EPIDEMIOLOGY OF VIRUSES

INFECTING HOP (*Humulus lupulus* L.)

IN AUSTRALIA

VOLUME TWO

By

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CHAPTER FIVE

DETECTION OF PNRSV IN AUSTRALIAN HOP CULTIVARS

5.1. INTRODUCTION

Viruses infecting hops in Australia are potential causes of significant reductions in yield and levels of bittering compounds (chapter one). To aid in control, these viruses must be accurately and rapidly identified to ensure the continued production of high yields and quality hop products.

Traditional methods of virus detection, such as mechanical inoculation to herbaceous indicator species, are unreliable without the use of purified preparations. In addition, there is often a significant delay between time of inoculation and symptom expression, which is often inconspicuous. Indexing is a commonly used technique for the detection of PNRSV-C in *Prunus* spp. Graft inoculation of infected samples to *P. serrulata* Lindl. (shirofugen flowering cherry) results in the development of characteristic tissue necrosis symptoms. It is unknown whether *P. serrulata* is sensitive to the PNRSV serotypes infecting hop. Immunological detection by ELISA (Clark and Adams, 1977) continues to be considered the most convenient and sensitive method for the routine detection of viruses infecting hop (Thresh *et al.*, 1977). This technique has made possible the rapid screening of mother plants prior to propagation and has provided a valuable tool for researchers studying the aetiology and epidemiology of hop viruses. The uptake of more recently developed nucleic acid based methods for the detection of viruses infecting hops

has been slowed by the lack of sequence information and expertise to handle nucleic acid extracts efficiently for routine screening, added cost, and they are more labour intensive.

Optimisation of ELISA for the detection of viruses infecting hop cultivars requires a detailed knowledge of fluctuations in virus titre throughout the growing season, relative levels in a range of tissues in chronically infected plants, and the variation in serological reactions within viruses.

To date, two serotypes of PNRSV have been identified infecting hops throughout the world (Bock, 1967; Thresh *et al.*, 1977). The apple (PNRSV-A) serotype is identical to apple mosaic virus (ApMV), found infecting *Malus* spp. The intermediate (PNRSV-I), or hop serotype is related to both ApMV, and the PNRSV cherry serotype infecting *Prunus* spp. (PNRSV-C) (Fulton, 1970; Barbara *et al.*, 1978), but more closely related to the former (Ong and Mink, 1989; Crosslin and Mink, 1992; Klein and Husfloen, 1995).

Several studies have shown the highest virus titres to occur in young hop shoots early in the season, and consequently virus testing was more reliable using succulent tissue following emergence (Thresh and Ormerod, 1974; Thresh, *et al.*, 1977; Anonymous, 1980). PNRSV has also been detected in hop roots and rhizomes with comparable reliability to young leaves (Anonymous, 1980). However, changes in virus titre throughout the season are likely to be cultivar specific and influenced by local climate.

The objective of this study was to determine which serotypes of PNRSV infected hop cultivars in commercial Australian gardens, and the most suitable time and types of tissue for detection of PNRSV by ELISA.

5.2. MATERIALS AND METHODS

Detection of PNRSV (A & I) was by DAS-ELISA (Appendix 2). Absorbance values from different microtitre plates were compared by standardisation to a common positive control. The positive control (dried infected leaves of *Cucumis sativus*) was kindly supplied by Dr. D.J. Barbara, HRI, UK, and was used to develop a standard curve for each antiserum (absorbance values at exponentially increasing dilutions from 1:10 to 1:1280, and subsequently used on each plate. The shape of the standard curve for each antiserum, was examined to determine whether data transformation was necessary.

PNRSV serotype survey

The incidence and serological variation within PNRSV serotypes in a range of cultivars at two commercial hop farms in Tasmania and one in Victoria, Australia was investigated.

Blocks one and two were planted in 1989 and 1994 respectively, with virus tested material of 'Victoria'. Block one (n=1275) was situated at Bushy Park and block two (n=500) at Myrtleford (chapter two). Block three was a cultivar x row spacing trial situated at Bushy Park, Tasmania. It was planted in 1989 with virus tested material of

cultivars 'T11', 'Opal', 'T25', and 'Victoria' (n>700 for each cultivar). 'T25' was not tested as a significant but unknown proportion of the planting material was infected by PNRSV through propagation.

Block four was a cultivar collection block (n=99), also situated at Bushy Park, consisting of cultivars of varying ages bred in Australia and overseas. The virus status of the material at planting was unknown. Surveys were also conducted in gardens of 'Pride of Ringwood' (n=20), 'Victoria' (n=20), 'Tettnang' (n=20), and 'Nugget' (n=20), ranging between seven and twenty years in age, at Forrester River hop farms (FRF) in north east Tasmania. Two further gardens of 'Pride of Ringwood' (n=40 at each site) and one 'Victoria' (n=40) were also sampled. These gardens were situated on private properties, also in north east Tasmania (X and Y). They were selected as the oldest 'Pride of Ringwood' gardens (planted in 1970's) in Tasmania, and considered most likely to be infected by a wide range of PNRSV serotypes.

Young leaves from individual plants in blocks one and three were collected in the spring of 1997, and in block two in the spring of 1998. Dormant buds from individual plants in block four, and gardens in north east Tasmania were collected in mid-winter of 1997. Dormant buds were tested by ELISA for PNRSV (A & I) (Appendix 2) concurrently using antisera to PNRSV-A, PNRSV-I, and rose mosaic virus 3 (RMV-3) (kindly supplied by Dr. D.J. Barbara) (Appendix 2). Leaf samples (blocks one, two, and three) were tested using antisera to PNRSV-A, and RMV-3. Plants infected by either PNRSV-A or PNRSV-I, from block one (n=100), block two (n=30), 'T11', 'Victoria', and 'Opal'

in block three (n=30 from each cultivar), and all infected plants in block four and gardens in north east Tasmania were included in comprisons to assess serotype variation within serotypes.

All positive samples were separated into serotypes (either PNRSV-A or PNRSV-I) by graphical comparison of the standardised absorbance values using PNRSV-A and RMV-3 antisera. Isolates with high values (>0.450) with PNRSV-A antiserum and low values (<0.300) with RMV-3 antiserum were considered PNRSV-A. Isolates with high values for RMV-3 antiserum (>0.450) and low values for PNRSV-A antiserum (<0.450) were considered PNRSV-I (Figure 5.4). The log ratio of standardised absorbance values of reactions to PNRSV-A and RMV-3 antisera was used to differentiate positive samples into groups. Those with ratios greater than 0.350 and less than 1.20 were considered PNRSV-A, while those with ratios less than -0.50 and greater than -0.55 were considered PNRSV-I. The ratios in the majority of positive samples ranged between 0.450 and 0.650, and between -0.20 and -0.30 with PNRSV-A and PNRSV-I, respectively (Figure 5.5). The mean standardised absorbance values for PNRSV-A serotypes were 0.614 - 0.825, 0.106 - 0.209, and 0.580 - 0.614 using antisera prepared against PNRSV-A, PNRSV-I, and RMV-3 respectively, while those for PNRSV-I serotypes were 0.296 - 0.424, 0.549 - 0.801, and 0.344 - 0.859.

Reliability of PNRSV detection

Thirty-three plants infected by PNRSV in block one in 1990 (chapter two) were selected. Dormant buds were taken in August 1996 and from April to July the following year. Young leaf samples from laterals were taken from August 1996 for the remainder of the growing season, in the first week of every month. Each time, three leaf samples were selected from bines trained upon each each string, and subsamples tested by ELISA. After twelve months, ten plants were selected (five infected by either PNRSV serotype). Young leaf tissue was collected from them at monthly intervals, for seven months. Each sample was tested concurrently using both PNRSV-A and RMV-3 antisera.

Detection of PNRSV in different tissues

Four plants each of 'Victoria' and 'Opal' infected by PNRSV (two of either serotype) were randomly selected from block three (chapter two). Strings were divided into four even sections (approximately 1.5 m long), and old and young leaf samples selected from each section, prior to harvest (March, 1998).

The following season, four plants each of 'Victoria', and of 'Nugget' (two of either serotype), two plants of 'Pride of Ringwood' infected with PNRSV-A, and two PNRSV-I infected 'Opal' plants were arbitrarily selected from chronically infected plants in the yield trial at Bushy Park (chapter six). Strings were divided vertically into three equal sections (approximately 1.8 m long). Samples of old and young leaves were selected from each section, along with four different portions of the basal growth, when plants had

reached the top of the trellis (late December 1998). These samples were tested by ELISA for PNRSV (A & I) (Appendix 2) concurrently using both PNRSV-A and RMV-3 antisera.

Shirofugen flowering cherry assay of PNRSV serotypes infecting hop

Budsticks (30 cm in length) were selected prior to harvest (February, 1998) from PNRSV (A & I) infected 'Victoria' and 'Pride of Ringwood' plants, and plants of both cultivars with no detectable infection by either serotype of PNRSV. Shirofugen cherry virus indexing was conducted by Dr. Michael Barkley, New South Wales Department of Agriculture, in Camden, New South Wales, Australia. Three chips per sample were graft inoculated by budding onto a vigorously growing branch. After six weeks the area surrounding the bark pieces was examined for tissue necrosis.

5.3. RESULTS

Standard curves for PNRSV-A, PNRSV-I, and RMV-3 are in Figures 5.1 to 5.3 respectively. All ELISA absorbance values from positive field samples fell within the linear section of the curves and therefore did not require transformation. Absorbance values between ELISA plates were standardised by dividing each by the absorbance of the common positive control dilution included on each plate.

PNRSV serotype survey

In 1997, nine years after planting block one, 92 % of plants were infected by PNRSV (A & I), and the incidence of PNRSV-I (62 %) was double that of PNRSV- A (30 %). In block two, four years after planting, 31 % of plants were infected by PNRSV (A & I). The incidence of PNRSV-A was higher (19 %) than that of PNRSV-I (13 %) (chapter two) (Table 5.1).

In cultivar 'Opal' in block three, the incidence of PNRSV-A (77 %) in 1996 was three times higher than that of PNRSV-I (23 %). However, in the following year the incidence of PNRSV-I had risen to 52 % of total PNRSV infection (11 %). In 'T11' at the same site, the incidence of PNRSV-A (73 %) in 1996 was also three times higher than PNRSV-I (28 %), and again in the following year the incidence of PNRSV-I increased giving an incidence of PNRSV-A double that of PNRSV-I. In 'Victoria',

Figure 5.1. Absorbance values (405nm) versus logistic dilution of PNRSV-A control using PNRSV-A antiserum.

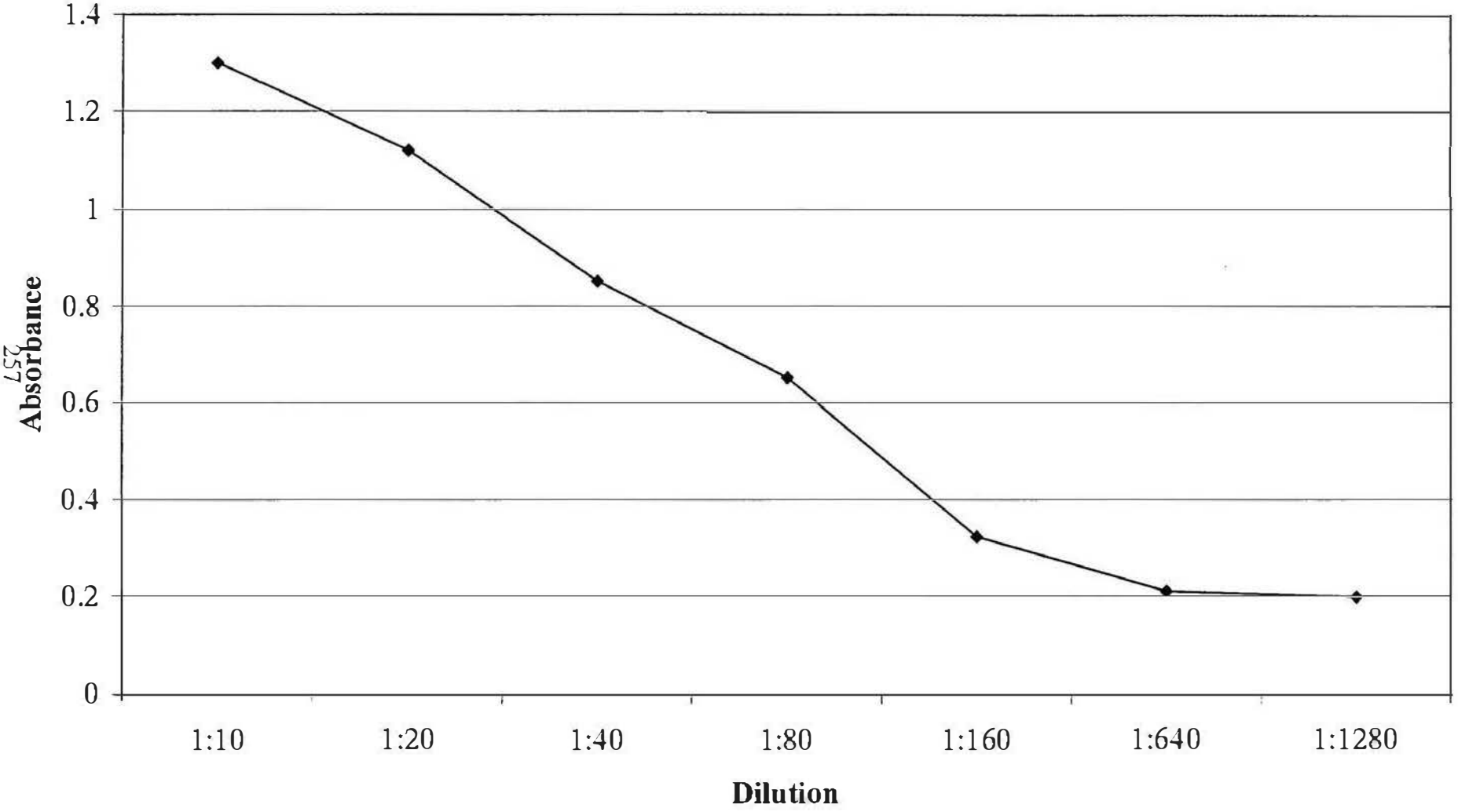


Figure 5.2. Absorbance values (405nm) versus logistic dilution of PNRSV-A control using PNRSV-I antiserum.

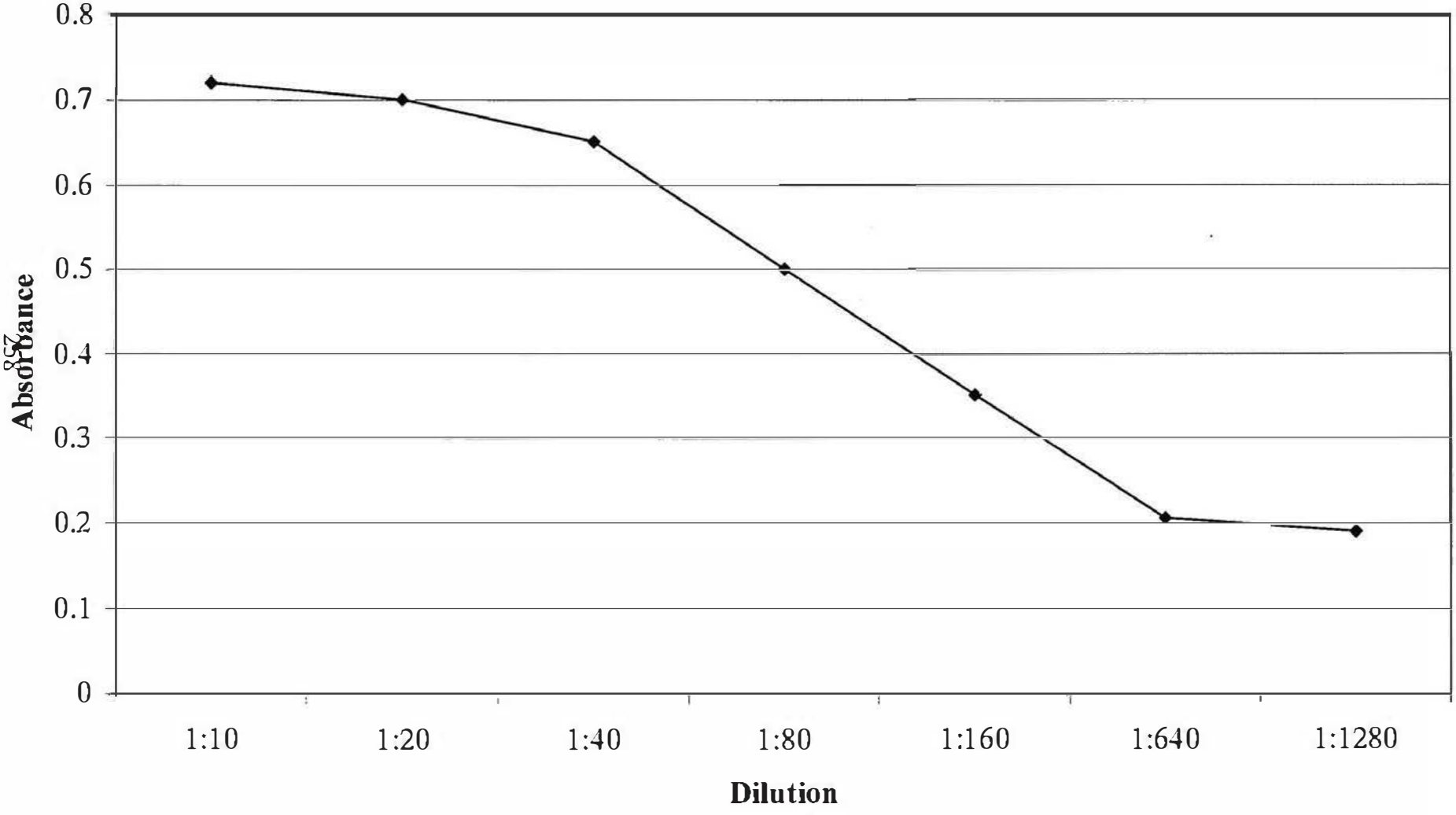
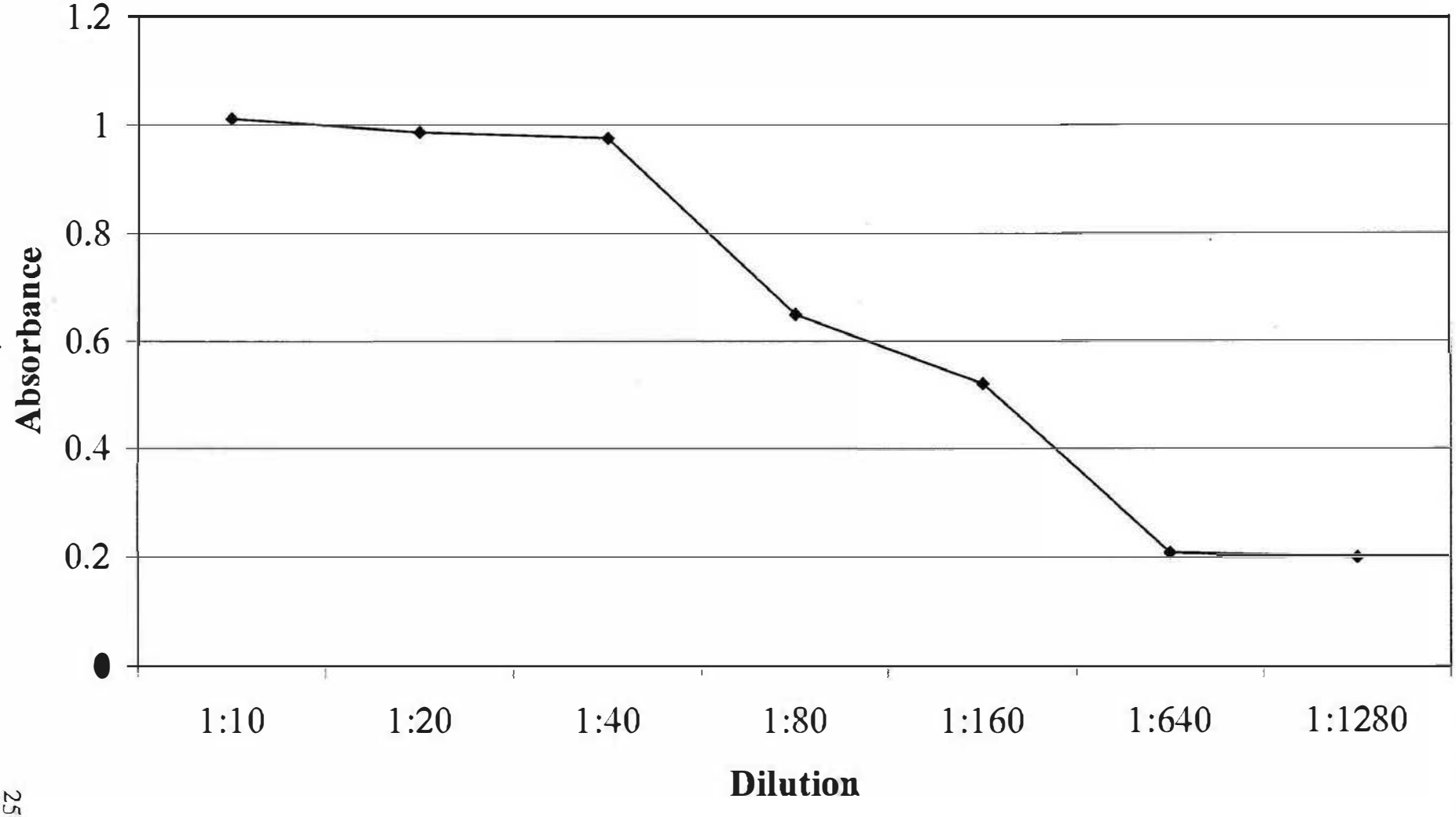


Figure 5.3. Absorbance values (405nm) versus logistic dilution of PNRSV-A control using RMV-3 antiserum.



the incidence of PNRSV-I was consistently higher than that of PNRSV-A in both years, with a ratio of PNRSV-A to PNRSV-I of 1:4 (Table 5.1).

Under half (44 %) of plants in the cultivar collection (block four) were infected by either serotype of PNRSV (Table 5.1). The majority of these infections were PNRSV-I (58 %). Surveys in hop gardens in north east Tasmania found differing levels of PNRSV incidence. In one 'Victoria' garden at Forrester River Hop Farms, PNRSV-I incidence was 70 %, however in a 'Victoria' garden on the property of private grower, 'X', only 1 % of plants were infected by PNRSV-A. A similar level of incidence (all PNRSV-A) in a 'Pride of Ringwood' garden at 'X' was also identified. In a 'Pride of Ringwood' garden at the property of private grower, 'Y', 43 % of plants were infected by PNRSV (A & I). The majority of these infections were PNRSV-A (82 %). A survey of gardens at Forrester River Hop Farms found 20% of 'Tettnang' plants were infected by PNRSV-I, and 30 % of 'Nugget' plants by PNRSV (A & I). Half of the 'Nugget' plants were infected by PNRSV-A and half by PNRSV-I (Table 5.1).

The smaller absorbance values from PNRSV-A and PNRSV-I antisera, and greater values with RMV-3 antiserum with PNRSV-I from 'Nugget' suggested it was serologically different from PNRSV-I infecting other cultivars (Table 5.2; Appendices 4.1 to 4.7).

Table 5.1. Incidence of PNRSV serotypes in a range of hop cultivars and sites.

Block (Cultivar)	Year	*n*	% Incidence PNRSV (A & I)	Incidence of each serotype (%)		Ratio of PNRSV-A to PNRSV-I
				PNRSV-A	PNRSV-I	
1 ('Victoria')	1996	1275	66	41	59	2 : 3
1 ('Victoria')	1997	1275	92	32	68	1 : 2
2 ('Victoria')	1998	500	31	60	40	3 : 2
3 ('●pal')	1996	764	5	77	23	3 : 1
3 ('●pal')	1997	764	11	48	52	1 : 1
3 ('Victoria')	1996	760	65	19	81	1 : 4
3 ('Victoria')	1997	760	91	19	81	1 : 4
3 ('T11')	1996	774	14	73	27	3 : 1
3 ('T11')	1997	774	17	67	33	2 : 1
4 ('Museum')	1997	99	44	43	58	2 : 3
FRF ('Victoria')	1997	20	70	0	100	0 : 1
X ('Victoria')	1997	40	1	100	0	1 : 0
FRF ('Tettnang')	1997	20	20	0	100	0 : 1
FRF ('Nugget')	1997	20	30	50	50	1 : 1
X ('Pride of Ringwood')	1997	40	43	82	18	5 : 1
Y ('Pride of Ringwood')	1997	40	1	100.	0	1 : 0

FRF – Forrester River Hop Farms; X = private grower 1; Y = private grower 2.

Table 5.2. Mean and standard deviation of standardised absorbance values for randomly selected plants infected by either PNRSV-A or PNRSV-I in a range of cultivars at different sites.

Plot (Cultivar)	PNRSV Serotype	*n*	Mean (standard deviation) of A_{405} values[1]		
			PNRSV-A	PNRSV-I	RMV-3
1 ('Victoria')	PNRSV-A	100	0.62 (0.12)	NA	0.20 (0.01)
1 ('Victoria')	PNRSV-I	100	0.36 (0.04)	NA	0.69 (0.02)
2 ('Victoria')	PNRSV-A	30	0.83(0.10)	NA	0.11(0.01)
2 ('Victoria')	PNRSV-I	30	0.41 (0.04)	NA	0.79(0.02)
3 ('●pal')	PNRSV-A	30	0.67 (0.03)	NA	0.21(0.02)
3 ('●pal')	PNRSV-I	30	0.29 (0.03)	NA	0.68(0.05)
3 ('Victoria')	PNRSV-A	30	0.81 (0.22)	NA	0.19 (0.01)
3 ('Victoria')	PNRSV-I	30	0.31 (0.03)	NA	0.78 (0.01)
3 ('Tl l')	PNRSV-A	30	0.76 (0.08)	NA	0.19 (0.01)
3 ('Tl l')	PNRSV-I	30	0.42 (0.04)	NA	0.78 (0.01)
4 ('Museum')	PNRSV-A	42	0.61 (0.08)	0.61 (0.07)	0.19 (0.03)
4 ('Museum')	PNRSV-I	42	0.29 (0.07)	0.79 (0.16)	0.55(0.17)
FRF ('Victoria')	PNRSV-I	14	0.37 (0.08)	0.77 (0.10)	0.68 (0.03)
X ('Victoria')	PNRSV-A	2	0.69 (0.04)	0.58 (0.04)	0.21 (0.01)
FRF ('Tettnang')	PNRSV-I	4	0.42 (0.04)	0.86 (0.01)	0.78 (0.02)
FRF ('Nugget')	PNRSV-I	6	0.28 (0.05)	0.34 (0.12)	0.80 (0.01)
X & Y ('Pride of Ringwood')	PNRSV-A	15	0.76 (0.09)	0.57 (0.06)	0.23 (0.03)

[1] values from each antiserum standardised to common positive controls (hop) on each plate.

FRF = Forrester River Hop Farms; X = private grower 1; Y = private grower 2; NA – not applicable.

Figure 5.4. Standardised absorbance reactions of PNRSV infections in a range of cultivars using PNRSV-A and RMV-3 antisera.

