of the monitoring showed that pest pressure and the need for action varied between regions, within regions and on individual farms.
- Collation of the results from this and preceding projects shows that some growers had recurring problems with the same virus.

Recommendations

- Virus testing become a permanent component of the seed schemes in Western Australia and be deployed to later generations in addition to generation two sown crops.
- Virus testing be considered for adoption by the National Standard for the Certification of Seed Potatoes to demonstrate the viral health of certified seed and improve the effectiveness of our National seed certification system.
- Future research to develop a cost effective, rapid, quantitative, PCR test and sampling procedure to measure virus level in dormant tubers. This contrasts with the current choice of testing leaf material to estimate virus level in tubers or waiting several weeks for tuber dormancy to break before testing can be conducted.
- Introduce tolerances for PVS and PVX in the Registered Seed Scheme to speed the eradication of the viruses from that scheme.
- The weekly reports of pest abundance and associated recommendations for action assist in keeping pest numbers below levels that could affect the quality and yield of crops. Seed growers should have access to this information either by monitoring themselves or employing a crop inspection service.

3. VIRUS TESTING

INTRODUCTION

Western Australia has only recently been settled by Europeans (1829) and remains isolated from the rest of Australia and the world. Strict quarantine regulations were introduced early and are continually enhanced. As a result WA is free of many of the world's most serious potato diseases. The WA seed potato industry has the potential to capitalise on this natural advantage by supplying high health status seed potatoes to domestic users, growers in other states of Australia and Asia.

To encourage greater market access to Asia, the Department of Agriculture Western Australia (DAWA) was approached by industry in 1996 to develop a Certified Seed Scheme and to take over management of the existing Approved Seed Scheme. The Certified Seed Scheme complies with all the requirements of the National Standard for the Certification of Seed Potatoes (2000) but has additional provisions for isolation between generations, aphid control and virus testing to provide more effective virus control.

The Approved Seed Scheme did not limit the number of generations seed could be reproduced in the scheme and was therefore prone to virus outbreaks. A Registered Seed Scheme was developed in 1997 and the Approved scheme phased out by 2000. The Registered Seed Scheme was developed primarily to accommodate the traditional seed potato growing area located around Albany. In this area, crops are grown without rotation on low lying ground that floods annually. Some swamps may have been in continuous seed potato production since 1911. It is testament to the relative freedom from disease that this system continues to produce healthy seed potatoes. Both the Registered and Certified Seed Schemes require Pathogen Tested source material and a limit the number of generations. The Registered Seed Scheme differs from the Certified Seed Scheme in many ways, including less isolation between generations and no rotation and it does not seek to control PVX and PVS.

Many virus diseases affect potato crops, reducing yield and/or adversely affecting the quality of tubers. It is important that seed potato crops have no or minimal levels of tuber-borne viruses to enable future commercial crops to perform to their potential. The principle virus diseases affecting Western Australian seed potato crops are potato leafroll virus (PLRV), tomato spotted wilt virus (TSWV), potato virus S (PVS) and potato virus X (PVX). Of these PLRV has caused the most serious losses in Western Australia in the past. Potato virus Y (PVY) had not been detected by serological methods in Western Australia prior to 2003. Unfortunately, outbreaks of PVY occurred in 2003 and 2004 that were subject to eradication. To monitor the success of the eradication program PVY, has been added to the suite of viruses tested by this project.

This project, PT 03061 evaluates the success of the schemes at controlling the five virus diseases of concern through virus testing to eliminate infected material and pest monitoring to assist in control of insect transmitted viruses. The assessment was made by testing all seed crops for virus late in their third field generation and monitoring for insect pests on each seed grower's property during the 2003/2004 season. They also demonstrated the value of testing and monitoring as a risk management tool for seed producers. PT03061 was preceded by three similar virus testing projects and two similar aphid monitoring projects. The collated

results of all projects give a clear picture of the virus status of individual growers, the two seed schemes and the general seed industry in Western Australia (Appendix 1). Recommendations are made at the end of this report.

MATERIALS AND METHODS

Field surveys

The survey was managed by DAWA. Crop inspection staff of the Department's seed certification service collected leaf samples while observing a strict hygiene protocol. The seed crops were located in Esperance, Albany, Rosa Brook, Manjimup and Scott River. For each property, a target of five hundred leaf samples were taken from generation two sown seed crops close to their senescence. Usually a sample was taken every five metres from several sites in the crop. The newest fully developed leaf was chosen from the randomly selected plant. The number of samples taken per crop was proportional to the total area sown with generation two plots but never below 50 leaves per plot. Staggered times of sowing required an estimation of the number of samples taken at any particular visit and this caused some inaccuracy in the total number of samples collected.

The origin of samples was recorded to pinpoint problem plots. Samples were couriered in cooled esky's to ensure that they arrived at the laboratory in good condition. Samples were tested in the DAWA's virology laboratory either by ELISA or Tissue Blot methods under the supervision of Dr Roger Jones.

Enzyme-linked immunosorbent assay (ELISA)

Tests for TSWV, PVS, PVX and PVY were conducted by ELISA. Leaves were extracted (1 g/20 mL) in phosphate buffered saline (10 mM potassium phosphate, 150 mM sodium chloride), pH 7.4, containing 5 ml/L Tween 20 and 20 g/L polyvinyl pyrrolidone, using a leaf press (Pollahne, Germany). The sample extracts were tested for infection by double antibody sandwich ELISA as described by Clark and Adams (1977). Each sample was tested in duplicate wells in microtitre plates and appropriate infected and healthy leaf samples were included in paired wells as controls. The substrate used was 0.6 mg/mL of p-nitrophenyl phosphate in 100 ml/L of diethanolamine, pH 9.8. Absorbance values (A405) were measured in a Multiskan plate reader (Labsystems, Finland). Positive absorbance values were always at least 10 times those of healthy sap. Positive controls were grown in an aphid proof glasshouse from infected tubers and Polyclonal antiserum to PVS, PVX, PVX and PVY were obtained from Loewe Biochemica, Germany, and polyclonal antiserum to TSWV from BioRad, France.

Tissue blot immunoassay (TBIA)

Tests for PLRV were conducted by TBIA. Potato shoot samples were bound in bundles of up to 10 with parafilm. A scapel was used to cut the ends off single shoot or shoot bundle samples and the cut surfaces then pressed twice onto 0.45 μm pore size nitrocellulose membrane (Schleicher and Schuell, Inc., Keene, New Hampshire, USA). The procedure for TBIA was as described by Coutts and Jones (2000) and Latham and Jones (2001). Tissue prints were blocked in phosphate buffered saline containing 40 g/L BSA for one hour. They were incubated with PLRV-specific primary antibodies and then with enzyme-labelled species specific (anti-rabbit) secondary antibodies conjugated to alkaline phosphatase (Southern Biotechnology Associates, USA) each for two hours. The substrate solution

contained 14 mg nitroblue tetrazolium and 7 mg 5-bromo-chloro-3-indolyl phosphate in 40 mL of substrate buffer consisting of 0.1M Tris, 0.1M NaCl, 5 mM $MgCl_2$, pH 9.5. Development of purple colour in the phloem was observed using a binocular microscope and revealed PLRV presence in tissue prints from infected samples. Positive controls were grown in an aphid proof glasshouse from infected tubers. Polyclonal antiserum to PLRV was obtained from Loewe Biochemica, Germany.

Interpretation of results

The percentage incidence from grouped sample results was estimated using the formula of Gibbs and Gower (1960). The TBIA method identifies infected samples individually. Results of the testing were sent to seed growers and the Seed Certification Authority within a week of sampling.

If virus incidence results were above a previously agreed critical limit, plots were rejected. If results were cause for concern, but below the critical limits, the Seed Certification Authority contacted the grower to discuss options for management or eradication of the problem. In this situation, any action was the responsibility of the seed grower.

RESULTS

It is estimated a total of 58 plants were infected by viruses of the 6,892 plants tested, at an infection rate of 0.84 per cent. An average of 492 plants were tested per grower. No virus was detected in seven of the fourteen growers properties surveyed. The most common virus detected was PVX (36 infected plants from two properties), followed by PLRV (ten infected plants from five properties), PVS (eight infected plants from two properties), TSWV (two infected plants from two properties) and finally PVY (two infected plants from one property).

Table 1. Summary of virus test results on leaf samples

Grower	Number of samples tested	Estimated number of infected samples				
		PLRV	TSWV	PVS	PVX	PVY
Certified Seed						
Grower 1	**640**	**3**	**0**	**0**	**0**	**0**
Grower 3	**500**	**0**	**0**	**0**	**0**	**0**
Grower 5	**469**	**0**	**0**	**0**	**0**	**0**
Grower 4	**810**	**0**	**1**	**0**	**0**	**0**
Grower 5	**400**	**0**	**0**	**0**	**0**	**0**
Grower 6	**240**	**0**	**0**	**0**	**0**	**0**
Grower 7	**170**	**0**	**0**	**0**	**0**	**0**
Registered Seed						
Grower 8	**642**	**2**	**0**	**0**	**1**	**0**
Grower 9	**560**	**3**	**0**	**5**	**0**	**2**
Grower 10	**480**	**0**	**0**	**0**	**0**	**0**
Grower 11	**560**	**1**	**1**	**0**	**0**	**0**
Grower 12	**601**	**0**	**0**	**3**	**35**	**0**
Grower 13	**420**	**0**	**0**	**0**	**0**	**0**
Grower 14	**400**	**1**	**0**	**0**	**0**	**0**
Sub Total - Certified Seed	**3229**	**3**	**1**	**0**	**0**	**0**
Sub Total - Registered Seed	**3663**	**7**	**1**	**8**	**36**	**2**
Grand Total - Both Schemes	**6892**	**10**	**2**	**8**	**36**	**2**
Infected Samples - Certified Seed (%)		**0.09**	**0.03**	**0**	**0**	**0**
Infected Samples - Registered Seed (%)		**0.20**	**0.03**	**0.22**	**0.98**	**0.05**
Infected Samples – Both Schemes (%)		**0.14**	**0.03**	**0.12**	**0.52**	**0.03**

DISCUSSION

Overall

An aim of this project was to estimate the virus status of seed crops after three field generations and prevent spread of virus in subsequent generations by the early identification and removal of plots carrying unacceptable levels of infection, or alternatively the application of intensive virus management strategies. The survey also sought to assist the seed certification authority to evaluate the effectiveness of the two schemes.