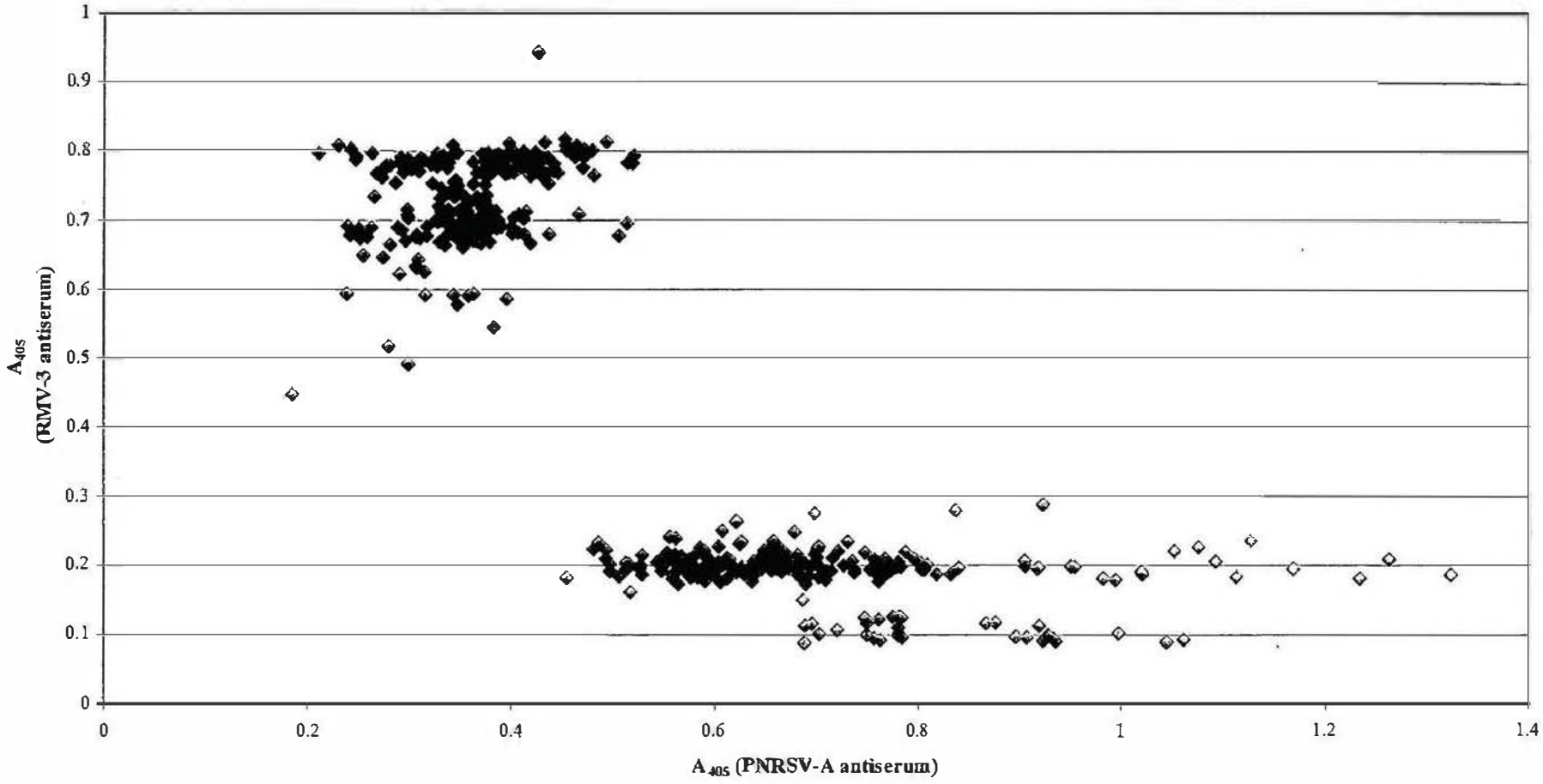
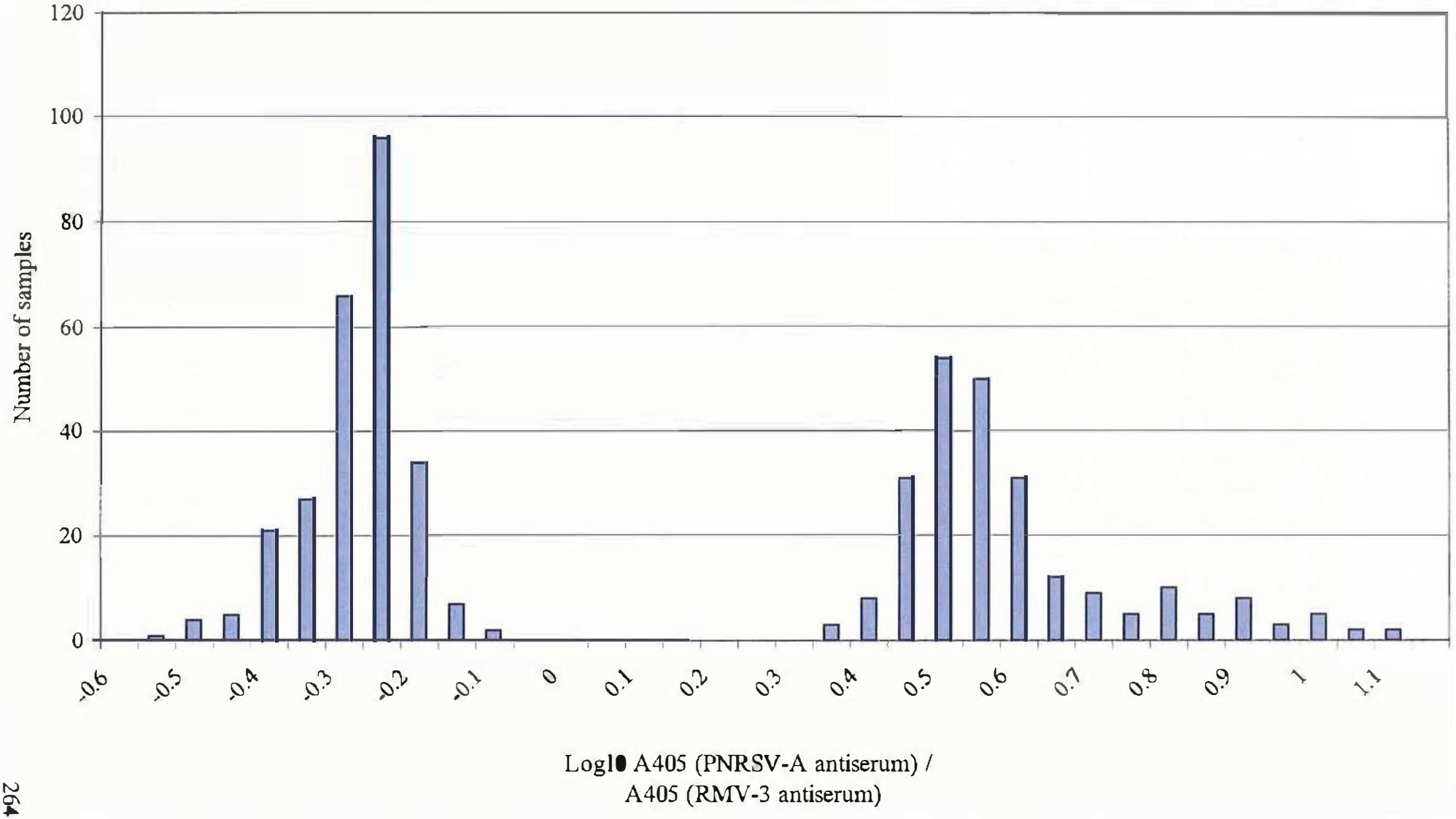


Figure 5.5. Frequency distribution of PNRSV serotypes in a range of hop cultivars.



Reliability of PNRSV detection

The temporal fluctuations in standardised absorbance values, corresponding to PNRSV levels in chronically infected 'Victoria' plants were monitored.

Changes in the levels of PNRSV-A were consistent in all plants in 1996/97 (Figures 5.6 & 5.7). Testing with RMV-3 antiserum produced average standardised absorbance values of 0.20 throughout the 12 month period. The levels of PNRSV-A were generally high, and equal to levels in young leaves in early spring (September-October). PNRSV levels in dormant buds were highest in the month following harvest (May). PNRSV levels remained high in young leaves throughout the growing season until prior to harvest (March), where no virus infection was detectable in some plants using PNRSV-A antiserum (Figures 5.6 & 5.7; Appendix 5.8).

Changes in the levels of PNRSV-I were also consistent in all plants in 1996/97 (Figures 5.8 & 5.9). Minimal fluctuations in standardised absorbance values were obtained with PNRSV-A and RMV-3 antisera. Dormant buds were also a reliable indicator of infection by PNRSV, but a slight decline in absorbance value was observed in June and July. Levels of PNRSV-I remained high throughout the growing season, with only a marginal decrease prior to harvest (March-April) with RMV-3 antiserum (Figures 5.8 & 5.9; Appendix 5.8).

Figure 5.6. Monthly fluctuations in mean standardised absorbance reactions (405nm) to PNRSV-A antiserum in PNRSV-A infected 'Victoria' plants at Bushy Park in 1996/97

(bars = standard error).

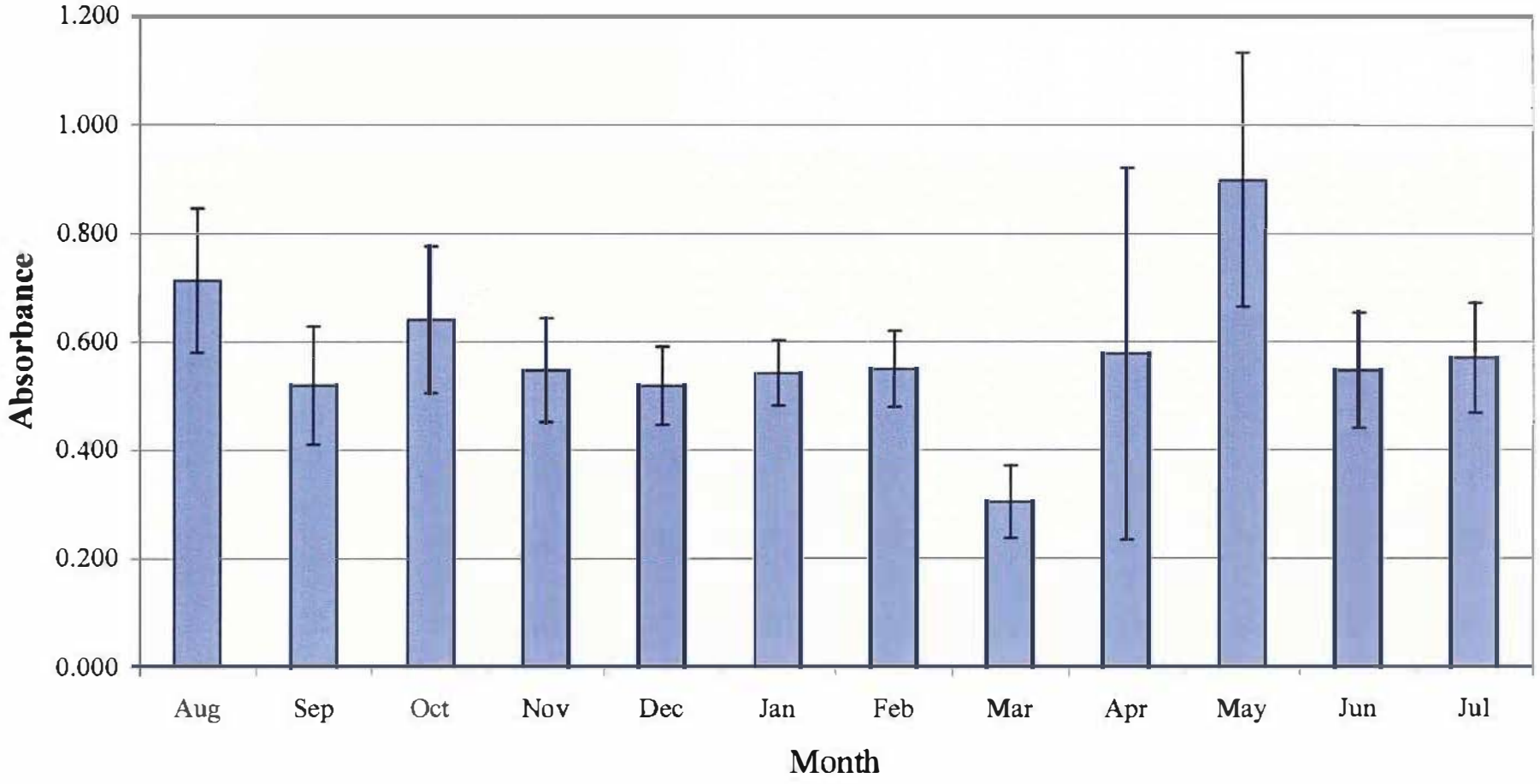


Figure 5.7. Monthly fluctuations in mean standardised absorbance reactions (405nm) to RMV-3 antiserum in PNRSV-A infected 'Victoria' plants.
(bars = standard error)

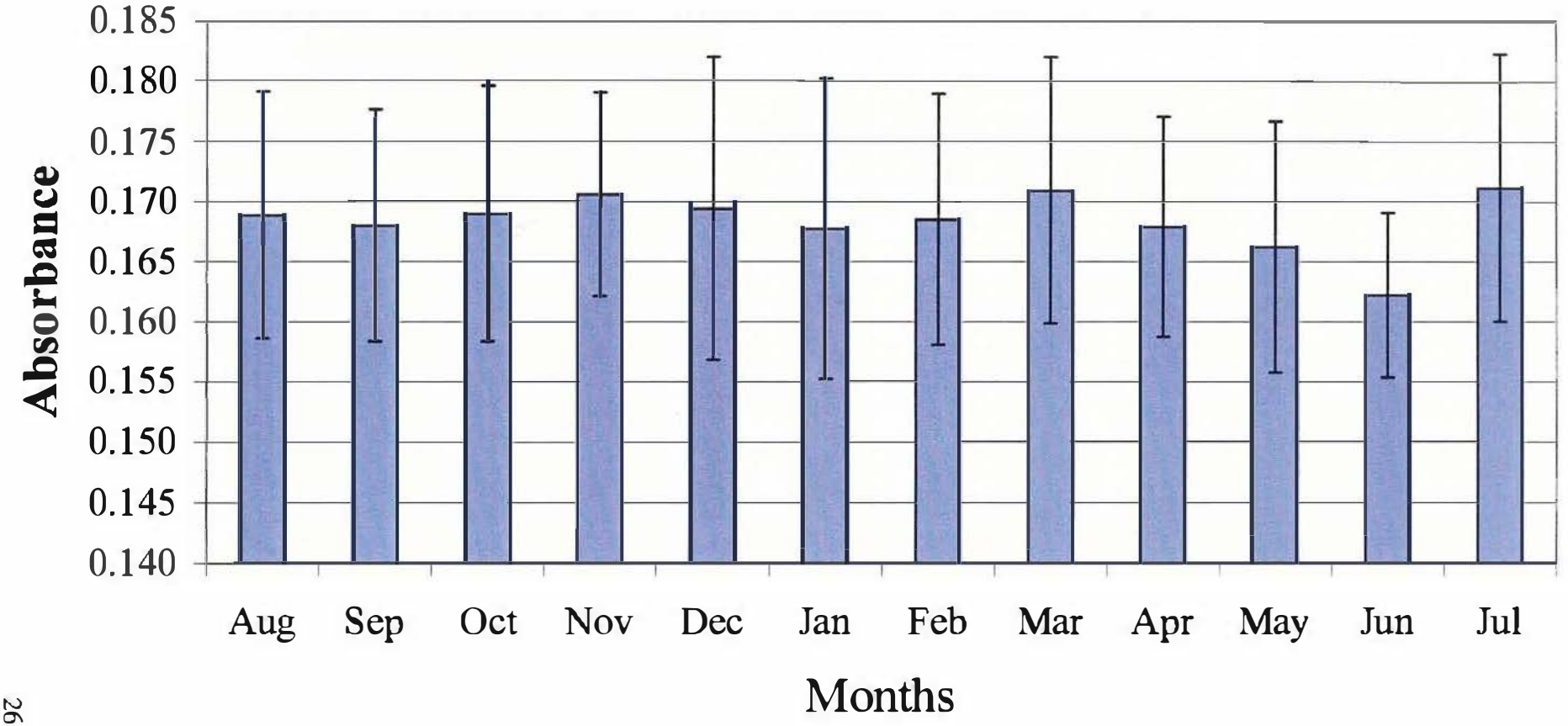


Figure 5.8. Monthly fluctuations in mean standardised absorbance reactions (405nm) to PNRSV-A antiserum in PNRSV-I infected plants
(bars = standard error).

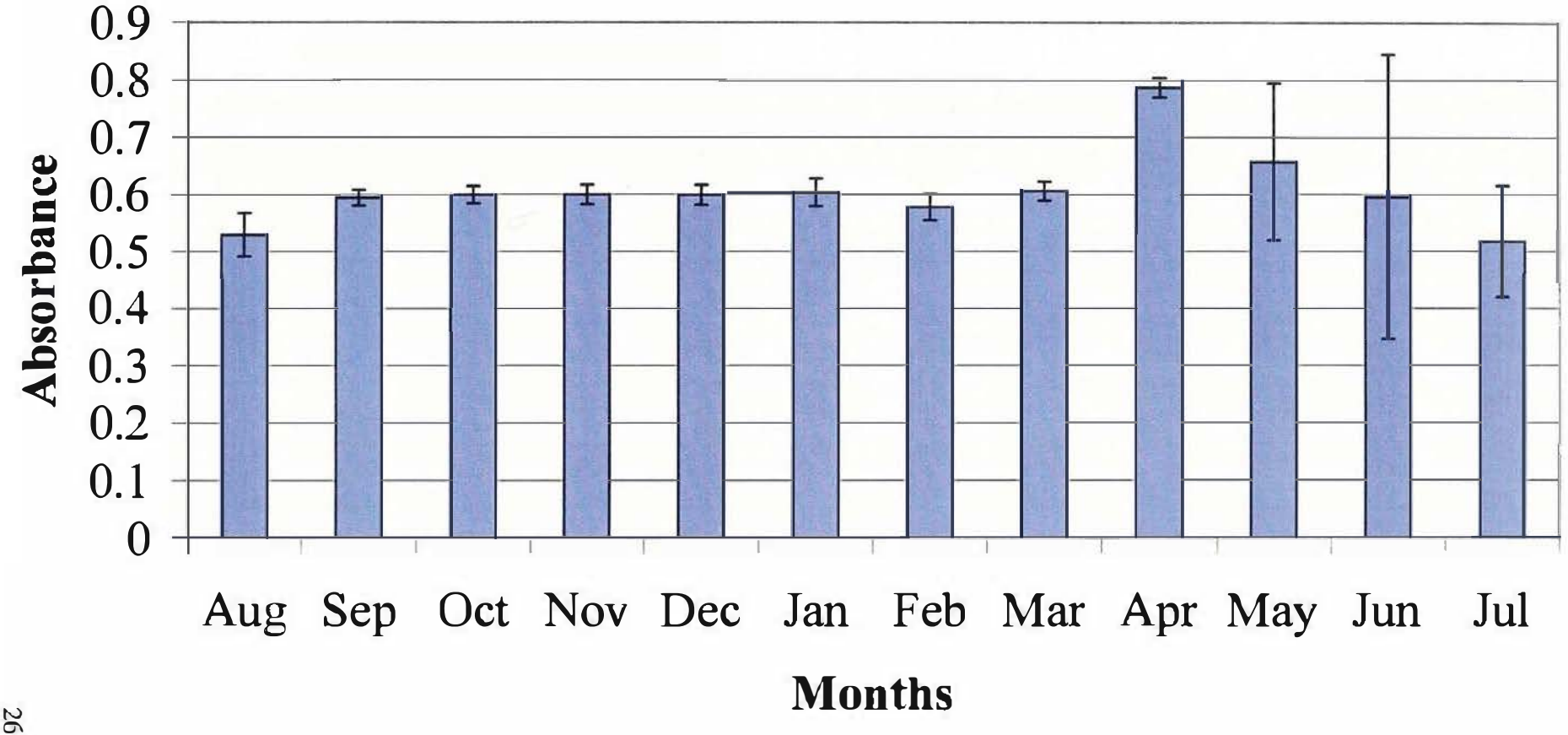


Figure 5.9. Monthly fluctuations in mean standardised absorbance reactions (405nm) to RMV-3 antiserum in PNRSV-I infected 'Victoria' plants

(bars = standard error).

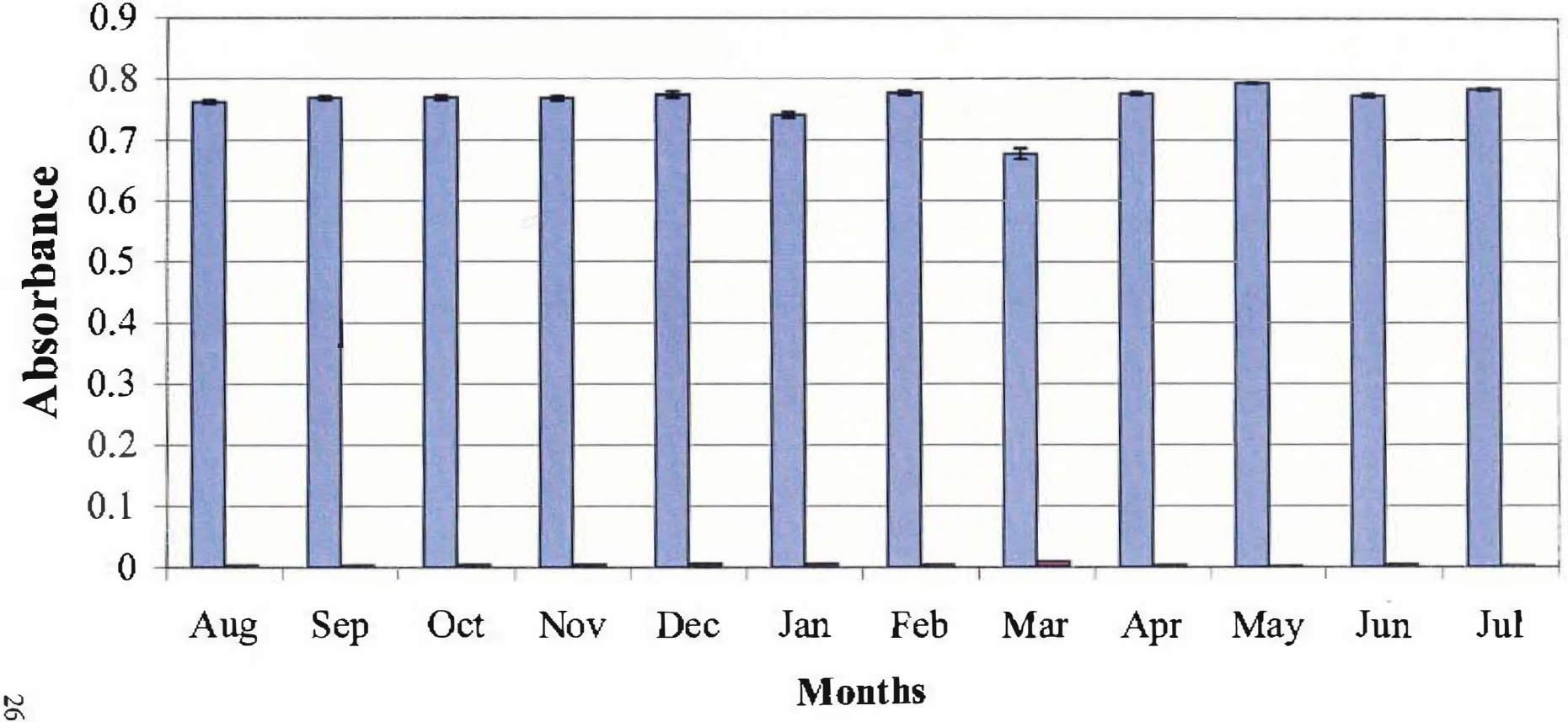


Figure 5.10. Monthly fluctuations in mean standardised absorbance reactions (405nm) to PNRSV-A antiserum in PNRSV-A(blue bars) and PNRSV-I (red bars) infected 'Victoria' plants in 1997-98 season

(Bar = standard error).

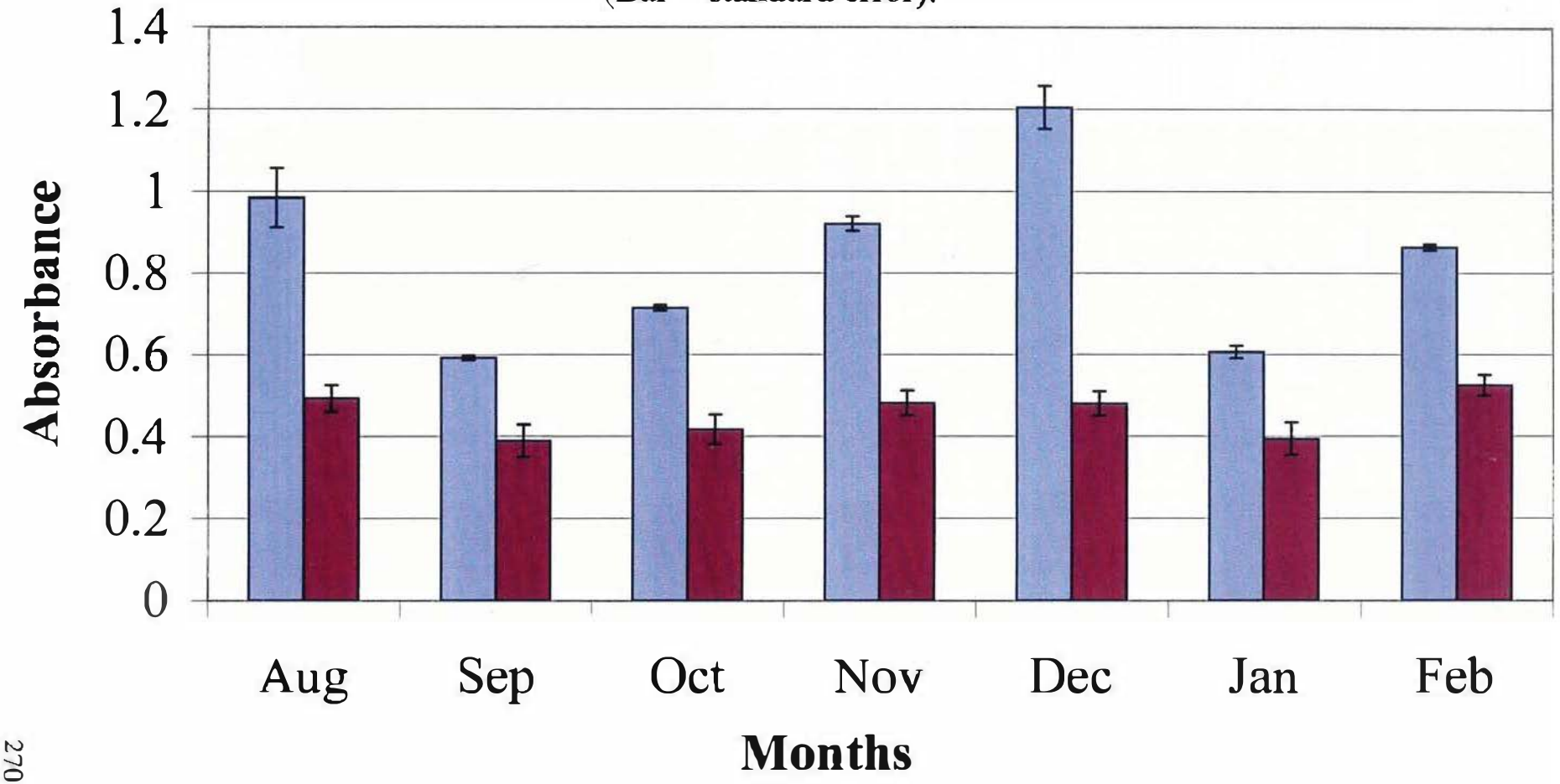
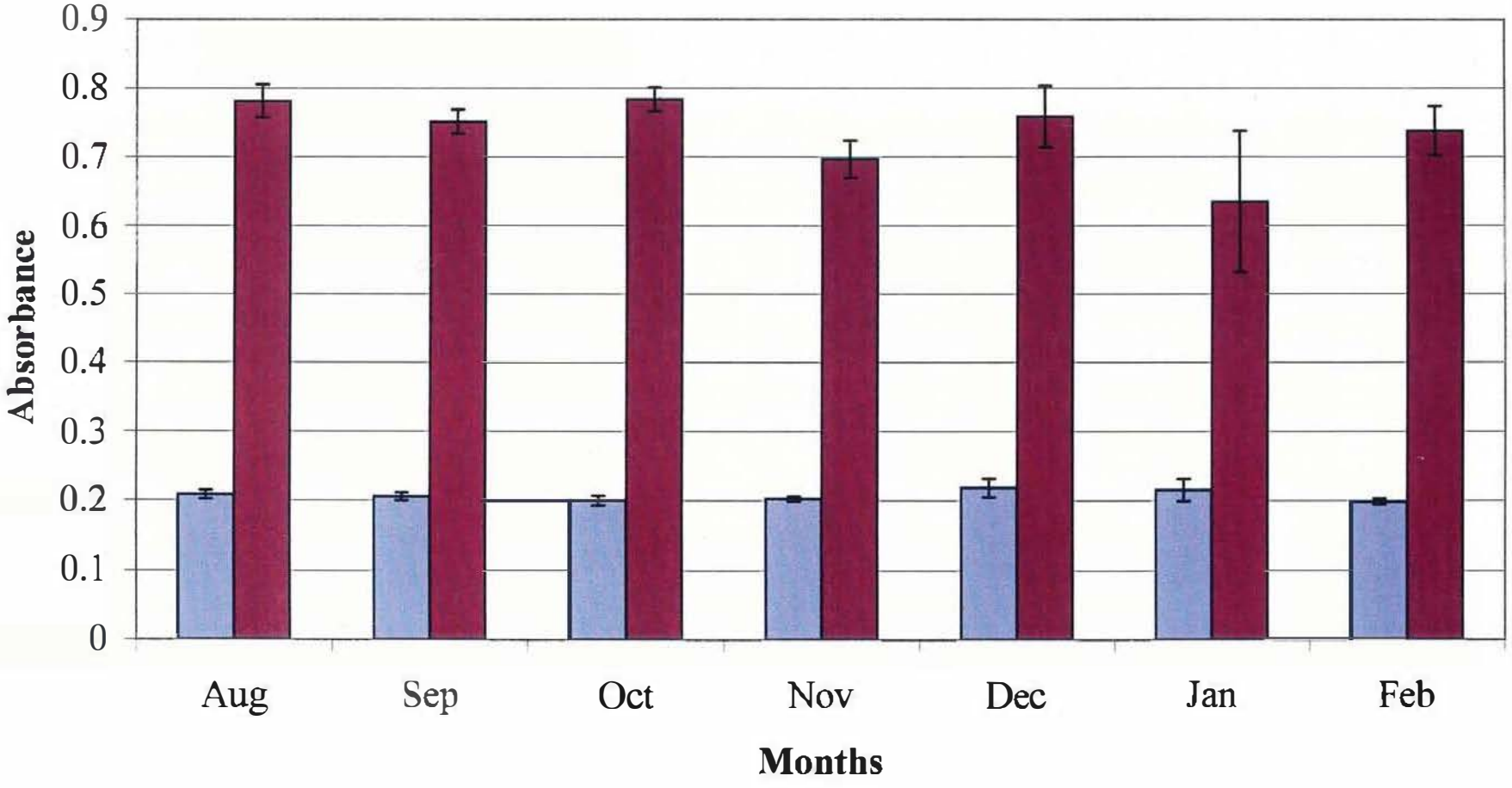


Figure 5.11. Monthly fluctuations in mean standardised absorbance reactions (405nm) to RMV-3 antiserum in PNRSV-A (blue bars) and PNRSV-I (red bars) infected plants in 1997 and 1998 (bars = standard error).