The results showed that both seed schemes were meeting their respective objectives. The percentage of plants infected with all viruses from all growers properties was 0.8 per cent. Overall, the level compares favourably with the visually determined national standard for virus in generation three seed, which is 0.1 per cent for seed for further multiplication and 1.0 per cent for certified seed for sale.

The WA Certified Seed Scheme is controlling the four endemic viruses quite well.

The Registered Seed Scheme is also satisfactorily controlling PLRV and TSWV. While it was not designed to control PVS and PVX, the Registered Seed Scheme, and seed growers have achieved a high degree of success in reducing these viruses from levels discovered by the 1987 survey (Jones and Wilson, 1989). The success should encourage registered seed growers to strengthen their seed scheme rules to eradicate PVS and PVX.

Potato Leafroll Virus

PLRV is both tuber-borne and introduced to and spread within crops by aphids. Identification of plants infected with PLRV within a growing season (current season infection) is particularly difficult and often beyond the ability of seed growers and seed certification inspectors. Plants growing from infected seed are usually easier to visually identify in the crop but can take several critical weeks to display symptoms.

The percentage of plants infected with PLRV (0.145%) compares favourably with the visually determined national standard for virus in generation three seed. However, PLRV infected plants were detected in five of the fourteen properties tested, indicating a source of infection will still exist in many of next season's crops. Research has shown potato plants are most susceptible to infection with PLRV early in the life of the crop (Loebenstein, 2001). Growers will need to control early aphid infestations with appropriate insecticides and ensure that thorough roguing occurs at the earliest expression of symptoms to guard against serious outbreaks of the disease next season.

Tomato Spotted Wilt Virus

TSWV is tuber-borne, spread by thrips and has an extensive host range. Identification of plants infected with TSWV is possible in the field although expression of symptoms and effectiveness of transmission of virus through tubers to the next generation is variety specific. In recent seasons few TSWV infections have resulted in plots being rejected from the seed schemes.

In the survey, TSWV was only detected in one plant at each of two properties, an incidence of 0.03 per cent. The disease does not appear to be a major threat to most seed growers. However, this and preceding projects (Appendix 1) show particular growers properties have recurrent infections, perhaps indicating a source of the virus is located nearby. Growers have been warned that thrip vectors may be introducing TSWV from nearby weeds.

Potato Virus Y

PVY is a common and serious disease of potatoes that is present in most seed growing areas of the world. It is tuber-borne and spread by aphids. Some varieties show obvious symptoms, particularly plants that grow from infected tubers, while others show few obvious symptoms. Identification in crops after row closure is problematical.

The survey detected two infected plants in the same plot. This followed an outbreak of PVY in the year before this project began. The PVY find detected by this project was notified to quarantine and dealt with by an eradication program.

Potato Virus S and Potato Virus X

PVS and PVX are 'contact transmitted viruses'. Infected plants infect healthy plants by such actions as the movement of personnel and equipment through a crop. Infection is also spread during seed cutting operations. Both viruses are tuber-borne and PVS can also be spread by aphids. Identification of infected plants from visual symptoms is very difficult in summer crops. Current Import Permits for seed potatoes to Sri Lanka stipulate a nil tolerance to PVX.

This project detected PVS at two of the fourteen sites. Using the Gibbs and Gower (1960) formula the infection rate is estimated to be eight plants of the 6,892 tested or 0.12 per cent. PVX was also detected at two sites. The infection rate of PVX was estimated by the same method at 36 infected plants of the 6892 tested, approximately 0.5 per cent. All finds were in the Registered Seed Scheme.

This and preceding projects show growers in the Certified Seed Scheme are maintaining very low levels of PVS and PVX.

The seed crops in the forerunner to the Registered Seed Scheme, the Approved Seed Scheme, were heavily infected with PVS (55% of plants infected) and PVX (73% of plants infected) (Jones and Wilson, 1989). The marked decline in PVS and PVX infection rates suggest that these viruses could be eradicated from the Registered Seed Scheme. PVS or PVX were only detected on three registered seed grower's properties. However, at one of these properties heavy infection was detected in three of four plots. Analysis of results over the four consecutive years, indicates that only two growers are having repeated difficulty with these diseases (Appendix 1).

The substantial reduction in infection since the 1987 survey is though to be a function of:

- exclusive use of Pathogen-Tested source seed;
- limiting the number of generations that seed can be propagated;
- more widespread knowledge of the methods of virus transmission among seed growers;
- improved hygienic practices in seed cutting operations;
- improved hygienic practices when staff and machinery move through crops.

Application of Virus Test Results

In Western Australia, the Seed Certification Authority consults with industry through an industry consultative committee (The Western Australian Seed Advisory Committee). The committee approved use of virus test and insect monitoring results by the Seed Certification Authority. Seed growers were advised of actions that the Authority would take at various levels of infection. Any crops exceeding the critical limits were rejected from the Seed Schemes. A statistical package was developed by DAWA's biometrican as part of a preceding project, PT 02047, to assist the certification service to determine if a result exceeded the critical limit for a range of sample sizes at the 95 per cent level of confidence. This 95% confidence limit is in concert with the level used in Organisation for Economic Cooperation and Development and United Nations seed schemes.

The testing program assists seed growers and the seed certifiers to identify emerging viral problems in specific plots. They then have the opportunity to focus risk and virus management strategies on the plots of concern including:

- investigate the source of the virus outbreak;
- dispose of the plot from the seed growers program as either wares, processing or seed depending on the severity of the infection level;
- retain the seed in the seed scheme but pay additional attention to the following crop in the form of early removal of virus infected plants and strict control of insect vectors by appropriate insecticides;
- alert seed certification officers to crops sown with low incidence infected seed;
- increase isolation of the following crop from healthy crops;
- pay extra attention to hygiene during seed cutting operations.

Customer confidence

The conduct of this and preceding projects has resulted in growers of seed potatoes and their customers having more confidence that seed will have acceptable levels of virus infected tubers.

Export markets are increasingly demanding laboratory testing of seed prior to import and placing restrictions on the level of virus permitted. For example Thailand is sensitive to PVY, Sri Lanka has a zero tolerance of PVX and Indonesia requires testing for the five viruses addressed in this project. Continued access to emerging markets like these requires successful management of viral infections. Presentation of survey results to importers clearly demonstrated our knowledge of the virus status of our seed and our capacity to manage virus.

Confidence in the virus test procedure is increasing after early scepticism by seed growers. The experience of seeing test results being reflected in the following years crop has improved acceptance of the technology. In 'end of season' reviews seed growers commended the testing program as providing timely and relevant information which enabled them to take appropriate action to identify and resolve threats to the production of healthy seed potatoes. Seed buyers have reported general improvement in the virus level of seed from seed schemes.

The Western Australian Seed Advisory Committee is continuing to fund virus testing program in 2004/2005.

This project found:

- In 2003/04 the incidence of the following viruses in samples from 6,892 generation two sown plants, sampled close to senescence was: PLRV 0.14 per cent, TSWV 0.03 per cent, PVY 0.03 per cent, PVS 0.12 per cent and PVX 0.52 per cent. The results showed that both seed schemes were meeting their respective objectives. Overall, this laboratory determined level compares favourably with the visually determined national standard for virus in generation three seed, which is 0.1 per cent for seed for further multiplication and 1.0 per cent for certified seed for sale. The PVY find resulted in steps being taken to eradicate the disease in WA.
- Australian seed certification schemes rely on visual identification of viruses. As many viruses are difficult to detect by symptoms alone, virus testing significantly enhances the effectiveness of the seed certification system by identifying and quantifying infection levels. Appropriate virus management strategies (Eyes on Potatoes, March 2004) can then be employed.
- When aphid monitoring and virus testing are applied in the Certified Seed Scheme all major viruses found in Western Australia were successfully controlled.
- Virus testing significantly improved the ability of seed growers and seed certification services to identify crops at risk of developing severe infections.
- The Registered Seed Scheme was controlling infection with PLRV and TSWV and that the incidence of PVS and PVX has improved significantly from levels of above 50 per cent of plants infected in a 1987 survey (Jones and Wilson, 1989) but that a few growers still had high levels of the viruses in some of their plots.
- PLRV was found to be under control in both seed schemes. However, it was still detected on five of the fourteen grower's properties tested, showing the virus has a presence in many seed crops and underlining the need for growers and seed certification inspectors to remain vigilant for aphid vectors and resulting spread of the virus.
- Collation of the results from this and preceding projects shows that some growers had recurring problems with the same virus.

4. APHID MONITORING

INTRODUCTION

The main insect transmitted virus diseases in WA potato crops are PLRV and TSWV. These viruses can be introduced into potato crops and spread within them by the insect vectors aphids and thrips respectively. Problems with virus infection of seed potato crops in WA in the past require that seed growers remain vigilant regarding the abundance of their insect vectors. For aphids, a threshhold for crop rejection, and therefore a guideline for applying insecticides to crops is included in the WA Certified Seed Potato Scheme rules (Anon (b), 2001). PVS and PVY can also be transmitted by aphids.

While there are other important aspects to producing seed potatoes with minimal virus levels, the object of monitoring was to minimise the abundance of insect virus vectors. In this way growers of seed potatoes and their customers will have more confidence that seed crops will have minimal virus spread by insects. The variation in aphid abundance in different cropping regions within WA, both within a season and among crops (Berlandier, 1997, 1999), indicates that individual crop monitoring for aphids is appropriate for growers to achieve effective aphid management. Apart from monitoring for disease vectors, other pests including disease could be assessed concurrently.

An aim of this project was to consolidate the positive response by WA seed growers to the previous two years of pest monitoring. The incorporation of a monitoring program in the WA seed potato industry is seen to be an important aspect in producing seed of high quality for both the domestic and increasingly important export sectors of the WA potato industry.