Fluctuations in PNRSV levels throughout the 1997-98 growing season were consistent with patterns in the previous season. Concurrent testing with PNRSV-A and RMV-3 antisera clearly differentiated isolates in all plants throughout the season. PNRSV levels in young leaves throughout the growing season remained high. In two of the five plants infected by PNRSV-A, a sharp increase in levels was detected in mid season (December) (Figures 5.10 & 5.11; Appendix 5.9).

Detection of PNRSV in different tissues

The relative ability to detect PNRSV in different tissues of chronically infected plants of a range of cultivars was studied.

Samples were collected from 'Victoria' and 'Opal' plants already known to be infected by either PNRSV-A or PNRSV-I prior to harvest in February 1997. PNRSV was detected in all samples collected from 'Victoria' plants infected by either serotype. PNRSV was detected in approximately half of samples collected from 'Opal' plants infected by either serotype. No spatial pattern could be attributed to the detection of either serotype of PNRSV in 'Opal' plants (Tables 5.3 & 5.4).

The following season, samples were collected from 'Victoria', 'Nugget', 'Opal', and 'Pride of Ringwood' plants infected by either PNRSV-A or PNRSV-I in December 1997 (mid-season). PNRSV was again detected in all samples collected from both 'Victoria' plants infected by either serotype. The detection of PNRSV-A in 'Nugget' plants varied

with the position of the sample. In one plant, samples testing positive were in the upper third of each string. In the second plant, samples testing positive originated from the upper third of one string, the middle third of the remaining strings, and from 75% of the basal growth. The detection of PNRSV-I in 'Nugget' plants also varied with sample position. Likewise, the detection of PNRSV-A in 'Pride of Ringwood' plants was also found to be tissue dependent. In one plant, the only sample testing positive to infection was from one portion of the basal growth, but in the second plant the only sample testing negative to infection was from the lower third of one string. The detection of PNRSV-I in 'Opal' plants also varied with sample position. In one plant, PNRSV-I was detected in the upper third of one string, and the middle third of another. In another plant, PNRSV-I was detected in the upper third of each string and in all samples taken from the basal growth (Tables 5.5 to 5.8).

Concurrent testing of plants with antisera to PNRSV-A and RMV-3 showed that samples from different areas of the same plant tested positive to both serotypes. All samples with no detectable virus infection gave low readings to both antisera.

Table 5.3. Mean standardised absorbance values for samples from various parts of 'Victoria' and 'Opal' plants in December 1996, infected by PNRSV-A and PNRSV-I, using antiserum produced against PNRSV-A.

Plant Section	Mean standardised absorbance values of A_{405} values[1] (PNRSV-A antiserum)			
	'Victoria' (PNRSV-A) $n = 2$	'Victoria' (PNRSV-I) $n = 2$	'Opal' (PNRSV-A) $n = 2$	'Opal' (PNRSV-I) $n = 2$
String 1 0 – 1.8m	0.71 (+)	0.39 (+)	0.73 (+)	0.54 (+)
String 1 1.8 – 3.6 m	0.85 (+)	0.41 (+)	0.72 (+)	0.19 (-)
String 1 3.6 – 5.5 m	0.78 (+)	0.42 (+)	0.13 (-)	0.08 (-)
String 2 0 – 1.8m	0.82 (+)	0.56 (+)	0.62 (+)	0.44 (+)
String 2 1.8 – 3.6 m	0.77 (+)	0.45 (+)	0.12 (-)	0.13 (-)
String 2 3.6 – 5.5m	0.79 (+)	0.37 (+)	0.65 (+)	0.12 (-)
String 3 0 – 1.8 m	0.80 (+)	0.39 (+)	0.21 (-)	0.42 (+)
String 3 1.8 – 3.6 m	0.82 (+)	0.44 (+)	0.72 (+)	0.18 (-)
String 3 3.6 – 5.5 m	0.77 (+)	0.52 (+)	0.63 (+)	0.54 (+)

[1] values from each antiserum standardised by dividing absorbance by common positive controls (hop).

Table 5.4. Mean standardised absorbance values for samples from various parts of 'Victoria' and 'Opal' plants in February 1996, infected by PNRSV-A and PNRSV-I, using antiserum produced against RMV-3.

Plant Section	Mean standardised absorbance values of A_{405} values[1] (RMV-3 antiserum)			
	'Victoria' (PNRSV-A) $n = 2$	'Victoria' (PNRSV-I) $n = 2$	'Opal' (PNRSV-A) $n = 2$	'Opal' (PNRSV-I) $n = 2$
String 1 0 – 1.8m	0.09 (-)	0.79 (+)	0.18 (-)	0.75 (+)
String 1 1.8 – 3.6 m	0.10 (-)	0.81 (+)	0.19 (-)	0.19 (-)
String 1 3.6 – 5.5 m	0.20 (-)	0.80 (+)	0.13 (-)	0.08 (-)
String 2 0 – 1.8 m	0.20 (-)	0.76 (+)	0.17 (-)	0.69 (+)
String 2 1.8 – 3.6 m	0.28 (-)	0.79 (+)	0.16 (-)	0.13 (-)
String 2 3.6 – 5.5 m	0.23 (-)	0.77 (+)	0.12 (-)	0.12 (-)
String 3 0 – 1.8 m	0.24 (-)	0.99 (+)	0.14 (-)	0.66 (+)
String 3 1.8 – 3.6 m	0.25 (-)	0.91 (+)	0.16 (-)	0.18 (-)
String 3 3.6 – 5.5 m	0.21 (-)	0.93 (+)	0.12 (-)	0.62 (+)

[1] values from each antiserum standardised by dividing absorbance by common positive controls (hop).

Table 5.5. Mean standardised absorbance values for samples from various parts of 'Victoria', 'Nugget', and 'Pride of Ringwood' plants in December 1997, infected by PNRSV-A, using antiserum produced against PNRSV-A.

Plant Section	Mean standardised absorbance values of A_{405} values[1] (PNRSV-A antiserum)					
	'Victoria' $n = 1$	'Victoria' $n = 1$	'Nugget' $n = 1$	'Nugget' $n = 1$	'Pride of Ringwood' $n = 1$	'Pride of Ringwood' $n = 1$
String 1 0 – 1.4 m	1.18 (+)	1.21 (+)	0.12 (-)	0.11 (-)	1.19 (+)	1.14 (+)
String 1 1.4 – 2.8 m	0.76 (+)	1.01 (+)	0.12 (-)	0.11 (-)	1.39 (+)	1.26 (+)
String 1 2.8 – 4.2 m	1.17 (+)	1.23 (+)	0.45 (+)	0.79 (+)	1.27 (+)	1.20 (+)
String 1 4.2 – 5.5 m	0.89 (+)	0.99 (+)	0.12 (-)	0.11 (-)	1.36 (+)	1.19 (+)
String 2 0 – 1.4 m	1.25 (+)	1.05 (+)	0.12 (-)	0.63 (+)	1.36 (+)	1.34 (+)
String 2 1.4 – 2.8 m	0.79 (+)	0.87 (+)	0.45 (+)	0.13 (-)	1.23 (+)	1.13 (+)
String 2 2.8 – 4.2 m	0.94 (+)	0.95 (+)	0.12 (-)	0.11 (-)	0.99 (+)	0.25(-)
String 2 4.2 – 5.5 m	0.90 (+)	0.89 (+)	0.12 (-)	0.67 (+)	1.10 (+)	1.28 (+)

String 3 0 – 1.4 m	1.14 (+)	1.06 (+)	0.57 (+)	0.11 (-)	1.26 (+)	0.77 (+)
String 3 1.4 – 2.8 m	1.12 (+)	1.10 (+)	0.11 (-)	0.13 (-)	1.10 (+)	1.24 (+)
String 3 2.8 – 4.2 m	1.16 (+)	1.02 (+)	0.11 (-)	0.28 (-)	1.01 (+)	1.19 (+)
String 3 4.2 – 5.5 m	1.19 (+)	1.20 (+)	0.20 (-)	0.39 (+)	1.04 (+)	1.05 (+)
Basal growth south	0.74 (+)	0.89 (+)	0.11 (-)	0.45 (+)	0.14 (-)	0.93 (+)
Basal growth north	0.78 (+)	0.74 (+)	0.56 (+)	0.47 (+)	0.17 (-)	0.16 (-)
Basal grow th east	0.79 (+)	0.87 (+)	0.12 (-)	0.19 (-)	0.19 (-)	0.23 (-)
Basal growth west	0.85 (+)	0.82 (+)	0.16 (-)	0.23(-)	0.21 (-)	0.25 (-)

[1] values from each antiserum standardised to common positive controls (hop) on each plate.

Table 5.6. Mean standardised absorbance values for samples from various parts of 'Victoria', 'Nugget', and 'Pride of Ringwood' plants in December 1997, infected by PNRSV-A, using antiserum produced against RMV-3.

Plant Section	Mean standardised absorbance values of A_{405} values[1] (RMV-3 antiserum)					
	'Victoria' $n = 1$	'Victoria' $n = 1$	'Nugget' $n = 1$	'Nugget' $n = 1$	'Pride of Ringwood' $n = 1$	'Pride of Ringwood' $n = 1$
String 1 0 – 1.4 m	0.10 (-)	0.20 (-)	0.12 (-)	0.11 (-)	0.12 (-)	0.16 (-)
String 1 1.4 – 2.8 m	0.14 (-)	0.09 (-)	0.12	0.11 (-)	0.11 (-)	0.12 (-)
String 1 2.8 – 4.2 m	0.14 (-)	0.09 (-)	0.13	0.16 (-)	0.13 (-)	0.14 (-)
String 1 4.2 – 5.5 m	0.11 (-)	0.08 (-)	0.12 (-)	0.11 (-)	0.15 (-)	0.15 (-)
String 2 0 – 1.4 m	0.19 (-)	0.16 (-)	0.12 (-)	0.19 (-)	0.16 (-)	0.16 (-)
String 2 1.4 – 2.8 m	0.16 (-)	0.17 (-)	0.16 (-)	0.13 (-)	0.12 (-)	0.15 (-)
String 2 2.8 – 4.2 m	0.16 (-)	0.19 (-)	0.12 (-)	0.11 (-)	0.09 (-)	0.20 (-)
String 2 4.2 – 5.5 m	0.12 (-)	0.20 (-)	0.12 (-)	0.18 (-)	0.19 (-)	0.13 (-)
String 3 0 – 1.4 m	0.15 (-)	0.18 (-)	0.19 (-)	0.11 (-)	0.15 (-)	0.14 (-)

String 3 1.4 – 2.8 m	0.15 (-)	0.17 (-)	0.11 (-)	0.13 (-)	0.17 (-)	0.14 (-)
String 3 2.8 – 4.2 m	0.14 (-)	0.20 (-)	0.11 (-)	0.14 (-)	0.18 (-)	0.19 (-)
String 3 4.2 – 5.5 m	0.14 (-)	0.21 (-)	0.20 (-)	0.15 (-)	0.13 (-)	0.18 (-)
Basal growth south	0.14 (-)	0.19 (-)	0.11 (-)	0.19 (-)	0.14 (-)	0.11 (-)
Basal growth north	0.15 (-)	0.21 (-)	0.23 (-)	0.25 (-)	0.24 (-)	0.21 (-)
Basal growth east	0.16 (-)	0.23 (-)	0.17 (-)	0.18 (-)	0.26 (-)	0.25 (-)
Basal growth west	0.17 (-)	0.19 (-)	0.15 (-)	0.14 (-)	0.16 (-)	0.17 (-)

[1] values from each antiserum standardised to common positive (hop) controls on each plate.

Table 5.7. Mean standardised absorbance values for samples from various parts of 'Victoria', 'Nugget', and 'Opal' plants in December 1997, infected by PNRSV-I, using antiserum produced against PNRSV-A.

Plant Section	Mean standardised absorbance values of A_{405} values[1] (PNRSV-A antiserum)					
	'Victoria' $n = 1$	'Victoria' $n = 1$	'Nugget' $n = 1$	'Nugget' $n = 1$	'Opal' $n = 1$	'Opal' $n = 1$
String 1 0 – 1.4 m	0.434 (+)	0.52 (+)	0.34 (+)	0.30 (+)	0.19 (-)	0.09 (-)
String 1 1.4 – 2.8 m	0.38 (+)	0.53 (+)	0.33 (+)	0.32 (+)	0.13 (-)	0.09 (-)
String 1 2.8 – 4.2 m	0.41 (+)	0.37 (+)	0.12 (-)	0.29 (+)	0.21 (-)	0.42 (+)
String 1 4.2 – 5.5 m	0.42 (+)	0.38 (+)	0.35 (+)	0.33 (+)	0.15 (-)	0.09 (-)
String 2 0 – 1.4 m	0.45 (+)	0.49 (+)	0.19 (-)	0.32 (+)	0.16 (-)	0.09 (-)
String 2 1.4 – 2.8 m	0.46 (+)	0.35 (+)	0.33 (+)	0.45 (+)	0.46 (+)	0.36 (+)
String 2 2.8 – 4.2 m	0.49 (+)	0.41 (+)	0.41 (+)	0.34 (+)	0.13 (-)	0.12 (-)
String 2 4.2 – 5.5 m	0.56 (+)	0.47 (+)	0.49 (+)	0.12 (-)	0.39 (+)	0.13 (-)
String 3 0 – 1.4 m	0.52 (+)	0.41 (+)	0.46 (+)	0.13 (-)	0.09 (-)	0.35 (+)
String 3 1.4 – 2.8 m	0.48 (+)	0.45 (+)	0.42 (+)	0.41 (+)	0.09 (-)	0.22 (+)

String 3 2.8 – 4.2 m	0.49 (+)	0.51 (+)	0.39 (+)	0.10 (-)	0.08 (-)	0.27 (+)
String 3 4.2 – 5.5 m	0.55 (+)	0.52 (+)	0.12 (-)	0.36 (+)	0.10 (-)	0.30 (+)
Basal growth south	0.56 (+)	0.50 (+)	0.11 (-)	0.35 (+)	0.12 (-)	0.31 (+)
Basal growth north	0.55 (+)	0.57 (+)	0.14 (-)	0.17 (-)	0.17 (-)	0.27 (+)
Basal growth east	0.54 (+)	0.59 (+)	0.15 (-)	0.12 (-)	0.18 (-)	0.21 (-)
Basal growth west	0.52 (+)	0.58 (+)	0.16 (-)	0.19 (-)	0.13 (-)	0.19 (-)

[1] values from each antiserum standardised by common positive controls (hop).

Table 5.8. Mean standardised absorbance values for samples from various parts of 'Victoria', 'Nugget', and 'Opal' plants in December 1997, infected by PNRSV-I, using antiserum produced against RMV-3.

Plant Section	Mean standardised absorbance values of A_{405} values[1] (RMV-3 antiserum)					
	'Victoria' $n = 1$	'Victoria' $n = 1$	'Nugget' $n = 1$	'Nugget' $n = 1$	'Opal' $n = 1$	'Opal' $n = 1$
String 1 0 – 1.4 m	0.41 (+)	0.43 (+)	0.53 (+)	0.59 (+)	0.12 (-)	0.16 (-)
String 1 1.4 – 2.8 m	0.42 (+)	0.42 (+)	0.56 (+)	0.48 (+)	0.11 (-)	0.12 (-)
String 1 2.8 – 4.2 m	0.39 (+)	0.42 (+)	0.13 (-)	0.52 (+)	0.13 (-)	0.42 (+)
String 1 4.2 – 5.5 m	0.29 (+)	0.43 (+)	0.62 (+)	0.43 (+)	0.15 (-)	0.15 (-)
String 2 0 – 1.4 m	0.41 (+)	0.44 (+)	0.12 (-)	0.50 (+)	0.16 (-)	0.16 (-)
String 2 1.4 – 2.8 m	0.49 (+)	0.46 (+)	0.65 (+)	0.49 (+)	0.44 (+)	0.46 (+)
String 2 2.8 – 4.2 m	0.32 (+)	0.38 (+)	0.55 (+)	0.54 (+)	0.99 (+)	0.20 (-)
String 2 4.2 – 5.5 m	0.46 (+)	0.39 (+)	0.56 (+)	0.18 (-)	0.42 (+)	0.13 (-)
String 3 0 – 1.4 m	0.43 (+)	0.41 (+)	0.63 (+)	0.11 (-)	0.15 (-)	0.44 (+)
String 3 1.4 – 2.8 m	0.39 (+)	0.40 (+)	0.61 (+)	0.13 (-)	0.17 (-)	0.42 (+)

String 3 2.8 – 4.2 m	0.47 (+)	0.42 (+)	0.62 (+)	0.60 (+)	0.18 (-)	0.31 (+)
String 3 4.2 – 5.5 m	0.55 (+)	0.43 (+)	0.20 (-)	0.63 (+)	0.13 (-)	0.37 (+)
Basal growth south	0.56 (+)	0.43 (+)	0.19 (-)	0.62 (+)	0.15 (-)	0.40 (+)
Basal growth north	0.55 (+)	0.48 (+)	0.15 (-)	0.17 (-)	0.19 (-)	0.18 (-)
Basal growth east	0.51 (+)	0.49 (+)	0.17 (-)	0.18 (-)	0.16 (-)	0.17 (-)
Basal growth west	0.54 (+)	0.44 (+)	0.11 (-)	0.19 (-)	0.14 (-)	0.37 (+)

[1] values from each antiserum standardised by common positive controls (hop).

Shirofugen flowering cherry assay of PNRSV serotypes infecting hop

The reaction of shirofugen flowering cherry to PNRSV serotypes infecting hop was investigated. After six weeks, only PNRSV-I samples from 'Victoria' induced tissue necrosis (Table 5.9).

Table 5.9. Shirofugen flowering cherry assay of ('Victoria' and 'Pride of Ringwood') budsticks infected by either PNRSV-A or PNRSV-I.

Cultivar (PNRSV serotype)	Shirofugen Index[1]
'Victoria' (PNRSV-A)	Negative
'Victoria' (PNRSV-A)	Negative
'Victoria' (PNRSV-I)	Positive
'Victoria' (PNRSV-I)	Positive
'Pride of Ringwood' (PNRSV-A)	Negative
' Pride of Ringwood' (PNRSV-A)	Negative
'Pride of Ringwood' (PNRSV-I)	Negative
'Pride of Ringwood' (PNRSV-I)	Negative
'Victoria' (virus free)	Negative
'Victoria' (virus free)	Negative
'Pride of Ringwood' (virus free)	Negative
'Pride of Ringwood' (virus free)	Negative

[1] Samples testing positive to shirofugen assay produced a necrotic reaction in tissue surrounding budchips.

5.4. DISCUSSION

This study focussed on optimising the ELISA procedure for routine diagnosis of PNRSV, by characterising sample variation within serotypes, temporal fluctuations in virus levels, and the relative efficiencies of virus detection in various tissues.

The relative incidence of PNRSV-A and PNRSV-I serotypes within gardens varied with cultivar and site, (e.g. the difference in the infection ratios of PNRSV-A to PNRSV-I between 'Victoria' gardens). Differences in the spread of either serotype may rely upon several factors including a) inoculum load, b) the initial inoculum assuming a certain percentage of the planting material was infected, c) local climatic effects influencing plant vigour and hence susceptibility to virus infection, d) cultural practices influencing the mode of virus transmission (chapter three), and e) the spatial arrangements of particular serotypes which may exclude spread by the other serotype or particular strains of each serotype. All positive samples were clearly separated into either PNRSV-A or PNRSV-I serotypes. PNRSV-A samples reacted strongly to PNRSV-A antiserum, weakly to PNRSV-I antiserum, and failed to react to RMV-3 antiserum. PNRSV-I samples reacted weakly to PNRSV-A antiserum, and strongly with PNRSV-I and RMV-3 antiserum. PNRSV-C samples would have reacted strongly to RMV-3 and failed to react to the PNRSV-A and PNRSV-I antiserum. The only serological variation to this pattern was with PNRSV-I infected samples from 'Nugget' plants in gardens in the north east of Tasmania. These gave no reaction to PNRSV-A antiserum, a relatively weak reaction

with PNRSV-I antiserum and a strong reaction to RMV-3 antiserum. Further surveys of 'Nugget' gardens are required to determine the extent of is serological variation.

Some PNRSV-A and PNRSV-I reactions to PNRSV-A antiserum overlapped at the lower end of the PNRSV-A group. Concurrent testing with RMV-3 was essential for the differentiation of these isolates into serotypes. Therefore, for the routine detection of PNRSV infection by either serotype, testing with PNRSV-A antisera would be suitable. Aetiological and epidemiological studies necessitating the differentiation of serotypes would require concurrent testing with at least one other antiserum able to detect PNRSV-I. Antiserum to RMV-3 was selected as the most suitable for concurrent use in further studies (chapter two) because of its failure to react to PNRSV-A, providing an easy method for determining whether weaker reactions to PNRSV-A antisera resulted from lower virus titre or serological differences.

The presence of mixed infections of PNRSV-A and PNRSV-I within a plant still cannot be resolved by serological testing using currently available antisera. Nucleic acid based studies rely upon the provision of sequence information for PNRSV-I. Probes designed specifically to target sequences unique to the individual serotypes would aid in the determination of mixed infections in the field. Nucleic acid based methods would also be useful in studying variation within serotypes for the preliminary identification of strain variants.

PNRSV was detected in all samples from 'Victoria' plants tested over a period of 20 months. Dormant buds were a reliable indicator of PNRSV infection. However, the need to dig up buds using clean blades in between each plant to prevent possible cross-contamination reduced the number of plants easily sampled. PNRSV was successfully detected in young leaves sampled throughout the season, despite a marginal decline in virus titre prior to harvest (march). This is unlike the decline in virus titre after mid-season (late December), in 'Pride of Ringwood', which made virus detection less reliable (D. Munro, pers. comm.). However, studies on cultivars grown in the United Kingdom (Thresh *et al.*, 1977) and New Zealand (F. Hay, pers. comm.) were equally successful in detecting PNRSV infection early and late in the season.

The ability to detect PNRSV reliably in different tissues varied with cultivar. PNRSV-A and PNRSV-I were detected in all tissues at various positions around chronically infected (infection detected the previous year) 'Victoria' plants, suggesting a symmetrical virus distribution within the plant. In chronically infected 'Nugget', 'Pride of Ringwood', and 'Opal' plants, infected by either serotype, the probability of detecting infection varied with the part of the plant sampled, suggesting an asymmetric virus distribution within the plant. The high levels of PNRSV throughout the season and even distribution within 'Victoria' may influence the rate of virus spread in this cultivar, by increasing the probability of PNRSV transmission. The asymmetric virus distributions in certain cultivars may also explain the typically "random" appearance of PNRSV infected plants (primary foci) in the first year following planting with virus tested material. For example, if mother plants for propagation were selected on the basis of test results using

only leaf samples from bines from one string, virus infection may be missed. Therefore, a certain proportion of the cuttings taken from the mother plant may be virus infected and planted out randomly in the field. These studies selected samples only from various zones on strings on which various numbers of bines were trained. To investigate virus distributions within plants in more detail, samples could be taken from specific bines. In addition, softwood cuttings could be taken from each section and rooted plantlets tested for virus infection following a dormancy period. This would verify that the sections with no detectable virus infection the previous season were infected but virus titre was not high enough for detection by ELISA or that some sections were healthy.

The PNRSV-I isolate infecting 'Victoria' was found to induce a tissue necrosis reaction in shirofugen flowering cherry, similar to that induced by samples infected by PNRSV-C. This was the first attempt at assessing the reactivity of PNRSV-I isolates to this assay and the first incidication of a close relationship between these isolates and PNRSV-C as suggested by immunology. The negative reaction to PNRSV-I isolates infecting 'Pride of Ringwood' may suggest isolate differences among cultivars, or lower virus titre in 'Pride of Ringwood' when budsticks were selected later in the season, or possibly selection of healthy bud sticks in 'Pride of Ringwood' due to an asymmetric distribution of the virus within the source plant. Considering the biophysical and serological differences between PNRSV-A and PNRSV-C (Ong & Mink, 1989), a negative reaction to PNRSV-A in either cultivar was expected. Further experiments are necessary to investigate the differences in reaction of both serotypes from different cultivars at various times throughout the season.

In conclusion, all PNRSV positive samples evaluated were clearly separated into either PNRSV-A or PNRSV-I serotypes by testing with PNRSV-A, PNRSV-I, and RMV-3 antisera. The titre of PNRSV remained high in all tissues in 'Victoria' throughout the season, which may contribute to increasing the probability of transmission between plants through the aerial tissues and at least partially explain the higher incidence of plants infected by PNRSV in this cultivar.

CHAPTER SIX

EFFECT OF VIRUSES ON YIELD AND QUALITY OF HOP CULTIVARS IN AUSTRALIA

6.1. INTRODUCTION

A detailed knowledge of the effect of viruses on the yield and levels of brewing acids of individual cultivars is vital for determining whether infection poses a significant constraint to Australian hop production, thereby requireing the implementation of control measures.

All three viruses found commonly infecting commercial hop gardens in Australia, HpLV, HpMV, and PNRSV (A & I), (chapter two) have been reported to reduce production in at least one hop cultivar overseas. PNRSV is the most damaging virus because it has been found to significantly reduce production and quality of hop cultivars grown in the United Kingdom (Neve and Thresh, 1984). Infection by PNRSV has also been associated with significant reductions in cone yield and alpha acid content in cultivars in Germany ('Northern Brewer') (Kremheller *et al.*, 1988), the U.S.A. ('Chinook') (Probasco and Murphey, 1996), and New Zealand ('Superalpha') (Hay *et al.*, 1992). HpMV has been reported to significantly reduce cone yields in sensitive cultivars in the United Kingdom (e.g. 'Goldings'), Australia ('J78'), the U.S.A. ('Chinook') (Probasco and Murphey, 1996), and in mixed infections in New Zealand ('Superalpha') (Hay *et al.*, 1992). The

sole report associating HpLV with significant reductions in cone yields and brewing organic acid levels is from the U.S.A. in 'Chinook' (Probasco and Murphey, 1996). Mixed infections between HpLV, PNRSV, HpMV, and ArMV also significantly reduced cone yields in cultivars grown in New Zealand (Hay *et al.*, 1992). Significant reductions in cone yields and alpha acid levels from virus infection have also been reported in two traditionally grown cultivars in Australia. PNRSV was found to significantly reduce cone yields of 'J78', while HpMV was associated with significant reductions in plant vigour, cone yields, and alpha acid levels in 'Pride of Ringwood'. Infections by PNRSV and HpLV + PNRSV have also been reported as detrimental to hop production of this cultivar (G. Leggett, pers. comm.). No study has been conducted to indicate whether these viruses pose an economically-significant constraint to the production of the newer, triploid varieties, such as 'Victoria'.

The objective of this study was to examine the effect of HpLV, HpMV, PNRSV-A, and PNRSV-I, and mixed infections of these viruses on the yield and brewing acid composition in four cultivars commonly grown in Australia.

6.2. MATERIALS AND METHODS

Propagation of planting material and trial design

Mother plants for propagation were selected from surveys in the 1995-96 season, and potted plants infected by a variety of virus combinations, established two years previously (1993/94) from softwood cuttings. Potted plants were grown in the glasshouse over winter under supplementary lighting. Plants infected by the same viruses were separated from plants infected by different combinations of viruses (HpLV, HpMV, HpLV + PNRSV-A, HpLV + PNRSV-I, HpMV + PNRSV-A, HpMV + PNRSV-I, HpLV + HpMV + PNRSV-A, HpLV + HpMV + PNRSV-I). Softwood cuttings were made from these plants throughout winter and early spring, depending upon material availability (Appendix 4). Samples from plants selected in the field in late summer were re-tested early the following spring to verify the virus status of plants had not altered between seasons. Softwood cuttings were taken from these plants in spring (Appendix 4; Plate 6.1). Mother plants free of detectable infection by all three viruses ("virus tested") were selected from field plants. These plants were separated from virus infected plants by at least two virus tested plants both along and across rows. Cuttings from virus tested plants were despatched to a commercial company, Hills Transplants Pty. Ltd., Devonport, Tasmania the same day for further propagation (Plate 6.2).

The effects of virus infection was studied in four economically important cultivars, 'Opal', 'Pride of Ringwood', 'Victoria', and 'Nugget'. Plots contained six virus infected

("treatment") plants, arranged with three plants along two rows (Plate 6.2). These were surrounded by 24 virus free plants of the same cultivar to monitor spread in the preceding years and to slow the rate of infection by all viruses in control plots (containing virus free treatment plants) and other virus infected treatment plants by other viruses. A lack of field space and the inability to identify certain virus combinations in some cultivars in the field, made the number of replicates for each combination different and resulted in the absence of some virus combinations. This made the trial design unbalanced and unorthogonal. The total number of plots was 151 and the overall field size was three hectares and was planted on 15th October 1996. The spacing between plants, both along and across rows, was 2.1 m.

Plant death in the first year after planting and insufficient material to plant a small proportion of plots meant that additional propagation was required the following season. These plants were excluded from analyses in subsequent years. Treatment plants infected by other viruses in subsequent years were also excluded from the analyses. All other operations (e.g. fertiliser and herbicide application) were as for a commercial hop garden.

Plate 6.1. Propagation of virus infected hop softwood cuttings for inclusion in yield trial.

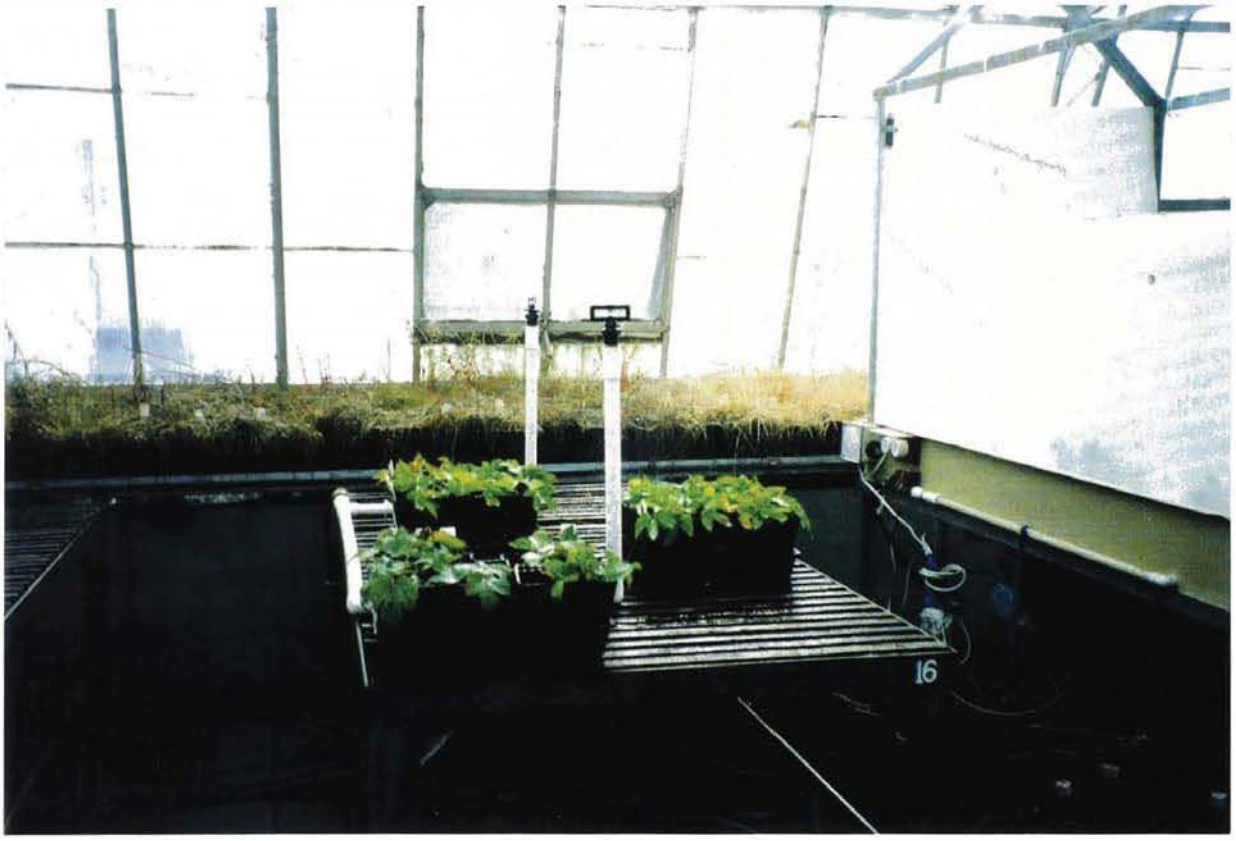


Plate 6.2. Experimental units following planting of hop yield trial at Bushy Park, Tasmania.

Serological assessment

'Treatment plants' (within each plot) were tested for infection by HpLV, HpMV, and PNRSV (A & I), in the spring of 1997 by DAS-ELISA (Appendix 1), to ensure the virus status had not altered from that in the original planting material. Randomly selected expanding leaves were sampled from treatment plants in each plot, and a representative sub-sample taken for serological testing. In the spring of 1998, treatment plants were tested for only those viruses absent the previous season to monitor virus reinfection. Randomly selected expanding leaves were sampled from each treatment plant in the spring of 1998, and a representative sub-sample by DAS-ELISA (Appendix 1).

Virus infection in plants within buffer rows were also monitored in 1998. Randomly selected expanding leaves were sampled from each of six buffer plants in rows one, four, and five, and the three buffer plants in rows two and three. Sub-samples were taken from each bulk sample for testing by DAS-ELISA for HpLV, HpMV, and PNRSV (A & I) (Appendix 1). When virus infection was detected, young leaves were re-sampled from individual plants and tested by DAS-ELISA. The spatial pattern of infected plants was mapped by row and column positions (Microsoft Excel^{R}) and virus incidence compared between cultivars (chapter two).

Agronomic assessments

Height of the plants within each plot was measured in the growing seasons (1st November 1997 and 2nd November 1998). The height of all treatment plants was measured (cm) to the highest bine on any string (Plate 6.3). The height of virus infected and virus plants were compared in both years by analysis of variance (Genstat 5; Version 3.1). Virus associated symptoms were also described.

Harvest

Time of harvest of 'treatment plants' varied with cultivar. 'Nugget' was the earliest maturing cultivar and plots were harvested in early March. A maximum of seven days separated harvest of 'Pride of Ringwood', 'Victoria', and 'Opal' plots in that order. Plants were manually removed from the top of the trellis (Plate 6.4) and all 6 treatment plants from each plot were transported together to an Allaeys picking machine. The cones were bagged and weighed using Joey Avery scales, accurate to 0.1kg. Plots were compared on the basis of green hops per string. The majority of plots were too immature to produce sufficient cones to harvest in 1998. Treatment plants within all plots were harvested in 1999.

Plate 6.3. Quantitative measurement of plant height early in season (measured to tallest trained bine).

Plate 6.4. Hand removal of hop plants for transport to picking machines.

Brewing organic acid analysis

Brewing acids were extracted from 10 gm of green hops. Samples were pulverized in 100 ml toluene by ball bearings in cylinders on a mechanical rotator. After 30 minutes, 0.5 ml of the solution was made up to 20 ml with methanol in a volumetric flask. A portion (2 ml) of this solution was then passed through a 0.45 μm filter into a high performance liquid chromatography (HPLC) vial (L.J. Sherriff, pers. comm).

Brewing acid composition was analysed by HPLC, on a liquid chromatograph equipped with a filter photometer at 314 nm. The column was a 150 x 4.6 mm Allsphere ODS-1, (5 micron). The guard column was a 7.5 x 4.6 mm Allsphere ODS-1 (5 micron). Both columns were manufactured by Alltech Associated, Inc. (Deerfield, Illinois, USA). The mobile phase was a solution of HPLC grade methanol:distilled water, and 88 % w/w orthophosphoric acid in the ratio of 85:15:0.25. The wash solvent was 75 % methanol solution. Both the mobile phase and wash solvent were filtered and degassed prior to use. An aliquot (10 μL) of sample was injected into the column at a rate of 0.55 ml min^{-1}. Concentrations were corrected for dilution in all four components by dividing the component concentration by the weight of the sample and multiplying by ten.

Values for samples were compared to those on a standard curve, constructed using the ICE-1 standard (composed of known quantities of cohumulone, humulone, colupulone, and lupulone). A known amount of the ICE-1 standard was weighed (to four decimal places) and dissolved in 150 ml methanol. Aliquots (3 ml, 4 ml, 5 ml, 6 ml, and 7 ml)

were then made up to 20 ml using methanol in volumetric flasks, and filtered through a 0.45 μm filter into HPLC autosampler vials (L.J. Sherriff, pers. comm.).

Statistical analyses

Agronomic assessment data able to be expressed in binary form (e.g. burr development) were analysed by likelihood chi-square analysis. A likelihood chi-square value was calculated for a two-way contingency table using the log-linear regression model for that data. A significant result, determined by comparison with the chi-square distribution, indicated dependence between the row and column classification factors of the contingency table (e.g. that the burr development response was dependent on the type of virus infection). Significant results were isolated by examination of the adjusted standardised residuals for the contigency table. Residuals with an absolute value greater than 1.96 indicated virus responses were significantly different (P=0.05) from expectations of the null hypothesis that there were no differences between the responses for the different virus groups (Genstat 5; Version 3.1). Continuous data (e.g. harvest and quality results) was analysed by analysis of variance (Genstat5; Version 3.1).

Single plant harvests

Assessments of the yield and quality of individual virus infected single 'Victoria' plants were made in 1997. Assessment of the effect of PNRSV-A and PNRSV-I infection on yield and quality of 'T11', and 'Opal' plants were made in 1998. Plants were randomly selected from surveys of commercial hop gardens at Bushy Park.

In 1997, eight year old 'Victoria' plants (n=6) infected by each of HpLV, HpMV, HpLV + HpMV, HpLV + PNRSV (A & I), HpMV + PNRSV (A & I), and HpLV + HpMV + PNRSV (A & I) and healthy plants were selected.

In 1998, nine year old 'Opal' and 'T11' plants (n=5) infected by each of PNRSV-A, PNRSV-I, and healthy plants were selected.

Each plant was removed manually from the trellis top and transported to an Allaeys picking machine. The cones per plant were bagged and weighed using Joey Avery scales, accurate to 0.1 kg. Yield results from 'Victoria' plants in 1997 were compared by the yield of cones per hectare (kg ha^{-1}). Yield results from 'Opal' and 'T11' plants in 1998 were compared by the weight of green hops per string. Analysis of the relative levels of brewing organic acids was performed by HPLC, as described previously.

6.3. RESULTS

Propagation of virus infected plants

Minor losses (<10%) occurred in virus tested plants of all cultivars during propagation (Table 6.1). In all cultivars, virus infection (except HpLV) reduced the number of plants surviving propagation. Losses occurred from failing to produce extensive roots and subsequent death in the hardening off process. Substantial losses of plants from failure to establish in the first year, were also associated with virus infection.

Infection by HpMV in 'Nugget' reduced the survival of cuttings by 44 %. Similar, reductions in the number of surviving cuttings were also demonstrated in those infected by PNRSV-I (36 %). The percentage of cuttings that survived, infected by HpLV and HpLV + PNRSV-I was 25 and 24 % respectively. Infection by HpMV (42 %) and HpLV + PNRSV-I (58 %) were also associated with losses in plants in the field. No losses were associated with infection by HpLV. Small proportions of plants infected by PNRSV-I (17 %) and virus free plants (8 %) also failed to establish in the field (Table 6.1).

The overall percentage of cuttings that did not survive of virus infected 'Opal' cuttings in propagation was approximately 40 % and in the field approximately 50 %. Infection by all three viruses had the greatest effect on cutting survival in propagation and plant survival in the field. For example, infection by HpLV + HpMV + PNRSV-A reduced cutting survival throughout propagation by 40 %, and a further 13 % failed to establish in the field. A similar percentage of cuttings that did not survive was observed in cuttings

infected by HpLV + HpMV + PNRSV-I, while a further 50 % failed to establish in the field. Infection by HpLV, HpMV, and PNRSV-I reduced survival of cuttings by 15 %, 27 %, and 17 %, respectively. A minority of plants infected by HpLV (7 %) and HpMV (7 %), respectively also failed to survive in the first year in the field. No deaths in the field were observed in plants infected by PNRSV-I. Infection by HpMV + PNRSV-I had a greater negative impact on cutting survival as 37 % of cuttings failed to produce roots (Table 6.1).

The incidence of dead 'Pride of Ringwood' cuttings was highest in those infected by HpMV (72 % over two propagation attempts). Nevertheless, only 22 % of HpMV plants failed to survive the first year in the field. The percentage of cuttings failing to survive propagation was also high when infected by HpMV + PNRSV-I (63 %), however, all plants established in the first year in the field. Infection by HpLV + HpMV resulted in losses in propagation (21 %) and in the field (25 %). Infection by HpLV was associated with only minor losses of cuttings in propagation (7 %) and in the field (6 %). The incidence of cuttings infected by PNRSV-A and PNRSV-I was 29 % and 35 % respectively. Minor losses were found in plants infected by PNRSV-A (3 %). All plants infected by PNRSV-I survived the first year. The incidence of cutting survival was low in cuttings infected by HpLV + HpMV + PNRSV-A (25 %), while a further 15 % of plants failed to survive the first year (Table 6.1).

The incidence of survival of 'Victoria' cuttings infected by HpLV, PNRSV-A, and PNRSV-I was 24 %, 16 %, and 19 % respectively. Minor losses were incurred in the field in plants infected by HpLV (10 %) and PNRSV-I (5 %). All plants infected by PNRSV-A established in the first year. Infection by HpMV + PNRSV-A substantially reduced incidence of cutting survival (56 %) and incidence of plant survival in the field (50 %). The incidence of surviving cuttings infected by HpLV + HpMV + PNRSV-A was low in both propagation (10 %) and in the field (8 %). Infection by HpLV + HpMV + PNRSV-I reduced cutting survival by 20 %, and plant survival in the field by 4 % (Table 6.1).

'# l x c i d e f o d e a c h e t a i t m i r i u n s f e a t h e d d v t i e r s u c s e d t i o n f t g h s e o p c u l t i ' v N a u r g s g ' e O t p ' a , ' l P ' r , i o d R e i n g o v d o à n , d V i c a t ' d , u i r i p n r g o p a g a n t d o n e s t a b l i s f t h m e t e r r t i a l e x e a f e r f c i t o s f v n i g r i u n s f e c o t n i y o i n e a l d d e v e l f s b r e w i o n r g g a n c i c d s .

"%#' $ #'	& "' ' &$$ #'	&$$ # '	&$$ #''
& $ '			
HpLV	100	25	0
HpMV	50	44	42
PNRSV-I	50	36	17
HpLV + PNRSV-I	50	24	58
Control	100	4	8
! '			
HpLV	82	15	7
HpMV	74	27	7
PNRSV-I	100	17	0
HpMV + PNRSV-I	65	37	0
HpLV + HpMV + PNRSV-A	95	40	13
HpLV + HpMV + PNRSV-I	100	39	50
Control	100	6	5

Virus combinations	Number of Cuttings	Incidence of dead plants (%)	Incidence of dead plants (%)
'Pride of Ringwood'			
HpLV	91	7	6
HpMV	150 [1]	72	22
HpMV + HpMV	100	21	25
PNRSV-A	85	29	3
PNRSV-I	85	35	0
HpLV + PNRSV-A	100	43	78
HpLV + PNRSV-I	95	24	0
HpMV + PNRSV-I	40	63	0
HpLV + HpMV + PNRSV-A	100	25	15
Control	100	9	7
'Victoria'			
HpLV	95	24	10
HpLV + HpMV	90	43	7
PNRSV-A	80	16	0
PNRSV-I	95	19	5
HpLV + PNRSV-A	95	19	0
HpLV + PNRSV-I	100	20	20
HpMV + PNRSV-A	45	56	50
HpLV + HpMV + PNRSV-A	80	10	8
HpLV + HpMV + PNRSV-I	100	20	4
Control	100	6	0

[1] Number of plants propagated over two batches of cuttings.

Yield trial

Agronomic assessments

Height – 1st November 1997

On 1st November 1997, significant reductions in plant height were associated with virus infection in all cultivars (Table 6.2).

In the hop cultivar, 'Nugget', single and mixed virus infections significantly reduced plant height compared to that of control plants. Infection by HpMV had the greatest effect on reducing plant height (14 % of virus tested plants), however these plants were not significantly different from those infected by HpLV + PNRSV-I. The height of HpLV infected plants was significantly less than control plants (61 % of virus tested plants). The height of plants infected by PNRSV-I was significantly less than control plants (35% of virus tested plants) and plants infected by HpLV, but significantly greater than plants infected by HpMV (Table 6.2).

'Opal' plants infected by HpLV + HpMV + PNRSV-A were significantly taller (13 % taller than control plants) than virus tested plants and plants infected by HpLV + HpMV + PNRSV-I, HpMV + PNRSV-I, PNRSV-I, HpMV, and HpLV. The height of plants infected by HpLV, HpMV, and PNRSV-I were not significantly different from virus tested plants. The height of plants infected by HpMV + PNRSV-I (67 % shorter than virus tested plants) were not significantly different from plants infected by HpLV + HpMV + PNRSV-I (75 % shorter than virus tested plants) (Table 6.2).

Plant height was significantly reduced in 'Pride of Ringwood' plants infected by all viruses and virus combinations compared to control plants (Table 6.2). Infection by HpLV + PNRSV-I had the greatest effect on plant height (10% of control plants), but was not significantly different from plants infected by HpMV + PNRSV-I, HpMV, HpLV + HpMV, PNRSV-A, and PNRSV-I (Table 6.2).

In 'Victoria', all viruses and virus combinations significantly reduced plant height compared to virus free plants (Table 6.2). Height was not significantly different between plants infected by PNRSV-A or PNRSV-I, and plants infected by HpLV, and HpLV + HpMV. Infection by HpMV + PNRSV-I (24 % of control plants) had the greatest effect on plant height, however these plants were not significantly different from those infected by either HpLV + PNRSV-A, HpLV + PNRSV-I, HpLV + HpMV + PNRSV-A, and HpLV + HpMV + PNRSV-I (Table 6.2).

Table h&AA=;[hTAchFW`XRA=[GTPThPbJ7P[h=FBE[>hM h FRh`BC=[hU7J /hVF<=ThAh

1FPBfTT< 8S<h4F;[TWFT7PhhY-\Thc=M9=Wh!" h

Virus	Cultivar			
	'Nugget'	'Opal'	'Pride of Ringwood'	'Victoria'
'U)4h	9hh	h9h	hh	h:>h
'U*4h	h<h	h9h	h9><h	-#h
'U)4 'U+4h	,#h	,#h	h9><h	h>h
0,124 #h	,#h	,#h	!9;<h	h?h
0,124 (h	!h;h	h9h	h9><h	!h?h
'U)4h , 0,134h #h	,#h	,#h	h9>h	!h:><h
'U)h4h, 0,134h (h	h;<h	,#h	hd	"h;<h
'U*4h , 0,134h #h	,#h	,#h	,#h	:@h
'U*4h , 0,134 (h	,#h	!hh	h:><h	+hh
'U)5h h'U+4h h/,124 #h	,#h	ha	!h9>h	h<h
'U)6h h'U+4h h/,126h (h	,#h	hh	,#h	!h:@<h
$TP[WTJh	8h	9hh	h7h	7h
	!h	h	h	h
) 2 % h	h	h	h	h
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-#h - ThNATWM7[_TPdh7HL7:J<=ah=h[J8hIhTAO7[=Q8khA=;]=<hghjE=Z=ehHW`Z=TZWdhVbZh

;TM:FS7[FTPXh

Symptom appearance and burr development – 30th December 1997

Virus infection had a significant effect on symptom appearance in ''Nugget' (Table 6.3), 'Opal' (Table 6.4), 'Pride of Ringwood' (Table 6.5), and 'Victoria' (Table 6.6). Virus infection failed to significantly effect burr development in any cultivar (Tables 6.3-6.6).

'Nugget'

Infections by HpMV, and PNRSV-I were associated with virus symptoms. Control plants exhibited significantly less virus symptoms than expected under the null hypothesis (Table 6.3). PNRSV-I infection induced necrotic spots and vein-clearing (Plate 6.5). Tissues of plants infected by either virus appeared brittle.

Plate 6.5. Typical symptoms of PNRSV-I infection in the hop cultivar, 'Nugget' (inter-veinal chlorosis and some necrotic spots).

Plate 6.6. Typical symptoms of HpMV infection in the hop cultivar, 'Pride of Ringwood' (downward rolling of leaves)

Table 6.3. Chi-square analysis of symptom incidence and burr development in virus infected and virus free 'Nugget' plants on 30th December 1997.

Virus	Symptom Incidence		Burr Incidence	
	Standardised Adjusted Residuals[1]		Counts	
	Absent	Present	Absent	Present
HpLV	-1.2	1.2	0	12
HpMV	-4.1	4.1	0	9
PNRSV-I	-3.2	3.2	0	6
HpLV + PNRSV-I	-1.5	1.5	1	5
Control	6.4	-6.4	3	24
X^2	56.8		3.3	
df	4		4	
P<	0.001		0.349 (ns)	

[1] Residuals greater than an absolute value of 1.96 were inconsistent with the assumption of independence in the null hypothesis.

'Opal'

Infections by HpLV + HpMV + PNRSV-A or HpLV + HpMV + PNRSV-I were associated with significant development of virus associated symptoms. Infection by HpLV, HpMV, PNRSV-A, or PNRSV-I failed to develop symptoms of virus infection (Table 6.4). Symptoms in plants infected by all three viruses were necrotic spots and larger angular lesions, downward rolling of the leaves, and bines which failed to climb on strings. Symptoms among plants infected by all three viruses and either PNRSV serotype were indistinguishable.

Table 6.4. Chi-square analysis of symptom incidence and burr development in virus infected 'Opal' plants on 30th December 1997.

Virus	Symptom Incidence		Burr Incidence	
	Standardised Adjusted Residuals[1]		Counts	
	Absent	Present	Absent	Present
HpLV	-1.4	1.4	4	8
HpMV	-1.1	1.1	4	11
PNRSV-I	1.5	-1.5	0	3
HpMV + PNRSV-I	-1.4	1.4	0	6
HpLV + HpMV + PNRSV-A	-3.3	3.3	0	7
HpLV + HpMV + PNRSV-I	-2.8	2.8	1	7
Control	5.0	-5.0	6	45
X^2	42.5		9.5	
df	6		6	
P<	0.001		0.146 (ns)	

[1] Residuals greater than an absolute value of 1.96 were inconsistent with the assumptions of independence in the null hypothesis.

'Pride of Ringwood'

Infection with HpMV was also associated with the development of significantly more severe virus associated symptoms than in the control (Table 6.5). Symptoms of HpMV infection included necrotic spots, downward rolling of the leaves, and vein clearing (Plate 6.7).

'Victoria'

Infection by HpLV alone, and HpLV + PNRSV-I were associated with significantly more severe virus associated symptoms than in the control (Table 6.6). Symptoms associated with infection by HpLV were necrotic spots. Downward rolling of the leaves was associated with infection by HpLV + PNRSV-I.

Table 6.5. Chi-square analysis of symptom incidence and burr development in virus infected 'Pride of Ringwood' plants on 30th December 1997.

Virus	Symptom Incidence		Burr Incidence	
	Standardised Adjusted Residuals[1]		Counts	
	Absent	Present	Absent	Present
HpLV	-0.9	0.9	0	18
HpMV	-2.7	2.7	5	13
PNRSV-A	-1.5	1.5	1	11
PNRSV-I	-1.5	1.5	0	6
HpLV + HpMV	-1.5	1.5	1	5
HpLV + PNRSV-A	-1.4	1.4	2	3
HpLV + PNRSV-I	-1.0	1.0	1	8
HpMV + PNRSV-A	1.4	-1.4	2	4
HpMV + PNRSV-I	-1.0	1.0	1	2
HpLV + HpMV + PNRSV-A	-0.2	0.2	3	18
HpLV + HpMV + PNRSV-I	-1.5	1.5	0	6
Control	8.5	-8.5	2	19
X^2	89.3		16.4	
df	11		11	
P<	0.001		0.127 (ns)	

[1] Residuals greater than an absolute value of 1.96 were inconsistent with the assumption of independence in the null hypothesis.

Table 6.6. Chi-square analysis of symptom incidence and burr development in virus infected 'Victoria' plants on 30th December 1997.

Virus	Symptom Incidence		Burr Incidence	
	Standardised Adjusted Residuals[1]		Counts	
	Absent	Present	Absent	Present
HpLV	2.5	-2.5	0	23
PNRSV-A	-1.7	1.7	0	7
PNRSV-I	1.0	-1.0	1	19
HpLV + PNRSV-A	-0.7	0.7	1	6
HpLV + PNRSV-I	-4.6	4.6	1	16
HpMV + PNRSV-I	1.5	-1.5	1	8
HpLV + HpMV + PNRSV-A	0.5	-0.5	0	15
HpLV + HpMV + PNRSV-I	0.2	-0.2	2	10
Control	2.4	-2.4	0	21
X^2	45.2		9.5	
df	8		8	
P<	0.001		0.389	

[1] residuals greater than an absolute value of 1.96 were inconsistent with the assumption of independence in the null hypothesis.

Height – 2nd November 1998

Table 6.7. Effect of virus infection on plant height in the hop cultivars, 'Nugget', 'Opal', 'Pride of Ringwood', and 'Victoria' measured on 2nd November 1998.