MATERIALS AND METHODS

The monitoring program was organised by the DAWA. Monitoring was undertaken by Department and casual staff.

For logistical reasons, the seed potato crops in WA were divided into four regions – Esperance, Albany (including Bremer Bay), Lower southwest (Manjimup, Pemberton and Scott River) and Southwest (Rosa Brook, Busselton and Metropolitan). Seed growers in each region were invited to be involved with the monitoring program.

For each seed grower, one crop was selected for monitoring from crop emergence to crop senescence or spray off. Crops selected for monitoring were the oldest generation, which usually coincided with the largest area of planting for that month. Monitoring was undertaken weekly and where practicable was on the same day of the week each time. Persons undertaking the monitoring observed a crop hygiene protocol. Rubber boots were cleaned after and before each site - loose dirt/mud was removed and boots disinfected with Farm Cleanse @ 10 mL/L water.

The procedure for monitoring was based on examining 50 lower leaves, tapping foliage over a 2 L plastic container at each of ten locations. Other observations were recorded including mobile insects not readily sampled by the other two methods and the presence of important diseases. This monitoring was along a predetermined path through the crop. This path commenced as a triangle through the crop up to row closure. After this, monitoring was undertaken by walking around the edge of the crop and stepping into the crop approximately 20 m on each side of it. From crop die down to senescence or spray off, the triangular monitoring path through the crop was resumed. Monitoring included looking for insects and symptoms to indicate disease outbreaks. In addition to this monitoring, pheromone traps for two species of looper (*Chrysodeixis argentifera* and *C. eriosoma*) were located at some of the crops being monitored to assess whether they were useful as a warning tool for pest occurrence.

Score sheets were faxed to DAWA Manjimup to review the monitoring data. Using the summary sheets, growers were advised of the main findings of the monitoring and any recommendations to take action against pests. This information was sent to the grower by facsimile or, when action was contemplated, the grower was telephoned to discuss control options.

RESULTS

The number of growers from each region involved in the monitoring and other information on the size of the monitoring program are given in Table 1. With a decline in seed potato production in the Albany area, there was a proportional reduction in monitoring. Most visits were made to crops in the Lower South West region (Manjimup/Pemberton), where the number of seed growers has been increasing over the last two seasons. Slightly more monitoring was undertaken in the South West region (Busselton, Margaret River and Kirup). Overall, the monitoring program was smaller than the two previous seasons.

The pests found for which either a recommendation to apply insecticide was made or where action was considered but not necessarily undertaken during the 2003/04 season were: aphids, loopers, potato moth and thrips. The number of times such occasions arose for:

Table 1. Information to indicate the size of the monitoring exercise undertaken in WA seed potato crops in the 2003/04 season, compared with the previous seasons

Region	Crops monitored			Weekly visits			Leaves checked		
	01/02	02/03	03/04	01/02	02/03	03/04	01/02	02/03	03/04
Esperance	7	2	**0**	46	12	**0**	2450	600	**0**
Albany	40	13	**8**	161	121	**70**	16300	6050	**3500**
Lower South West	8	21	**17**	74	193	**156**	3950	9650	**7800**
South West	9	9	**11**	79	85	**109**	4950	4250	**5450**
All areas	*64*	*45*	***36***	*360*	*411*	***335***	*27650*	*20550*	***16750***

Aphids, and the rest of the pests mentioned is shown in Tables 2 and 3 respectively. Also included in the information on aphids in Table 2 is the proportional representation of these occasions in relation to the total number of farm visits made within each region, to take into account differences in crop visits among the regions. Most recommendations for action against aphids were for the south-west region. This was due largely to the prevalence of aphids in spring in almost all crops grown there at that time (see Fig. 1).

Table 2. Number of times recommendations to protect seed potato crops from aphids were made to WA seed potato growers in the past three seasons and the percentage of crops monitored

Region	No. recommendations			% Plots monitored		
	01/02	02/03	03/04	01/02	02/03	03/04
Esperance	5	0	**-**	10	0	**-**
Albany	27	7	**0**	8	6	**0**
Lower South West	4	12	**6**	5	6	**4**
South West	0	7	**16**	0	8	**15**
All areas	*36*	*26*	***22***	*6*	*5*	*7*

Apart from recommendations for controlling aphids, the only other pest problems recommended for action in the 2003/04 season were in the South West region. For all other regions, alternative pests were either absent or at levels too low to consider control action was required. Compared to the previous two seasons, 2003/04 was a season in which pests other than aphids were in lower numbers.