Virus	Cultivar			
	'Nugget'	**'Opal'**	**'Pride of Ringwood'**	**'Victoria'**
HpLV	155 abc	208	251 a	349 a
HpMV	165 abc	NA	226 abc	NA
PNRSV-A	NA	NA	216 abc	263 bc
PNRSV-I	135 bc	234	167 acd	293 abc
HpLV + HpMV	NA	NA	184 abc	272 abc
HpLV + PNRSV-A	NA	NA	256 a	271 abc
HpLV + PNRSV-I	161 abc	NA	200 abc	245 c
HpMV + PNRSV-A	NA	NA	NA	312 ab
HpMV + PNRSV-I	NA	211	113 d	NA
HpLV + HpMV + PNRSV-A	NA	214	232 ab	253 bc
HpLV + HpMV + PNRSV-I	NA	206	NA	295 abc
Virus tested	117 a	251	227 abc	312 a
df	239	509	654	684
L.S.D.	51	50	63	63
P <	0.001	0.05 (ns)	0.001	0.001

Significant reductions in plant height were associated with virus infection in all cultivars, except 'Opal' on 2nd November 1998 (Table 6.7). Infection by PNRSV-I significantly increased plant height of 'Nugget' compared to virus free plants. Height in plants infected by HpLV + PNRSV-I was not significantly different from plants infected by only HpLV or PNRSV-I. The most deleterious infection in 'Pride of Ringwood' plants was HpMV + PNRSV-I, which significantly reduced plant height by 48 % compared to control plants. Infection by PNRSV-A, HpLV + PNRSV-I, and HpLV + HpMV + PNRSV-A significantly reduced height compared to 'Victoria' control plants (Table 6.7)

Table 6.8. Incidence of virus symptoms in virus infected 'Nugget', 'Opal', 'Pride of Ringwood', and 'Victoria' plants on 2nd November 1998.

Virus	'Nugget'		'Opal'	
			Standardised Adjusted Residuals[1]	
	Absent	Present	Absent	Present
HpLV	2.2	-2.2	1.6	-1.6
HpMV	-0.6	0.6	2.1	-2.1
PNRSV-A	NA	NA	NA	NA
PNRSV-I	-0.9	0.9	-0.7	0.7
HpLV + HpMV	NA	NA	NA	NA
HpLV + PNRSV-A	NA	NA	NA	NA
HpLV + PNRSV-I	-1.3	1.3	NA	NA
HpMV + PNRSV-A	NA	NA	NA	NA
HpMV + PNRSV-I	NA	NA	-4.5	4.5
HpLV + HpMV + PNRSV-A	NA	NA	2.1	-2.1
HpLV + HpMV + PNRSV-I	NA	NA	-1.1	1.1
X^2	11.1		34.8	
df	3		5	
P<	0.026		0.001	

Virus	'Pride of Ringwood'		'Victoria'	
	Absent	**Present**	**Absent**	**Present**
HpLV	2.4	-2.4	0.3	-0.3
HpMV	-2.3	2.3	NA	NA
PNRSV-A	-1.6	1.6	1.1	-1.1
PNRSV-I	1.0	-1.0	0.6	-0.6
HpLV + HpMV	4.3	-4.3	0	0
HpLV + PNRSV-A	-0.1	0.1	0.7	-0.7
HpLV + PNRSV-I	-0.9	0.9	2.8	-2.8
HpMV + PNRSV-A	NA	NA	0.7	-0.7
HpMV + PNRSV-I	0.6	-0.6	NA	NA
HpLV + HpMV + PNRSV-A	-1.7	1.7	1.0	-1.0
HpLV + HpMV + PNRSV-I	NA	NA	-5.9	5.9
df	8		8	
χ^2	36.8		35.9	
P **<**	0.001		0.001	

[1] Residual greater than an absolute value of 1.96 were inconsistent with the assumption of independence in the null hypothesis.

Significant differences were identified in symptom appearance between the different virus combinations for all cultivars (Table 6.8). No virus associated symptoms were observed in virus free plants of any cultivar. Infection by HpLV was associated with the development of virus symptoms in 'Nugget'. Symptoms of HpLV in 'Nugget' were downward rolling of the leaves and marginal chlorosis. Symptoms of PNRSV-A in 'Nugget' were vein-associated necrotic spots and vein clearing. Infection by HpMV, HpMV + PNRSV-I, and HpLV + HpMV + PNRSV-A were associated with the development of virus associated symptoms in 'Opal'. The most common symptom associated with all these infections was downward rolling of the leaves. 'Pride of Ringwood' plants infected by HpLV, HpMV, and HpLV + HpMV showed significantly more symptoms than expected. Symptoms of HpLV and HpLV + HpMV infection were a subtle interveinal chlorosis. Symptoms of HpMV infections were strongly pronounced downward rolling of the leaves, failure of the bines to climb on the string, necrotic spots, and interveinal chlorosis. In 'Victoria' infection by HpLV + PNRSV-I, and HpLV + HpMV + PNRSV-I were associated with the significant development of virus associated symptoms. Symptoms of both types of infection were mild interveinal chlorosis (Table 6.8).

Significant changes in the beta acid congeners were also associated with virus infection in 'Opal'. Plants infected by HpLV + HpMV + PNRSV-A (19 %) and HpLV + HpMV + PNRSV-I (13 %) had significantly higher colupulone levels than those in control plants. Lupulone levels were marginally reduced in plants infected by HpLV (11 %), PNRSV-I (9 %), and HpMV (4 %). No significant difference in lupulone levels was demonstrated in plants infected by HpLV + HpMV + PNRSV-I, HpLV + HpMV + PNRSV-A, HpMV + PNRSV-I, HpMV, and PNRSV-I, compared to levels in control plants. Lupulone levels were significantly lower in plants infected by HpLV (18 %) than in plants also infected by HpMV and PNRSV-I. Subsequently, plants infected by HpLV + HpMV + PNRSV-A (15 %) and HpLV + HpMV + PNRSV-I (14 %) had significantly higher beta acid content than control plants. Beta acid levels were not significantly different from those of control plants in plants infected by HpMV + PNRSV-I, HpMV, PNRSV-I, and HpLV. Consequently, the alpha to beta acid ratio were significantly lower in plants infected by HpLV + HpMV + PNRSV-A (35 %) and HpLV + HpMV + PNRSV-I (41 %) (Table 6.9).

Table 6.9. Effect of virus infection on yield (green weight per string) and levels of brewing organic acids in 'Opal' in 1999.

Virus	Gr.Wt./ String[1]	CoH[2]	Hum.[3]	% alpha[4]	CoL[5]	Lup.[6]	% beta[7]	a/b[8]
HpLV	0.7b	84.7bc	215.9ab	12.0ab	71.8d	64.6c	5.5b	2.2a
PNRSV- I	1.2a	87.5a	228.7a	12.7a	73.9cd	67.0bc	5.6b	2.3a
HpMV	1.0a	91.0a	223.4a	12.6a	78.1bcd	67.5bc	5.8b	2.2a
HpMV + PNRSV-I	1.2a	87.4ab	230.4a	12.7a	79.1bc	70.5abc	5.9b	2.1a
HpLV + HpMV + PNRSV – A	0.5b	77.7cd	164.4b	9.7bc	97.1a	77.3a	6.9a	1.4b
HpLV + HpMV + PNRSV-I	0.6b	66.3d	145.5b	8.5c	91.1a	79.0a	6.8a	1.3b
Control	1.2a	92.1a	223.9a	12.6a	80.9b	68.2abc	5.9b	2.1a
d f	23	23	23	23	23	23	23	23
L.S.D.	0.4	5.3	27.4	1.2	6.6	11.3	0.6	0.2
P <	0.001	0.001	0.001	0.001	0.001	0.043	0.001	0.001

[1]Gr.Wt./String – Green weight per string (kg); [2]CoH – cohumulone; [3]Hum. – humulone; [4] % alpha – alpha acid congeners (per plant basis); [5]CoL – colupulone; [6]Lup. – lupulone; [7] % beta – beta acid congeners (per plant basis); [8]a/b – alpha to beta acid ratio – per plant basis.

'Nugget'

Infection by HpLV, PNRSV-I, HpMV, and HpLV + PNRSV-I had no significant effect on yield (green weight per string), levels of cohumulone, humulone, colupulone, lupulone, and subsequent proportions of alpha and beta acid in 'Nugget' (Table 6.10).

Table 6.10. Effect of virus infection on yield (green weight per string) and levels of brewing organic acids in 'Nugget' in 1999.

Virus	Gr.Wt./ String[1]	CoH[2]	Hum.[3]	% alpha[4]	CoL[5]	Lup.[6]	% beta[7]	a/b[8]
HpLV	1.8	81.0	215.9	11.9	62.3	50.7	4.5	2.6
PNRSV- I	1.3	82.8	221.6	12.2	65.2	55.5	4.8	2.5
HpMV	1.8	82.9	234.0	12.7	65.4	56.5	4.9	2.6
HpLV + PNRSV-I	1.9	82.8	227.9	12.4	68.5	59.3	5.1	2.4
Control	2.2	84.0	226.2	12.4	67.2	56.8	4.9	2.5
df	17	17	17	17	17	17	17	17
L.S.D.	1.0	5.8	23.1	1.1	4.5	5.7	0.4	0.2
P <	0.240 (ns)	0.776 (ns)	0.503 (ns)	0.613 (ns)	0.074 (ns)	0.145 (ns)	0.082 (ns)	0.367 (ns)

[1]Gr.Wt./String – Green weight per string (kg); [2]CoH – cohumulone; [3]Hum. – humulone; [4] % alpha – alpha acid congeners (per plant basis); [5]CoL – colupulone; [6]Lup. – lupulone; [7] % beta – beta acid congeners (per plant basis);[8]a/b – alpha to beta acid ratio – per plant basis.

'Victoria'

Infection by HpLV, PNRSV-A, PNRSV-I, HpLV + HpMV, HpLV + PNRSV-A, HpLV + PNRSV-I, HpMV + PNRSV-I, HpLV + HpMV + PNRSV-A, and HpLV + HpMV + PNRSV-I had no significant effect on the yield (green weight per string or other parameters measured) of 'Victoria' plants in 1999. Furthermore, infection by one or more viruses was not associated with significant reductions in alpha and beta acid congener levels or the ratio between them (Table 6.11).

'Pride of Ringwood'

Significant reductions in yield and levels of brewing organic acids were associated with virus infection in 'Pride of Ringwood' (Table 6.12). Infection by HpMV alone and PNRSV-I alone significantly reduced yield (green weight per string) by 55 % and 51 % respectively. However, the yields of plants infected by HpMV and PNRSV-I, were not significantly different from those of plants infected by other viruses or virus combinations. Yield of plants infected by HpLV, HpLV + HpMV + PNRSV-A, HpLV + PNRSV-I, HpLV + PNRSV-A, HpLV + HpMV, and PNRSV-A was not significantly different from that of control plants.

Plants infected by HpMV alone had significantly reduced cohumulone (14 %) and humulone (12 %) levels compared to those of control plants. Infection by PNRSV-A (9

%), HpMV + PNRSV-I (9 %), and HpLV + PNRSV-I (11 %) also significantly reduced cohumulone levels compared to those of control plants. Significant reductions in humulone levels were associated with infection by HpMV + PNRSV-I (14 %), HpLV + HpMV + PNRSV-A (14 %), and PNRSV-A (18 %). Consequently, significant reductions in alpha acid levels were observed in plants infected by HpMV (19 %), PNRSV-A (15 %), HpMV + PNRSV-I (12 %), HpLV + HpMV + PNRSV-A (12 %), and HpLV + PNRSV-I (12 %) compared to those of control plants. The alpha acid levels of plants infected by HpLV, HpLV + HpMV, PNRSV-I, and HpLV + PNRSV-I were not significantly different from those in control plants.

Significant reductions in beta acid congener levels were also associated with virus infection. HpMV infected plants had significantly reduced colupulone (13 %) and lupulone (16 %) levels compared to those of control plants. This resulted in a significant reduction (14 %) in the proportion of beta acid compared to those of control plants. Infection by PNRSV-I, HpLV, HpMV + PNRSV-I, HpLV + HpMV + PNRSV-A, HpLV + HpMV, HpLV + PNRSV-I, PNRSV-A, and HpLV + PNRSV-A failed to significantly change beta acid congener levels compared to those of control plants. No significant change in the ratio of alpha to beta acids was associated with any virus or virus combination (Table 6.12).

Table 6.11. Effect of virus infection on yield (green weight per string) and levels of brewing organic acids in 'Victoria' in 1999.

Virus	Gr.Wt./String[1]	CoH[2]	Hum.[3]	% alpha[4]	CoL[5]	Lup.[6]	% beta[7]	a/b[8]
HpLV	1.4	103.0	162.2	10.6	94.0	52.1	5.8	1.8
PNRSV- A	2.7	106.6	167.7	10.9	96.1	52.5	5.9	1.8
PNRSV-I	1.4	106.0	166.7	10.9	96.9	53.6	6.0	1.8
HpLV + HpMV	1.4	108.8	174.0	11.3	98.2	55.0	6.1	1.9
HpLV + PNRSV-A	1.9	92.6	181.1	10.9	80.1	52.4	5.3	2.1
HpLV + PNRSV-I	1.4	105.4	169.5	11.0	94.3	53.0	5.9	1.9
HpMV + PNRSV-A	2.0	90.3	163.8	10.2	91.8	64.1	6.2	1.6
HpLV + HpMV + PNRSV – A	1.6	107.3	166.7	10.9	93.9	50.2	5.8	1.9
HpLV + HpMV + PNRSV-I	1.6	113.2	178.0	11.7	103.8	57.4	6.5	1.8
Control	1.7	114.7	196.7	12.5	99.8	58.3	6.3	1.9
df	45	45	45	45	45	45	45	45
L.S.D.	0.5	17.0	22.9	1.5	13.4	7.2	0.8	0.19
P <	0.083 (ns)	0.754 (ns)	0.227 (ns)	0.608 (ns)	0.601 (ns)	0.513 (ns)	0.775 (ns)	0.156 (ns)

[1]Gr.Wt./String – Green weight per string (kg); [2]CoH – cohumulone; [3]Hum. – humulone;

[4] % alpha – alpha acid congeners (per plant basis); [5]CoL – colupulone; [6]Lup. – lupulone; [7] % beta – beta acid congeners (per plant basis);[8]a/b – alpha to beta acid ratio – per plant basis.

Table 6.12. Effect of virus infection on yield (green weight per string) and levels of brewing organic acids in 'Pride of Ringwood' in 1999.

Virus	Gr.Wt./String[1]	CoH[2]	Hum.[3]	% alpha[4]	CoL[5]	Lup.[6]	% beta[7]	a/b[8]
HpLV	1.4ab	77.6a	181.0ab	10.3a	94.4ab	87.2ab	7.3ab	1.4
HpMV	0.9b	64.6d	144.3d	8.4d	77.0d	71.0c	5.9d	1.4
PNRSV-A	1.2ab	68.3cd	151.4cd	8.8cd	86.4abcd	76.9bc	6.5cd	1.3
PNRSV-I	0.9b	76.8ab	166.0abcd	9.7abc	99.1a	88.2a	7.5a	1.3
HpLV + HpMV	1.2ab	72.1abcd	173.1abc	9.8abc	85.9abcd	80.6abc	6.7bc	1.5
HpLV + PNRSV-A	1.3ab	72.3abcd	167.1abcd	9.6abc	86.2abcd	79.3abc	6.6bcd	1.5
HpLV + PNRSV-I	1.4ab	66.9cd	160.4abcd	9.1cd	81.5cd	76.9bc	6.3cd	1.4
HpMV + PNRSV-I	1.04ab	68.2cd	158.7bcd	9.1cd	85.9abcd	83.0ab	6.8abc	1.3
HpLV + HpMV + PNRSV – A	1.13ab	69.6abcd	157.6cd	9.1cd	86.9abc	79.9abc	6.8abc	1.4
Control	1.8a	74.7abc	183.6a	10.3ab	88.0abc	84.1ab	6.9abc	1.5
df	39	39	39	39	39	39	39	39
L.S.D.	0.7	8.3	23.0	1.2	9.8	11.1	0.7	0.2
***P* <**	0.019	0.001	0.001	0.001	0.001	0.002	0.001	0.224 (ns)

[1]Gr.Wt./String – Green weight per string (kg); [2]CoH – cohumulone; [3]Hum. – humulone;

[4] % alpha – alpha acid congeners (per plant basis); [5]CoL – colupulone; [6]Lup. – lupulone; [7] % beta – beta acid congeners (per plant basis);[8]a/b – alpha to beta acid ratio – per plant basis.

Single plant harvests

No significant effect on yield of cones per hectare (Table 6.13), the levels of cohumulone, humulone, colupulone, lupulone, alpha and beta acid content, and the alpha to beta ratio were identified from infection by any virus or virus combination in eight year old 'Victoria' plants (Table 6.14).

Table 6.13. Effect of virus infection on cone yield per hectare (kg ha^{-1}) in eight year old 'Victoria' plants in 1997.

Virus	Yield (kg ha^{-1})
HpLV	2883
HpMV	3022
PNRSV (A & I)	3600
HpLV & HpMV	2490
HpLV & PNRSV (A & I)	2711
HpMV & PNRSV (A & I)	3730
HpLV & HpMV & PNRSV (A & I)	3428
Control	3248
df	47
L.S.D.	998
P <	0.17 (ns)

Table 6.14. Effect of virus infection on brewing organic acid composition in eight year old 'Victoria' hop plants in 1997.

Virus	CoH	Humulone	% alpha	CoL	Lupulone	% beta	a/b
HpLV	168.5	233.0	16.0	131.6	75.7	8.3	1.9
HpMV	172.3	238.4	16.4	129.9	74.5	8.2	2.0
PNRSV (A & I)	165.9	230.3	15.9	128.4	73.9	8.0	1.9
HpLV & HpMV	160.7	218.7	15.2	128.9	72.9	8.1	1.9
HpLV & PNRSV (A & I)	167.1	226.1	15.7	132.5	75.5	8.4	1.9
HpMV & PNRSV (A & I)	169.2	230.7	16.0	130.8	73.8	8.2	1.9
HpLV & HpMV & PNRSV (A & I)	161.3	218.5	15.2	128.1	72.4	8.0	1.9
Control	169.0	235.4	16.2	127.5	72.9	8.0	2.0
df	47	47	47	47	47	47	47
L.S.D.	15.8	20.3	1.4	10.0	5.8	0.6	0.1
P <	0.81 (ns)	0.40 (ns)	0.57 (ns)	0.97 (ns)	0.92 (ns)	0.95 (ns)	0.35 (ns)

Table 6.15. Effect of PNRSV-A and PNRSV-I infection on yield (green weight per string) of nine year old 'Opal' and 'T11' hop plants in 1998.

Cultivar	Virus	Green weight/string
'Opal'	PNRSV-A	1.5
'Opal'	PNRSV-I	1.0
'Opal'	Control	1.2
'Opal'	*df*	14
'Opal'	L.S.D.	0.5
'Opal'	*P* <	0.61 (ns)
'T11'	PNRSV-A	1.5
'T11'	PNRSV-I	1.7
'T11'	Control	1.2
'T11'	*df*	14
'T11'	L.S.D.	0.5
'T11'	*P* <	0.63 (ns)

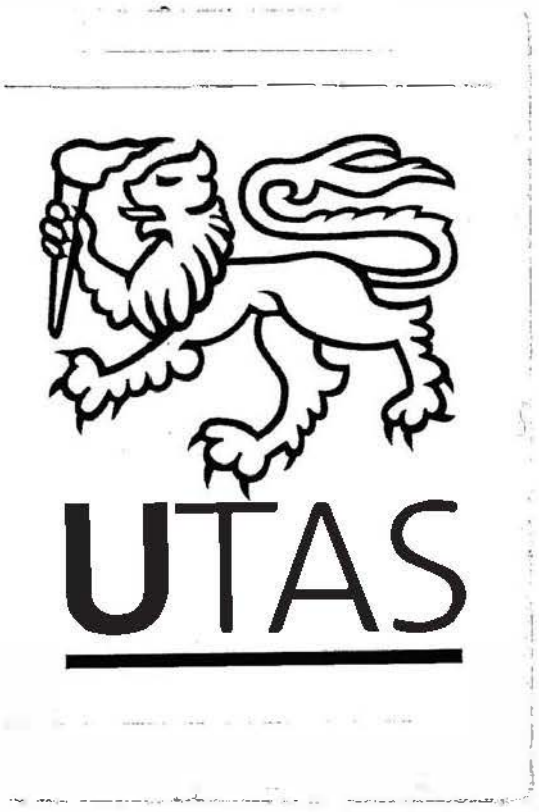
Table 6.16. Effect of PNRSV-A and PNRSV-I infection on brewing organic acid composition of nine year old 'Opal' plants in 1998.

Virus	% coHum	% alpha	% beta	a/b
PNRSV-A	28.7	11.1 b	6.4	1.8 b
PNRSV-I	29.9	12.2 b	5.8	2.1 a
Control	28.0	13.8 a	6.8	2.0 a
df	14	14	14	14
L.S.D.	3.9	1.1	1.1	0.3
P <	0.552 (ns)	0.001	0.249 (ns)	0.035

Table 6.17. Effect of PNRSV-A and PNRSV-I infection on brewing organic acid composition of nine year old 'T11' plants in 1998.

Virus	% coHum	% alpha	% beta	a/b
PNRSV-A	30.4	11.3 b	10.2	1.1
PNRSV-I	30.4	11.2 b	10.3	1.1
Control	31.4	12.8 a	11.5	1.1
df	14	14	14	14
L.S.D.	4.1	1.0	1.4	0.1
P <	0.82 (ns)	0.006	0.14 (ns)	0.76 (ns)

Infection by either PNRSV-A or PNRSV-I did not significantly reduce yield (green weight per string) in 'T11' or 'Opal' plants (Table 6.15). Alpha acid content was significantly reduced in 'Opal' plants infected by PNRSV-A (20 %) and PNRSV-I (12%) (Table 6.16). Similar reductions were also found in 'T11' plants infected by PNRSV-A (12 %) and PNRSV-I (13 %) (Table 6.17). PNRSV-A infection was also associated with a 13 % reduction in the alpha to beta acid ratio compared to that of control 'Opal' plants (Table 6.16). No significant change in the alpha to beta acid ratio was demonstrated in 'T11' plants (Table 6.17).



6.4. DISCUSSION

Virus infection was associated with significant decreases in yield (green weight per string) and levels of brewing organic acids in cultivars 'Opal' and 'Pride of Ringwood'.

In 'Opal', multiple infection by all three viruses was most detrimental to the success of propagation and survival in the first year in the field, yield, levels of brewing organic acids, and the alpha to beta acid ratio. Infection by any one of these viruses in isolation failed to significantly reduce the latter two characteristics. However, HpLV alone and in combination significantly reduced yield. Infection by HpLV + HpMV + PNRSV-A and HpLV + HpMV + PNRSV-I was also associated with virus symptom development early in both seasons. Significant reductions in plant height were not associated with infection by any of the three viruses until the 1998/99 season. This was demonstrated in late February of the same season. Harvest of single nine-year old 'Opal' plants infected by either PNRSV-A or PNRSV-I produced similar results. Unfortunately in the main trial the effect of infection by PNRSV-A was unable to be assessed. It is also likely that multiple infection by all three viruses would significantly reduce yield and brewing organic acid levels in older plants, and perhaps to a greater extent than in three year old plants from compounded effects of infection on reducing vigour and carbohydrate storage over winter. Alternatively, older established plants may be more tolerant.

The effects of only a restricted number of virus combinations could be assessed in 'Nugget', due to the inability to identify suitable infected plants in the field. Infection by HpLV, HpMV, PNRSV-I, and HpLV + PNRSV-I did not significantly effect yield and the levels of brewing organic acids in three year old plants. However, other viruses (e.g. PNRSV-A) and virus combinations may have a significant effect on production. The lack of observations of virus effects in older plants is also unfortunate. Viruses and virus combinations found to have no significant effect on production and quality in younger plants may have an effect on older plants, weakened from chronic virus infection. Despite the absence of significant effects on yield and levels of brewing organic acids, virus infection was associated with significant changes in some agronomic characteristics through the two seasons. Early in the first season, HpMV had the greatest effect on plant height.

Findings of significant reductions in yield and levels of brewing organic acids from infection by HpMV, PNRSV-A, HpLV + PNRSV-I, HpMV + PNRSV-I, and HpLV + HpMV + PNRSV-A confirmed previous qualitative observations suggesting viruses posed a significant constraint to production of 'Pride of Ringwood' (G. Leggett, pers. comm.). HpMV was the most deleterious to production as anticipated. Unfortunately, no assessments could be made on older plants. Infection by HpMV was also associated with significant losses throughout propagation and in the first year in the field, and height reductions early and half way throughout the first half of the season. However, later in the first season no effect was identified from virus infection on qualitative ratings of healthy, shape, height, and cone distribution. Lateral development was significantly

altered by HpMV, and PNRSV-A suggesting that infection changes the plants metabolite distribution. Flower development was hastened by HpMV + PNRSV-A. In the second season, significant effects on height early in the season were associated only with infection by HpMV + PNRSV-I, HpLV + HpMV, and PNRSV-I. Studies in 'Chinook' have also identified HpMV as posing a significant constraint to production (Probasco and Murphey, 1996), and in related cultivars, 'J78' (G. Leggett, pers. comm.), and 'Goldings' (Keyworth, 1943).

The lack of significant effects on yield and levels of brewing organic acids in both three and nine year old 'Victoria' plants from infection by HpLV, HpMV, PNRSV (A & I), and virus combinations examined is encouraging for the hop industry. Despite this, infection by HpMV + PNRSV-I reduced height early in the first season, In the second season, the majority of virus infection (PNRSV-A, PNRSV-I, HpLV + HpMV, HpLV + PNRSV-A, HpLV + PNRSV-I, and HpLV + HpMV + PNRSV-A) significantly reduced plant height, however later in the season these plants recovered and yield was not significantly different from control plants. This is the first quantitative report of a hop cultivar, being both highly susceptible to virus infection (chapter two) and highly tolerant (no effect on yield and levels of brewing organic acids). The absence of effects on yield and brewing organic acid levels is surprising considering the close relationship between 'Pride of Ringwood' and 'Victoria'.

In both trials the presence of hop latent viroid (HLVd) was assumed to be ubiquitous. Although no formal surveys were conducted in these trials, recent surveys conducted in

Tasmanian hop gardens failed to find any transplant material free of HLVd (T. McGee, pers. comm.), and considering, at least the designed yield trial were propagated from a small number of plants infection is likely to be widespread. Considering that in some cultivars infection by HLVd has been associated with 30 % decreases in yield, future trials may should examine its effects on Australian cultivars and other cultivars grown in Australian conditions and if the potential effects of mixed infections between viruses and HLVd are synergistic on yield reductions. However, comparison of high yield and bittering compound levels in virus tested plants of Australian cultivars compared to those of overseas cultivars suggests that if HLVd is ubiquitous it is having a minimal impact on production. Another limitation of the major trial is the assessment of only a small number of virus variants and the possible presence of a wide spectrum of isolates varying in pathogenicity. Further trials should assess whether different virus exist in Australian hop gardens and their various effects on production and quality of hop products. If mild and severe isolates could be identified this might also provide the basis for the assessment and use of cross-protection as a potential control method in cultivars where the incidence in infected plants following planting with virus tested material rapid.

CHAPTER 7

INTERACTIONS OF PLANT VIRUSES AFFECTING COMMERCIAL HOP GARDENS IN AUSTRALIA

7.1. INTRODUCTION

Patterns of association may result from interrelationships between organisms and from environmental factors. They depend upon whether pathogens colonize or avoid the same habitat, have some mutual attraction or repulsion, or have any interaction. Prior knowledge of the ecology of host-pathogen interactions in a complex pathosystem is vital to fully understanding how these interactions may influence the spatiotemporal characteristics of epidemics and for implementation of economical control measures.

Interspecific (between viruses) associations quantify the frequency that two species are found in the same ecological niche (host), and measure the affinity (or lack of it) for coexistence between two species. Extrapolating this concept to viral epidemiology, we refer to the frequency of mixed infections by two of more different viruses compared to the frequency of plants infected by only one virus. An association between two viruses may occur because; 1) both select or avoid the same host; 2) of similar abiotic and biotic environmental requirements; or 3) individuals in one or both of the populations have an affinity (either attraction or repulsion) for the other (Ludwig and Reynolds, 1988)

If there is no association between two species and the interaction is negative they may have resource requirements that are independent. If the process is positive the species may have a common response to a supply of unlimited resources. If the interaction between species is mutualistic and the process is positive the species enhance the probability of each others survival. However, if the interaction is negative, a resource factor is limiting. Competition interactions reflect an interference between species, producing occasional exclusion, or population fluctuations in unison, responding to limited resources (Schulter, 1984).

Multiple species comparisons, using statistical methods, test whether species in a group are associated, and assess the strength of any association between two species (Ludwig and Reynolds, 1988; Savary, *et.al.*, 1988; Schulter, 1984). The technique proposed by Schulter (1984) compare the observed variance in total number of species in samples with the variance expected under the null hypothesis. The null hypothesis states that the density or occurrence of each species in independent of other species. This method has been applied to quantify the ecology of arthropods and verterbrate species (Savary, *et.al.*, 1988) and to assess associations between pathogens in a leaf spot fungal disease complex on *Trifolium repens* L. (white clover) (Nelson and Campbell, 1992).

The aim of this study was to investigate whether the incidence of HpLV, HpMV, and PNRSV in commercial hop gardens in Australia suggests the presence of the concept of intervirus (interspecies) associations among any of these viruses and to discuss the

epidemiological implications.

7.2. MATERIALS AND METHODS

Field surveys

The status of individual plants, infected by HpLV, HpMV, and PNRSV was converted to binary (presence/absence) data in surveys conducted at three different sites each involving a range of cultivars. To ensure that every plant had an equal probability of becoming infected by each virus, the apple and intermediate serotypes of PNRSV were not differentiated. Field blocks one to three were those described in chapter two.

Block one was located at Bushy Park and was planted in 1989 with virus tested material of 'Victoria' (n=1275). In 1990, low disease incidence and lack of information regarding the incidence of HpLV made assessment of intervirus associations impossible. Incidence data from 1996 and 1997 was used to test for the presence of intervirus associations.

Block two was located at Myrtleford, Victoria, Australia, and was planted in 1994 with virus tested material 'Victoria' (n=500). Incidence data from 1998 was assessed for the presence of intervirus associations.

Block three was a replicated cultivar x row spacing trial, situated at Bushy Park, Tasmania, Australia. The trial consisted of three Australian bred triploid commercial cultivars, 'T25' (n=774), 'T11' (n=774), 'Victoria' (n=760), and a diploid sterile female,

'Opal' (n=764). All plots were planted in 1989 with virus tested material, except for 'T25', where an unknown proportion of the planting material was infected with PNRSV. Plots of each cultivar were duplicated in four different spacing treatments of four (three rows per bay), three (four rows per bay), two and a half (five rows per bay), and two (six rows per bay) metres between plants across rows, and a further treatment where row spacing alternated between two and three (3 x 2 rows per bay) metres (Figure 1.1). Spacing between plants within rows was two metres in all plots. Data was not analysed for individual plants if disease incidence of one of more of the viruses was 100% or 0% (indeterminate species).

Data analysis

Intervirus associations between the three viruses were assessed from binary (presence/absence) data from individual plants (sampling units, SU) in each survey. Analyses were performed by microcomputer software, SPASSOC, using the GWBASIC language. This programme calculated overall associations between the three species (multiple species case). If the overall association was significant, comparisons can be made between each pair of viruses (two species case). The relative strength of each pair-wise association is indicated.

The null hypothesis was that there was no association among the three viruses. This was true if the viruses were independent or the positive and negative associations among viruses were equal. Rejection of the null hypothesis infers a net positive or negative

association exists among the viruses. The total sample variance (2) was calculated by Equation 7.1.

$$\sigma^2 = \sum_{i=1}^{s} p_i(1-p_i) \qquad \text{Equation 7.1}$$

s = number of species (1...s)

$p_i = n_i/n$ (n_I = number of SU in which i species occurs; i = 1....s)

N = number of SU (i.e. plants)

Variance in total species number was calculated by Equation 7.2

$$s^2 = N^{-1} \sum_{j=1}^{N} (T_j - t)^2 \qquad \text{Equation 7.2}$$

T_j = number of species found in each SU (j = 1....N)

T = mean number of species per sample

The variance ratio (VR) (Equation 7.3) combined equations 7.1 and 7.2, and depicted an overall index of association.

$$VR = S^2 / \sigma^2 \qquad \text{Equation 7.3}$$

Under the null hypothesis, the VR equaled one. Deviations greater than one suggest a net positive association between viruses. Deviations less than one suggested a net negative association between viruses. Examination of the W statistic (Equation 7.4) determines whether a deviation from one was significant.

$$W = (N)(VR) \qquad \text{Equation 7.4}$$

This statistic approximates a chi-squared variate with $N-1$ degrees of freedom (Equation 7.5) (Mudva and Keyno 1988).

$$A\chi^2_{0.95,N} < W < \chi^2_{0.95,N} \qquad \text{Equation 7.5}$$

Pair-wise associations

If the deviation is assigned ($P<0.05$), then pairwise comparisons for each variable, X and Y, state effects of the number of SU infected by both viruses (a), the number of SU infected by X only (b), the number of SU infected by Y only (c), the number of SU free of both (d), and the total number of SU were calculated. The null hypothesis was that the two viruses behaved independently of the chi-squared statistic X_t^2 in equation 7.6.

$$X_t^2 = N(ad - bc)^2 / mnrs \qquad \text{Equation 7.6}$$

$m = a + b \qquad n = c + d \qquad r = a + c \qquad s = b + d$

Expected values for each of the 2 by 2 contingency table were calculated by equation 7.7.

$$E(a) = (a + b)(a + c) / N = rm / n \qquad \text{Equation 7.7}$$

This equation was simplified to Equation 7.8, and hence did not require comparison of expected values assessed and derived using inherent errors. Comparisons of observed and expected values.

$$X_t^2 = N(ad - bc)^2 / mnrs \qquad \text{Equation 7.8}$$

The theoretical chi-square value for one degree of freedom is 3.84 (Sokal & Rohlf, 1981). If the calculated value was greater than 3.84 ($X_t^2 > 3.84$), the null hypothesis was rejected in favour of the alternative, a significant ($P<0.05$) association between the two viruses. Positive associations between the two viruses were indicated if the observed number of co-infections by the two viruses were greater than expected by random chance. If the observed number of co-infections was less than expected by random chance this suggested the association was negative (Equation 7.9).

$$a - E(a) = (ad - bc)/N \qquad \text{Equation 7.9}$$

If any cell of the 2 x 2 contingency table had an expected frequency less than one, or if more than two of the cells had expected frequencies less than five the chi-square statistic was concluded as biased. In these cases the Yate's correction for continuity was applied to ensure a closer fit to the chi-square distribution (Equation 7.10).

$$X_t^2 = N\left[\left|(ad) - (bc) - (N/2)\right]^2\right| / mnrs \quad \text{Equation 7.10}$$

Strength of pair-wise associations

Three indices can be used to quantify the strength of significant pair-wise associations. All indices were equal to zero when there was no association between the viruses and one at "maximum association", implying that a significant positive pair-wise comparison was strongest when the indices approach one. Alternatively, when the pair-wise association was strongly negative the indices approach zero. The Ochiai Index (*OI*) was the geometric mean of a/m and a/r (Equation 7.11). The Dice Index (*DI*) was the harmonic

mean of the same parameters (Equation (7.12). The Jaccard Index (*JI*) expressed the proportion of SU infected by both viruses to the total number of SU infected by only one virus (Equation 7.13).

$$OI = a / \sqrt{a+b}\sqrt{a+c} \qquad \text{Equation 7.11}$$

$$DI = 2a / 2a + b + c \qquad \text{Equation 7.12}$$

$$JI = a / a + b + c \qquad \text{Equation 7.13}$$

The Jaccard and Dice Indices are biased when the number of SU is less than 10 and 20 respectively. Therefore, the Ochiai Index was used to report the strength of pair-wise association in this study.

RESULTS

Significant overall associations between HpLV, HpMV, and PNRSV were identified in block one at Bushy Park, Tasmania in 1996 and 1997. In both years, the overall association between the three viruses was positive. Virus incidence levels ranged from 60 % (HpMV) and 66 % (PNRSV). Virus incidence in 1997 was higher, ranging between 77 % (HpLV) and 92 % (PNRSV) (Table 7.1). In 1996 and 1997, pair-wise comparisons between the three viruses identified a significant positive association between HpLV and HpMV, and HpLV and PNRSV. In all cases, the Ochiai index was greater than 0.69 (Table 7.2).

A significant positive association between HpLV, HpMV, and PNRSV was also identified in 'Victoria', in plot two at Myrtleford in 1998. Virus incidence in this block

was lower than block one and ranged between 12 % (HpMV to 31 % (PNRSV) (Table 7.1). Pair-wise comparisons between the three viruses identified a significant positive association between HpLV and HpMV (Table 7.2).

Block three was segregated according to cultivar. When considering all 'Victoria' plots, overall associations between the three viruses were significant and positive in 1996 and 1997 (Table 7.3). Pair-wise comparisons identified significant positive relationships between HpLV and HpMV, and HpMV and PNRSV in both years. The strength of association between HpLV and HpMV was high (>0.77) in both years. The strength of association between HpMV and PNRSV was lower, ranging between 0.57 and 0.71 (Table 7.4).

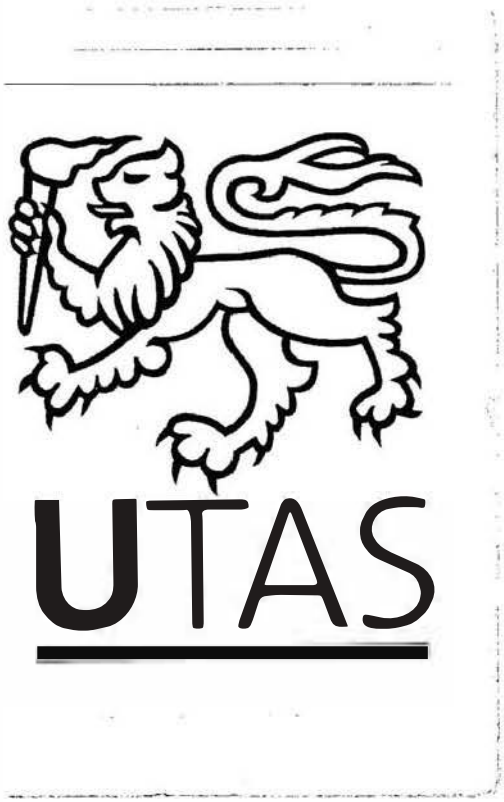


Table 7.1. Overall associations between HpLV, HpMV and PNRSV in hop blocks one (1996 and 1997) and 2 (1998).

Plot (Cv.)	Year	*n*	W-Statistic[1]	Variance Ratio[2]	Virus Incidence (%)		
					HpLV	HpMV	PNRSV[3]
1 (Victoria)	1996	1274	1856.09*	1.46	65	60	66
1 (Victoria)	1997	1274	1823.22*	0.89	77	88	92
2 (Victoria)	1998	500	854.36	1.71	17	13	31

[1] If the W-statistic is greater than the critical chi-square value at (n-1) degrees of freedom the overall association between the three viruses is significant, '*' (P<0.05).

[2] If the association is significant variance ratios (VR) greater than one indicate a possible positive association. Variance ratios less than one indicate a negative overall association.

[3] apple, 'A' and intermediate, 'I' serotypes of PNRSV were not differentiated

Table % PXa i r - cwoimspea r bs e t w e e HpnLV , HpaMnVd P,NRSV(a p p l'Aea,n d i n t e r i m a e t'd e l s , e o r t y p i e m s l) o o t n s (1 9 9 6 a n d 1 9 9 7) a n d (1 9 9 8) .

Block (Cultivar)	Year	Chi-square values for Pair-wise Comparisons[1] (Association; Ochiai Index[2])		
		HpLV/HpMV	HpLV/PNRSV[3]	HpMV/PNRSV
One ('Victoria')	1996	237.61 * Positive (0.79)	12.20 * Positive (0.69)	NS[4]
One ('Victoria')	1997	136.21 * Positive (0.88)	5.81 * Positive (0.91)	NS
Two ('Victoria')	1998	241.89 * Positive (0.74)	NS	NS

[1] Chi-square values greater than 3.84 ($\chi^2 > \chi^2_{0.05,1}$) were significant ($P<0.05$).

X5 FtXhe a s s o c i w a a t s p i o o s n i t t i h v e e e , l a t i v w a s s h t i r p o n a g s t e h r e O c h i a n i d e x a p p r o a c h e d o a n s e s . o c i w a f a t s n i t e d g r e a t t i h v e e e , l a t i v w a s s h t i r p o n a g s t e h r e o c h i a d i a e p x p r o a z c e h r e o d .

[3] a p p l e A a , n d i n t e d r i m a e t ' k s e , e o t y w p e e r s m e o d t i e f r f e n t t e i d a

[4] n o s t i g n i f a P < 0 . 0 5 n t

Multiple species comparisons of individual plots detected a significant and positive association between all three viruses in all plots (Table 7.3). Pair-wise comparisons identified significant positive associations between HpLV and HpMV in 85 % of plots. The strength of association between HpLV and HpMV was high in both years, ranging between 0.59 and 1.00 in 1996, and 0.56 and 0.94 in 1997. A significant positive association was detected between HpMV and PNRSV in one plot with two metres between plants across rows (six rows per bay) in 1996. A significant positive association was detected between HpLV and PNRSV in two plots in 1997. The associations between HpLV and PNRSV (0.71), and HpMV and PNRSV (0.59) were not as strong as the associations between the two *carlaviruses* (Table 7.4).

Significant, positive associations were detected between the three viruses when combining 'T11' plots in 1996 and 1997 (Table 7.5). Pair-wise comparisons identified significant positive associations between HpLV and HpMV in both years. The association between the two viruses was stronger in 1997 (0.82) than 1996 (0.61) (Table 7.6). Significant positive associations between the three viruses were identified in 88 % of individual plots (Table 7.5). Pair-wise comparisons identified significant positive associations between HpLV and HpMV in all individual plots in both years. The relative strength of the association (Ochiai index) ranged between 0.53 to 0.89 in 1996, and 0.43 to 96 in 1997. No significant associations were detected between either *carlavirus* and PNRSV (Table 7.6).

When combining 'Opal' plots in 1996 and 1997 (Table 7.7). Pair-wise comparisons of contiguous 'Opal' plots identified a significant positive association between HpLV and HpMV, and HpMV and PNRSV in 1996. However, the association between the latter two viruses was somewhat weak (0.31). In 1997, pair-wise comparisons identified a significant positive association between HpLV and HpMV, HpLV and PNRSV, and HpMV and PNRSV. The association between the two *carlaviruses* was stronger in 1996 (0.57) than 1997 (0.49). Ochiai indices were weak (less than 0.50) for both associations between HpLV and PNRSV, and HpMV and PNRSV (Table 7.8). Significant positive associations between the three viruses were identified in all individual plots (Table 7.7). Pair-wise comparisons identified a significant positive association between HpLV and HpMV in all plots in 1996 and 71 % of plots in 1997. The strength of the association ranged between 0.29 and 1.00 in 1996, and 0.35 and 0.74 in 1997. A significant positive association was detected between HpLV and PNRSV in 18 % of plots in 1997. These plots had close spacings between plants across rows, two with equal spacings between plants in either direction. The strength of association between HpLV and PNRSV was low in all plots, ranging between 0.26 and 0.38. A significant positive association was also detected between HpMV and PNRSV in 24 % of plots in 1997. These plots also had close spacings between plants across rows, two with equal spacings between plants in either direction, and one in plots with two (six rows per bay) and three (five rows per bay) metres between plants across rows. The strength of association ranged between 0.28 and 0.53 (Table 7.8).

Table 7.3. Overall associations between HpLV, HpMV, and PNRSV in 'Victoria' plots (cultivar x row spacing trial) at Bushy Park, Tasmania in 1996 and 1997.

Plot (Replicate)	Year	N	W-stat.[1]	Variance Ratio [2]	Disease Incidence (%)		
					HpLV	HpMV	PNRSV[3]
3 x 2 (1)	1996	90	133.82 *	1.49	43	57	93
3 x 2 (1)	1997	90	NA[4]	NA	50	67	100
3 x 2 (2)	1996	90	142.51 *	1.58	24	10	23
3 x 2 (2)	1997	90	167.38 *	1.86	29	54	62
6 (1)	1996	102	142.64 *	1.39	86	68	79
6 (1)	1997	102	126.12 *	1.24	88	85	86
6 (2)	1996	102	187.49 *	1.84	57	19	43
6 (2)	1997	102	NA	NA	74	66	100
5 (1)	1996	75	101.00 *	1.35	21	12	93
5 (1)	1997	75	106.59 *	1.42	39	40	99
5 (2)	1996	85	122.79 *	1.45	15	24	80
5 (2)	1997	85	94.08	NA	25	41	98
4 (1)	1996	60	106.31 *	1.77	27	10	72
4 (1)	1997	60	72.24	NA	45	43	98
4 (2)	1996	60	128.68 *	2.15	37	37	43
4 (2)	1997	60	116.95 *	1.95	43	42	67
3 (1)	1996	51	96.69 *	1.89	24	33	73
3 (1)	1997	51	62.58	NA	31	45	98
3 (2)	1996	45	99.11 *	2.21	49	53	71
3 (2)	1997	45	79.93 *	1.78	60	56	87
Contiguous Plots	1996	760	1332.74 *	1.75	41	33	67
Contiguous Plots	1997	568	866.56 *	1.53	46	53	86

[1] If the W-statistic was greater than the critical chi-square value at (n-1) degrees of freedom, the overall association between the three viruses was significant, '*' (P<0.05).

[2] If the association was significant, variance ratios greater than one indicate a possible positive association. Variance ratios less than one indicate a negative association.

[3] apple, 'A' and intermediate, 'I' serotypes of PNRSV were not differentiated.

[4] NA = not assessed due to one or more viruses being 'indeterminate' (disease incidence of one or more viruses was 0 or 100%).

Table 7.4. Pair-wise associations between HpLV, HpMV, and PNRSV in 'Victoria' plots (cultivar x row spacing trial) at Bushy Park, Tasmania in 1996 and 1997.

Plot (Replicate)	Year	Chi-square values for Pair-wise Comparisons[1] (Association; Ochiai Index[2])		
		HpLV/HpMV	HpLV/PNRSV[3]	HpMV/PNRSV
3 x 2 (1)	1996	26.09 * Positive (0.76)	NS	NS
3 x 2 (1)	1997	NA	NA	NA
3 x 2 (2)	1996	30.91 * Positive (0.64)	NS	NS
3 x 2 (2)	1997	7.45 * Positive (0.56)	8.08 * Positive (0.71)	NS
6 (1)	1996	28.49 * Positive (0.88)	NS	NS
6 (1)	1997	47.09 * Positive (0.96)	NS	NS
6 (2)	1996	14.21 * Positive (0.59)	NS	16.24 * Positive (0.59)
6 (2)	1997	NA[3]	NA	NA
5 (1)	1996	27.81 * Positive (0.67)	NS	NS
5 (1)	1997	55.57 * Positive (0.92)	NS	NS
5 (2)	1996	49.88 * Positive (0.81)	NS	NS
5 (2)	1997	NS	NS	NS

4 (1)	1996	14.41 * Positive (0.61)	NS	NS
4 (1)	1997	NS	NS	NS
4 (2)	1996	60.00 * Positive (1.00)	NS	NS
4 (2)	1997	48.41 * Positive (0.94)	4.10 * Positive (0.65)	NS
3 (1)	1996	17.65 * Positive (0.70)	NS	NS
3 (1)	1997	NS	NS	NS
3 (2)	1996	24.41 * Positive (0.87)	NS	NS
3 (2)	1997	30.38 * Positive (0.92)	NS	NS
Contiguous Plots	1996	302.63 * Positive (0.77)	NS	34.23 * Positive (0.57)
Contiguous Plots	1997	267.87 * Positive (0.84)	NS	5.91 * Positive (0.71)

[1] Chi-square values greater than 3.84 ($\chi^2 > \chi^2_{0.05,1}$) were significant, '*' ($P<0.05$).

[2] If the association was positive, the relationship was stronger as the ochiai index approached one. If the association was negative, the relationship was stronger as the ochiai index approached zero.

[3] apple, 'A' and intermediate, 'I' serotypes were not differentiated

[4] NS = not significant at $P<0.05$

[5] NA = not assessed due to one or more viruses being 'indeterminate' (disease incidence of one or more viruses was 0 or 100%).

Table 7.5. Overall associations between HpLV, HpMV, and PNRSV in 'T11' plots (cultivar x row spacing trial) at Bushy Park, Tasmania in 1996 and 1997.

Plot (Replicate)	Year	N	W-stat.[1]	Variance Ratio [2]	Disease Incidence (%)		
					HpLV	HpMV	PNRSV[3]
3 x 2 (1)	1996	102	163.87 *	1.61	27	38	2
3 x 2 (1)	1997	102	172.82 *	1.69	38	51	7
3 x 2 (2)	1996	102	NA	NA	11	8	0
3 x 2 (2)	1997	102	140.89 *	1.38	18	35	7
6 (1)	1996	102	139.23 *	1.36	65	44	6
6 (1)	1997	102	170.19 *	1.67	84	78	19
6 (2)	1996	102	142.13 *	1.39	48	28	21
6 (2)	1997	102	139.16 *	1.36	59	42	43
5 (1)	1996	75	NA	NA	20	9	0
5 (1)	1997	75	149.17 *	1.99	32	32	1
5 (2)	1996	75	NA	NA	9	11	0
5 (2)	1997	75	147.54 *	1.97	13	19	5
4 (1)	1996	60	89.39 *	1.49	28	17	8
4 (1)	1997	60	124.77 *	2.08	40	37	10
4 (2)	1996	60	89.78 *	1.49	13	7	7
4 (2)	1997	60	91.45 *	1.52	25	13	10
3 (1)	1996	45	59.52	1.32	40	4	24
3 (1)	1997	45	40.83	0.91	62	44	47
3 (2)	1996	51	NA	NA	63	6	0
3 (2)	1997	51	95.98 *	1.89	75	63	20
Contiguous Plots	1996	471	684.47 *	1.45	53	39	11
Contiguous Plots	1997	774	1352.16 *	1.75	44	43	15

[1] If the W-statistic was greater than the critical chi-square value at (n-1) degrees of freedom, the overall association between the three viruses was significant, '*' (P<0.05).

[2] If the association was significant, variance ratios greater than one indicate a possible positive association. Variance ratios less than one indicate a negative association.

[3] apple, 'A' and intermediate, 'I' serotypes of PNRSV were not differentiated.

[4] NA = not assessed due to one or more viruses being 'indeterminate' (disease incidence of one or more viruses was 0 or 100%).

Table 7.6. Pair-wise associations between HpLV, HpMV, and PNRSV in 'T11' plots (cultivar x row spacing trial) at Bushy Park, Tasmania in 1996 and 1997.

Plot (Replicate)	Year	Chi-square values for Pair-wise Comparisons[1] (Association; Ochiai Index[2])		
		HpLV/HpMV	HpLV/PNRSV[3]	HpMV/PNRSV
3 x 2 (1)	1996	42.59 * Positive (0.76)	NS	NA
3 x 2 (1)	1997	54.67 * Positive (0.85)	NS	NA
3 x 2 (2)	1996	NA	NA	NA
3 x 2 (2)	1997	6.38 * Positive (0.43)	NS	NS
6 (1)	1996	NS	NS	NS
6 (1)	1997	31.19 * Positive (0.92)	NS	NS
6 (2)	1996	11.68 * Positive (0.58)	NS	NS
6 (2)	1997	25.24 * Positive (0.74)	NS	NS
5 (1)	1996	NA	NA	NA
5 (1)	1997	52.94 * Positive (0.89)	NS	NS
5 (2)	1996	NA	NA	NA
5 (2)	1997	50.28 * Positive (0.85)	NS	NS

4 (1)	1996	25.39 * Positive (0.71)	NS	NS
4 (1)	1997	52.11 * Positive (0.96)	NS	NS
4 (2)	1996	14.11 * Positive (0.53)	NS	NS
4 (2)	1997	19.23 * Positive (0.63)	NS	NS
3 (1)	1996	NS	NS	NS
3 (1)	1997	NS	NS	NS
3 (2)	1996	NA	NA	NA
3 (2)	1997	29.39 * Positive (0.92)	NS	
Contiguous Plots	1996	81.64 * Positive (0.61)	NS	NS
Contiguous Plots	1997	353.42 * Positive (0.82)	NS	NS

[1] Chi-square values greater than 3.84 ($\chi^2 > \chi^2_{0.05,1}$) were significant, '*' (P<0.05).

[2] If the association was positive, the relationship was stronger as the ochiai index approached one. If the association was negative, the relationship was stronger as the ochiai index approached zero.

[3] apple, 'A' and intermediate, 'I' serotypes were not differentiated

[4] NS = not significant at P<0.05

[5] NA = not assessed due to one or more viruses being 'indeterminate' (disease incidence of one or more viruses was 0 or 100%).

Table 7.7. Overall associations between HpLV, HpMV, and PNRSV in 'Opal' plots (cultivar x row spacing trial) at Bushy Park, Tasmania in 1996 and 1997.

Plot (Replicate)	Year	N	W-stat.[1]	Variance Ratio[2]	Disease Incidence (%)		
					HpLV	HpMV	PNRSV[3]
3 x 2 (1)	1996	102	207.59 *	2.04	5	21	9
3 x 2 (1)	1997	102	208.14 *	2.04	12	27	26
3 x 2 (2)	1996	90	NA[4]	NA	0	2	0
3 x 2 (2)	1997	90	151.49 *	1.68	3	26	6
6 (1)	1996	90	151.21 *	1.68	7	24	3
6 (1)	1997	90	204.88 *	2.28	11	29	8
6 (2)	1996	90	160.94 *	1.79	7	7	3
6 (2)	1997	90	149.84 *	1.66	9	22	13
5 (1)	1996	85	NA	NA	0	1	0
5 (1)	1997	85	NA	NA	0	5	0
5 (2)	1996	75	NA	NA	2	5	3
5 (2)	1997	75	161.36 *	2.15	3	21	12
4 (1)	1996	68	NA	NA	7	9	3
4 (1)	1997	68	104.35 *	1.54	9	21	10
4 (2)	1996	68	137.38 *	2.02	10	10	12
4 (2)	1997	68	140.26 *	2.06	13	19	16
3 (1)	1996	45	NA	NA	0	33	0
3 (1)	1997	45	NA	NA	0	33	0
3 (2)	1996	51	105.62 *	2.07	18	31	22
3 (2)	1997	51	106.57 *	2.09	22	35	71
Contiguous Plots	1996	401	799.05 *	1.99	10	25	9
Contiguous Plots	1997	634	1262.41 *	1.99	10	30	18

[1] If the W-statistic was greater than the critical chi-square value at (n-1) degrees of freedom, the overall association between the three viruses was significant, '*' (P<0.05).

[2] If the association was significant, variance ratios greater than one indicate a possible positive association. Variance ratios less than one indicate a negative association.

[3] apple, 'A' and intermediate, 'I' serotypes of PNRSV were not differentiated.

[4] NA = not assessed due to one or more viruses being 'indeterminate' (disease incidence of one or more viruses was 0 or 100%).

Table 7.8. Pair-wise associations between HpLV, HpMV, and PNRSV in 'Opal' plots (cultivar x row spacing trial) at Bushy Park, Tasmania in 1996 and 1997.