Table 3. Number of times recommendations to protect seed potato crops from a range of non-aphid foliage pests were made to WA seed potato growers in the past three seasons

Region	Pest	Heliothis	Loopers	Potato moth	Thrips	Wingless grasshopper	Rutherglen Bug
Esperance	01/02	0	0	0	0	1	0
	02/03	0	0	0	0	2	0
	03/04	-	-	-	-	-	-
Albany	01/02	4	15	0	3	0	13
	02/03	0	0	0	0	3	0
	03/04	0	0	0	0	0	0
Lower South West	01/02	0	0	0	0	0	2
	02/03	0	3	0	0	0	0
	03/04	0	0	0	0	0	0
South West	01/02	0	0	1	3	0	0
	02/03	0	0	2	2	0	3
	03/04	0	1	1	2	0	0
All areas	*01/02*	*4*	*15*	*1*	*6*	*1*	*15*
	02/03	*0*	*3*	*2*	*2*	*5*	*3*
	03/04	*0*	*1*	*1*	*2*	*0*	*0*

In general terms, aphids were most abundant in spring and autumn, with some crops infested during summer. This seasonal abundance of aphids is consistent with results from monitoring WA potato crops in other years (Berlandier, 1997, 1999). In general, aphid abundance was held in check at levels below the WA Seed Potato Scheme threshhold (Anon. (b), 2003).

As was the case for the previous two seasons, information from the monitoring was sent to growers in a timely manner.

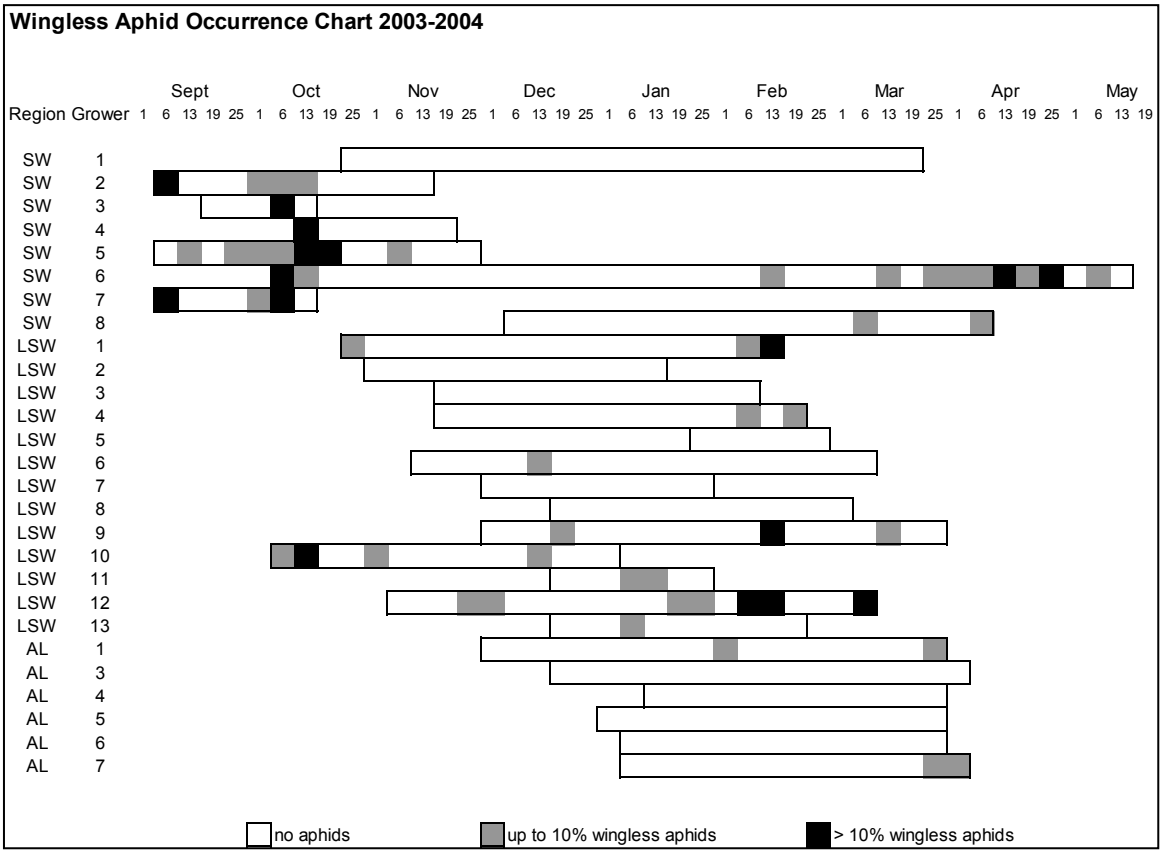
During the 2003/04 season, no seed potato crops were rejected by seed inspectors on the basis of excessive aphid numbers. In the situations where aphid pressure was present at excessive levels during crop growth, numbers were reduced as soon as practicable. This is considered acceptable in view of the fact that aphids acquire and transmit PLRV over a few days.

There were no occasions where foliar diseases that might have been important to crop health were noted.

A review of the monitoring was held with the WA Seed Potato Advisory Committee. Growers felt the monitoring program was achieving the objectives of timely warnings for pest control and maintaining high quality of seed potatoes. At this review, growers recommended that funding be made available so that monitoring can be undertaken for a further season, but with the recommendation that crops being monitored were the same as those being tested for virus levels.

Figure 1. Abundance of wingless aphids in seed potato regions in WA in 2003/04. The regions are: SW = south west (Kirup, Busselton and Margaret River); LSW = lower south west (Manjimup and Pemberton); AL = Albany.

DISCUSSION



For PLRV, which has been of concern in the WA seed potato scheme in the past, monitoring and controlling the most important vector, green peach aphid (*Myzus persicae*) is a mandatory part of growing quality seed. The current project sought to reinforce to existing seed growers and to introduce to a large group of new growers the value of a monitoring service to assist in growing crops with aphid levels below the locally accepted threshhold of aphid abundance (Anon. 2003).

The monitoring of seed potato crops in WA in the 2002/03 season showed that aphids were the main insect pest. Other foliar pests were present, but at low numbers with few recommendations for control being made to seed growers.

Seed growers were advised of the occurrence of foliar pests in a timely fashion that allowed them to take action to prevent sustained pest presence. The modified protocol for the monitoring where a skip week was introduced worked well but with one notable exception. An aphid infestation occurred during that week in one crop. The infestation was not detected. This occurred at a time when aphids were generally at a low level of abundance in the region, but emphasises the need for regular crop monitoring.

With the exception of a few crops where aphid pressure was high and required multiple insecticide applications to achieve these desired aphid levels, normal grower practice resulted in good aphid control. This was achieved both in response to the monitoring and by growers adopting their usual aphid control program. In the case of the latter approach, the monitoring scheme is building confidence in seed growers that reduced levels of spraying can be introduced without compromising seed potato quality. Sprays early in the crops life are considered most important.

By continuing such an industry funded program, growers' confidence can be enhanced to the point where spray on demand becomes an acceptable practice. This not only applies to managing aphids, but for other pests as well. The importance of this aspect is seen where other pests invade crops and other control options, including different insecticides, can be selected. In this way, treatment on demand can incorporate the specialist approach to pest management with the objective of reduced use of broad spectrum insecticide use.

This survey found:

- The weekly reports of pest abundance and associated recommendation for action kept pest numbers below levels that could affect the quality and yield of crops.
- The results of the monitoring showed that pest pressure and the need for action varied between regions and within regions on individual farms.
- Pest control recommendations were implemented in a timely manner and excessive levels of pests were extremely rare. Although not all seed crops were monitored, seed growers reported that the information provided was useful in decision making for other nearby crops.

5. TECHNOLOGY TRANSFER

This project was devised for the benefit of seed growers and the wider potato industry. Results of the project have state, national and international implications and have been transferred by the following methods.

STATE

Seed growers and the Seed Certification Authority were provided with their results of virus testing, pest abundance and related management recommendations within a week of sampling. The seed growers, seed potato industry and the WA Seed Advisory Committee were provided summaries of the testing during regular meetings. This has allowed seed growers to benchmark the effectiveness of virus management strategies against the performance of other growers. For legal reasons it is essential that seed growers confidentiality be preserved.

The results of the PVS and PVX testing will be used to propose the introduction of more vigorous virus standards to the Registered Seed Scheme. This will enable rejection of crops identified with excessive levels PVS or PVX for the first time. Continued testing to identify disease outbreaks could see these viruses eliminated from the Registered Seed Scheme in the near future.

The results of the PVY testing program were made available (along with the results of other tests conducted for the purpose) to the WA quarantine service to monitor the success of the PVY virus eradication program.

NATIONAL

The project leader has been invited to present the results of this and preceding projects at the Potato 2005 Conference.

The results of this and preceding projects have been collated and presented to potato seed scheme managers in Australia through the operations of the Seed Potatoes Australia Group (SPAG).

A survey has shown Tasmanian certified seed potatoes to be infected with PVS and PVX. Dr Roger Jones was invited to Tasmania to present virus control strategies to industry. His presentations used the results from this and preceding projects in WA to reinforce the effectiveness of the control strategies he proposed for use in Tasmania.

Virus management strategies have been published in 'Eyes on Potatoes' magazine.

INTERNATIONAL

The information generated by this project, and preceding projects, has been presented to government and private sector delegations from China, Sri Lanka, Mauritius, Thailand and Indonesia to demonstrate our knowledge and control of viruses in WA seed potato schemes. Delegates have been impressed with the extent of the virus indexing undertaken in the schemes and the low levels of virus present. The acceptance of the leaf test to satisfy import requirements (as opposed to tubers being tested) by quarantine authorities from these countries indicates their acceptance of our technical abilities.

6. RECOMMENDATIONS

The following recommendations are made:

1. That virus testing become a permanent component of the seed schemes in Western Australia and be deployed to later generations in addition to G2 sown crops.
2. Virus testing be considered for adoption by the National Standard for the Certification of Seed Potatoes to improve the effectiveness of National seed certification system and demonstrate the viral health of certified seed.
3. Future research to develop a cost effective, rapid, quantitative, PCR test and sampling procedure to measure virus level in dormant tubers. This contrasts with the current choice of testing leaf material to estimate virus level in tubers or waiting several weeks for tuber dormancy to break before testing can be conducted.
4. PVY testing continue to monitor the success of the current eradication program.
5. The Registered Seed Scheme has significantly reduced the level of PVX and PVS. However, considering the growing importance of these viruses in some export markets, particularly Sri Lanka which has a nil tolerance to PVX, it is recommended that

registered seed growers consider mounting concentrated efforts to eradicate these viruses from their scheme. Possible measures include:

- mandatory rejection of Registered Seed Scheme crops showing high levels of PVS and PVX;
- adopt effective inter generational isolation requirements;
- mandatory testing of seed for further multiplication being traded between seed growers.