Plot (Replicate)	Year	Chi-square values for Pair-wise Comparisons[1] (Association; Ochiai Index[2])		
		HpLV/HpMV	HpLV/PNRSV[3]	HpMV/PNRSV
3 x 2 (1)	1996	4.99 * Positive (0.29)	NS	NS
3 x 2 (1)	1997	NS	6.51 * Positive (0.38)	6.98 * Positive (0.46)
3 x 2 (2)	1996	NA	NA	NA
3 x 2 (2)	1997	NS	4.56 * Positive (0.26)	3.31 * Positive (0.28)
6 (1)	1996	6.21 * Positive (0.35)	NS	NS
6 (1)	1997	27.69 * Positive (0.62)	7.75 * Positive (0.36)	11.93 * Positive (0.45)
6 (2)	1996	60.73 * Positive (0.83)	NS	NS
6 (2)	1997	30.74 * Positive (0.63)	NS	NS
5 (1)	1996	NA	NA	NA
5 (1)	1997	NA	NA	NA
5 (2)	1996	NA	NA	NA
5 (2)	1997	7.58 * Positive (0.35)	NS	13.75 * Positive (0.53)
4 (1)	1996	NA	NA	NA

4 (1)	1997	NS	NS	NS
4 (2)	1996	57.61 * Positive (1.00)	NS	NS
4 (2)	1997	32.66 * Positive (0.74)	NS	NS
3 (1)	1996	NA	NA	NA
3 (1)	1997	NA	NA	NA
3 (2)	1996	23.91 * Positive (0.75)	NS	NS
3 (2)	1997	18.99 * Positive (0.71)	NS	NS
Contiguous Plots	1996	109.23 * Positive (0.57)	NS	18.84 * Positive (0.31)
Contiguous Plots	1997	104.28 * Positive (0.49)	10.84 * Positive (0.24)	29.14 * Positive (0.37)

[1] Chi-square values greater than 3.84 ($\chi^2 > \chi^2_{0.05,1}$) were significant, '*' (P<0.05).

[2] If the association was positive, the relationship was stronger as the ochiai index approached one. If the association was negative, the relationship was stronger as the ochiai index approached zero.

[3] apple, 'A' and intermediate, 'I' serotypes were not differentiated

[4] NS = not significant at P<0.05

[5] NA = not assessed due to one or more viruses being 'indeterminate' (disease incidence of one or more viruses was 0 or 100%).

DISCUSSION

Significant positive associations were detected between HpLV and HpMV in all 'Victoria', and over 85 % of all 'T11' and 'Opal' plots. The absence of the dominant aphid vector of HpLV and HpMV in Australia, *Phorodon humuli* Shrank, suggests that spread of these viruses relies upon alternative aphid vectors. Alate and apterous forms of *Macrosiphum euphorbiae* Thomas and *Myzus persicae* Sulzer (Adams & Barbara, 1980), both commonly found species in Australia, also transmit HpMV. However, these aphids have not shown to be vectors of HpLV (Adams and Barbara, 1980), and the mode of spread of this virus in Australian hop gardens remains unknown. The random spatial characteristics of *carlavirus* epidemics in block two (chapter three) strongly suggests spread by alate aphid vectors. Autocorrelated along row spread of both HpLV and HpMV in plot one in 1997 may also be indicative of common apterous or alate aphid vectors. No studies have been conducted to identify aphid species in Australian hop gardens and surrounding hosts throughout the year, nor detailed vector studies with local virus variants and aphid biotypes. However, the results suggest, that in local hop gardens, HpLV and HpMV have a common aphid vectors.

The presence of one virus may enhance the ability of the aphid vectors to acquire another virus and/or may also enhance the successful transmission of the other or both viruses. Possible mechanisms include an encapsidation relationship between the two viruses or influences on virus titre and virus acquisition efficiency. Transencapsidation involves the

encapsidation of one virus by the coat protein of another virus, or one virus may incorporate coat protein subunits of the second virus into its capsid, resulting in successful transmission by vectors of the second virus. In the case of HpMV and HpLV, if HpLV is not naturally vectored by *M. persicae* and *M. euphorbiae*, whilst HpMV is, a transencapsidation event could allow successful transmission of HpLV in co-infected plants. However, the presence of HpLV infected plants alone suggest that transencapsidation may not be the mechanism involved in HpLV transmission. Mixed infections between the two viruses in the same plant may result in higher virus titres of one or both viruses, increasing acquisition efficiency or spread by other means (i.e. contact transmission).

The positive association between HpLV and HpMV may also reflect enhanced susceptibility of the plant to infection with one virus following previous infection of the other, or that virus infected plants are more attractive to virus vectors. The former case may result from either a drop in the level of inhibitors of transmission and increase in virus titre, or drop in enzymes involved in the natural plant defence mechanisms.

Positive associations were also found between HpLV and PNRSV in 'Victoria' (blocks one and three) and in 'Opal' (block three) in 1997. This association is surprising considering HpLV and PNRSV are from two very different genera vectored in different ways. Analysis of the spatial characteristics of HpLV and PNRSV epidemics in blocks one and three (chapter three) suggested spread of both viruses along rows. PNRSV spread is almost certainly (chapters three and four) related to cultural practices such as

mowing and plant contact. Alate or apterous aphids may be responsible for the spread of HpLV, preferentially directed along rows by basal growth "bridges". The positive association between the two viruses may be because HpLV is also contact transmitted with greater ease. Alternatively, the association may suggest an enhanced susceptibility of specific hop cultivars to infection with either PNRSV or HpLV following previous infection with the other virus.

The positive association between HpMV and PNRSV in 'Victoria' in block three in 1997, and 'Opal' in 1996 and 1997 may also reflect an enhanced susceptibility of plants to infection by other viruses from after infection by one virus. However, the lack of association of both associations between HpLV and PNRSV, and HpMV and PNRSV with cultivar and individual plots infers these results should be interpreted with caution.

More weight could be attributed to these observations if trials conducted under controlled conditions had been done to mimick those found in the field. Further investigations into the identification of aphid species in Australian hop gardens and virus transmission studies are needed to increase our knowledge regarding hop *carlavirus* transmission in the absence of *P. humuli*. Transencapsidation hypotheses for HpLV and HpMV would also be interesting to pursue, particularly if alternative vectors for HpLV cannot be identified. Enhanced susceptibility with PNRSV due to previous infection between the *carlaviruses* and PNRSV could be studied by inoculation of a range of hop cultivars, propagated from HpLV or HpMV infected material. Virus tested plants of the same cultivar and age could be used as controls. A significant increase in the rate of PNRSV

infection in those plants pre-infected with either *carlavirus* would support the theory of enhanced susceptibility to virus infection when previously infected by other related or unrelated viruses.

To my knowledge, this study is the first to adopt the ecological principles of intervirus associations and apply them to the incidence of virus infection of plants. The advantage of this method is that it measures an association, from which interactions can be hypothesized without measuring the association directly. Intervirus associations between viral pathogens imply spatial relationships between them. However, this may also reflect environmental and/or dispersal gradients. The relationships suggested from this study support the need for detailed aphid vector studies and molecular investigations into the mechanisms underlying associations such as transencapsidation.

8. DISCUSSION

High virus incidence was consistently demonstrated in 'Victoria' hop gardens (the most important triploid cultivar in Australia) at sites in Tasmania and Victoria, Australia. In block plot at Bushy Park, infection by all three viruses approached 100 %, and PNRSV infected 92 % of plants only eight years after planting with virus tested material. The incidence of viruses in this cultivar was significantly higher than in 'Opal', 'T11', and the traditionally grown cultivar 'Pride of Ringwood'. This made traditional control methods such as establishing gardens with virus tested material and roguing of infected plants soon after establishment ineffective. The lack of conspicuous symptoms associated with virus infection in 'Victoria' also makes rapid and reliable identification of infected plants for roguing difficult.

Mechanical inoculation of PNRSV to a range of hop cultivars suggested 'Victoria' was significantly more susceptible to infection by PNRSV and that the rapid spread was likely to result from the highly susceptible nature of this cultivar to infection. The ability to detect PNRSV in 'Victoria' throughout the growing season may influence the ability to transmit for longer periods throughout the growing season, and a higher virus titre. This is unlike other cultivars where detection is often unreliable after mid-season, which is assumed to result from a drop in virus titre (Munro, pers. comm.). PNRSV was also detected in all tissues tested from various positions around the plant in 'Victoria' suggesting a symmetrical virus distribution. However, in less suceptible cultivars, the detection of PNRSV indicated an asymmetric distribution. These factors may increase

the probability of virus transmission from infected to virus tested plants by extending the window of opportunity for transmission and increasing the amount of available tissue with levels that are sufficent for effective transmission.

The lack of significant reductions in yield and levels of brewing organic acids from infections by HpLV, HpMV, PNRSV (A & I), and from various co-infections between them in both three and nine year old 'Victoria' plants, suggested that this cultivar may be tolerant to virus infection. These findings question the continued need for virus control by planting with virus tested material of 'Victoria'. However, the significant effects of virus infection on propagation suggest the use of virus tested material as mother plants would be beneficial. Nevertheless, establishing gardens with virus tested material would reduce the number of infected plants, able to act as sources of inoculum for adjacent gardens containing cultivars in which viruses pose a significant constraint to production.

'Opal' was the least susceptible of the newly developed hop cultivars. Consistently low incidence of all three viruses was recorded in 'Opal' gardens of varying ages. Virus incidence in 'Opal' was similar to that of 'Pride of Ringwood' gardens, both in this and previous studies in Tasmania (Munro, 1987). Mechanical inoculation with PNRSV failed to establish infection of 'Opal' plants, which suggests that low PNRSV incidence in the field may decrease infection of new plants. Testing in mid-season of a range of tissues of 'Opal' plants, chronically infected by either PNRSV-A or PNRSV-I, failed to detect virus in some sections within the plant. This suggested an asymmetric distribution and

possibly it indicates translocation resistance, decreasing further the probability of virus transmission, by decreasing the amount of tissues containing sufficient inoculum.

Significant reductions in three year old 'Opal' plants, in yield, alpha acid content, and the alpha to beta acid ratio, and increases in beta acid content were demonstrated in plants infected by HpLV + HpMV + PNRSV-A, and HpLV + HpMV + PNRSV-I. Plants infected by only HpLV had significantly reduced yield and alpha acid levels compared to healthy plants. However, significant reductions in beta acid content in nine year old plants infected by PNRSV-A or PNRSV-I alone suggested with time other virus infection may become detrimental to levels of brewing organic acids. This information, together with the slow rate of virus infection after establishing gardens with virus tested material suggested that virus control by traditional methods in this cultivar remains important to minimise losses.

The transmission of PNRSV in Australian hop gardens was associated with mowing of basal growth and contact between plants early in the growing season. Glasshouse trials were successful at transmitting PNRSV between plants by simulating mowing and plant contact. However, the low infection frequencies in these trials meant that the results were difficult to extrapolate to the field because of differences in inoculum pressure, tissue type, susceptibility to infection, and environmental factors. The reduction in spread when plant contact was restricted early in the season suggested that plant contact from midseason onwards at the trellis top did not significantly contribute to virus transmission. The lack of significant temporal fluctuations in virus titre during the season and ability to

detect PNRSV in all tissues in 'Victoria' suggested this may be related to a rise in inhibitors or decrease in the physical succulence of tissues, making mechanical transmission through plant contact less likely. This also suggested that the majority of transmission occurred because of the large amount of succulent basal growth present until sheep grazing, starting approximately in mid season. Superfluous intertwined basal growth between adjacent plants may increase the probability of virus transmission by providing a large source of inoculum, and by ensuring intimate contact between infected and healthy plants through contact transmission. Contact transmission may occur from small wounds created through the action of mowing blades or the indirect and direct result of plant contact.

Transmission of PNRSV between plants through root grafting in the glasshouse suggested this may also provide a route for virus transmission. The existence of root grafts in Australian hop gardens was suggested by injection of a translocatable herbicide marker. However, the extent to which root grafts contribute to transmission of all three viruses is yet to be confirmed. It is unlikely that sufficient time would elapse for root grafts to form in the annual roots, leaving only the chance of their formation in the perennial roots. This may be a random event and is likely to be influenced by the ability of certain cultivars to form grafts, and external factors such as inconsistencies in the soil profile forcing roots from two plants together. As root grafts are direct links between the vascular tissue of two plants, this would be expected to prove sufficient for virus transmission. However, in some cultivars asymmetric virus distributions within the plant

and the inability of certain virus strains to cross phloem links may reduce the effectiveness of root grafts in virus transmission.

Despite the apparent lack of aphid species capable of transmitting HpLV in Australia, the temporal increase in incidence in several cultivars suggested a vector species exists. The significant prevalence in hop plants of mixed infections between HpLV and HpMV compared to plants infected by these two *carlaviruses* alone suggested these aphid species may be the same as those responsible for the transmission of HpMV. *M. euphorbiae* and *M. persicae* are vectors of HpMV in other countries and are assumed to be vectors in Australia. However, aphid biotypes may differ in their efficiencies in transmission and the capability of Australian biotypes to transmit HpLV and HpMV needs to be investigated. The predominance of co-infections may also suggest spread of HpLV by transencapsidation with HpMV, allowing spread of HpLV by vectors of HpMV. This mechanism would involve either the incorporation of HpLV nucleic acid within the coat protein of HpMV, or the incorporation of HpLV coat protein subunits into the capsid of HpMV. However, the existence of a small proportion of plants infected by HpLV alone suggested a vector for HpLV may also exist.

Spatial analyses of *carlavirus* epidemics at hop garden sites in Bushy Park and Myrtleford depicted different distributions, which may reflect differences in dominant spread mechanisms. Random distributions of both HpLV and HpMV in a 'Victoria' garden in Myrtleford suggested the dominant vectors were alate aphid species. However, the autocorrelated along row spread of HpLV and HpMV in 1997 in a 'Victoria' garden

in Tasmania was indicative of contact transmission by basal growth mowing, or spread by either apterous or alate aphid vectors preferentially directed along rows by extensive basal growth contact early in the season.

In conclusion, this study was apparently the first to quantify the accelerated spread rate of viruses in a newly developed Australian hop cultivar, to spatially characterise viral epidemics in hop gardens, to identify a hop cultivar tolerant to virus infection and other cultivars with low susceptibility, and to use the principles of interspecific association analysis in plant viral epidemiology. The spread of PNRSV in hop gardens in Australia was associated with basal growth mowing and resulting extensive plant contact early in the season. In Tasmania, the spread of *carlaviruses* may also be influenced by mowing. Significant reductions in yield and levels of brewing organic acids in three year old plants from infection by HpMV of 'Pride of Ringwood' and 'Nugget', and from infection by all three viruses in 'Opal' demonstrated that viruses continue to pose significant constraints to production. Slow rates of virus infection following planting with virus tested material in 'Pride of Ringwood' and 'Opal' gardens means that the continued policy of establishing gardens with virus tested material should provide adequate control. However, as both three and nine year old 'Victoria' plants appear tolerant of infection by individual viruses and co-infections between them, this suggests that for this cultivar the higher costs involved in establishing gardens with virus tested material appear not worthwhile for this cultivar. However, the continued use of this policy reduces inoculum for other cultivars.

9. SUGGESTIONS FOR FURTHER STUDIES

Some aspects of the epidemiology of viruses infecting hops in Australia were not covered in detail. Findings from these studies may also be the precursor for further trials.

9.1. Effect of viruses on yield and quality

Despite the time and costs involved in establishing the yield trial, the benefits of having a properly, designed layout with controls, trial outweighed these factors. The trial made possible examination of certain virus combinations in particular cultivars, inclusion of buffer plants to reduce the rate of infection of control plots, ability to assess effects of agronomic characteristics and production over an extended period of time, and the capability to measure virus spread to plants of several cultivars. However, the inability to detect certain virus infections in some cultivars and field size constraints made the trial design unbalanced and unorthogonal, making results difficult to statistically analyse. An insufficient supply of virus tested plants and varying mortality rates of virus infected plants also made the trial difficult to analyse because of the requirement to exclude plots containing plants of different ages. Time constraints over the course of this study also meant that only one preliminary harvest of suitable plots, and one full harvest the following season could be conducted. Further investigationsof the significant effects of viruses found in some cultivars requires the continued monitoring of this trial for at least an additional two seasons, depending upon virus re-infection in control plots. Moreover, considering the commercial life of a hop garden can extend to approximately 20 years,

full assessment of the effects of virus infection requires additional assessments in older plants. Viruses may have a significant effect on yield and quality of products early when plants are becoming established or later in life when plants have become debilitated by chronic infection and other stresses. This was overcome to some extent with harvests of single plants infected by certain virus combinations in older 'Victoria', 'Opal', and 'T11' plants.

This study assessed the effect of viruses on brewing organic acids. Further studies should include the effect on essential oil profiles of aroma hop cultivars and the effect of viruses on new and promising hop cultivars prior to wide scale release. Due to the poor mechanical inoculation techniques for all three viruses in the majority of cultivars, the latter would rely upon the detection of infection in the field, and either subsequent single plant harvests or propagation of specific virus combinations for small scale screening. Mechanical inoculation could also be used to assess relative susceptibility of breeding material to virus infection prior to further investigation. However, as found in 'Victoria' greater susceptibility to virus infection does not necessarily translate into significant effects on yield and quality of products.

9.2. Transmission of PNRSV in hop

The transmission of PNRSV was strongly associated with basal growth mowing in hop gardens, and in Tasmania, mowing is exclusively used for basal growth control. Further trials could examine alternative methods for basal growth control, which might reduce

the spread of PNRSV. Methods used in hop gardens in other parts of the world, such as dessicant herbicide sprays (United Kingdom and Europe) or cross-cultivation (U.S.A., and Victoria, Australia) could be trialled.

However, the prohibitively high fixed costs involved in changing trellis systems and the lack of significant reduction in yield and quality of products in cultivars where PNRSV spread is rapid (e.g. 'Victoria) tends to negate the usefulness of such investigations to the Australian hop industry. Nevertheless, 'Victoria' would provide a useful model system for assessment of other basal growth control options, which could be implemented if a cultivar were to be bred in the future in which PNRSV transmission is rapid and infection poses a significant constraint to production.

This project has detailed the intra-garden transmission of PNRSV, however we still know very little regarding the appearance of primary PNRSV foci in the field. We hypothesise that this appearance may relate to a certain proportion of cuttings from mother plants being infected, which despite testing negative to virus infection, the asymmetric distribution of the virus and selection of only one leaf from one bine for routine testing. Further studies into virus distribution within the hop plant would be useful to determine the best means of sampling. Leaf samples could be taken at various positions from the same bine and tested by ELISA. Softwood cuttings could then be made from each section and rooted plants tested. This would help determine whether plant parts testing negative to infection prior to propagation remained negative or had undetectable virus infection that subsequently multiplied to detectable levels following propagation.

The extent to which viruses are transmitted by root grafting in hops is also an issue. Further trials could involve glyphosate injection of plants of a range of cultivars and ages. Trials could also be designed to monitor PNRSV transmission in plots where root contact was discouraged using barriers such as herbicide impregnanted mesh. However, even if root grafting was shown to contribute significantly to virus transmission, the design of economically viable control measures would prove difficult. Root grafting may be discouraged by changing from a minimum tillage system to weed control by ground disturbance through minimising soil compaction. However, the depth of ground disturbance required is difficult to estimate. Cross-cultivation systems would further discourage anisotropic soil compaction and allow the roots to grow equally in all directions, which could improve nutrient uptake by increasing the exploitable volume of soil available to the plant.

Further field trials could extend some of the findings from glasshouse trials. All successful transmissions of PNRSV by mechanical inoculation in this study were between plants of like cultivar. This may reflect the presence of different virus variants adapted to certain cultivars, making transmission between plants of one cultivar easier than between different cultivars. Such trials would mechanically inoculate PNRSV (A or I) between like and unlike cultivars and compare inoculation success. Examinations of strain variation (serological and molecular) between Australian isolates and comparisons between Australian and overseas isolates of PNRSV would also be beneficial. Identification of severe isolates would justify a re-examination of the effects of PNRSV

on yield and quality. Should severe isolates be shown to have a significant effect on production control by cross-protection by a mild isolate could be investigated. However, this cross-protection phenomena may already exist naturally in the field.

Molecular characterisation of the PNRSV-A and PNRSV-I serotypes infecting hop would increase our understanding of the relationships between these serotypes and those infecting other species (e.g. PNRSV-C infecting *Prunus* spp.). Clarification of whether mixed infections between PNRSV-A and PNRSV-I occur together in the same plant would greatly increase our epidemiological and aetiological knowledge of these viruses.

9.3. *Carlavirus* spread

Significant deficiencies exist in our knowledge regarding the spread of both *carlaviruses* in Australian hop gardens. Further studies are needed to determine a) which aphid species are responsible for virus transmission, b) if a transencapsidation relationship exists between the two, c) if transmission occurs from contact transmission as a result of basal growth mowing, d) when spread by aphids is occurring, and e) the factors influencing spread. Field work investigating such issues should include a) intensive monitoring of infection in a range of cultivars at different sites, b) tracking of aphid populations throughout the growing season on hops, c) identification of alternative hosts outside the growing season surrounding the gardens.

9.4. **Role of viroid pathogens**

Recent surveys in Australian hop gardens suggested infection with HLVd was ubiquitous (G. Leggett, pers. comm.), and hence it was assumed to be present in all hop planting material used in the yield comparison (chapter six). Recent studies in the United Kingdom found HLVd significantly reduced yield of 'Omega' (Barbara, *et al.*, 1992). Trials investigating the effect of HLVd on production and quality of Australian hop cultivars are vital to determine whether the use of viroid tested material may significantly increase production. Assuming the viroid did pose a significant constraint to production, elucidation of which cultural practices are involved in transmission, and/or transmission by transencapsidation involving any of three viruses commonly found would prove useful.

It would also be helpful to determine whether Australian HSVd strains ubiquitously infecting grapes are capable of infecting and causing significant reductions in yield of hop. This is particularly important because of the exponential increase in vineyards in Tasmania and Victoria in recent years. In some cases both are on the same properties.

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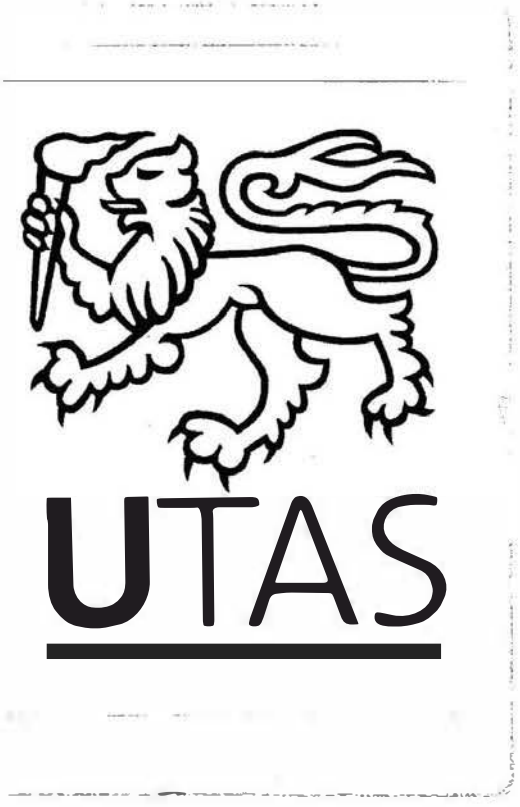


APPENDIX 1:

IMMUNOGLOBULIN PURIFICATION AND ENZYME CONJUGATION

Raw polyclonal antiserum to HpMV was kindly supplied by R. Klein, Washington State University, U.S.A. Antisera to HpLV, chestnut mosaic virus (ChMV), and rose mosaic virus 3 (RMV 3), used to detect both serotypes of PNRSV, were kindly supplied by D. Barbara, Horticultural Research International,U.K.

Immunoglobulins were purified from crude antiserum using a two-step procedure, CM Affi-Gel Blue followed by ammonium sulphate precipitation (Clark & Bar-Joseph, 1984). The first step involved chromatography aimed at selectively absorbing both albumin and plasminogen from the serum to prepare antiserum free from albumin and protease activity. A column was prepared with a bed volume of 3.1 mL per mL of crude antiserum. The column was prewashed with five bed volumes of pre-wash buffer, followed by seven bed volumes of deionised water and equilibrated with two bed volumes of running buffer. Serum was eluted from the column with one bed volume of running buffer. The optical densities (OD) of individual eluted fractions were read by a spectrophotometer using an absorbance of 280 nm. Concentrated fractions (those with high readings) were pooled and diluted using running buffer to 1 mg per mL (OD=1.4). The column was regenerated by two bed volumes of 1.5 M sodium thiocyanate, followed by five bed volumes of buffer A and two bed volumes of running buffer.

Ammonium sulphate precipitation was used subsequently to obtain a globulin fraction free of protease and serum complement proteins. Ammonium sulphate was added to the eluted protein, forming a 45 % saturated solution. The solution was stirred at room temperature for one hour prior to centrifugation at 4°C at 1000 g for 20 minutes. Pellets were resuspended in 45 % saturated ammonium sulphate and centifugation repeated. Pellets were resuspended in a minimum volume of PBS (Appendix 2). The remaining ammonium sulphate was removed by dialysis to half strength PBS (1mL:1L).

Antisera were stored in silicone-coated bottles at 4 °C. Sodium azide (0.02 %) was added as a preservative.

ENZYME CONJUGATION OF PURIFIED IMMUNOGLOBULINS

Enzyme conjugates were prepared by linking purified immunoglobulins to alkaline phosphatase. This enzyme has advantages of stability, the simple conjugation process to protein by a glutaraldehyde connection and linear reaction kinetics with substrates (Clark & Bar-Joseph, 1984). Immunoglobulins (0.8 mL) were combined with 2 mg of alkaline phosphatase, prior to addition of 25 % glutaraldehyde (2.4 μL), forming a final concentration of 0.06%. The solution was incubated at 37 °C for four hours prior to dialysis with three washes of half strength PBS (1mL:1L). Enzyme conjugates were stored in silicone coated bottles with 5mg per mL of bovine serum albumin and 20 μL per mL of 1 % sodium azide solution.

BUFFERS FOR IMMUNOGLOBULIN PURIFICATION

1. Pre-wash buffer

Glacial acetic acid (CH_3COOH)	57.35 ml
Sodium chloride (NaCl)	81.82 g
Isoproponal	400.00 ml
Distilled Water	542.65 ml

A few drops of 10N sodium hydroxide (NaOH) was added to alter the pH from 2.55 to 3.0.

2. Running Buffer

Potassium phosphate dibasic (K_2HPO_4)	1.10 g
Potassium phosphate monobasic (KH_2PO_4)	0.50 g
Sodium chloride (NaCl)	8.77 g
Sodium azide (preservative) (NaN_3)	0.10 g

Make up to one litre with distilled water

APPENDIX 2:

SEROLOGICAL TESTING

Tissues were tested for virus infection by double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) (Clark & Adams, 1977), using polyclonal antisera to HpMV, HpLV, ChMV, and RMV-3 (Barbara *et al.*, 1978). Microtitre plates (Greiner Labortechnik) were coated with polyclonal antisera diluted in carbonate coating buffer and incubated at either 4 °C for 12 hours or 37 °C for four hours. The volume of reactant in each step was 100 μL per well. Leaf tissue was subsampled and 0.1 g of plant material homogenised in a smooth rotary press in 1 mL phosphate-buffer saline (PBS) extraction buffer (1:10 dilution). Samples were tested in duplicate in a "criss-cross" format to decrease within-plate variation, with appropriate hop positive, negative, and buffer only controls on individual plates. Enzyme conjugates were diluted in PBS extraction buffer and incubated at either 4 °C for 12 hours or 37 °C for four hours. Substrate (4, p-nitrophenol phosphate, Sigma 104 - 5mg tablets) was diluted at 5 mg per 10 mL of substrate buffer. Between steps plates were washed three times with PBS-Tween wash buffer containing 1 % skim milk powder as a blocking agent.

ELISA results were recorded using a Titertek Multiskan MCC spectrophotometer (405 nm filter). Results were summarised graphically through "Genesis" software, version 2.12 (Life Sciences [UK] Ltd) through an IBM PC. Positive samples were those with absorbance values greater than the upper negative critical limit, defined as the mean of

the negative controls plus three times the standard deviation of the negative samples. Samples reacting to ChMV antisera with absorbance values greater than 35 times the mean of the negative controls (O.D.>1.0) and failing to react to RMV-3 antisera were considered the apple serotype. Samples reacting with absorbance values 15 to 25 times the mean of the negative controls (1.4<O.D<1.6) to both RMV-3 and ChMV antisera were considered the intermediate serotype (Barbara *et al.*, 1978).