6. Encourage WA seed growers to employ a pest monitoring program, in conjunction with other virus management strategies, as a basis for producing quality seed potatoes with minimal virus levels. A manual for production of seed potatoes is being developed as part of another project.

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APPENDIX 1: VIRUS TEST RESULTS ON 2001-2004 BY GROWER

Grower	Year	No. samples	PLRV (%)	TSWV (%)	PVS (%)	PVX (%)	PVY (%)
Grower 1 Total	2001	420	0.3	0.3	0	0	nt
Grower 1 Total	2002	430	0.5	0	0	0	nt
Grower 1 Total	2003	280	0.7	0	0	0	0
Grower 1 Total	2004	642	0.3	0	0	0.2	0
Grower 2 Total	2001	480	0	0	0	0	nt
Grower 2 Total	2002	500	0	0	0	0	nt
Grower 2 Total	2003	540	0	0	0	0	0
Grower 3 Total	2001	620	6.5	0	0.2	0.2	nt
Grower 3 Total	2002	500	0.2	0	0	0	nt
Grower 3 Total	2003	400	0	0	0	0	0
Grower 3 Total	2004	480	0	0	0	0	0
Grower 4 Total	2001	550	0.7	0	1.6	0.1	nt
Grower 4 Total	2002	379	0	0	1.8	0	nt
Grower 4 Total	2003	645	0	0	0.3	0	0
Grower 4 Total	2004	560	0.5	0	0.9	0	0.4
Grower 5 Total	2001	540	0	0	0	0	nt
Grower 5 Total	2002	200	0	0	0	0	nt
Grower 5 Total	2003	510	0.2	0	0	0	0
Grower 5 Total	2004	469	0	0	0	0	0
Grower 6 Total	2001	645	0.6	0	0	0	nt
Grower 6 Total	2002	782	0.1	0.1	0	0	nt
Grower 6 Total	2003	625	0	0	0	0	nt
Grower 7 Total	2003	800	0	3.3	0	0	0
Grower 8 Total	2002	675	0	0	0.2	0	nt
Grower 8 Total	2003	510	1.6	0	0	0	0
Grower 8 Total	2004	500	0	0	0	0	0
Grower 9 Total	2001	520	0.6	0.8	0	0	nt
Grower 9 Total	2002	496	0.2	0	0	0	nt
Grower 9 Total	2003	300	0	0.7	0	0	0
Grower 9 Total	2004	560	0.2	0.2	0	0	0
Grower 10 Total	2003	500	0	0	0	0	0
Grower 11 Total	2001	1060	0	0	0.1	0	nt
Grower 11 Total	2002	615	0	0	0	0	nt
Grower 11 Total	2003	880	2.7	0	0	0.3	0
Grower 11 Total	2004	640	0.5	0	0	0	0
Grower 12 Total	2001	200	0	0	0	0	nt
Grower 12 Total	2003	500	0	0	0	0	0
Grower 13 Total	2001	300	0	0	0.3	3.6	nt
Grower 13 Total	2002	500	0.4	0	3	55.0	nt
Grower 13 Total	2003	500	5.0	0	0	0	0
Grower 13 Total	2004	601	0	0	0.5	5.8	nt

Appendix 1 continued …

Grower	Year	No. samples	PLRV (%)	TSWV (%)	PVS (%)	PVX (%)	PVY (%)
Grower 14 Total	2002	550	0	0	0	0	nt
Grower 14 Total	2003	750	0.1	0	0	0	0
Grower 14 Total	2004	810	0	0.1	0	0	0
Grower 15 Total	2001	435	0	0	0	0	nt
Grower 15 Total	2003	510	0	0	0	0	0
Grower 15 Total	2004	400	0	0	0	0	0
Grower 16 Total	2003	500	nt	0	0	0	nt
Grower 17 Total	2003	430	0	0	0	0	0
Grower 17 Total	2004	240	0	0	0	0	0
Grower 18 Total	2003	500	0.6	0	0.4	0	0
Grower 19 Total	2001	515	0	0.7	0	0	nt
Grower 19 Total	2002	500	0	0	0	0	nt
Grower 19 Total	2003	200	0	0	0	0	0
Grower 20 Total	2003	220	0	0	0	0	0
Grower 20 Total	2004	420	0	0	0	0	0
Grower 21 Total	2001	500	0	0	0	0	nt
Grower 21 Total	2002	500	0	0	0	0	nt
Grower 21 Total	2003	350	0.6	0	0	0	0
Grower 21 Total	2004	400	0.2	0	0	0	0
Grower 22 Total	2002	480	0.2	0	0	0	nt
Grower 23 Total	2001	370	0	0	0.3	0	nt
Grower 23 Total	2002	500	0	0	0	0	nt
Grower 24 Total	2001	100	0	0	0	0	nt
Grower 25 Total	2001	190	0	0	nt	nt	nt
Grower 26 Total	2004	170	0	0	0	0	0

nt – not tested.