Criss-cross plate layout (boxes represent wells on ELISA plate):

Numbers represent individual samples;VF = negative hop controls; BF = buffer only controls; PS = positive hop controls (4 dilutions at 1:10, 1:30, 1:90, 1:270)

1	2	3	4	5	6	7	8	9	10	11	12
VF	BF	PS1	PS2	13	14	15	16	PS3	PS4	BF	VF
17	18	19	20	21	22	23	24	25	26	27	28
29	30	31	32	33	34	35	36	37	38	39	40
7	8	9	10	11	12	1	2	3	4	5	6
15	16	PS3	PS4	BF	VF	VF	BF	PS1	PS2	13	14
23	24	25	26	27	28	17	18	19	20	21	22
35	36	37	38	39	40	29	30	31	32	33	34

In this layout, duplicate samples never appear in the same row or column, all samples appear in peripheral wells no more than once (except for samples 6,7, 29 and 40), and allows healthy controls to appear in peripheral wells.

BUFFERS FOR SEROLOGICAL TESTING

1. Carbonate Coating Buffer (pH 9.6)

Sodium carbonate (Na_2CO_3)	1.59 g
Sodium bicarbonate ($NaHCO_3$)	2.93 g

Make up to one litre with distilled water.

2. Phosphate buffer saline (PBS) (pH 7.4)

Sodium chloride (NaCl)	8.00 g
Potassium phosphate monobasic (KH_2PO_4)	0.20 g
Disodium hydrogen phosphate dibasic ($Na_2HPO_4.2H_2O$)	1.44 g
Potassium chloride (KCl)	0.20 g

Make up to one litre with distilled water. PBS was made up as a 10x concentrate and diluted as necessary.

3. PBS Extraction Buffer (pH 7.4)

PVP 40,000 (Polyvinyl pyrrolidone)	20.00 g
Bovine Serum Albumin (BSA)	2.00 g

Make up to one litre with PBS.

4. Washing Buffer (pH 7.4)

Tween 20 (Polyoxyethylene (20) sorbitan monolaurate)	0.50 ml
Skim Milk Powder (Sunshine Brand)	1.00 g

Make up to one litre with PBS.

5. Substrate Buffer (pH 9.8)

Diethanolamine [$CH_2(OH)CH_2$]	97.00 ml

Add 5N hydrochloric acid (HCl) drop-wise until pH falls to 9.8. Make up to one litre with distilled water.

APPENDIX 3:

SPATIAL ANALYSIS OF VIRUS EPIDEMICS IN AUSTRALIAN HOP GARDENS

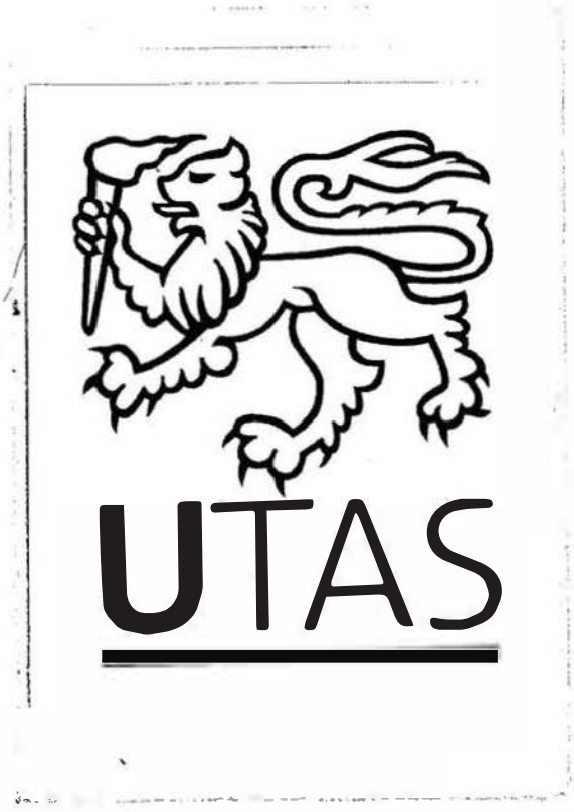
Appendix 3.1. Ordinary runs analysis of HpLV distribution in block one ('Victoria') in Bushy Park, Tasmania on 10th October 1996.

AlongRows (Individually)	Virus	m	N	E(U)	O(U)	S(U)	Z
1	HpLV	28	51	26.25	21	3.50	-1.36
2	HpLV	28	51	26.25	25	3.50	-0.22
3	HpLV	27	51	26.41	19	3.52	-1.96*
4	HpLV	22	51	26.02	18	3.47	-2.17*
5	HpLV	30	51	26.02	20	3.47	-1.59
6	HpLV	34	51	22.96	18	3.03	-1.47
7	HpLV	37	51	21.31	19	2.80	-0.65
8	HpLV	29	51	26.02	18	3.47	-2.17*
9	HpLV	29	51	26.02	22	3.47	-1.02
10	HpLV	13	51	21.31	15	2.80	-2.08*
11	HpLV	38	51	20.37	15	2.67	-1.83*
12	HpLV	43	51	14.49	17	1.84	1.64
13	HpLV	43	51	14.49	13	1.84	-0.54
14	HpLV	43	51	14.49	12	1.84	-1.08
15	HpLV	47	51	8.37	8	0.97	0.13
16	HpLV	43	51	14.49	16	1.84	1.09
17	HpLV	31	51	25.31	17	3.37	-2.32*
18	HpLV	38	51	20.37	13	2.67	-2.58*
19	HpLV	42	51	15.82	13	2.02	-1.15
20	HpLV	40	51	19.35	17	2.52	-0.73
21	HpLV	30	51	26.02	20	3.47	-1.59
22	HpLV	31	51	25.31	27	3.37	0.65
23	HpLV	24	51	26.41	23	3.52	-0.83
24	HpLV	31	51	25.31	18	3.37	-2.02*
25	HpLV	23	51	26.25	16	3.50	-2.79*
Along Rows (Contiguous[1])	HpLV	824	1275	584.52	425	16.33	-9.74*

Across Rows (Individually)	Virus	m	N	E(U)	O(U)	S(U)	Z
1	HpLV	18	25	11.08	12	1.95	0.73
2	HpLV	12	25	13.48	12	2.44	-0.40
3	HpLV	14	25	13.32	12	2.41	-0.34
4	HpLV	16	25	12.52	10	2.25	-0.90
5	HpLV	14	25	13.32	14	2.41	0.49
6	HpLV	18	25	11.08	6	1.95	-2.35*
7	HpLV	19	25	10.12	8	1.76	-0.92
8	HpLV	16	25	12.52	11	2.25	-0.45
9	HpLV	17	25	11.88	8	2.12	-1.60
10	HpLV	11	25	13.32	16	2.41	1.32
11	HpLV	14	25	13.32	11	2.41	-0.76
12	HpLV	11	25	13.32	13	2.41	0.07
13	HpLV	15	25	13.00	10	2.35	-1.07
14	HpLV	20	25	9.00	9	1.53	0.33
15	HpLV	17	25	11.88	11	2.12	-0.18
16	HpLV	17	25	11.88	10	2.12	-0.65
17	HpLV	19	25	10.12	12	1.76	1.35
18	HpLV	17	25	11.88	12	2.12	0.29
19	HpLV	16	25	12.52	12	2.25	-0.01
20	HpLV	17	25	11.88	11	2.12	-0.18
21	HpLV	15	25	13.00	12	2.35	-0.21
22	HpLV	14	25	13.32	11	2.41	-0.76
23	HpLV	12	25	13.48	11	2.44	-0.81
24	HpLV	15	25	13.00	9	2.35	-1.49
25	HpLV	16	25	12.52	12	2.25	-0.01
26	HpLV	14	25	13.32	9	2.41	-1.58
27	HpLV	17	25	11.88	11	2.12	-0.18
28	HpLV	16	25	12.52	11	2.25	-0.45
29	HpLV	14	25	13.32	13	2.41	0.07
30	HpLV	16	25	12.52	10	2.25	-0.90
31	HpLV	18	25	11.08	10	1.95	-0.30
32	HpLV	17	25	11.88	14	2.12	1.24
33	HpLV	18	25	11.08	10	1.95	-0.30
34	HpLV	16	25	12.52	12	2.25	-0.01
35	HpLV	15	25	13.00	13	2.35	0.21
36	HpLV	17	25	11.88	10	2.12	-0.65
37	HpLV	18	25	11.08	12	1.95	0.73
38	HpLV	18	25	11.08	9	1.95	-0.81
39	HpLV	14	25	13.32	14	2.41	0.49
40	HpLV	16	25	12.52	10	2.25	-0.90
41	HpLV	15	25	13.00	9	2.35	-1.49
42	HpLV	15	25	13.00	11	2.35	-0.64
43	HpLV	17	25	11.88	15	2.12	1.71
44	HpLV	13	25	13.48	11	2.44	-0.81
45	HpLV	19	25	10.12	8	1.76	-0.92
46	HpLV	16	25	12.52	13	2.25	0.44
47	HpLV	14	25	13.32	12	2.41	-0.34

48	HpLV	17	25	11.88	12	2.12	0.29
49	HpLV	22	25	6.28	7	0.97	1.26
50	HpLV	22	25	6.28	6	0.97	0.23
51	HpLV	19	25	10.12	11	1.76	0.79
Across rows (Contiguous	HpLV X	823	1275	584.52	523	16.33	-3.74*

rows combined by last plant of row i contiguous with first plant of row $i+1$
* Z-statistics less than –1.64 indicate significant aggregation of infected plants (P=0.05)
m = number of infected plants; N = total number of plants; E(U) = expected number of runs; O(U) = observed number of runs; S(U) = standard deviation of runs; Z = z-statistic



Appendix 3.2. Ordinary runs analysis of HpMV distribution in block one ('Victoria') in Bushy Park, Tasmania on 10th October 1996.

Along Rows (Individually)	Virus	m	N	E(U)	O(U)	S(U)	Z
1	HpMV	45	51	11.59	11	1.42	-0.06
2	HpMV	31	51	25.31	21	3.37	-1.13
3	HpMV	27	51	26.41	27	3.52	0.31
4	HpMV	24	51	26.41	21	3.52	-1.39
5	HpMV	21	51	25.71	25	3.42	-0.06
6	HpMV	42	51	15.82	16	2.02	0.33
7	HpMV	32	51	24.84	26	3.30	0.50
8	HpMV	30	51	25.71	22	3.42	-0.94
9	HpMV	31	51	25.31	19	3.37	-1.73*
10	HpMV	24	51	26.41	23	3.52	-0.83
11	HpMV	38	51	20.37	16	2.67	-1.45
12	HpMV	42	51	15.82	15	2.02	-0.16
13	HpMV	46	51	10.02	9	1.20	-0.43
14	HpMV	40	51	18.25	19	2.37	0.53
15	HpMV	42	51	15.82	12	2.02	-1.64
16	HpMV	22	51	26.02	24	3.47	-0.44
17	HpMV	11	51	18.25	14	2.37	-1.59
18	HpMV	35	51	22.96	13	3.03	-3.12*
19	HpMV	38	51	20.37	15	2.67	-1.83*
20	HpMV	37	51	21.31	17	2.80	-1.36
21	HpMV	36	51	22.18	20	2.92	-0.57
22	HpMV	35	51	22.96	21	3.03	-0.48
23	HpMV	24	51	26.41	13	3.52	-3.67*
24	HpMV	11	51	17.08	13	2.20	-1.63
25	HpMV	1	51	2.96	3	0.19	2.78
Along Rows (Contiguous[1])	HpMV	765	1275	613.40	422	17.14	-11.14*

Across Rows (Individually)	Virus	m	N	E(U)	O(U)	S(U)	Z
1	HpMV	16	25	12.52	10	2.25	-0.90
2	HpMV	20	25	9.00	6	1.53	-1.64
3	HpMV	16	25	12.52	10	2.25	-0.90
4	HpMV	14	25	13.32	14	2.41	0.49
5	HpMV	10	25	13.00	12	2.35	-0.21
6	HpMV	13	25	13.48	16	2.44	1.24
7	HpMV	14	25	13.32	9	2.41	-1.58
8	HpMV	14	25	13.32	14	2.41	0.49
9	HpMV	16	25	12.52	12	2.25	-0.01
10	HpMV	11	25	13.32	13	2.41	0.07
11	HpMV	15	25	13.00	12	2.35	-0.21
12	HpMV	14	25	13.32	10	2.41	-1.17
13	HpMV	16	25	12.52	12	2.25	-0.01
14	HpMV	16	25	12.52	10	2.25	-0.90
15	HpMV	17	25	11.88	10	2.12	-0.65
16	HpMV	14	25	13.32	12	2.41	-0.34
17	HpMV	20	25	9.00	8	1.53	-0.33
18	HpMV	18	25	11.08	11	1.95	0.22
19	HpMV	15	25	13.00	12	2.35	-0.21
20	HpMV	16	25	12.52	14	2.25	0.88
21	HpMV	17	25	11.88	16	2.12	2.18
22	HpMV	15	25	13.00	14	2.35	0.64
23	HpMV	17	25	11.88	12	2.12	0.29
24	HpMV	15	25	13.00	14	2.35	0.64
25	HpMV	18	25	11.08	14	1.95	1.75
26	HpMV	15	25	13.00	12	2.35	-0.21
27	HpMV	15	25	13.00	14	2.35	0.64
28	HpMV	16	25	12.52	14	2.25	0.88
29	HpMV	16	25	12.52	12	2.25	-0.01
30	HpMV	15	25	13.00	8	2.35	-1.92*
31	HpMV	16	25	12.52	11	2.25	-0.45
32	HpMV	15	25	13.00	11	2.35	-0.64
33	HpMV	13	25	13.48	12	2.44	-0.40
34	HpMV	16	25	12.52	6	2.25	-2.68*
35	HpMV	16	25	12.52	12	2.25	-0.01
36	HpMV	17	25	11.88	9	2.12	-1.12
37	HpMV	13	25	13.48	14	2.44	0.42
38	HpMV	15	25	13.00	12	2.35	-0.21
39	HpMV	12	25	13.48	14	2.44	0.42
40	HpMV	14	25	13.32	12	2.41	-0.34
41	HpMV	12	25	13.48	14	2.44	0.42
42	HpMV	13	25	13.48	12	2.44	-0.40
43	HpMV	13	25	13.48	14	2.44	0.42
44	HpMV	10	25	13.00	14	2.35	0.64
45	HpMV	17	25	12.52	10	2.25	-0.90
46	HpMV	12	25	13.48	11	2.44	-0.81
47	HpMV	14	25	13.32	10	2.41	-1.17
48	HpMV	13	25	13.48	12	2.44	-0.40
49	HpMV	14	25	13.32	12	2.41	-0.34
50	HpMV	20	25	9.00	6	1.53	-1.64
51	HpMV	16	25	12.52	12	2.25	-0.01
Across Rows (Contiguous[1])	HpMV	765	1275	613.40	554	17.14	-3.44*

[1] rows combined by last plant of row i contiguous with first plant of row $i+1$

* Z-statistics less than −1.64 indicate significant aggregation of infected plants (P=0.05)

m = number of infected plants; N = total number of plants; E (U) = expected number of runs; O(U) = observed number of runs; S(U) = standard deviation of runs; Z – z-statistic

. Q Q E M.C. Ordinary runs analysis of PNRSV (A & I) distribution in block one ('Victoria') in Bushy Park, Tasmania on 10th October 1996.

/KP Rows NDIUID	@HR TBKKW	S X	N	2 ? X	9 X	; ? X	Z
1	PNRSV[2]	19	51	24.84	13	3.30	-3.44*
2	PNRSV	18	51	24.29	18	3.22	-1.80*
3	PNRSV	27	51	26.41	21	3.52	-1.39
4	PNRSV	31	51	25.31	24	3.37	-0.24
5	PNRSV	26	51	26.49	29	3.53	0.85
6	PNRSV	40	51	18.25	19	2.37	0.53
7	PNRSV	31	51	25.31	18	3.37	-2.02*
8	PNRSV	24	51	26.41	17	3.52	-2.53*
9	PNRSV	30	51	25.71	22	3.42	-0.94
10	PNRSV	32	51	24.84	16	3.30	-2.53*
11	PNRSV	37	51	21.31	24	2.80	1.14
12	PNRSV	42	51	15.82	6	2.02	-4.61*
13	PNRSV	46	51	10.02	11	1.20	1.23
14	PNRSV	44	51	13.08	13	1.64	0.26
15	PNRSV	48	51	6.65	7	0.72	1.18
16	PNRSV	31	51	25.31	24	3.37	-0.24
17	PNRSV	31	51	25.31	15	3.37	-2.91*
18	PNRSV	47	51	8.37	8	0.97	0.13
19	PNRSV	31	51	25.31	12	3.37	-3.81*
20	PNRSV	28	51	26.25	16	3.50	-2.79*
21	PNRSV	33	51	24.29	14	3.22	-3.04*
22	PNRSV	21	51	25.71	19	3.42	-1.81
23	PNRSV	35	51	22.96	15	3.03	-2.46*
24	PNRSV	45	51	11.59	11	1.42	-0.06
25	PNRSV	50	51	2.96	3	0.19	2.78
Along Rows (Contiguous[1])	PNRSV	847	1275	569.65	384	15.92	-11.63*

Across Rows (Individually)	Virus	m	N	E(U)	O(U)	S(U)	Z
1	PNRSV	15	25	13.00	8	2.35	-1.92*
2	PNRSV	14	25	13.32	10	2.41	-1.17
3	PNRSV	14	25	13.32	12	2.41	-0.34
4	PNRSV	18	25	11.08	10	1.95	-0.30
5	PNRSV	19	25	10.12	10	1.76	0.22
6	PNRSV	15	25	13.00	8	2.35	-1.92*
7	PNRSV	17	25	11.88	12	2.12	0.29
8	PNRSV	16	25	12.52	10	2.25	-0.90
9	PNRSV	13	25	13.48	12	2.44	-0.40
10	PNRSV	15	25	13.00	14	2.35	0.64
11	PNRSV	16	25	12.52	12	2.25	-0.01
12	PNRSV	14	25	13.32	14	2.41	0.49
13	PNRSV	16	25	12.52	8	2.25	-1.79*
14	PNRSV	18	25	11.08	11	1.95	0.22
15	PNRSV	17	25	11.88	10	2.12	-0.65
16	PNRSV	18	25	11.08	11	1.95	0.22
17	PNRSV	15	25	13.00	12	2.35	-0.21
18	PNRSV	16	25	12.52	12	2.25	-0.01
19	PNRSV	20	25	9.00	11	1.53	1.64
20	PNRSV	17	25	11.88	11	2.12	-0.18
21	PNRSV	14	25	13.32	16	2.41	1.32
22	PNRSV	17	25	11.88	10	2.12	-0.65
23	PNRSV	17	25	11.88	8	2.12	-1.60
24	PNRSV	12	25	13.48	12	2.44	-0.40
25	PNRSV	14	25	13.32	10	2.41	-1.17
26	PNRSV	17	25	11.88	8	2.12	-1.60
27	PNRSV	15	25	13.00	11	2.35	-0.64
28	PNRSV	20	25	9.00	5	1.53	-2.29*
29	PNRSV	15	25	13.00	9	2.35	-1.49
30	PNRSV	12	25	13.48	9	2.44	-1.63
31	PNRSV	16	25	12.52	7	2.25	-2.23*
32	PNRSV	22	25	6.28	7	0.97	1.26
33	PNRSV	17	25	11.88	13	2.12	0.77
34	PNRSV	16	25	12.52	11	2.25	-0.45
35	PNRSV	19	25	10.12	11	1.76	0.79
36	PNRSV	16	25	12.52	11	2.25	-0.45
37	PNRSV	20	25	9.00	7	1.53	-0.98
38	PNRSV	21	25	7.72	9	1.27	1.41
39	PNRSV	20	25	9.00	7	1.53	-0.98
40	PNRSV	19	25	10.12	10	1.76	0.22
41	PNRSV	17	25	11.88	12	2.12	0.29
42	PNRSV	17	25	11.88	11	2.12	-0.18
43	PNRSV	20	25	9.00	8	1.53	-0.33
44	PNRSV	17	25	11.88	6	2.12	-2.54*
45	PNRSV	14	25	13.32	10	2.41	-1.17
46	PNRSV	14	25	13.32	16	2.41	1.32
47	PNRSV	17	25	11.88	11	2.12	-0.18
48	PNRSV	20	25	9.00	11	1.53	1.64
49	PNRSV	17	25	11.88	8	2.12	-1.60
50	PNRSV	17	25	11.88	10	2.12	-0.65
51	PNRSV	15	25	13.00	14	2.35	0.64
Across Rows (Contiguous[1])	PNRSV	847	1275	569.65	484	15.92	-5.35*

[1] rows combined by last plant of row *i* contiguous with first plant of row *i*+1
[2] apple and intermediate serotypes not differentiated
* Z-statistics less than −1.64 indicate significant aggregation of infected plants (P=0.05)
NA results from ordinary run analysis invalid due to either low (<5%) or high (>95%) disease incidence
M – number of infected plants; N – total number of plants; E(U) – expected number of runs; O(U) – observed number of runs, S(U) – standard deviation of runs; Z – z-statistic

. Q Q E C. HDMixary runs analysis of HpLV distribution in block one ('Victoria') in Bushy Park, Tasmania on 15th October 1997.

Across Rows (Individually)	Virus	m	N	E(U)	O(U)	S(U)	Z
1	HpLV	42	25	15.82	13	2.02	-1.15
2	HpLV	44	25	13.08	11	1.64	-0.96
3	HpLV	44	25	13.08	9	1.64	-2.19*
4	HpLV	39	25	19.35	12	2.52	-2.72*
5	HpLV	42	25	15.82	10	2.02	-2.63*
6	HpLV	47	25	8.37	9	0.97	1.16
7	HpLV	47	25	8.37	9	0.97	1.16
8	HpLV	44	25	13.08	7	1.64	-3.41*
9	HpLV	43	25	14.49	10	1.84	-2.17*
10	HpLV	28	25	26.25	10	3.50	-4.50*
11	HpLV	47	25	8.37	9	0.97	1.16
12	HpLV	51	25	NA	1	NA	NA
13	HpLV	49	25	4.84	5	0.47	1.41
14	HpLV	49	25	4.84	2	0.47	-5.01*
15	HpLV	50	25	2.96	2	0.19	-2.37*
16	HpLV	49	25	4.84	5	0.47	1.41
17	HpLV	46	25	10.02	11	1.20	1.23
18	HpLV	45	25	11.59	11	1.42	-0.06
19	HpLV	48	25	6.65	3	0.72	-4.34*
20	HpLV	48	25	6.65	5	0.72	-1.58
21	HpLV	49	25	4.84	3	0.47	-2.87*
22	HpLV	47	25	8.37	7	0.97	-0.90
23	HpLV	42	25	15.82	11	2.02	-2.14*
24	HpLV	43	25	14.49	12	1.84	-1.08
25	HpLV	40	25	18.25	7	2.37	-4.54*
Along Rows (Contiguous')	HpLV	1123	25	268.76	175	7.49	-12.46*

Across Rows (Individually)	Virus	m	N	E(U)	O(U)	S(U)	Z
1	HpLV	21	25	7.72	7	1.27	-0.17
2	HpLV	21	25	7.72	9	1.27	1.41
3	HpLV	17	25	11.88	10	2.12	-0.65
4	HpLV	21	25	7.72	8	1.27	0.62
5	HpLV	22	25	6.28	7	0.97	1.26
6	HpLV	22	25	6.28	7	0.97	1.26
7	HpLV	24	25	2.92	3	0.27	2.14
8	HpLV	23	25	4.68	5	0.64	1.28
9	HpLV	22	25	6.28	5	0.97	-0.80
10	HpLV	19	25	10.12	8	1.76	-0.92
11	HpLV	21	25	7.72	9	1.27	1.41
12	HpLV	18	25	11.08	9	1.95	-0.81
13	HpLV	20	25	9.00	8	1.53	-0.33
14	HpLV	22	25	6.28	7	0.97	1.26
15	HpLV	24	25	2.92	3	0.27	2.14
16	HpLV	22	25	6.28	5	0.97	-0.80
17	HpLV	22	25	6.28	7	0.97	1.26
18	HpLV	23	25	4.68	3	0.64	-1.84*
19	HpLV	22	25	6.28	5	0.97	-0.80
20	HpLV	22	25	6.28	6	0.97	0.23
21	HpLV	20	25	9.00	8	1.53	-0.33
22	HpLV	18	25	11.08	7	1.95	-1.83*
23	HpLV	18	25	11.08	13	1.95	1.24
24	HpLV	18	25	11.08	11	1.95	0.22
25	HpLV	22	25	6.28	6	0.97	0.23
26	HpLV	22	25	6.28	4	0.97	-1.83*
27	HpLV	23	25	4.68	4	0.64	-0.28
28	HpLV	21	25	7.72	8	1.27	0.62
29	HpLV	20	25	9.00	9	1.53	0.33
30	HpLV	23	25	4.68	5	0.64	1.28
31	HpLV	23	25	4.68	5	0.64	1.28
32	HpLV	23	25	4.68	5	0.64	1.28
33	HpLV	23	25	4.68	4	0.64	-0.28
34	HpLV	24	25	2.92	2	0.27	-1.55
35	HpLV	22	25	6.28	5	0.97	-0.80
36	HpLV	24	25	2.92	3	0.27	2.14
37	HpLV	24	25	2.92	3	0.27	2.14
38	HpLV	24	25	2.92	3	0.27	2.14
39	HpLV	22	25	6.28	5	0.97	-0.80
40	HpLV	22	25	6.28	7	0.97	1.26
41	HpLV	22	25	6.28	3	0.97	-2.86*
42	HpLV	23	25	4.68	3	0.64	-1.84*
43	HpLV	23	25	4.68	3	0.64	-1.84*
44	HpLV	23	25	4.68	5	0.64	1.28
45	HpLV	25	25	NA	1	NA	NA
46	HpLV	24	25	2.92	3	0.27	2.14
47	HpLV	24	25	2.92	3	0.27	2.14
48	HpLV	25	25	NA	1	NA	NA
49	HpLV	24	25	2.92	3	0.27	2.14
50	HpLV	24	25	2.92	3	0.27	2.14
51	HpLV	22	25	6.28	7	0.97	1.26
Across Rows (Contiguous[1])	HpLV	1123	1275	268.76	243	7.49	-3.37*

[1] rows combined by last plant of row *i* contiguous with first plant of row *i*+1

* Z-statistics less than –1.64 indicate significant aggregation of infected plants (P=0.05)

NA results from ordinary run analysis invalid due to either low (<5%) or high (>95%) disease incidence

m = number of infected plants; N = total number of plants; E (U) = expected number of runs; O(U) = observed number of runs; S(U) = standard deviation of runs; Z – z-statistic

Appendix 3.5. Ordinary runs analysis of HpMV distribution in block one ('Victoria') in Bushy Park, Tasmania on 15th October 1997.

Along Rows (Individually)	Virus	m	N	E(U)	O(U)	S(U)	Z
1	HpMV	48	51	6.65	7	0.72	1.18
2	HpMV	40	51	18.25	17	2.37	-0.32
3	HpMV	32	51	24.84	19	3.30	-1.62
4	HpMV	42	51	15.82	10	2.02	-2.63*
5	HpMV	34	51	23.67	19	3.13	-1.33
6	HpMV	47	51	8.37	9	0.97	1.16
7	HpMV	44	51	13.08	12	1.64	-0.35
8	HpMV	33	51	24.29	17	3.22	-2.11*
9	HpMV	35	51	22.96	19	3.03	-1.14
10	HpMV	43	51	14.49	11	1.84	-1.63
11	HpMV	45	51	11.59	10	1.42	-0.76
12	HpMV	46	51	10.02	11	1.20	1.23
13	HpMV	46	51	10.02	9	1.20	-0.43
14	HpMV	44	51	13.08	12	1.64	-0.35
15	HpMV	48	51	6.65	6	0.72	-0.20
16	HpMV	36	51	22.18	21	2.92	-0.23
17	HpMV	28	51	26.25	19	3.50	-1.93*
18	HpMV	40	51	18.25	12	2.37	-2.43*
19	HpMV	42	51	15.82	11	2.02	-2.14*
20	HpMV	45	51	11.59	9	1.42	-1.47
21	HpMV	41	51	17.08	13	2.20	-1.63
22	HpMV	39	51	19.35	17	2.52	-0.73
23	HpMV	37	51	21.31	23	2.80	0.78
24	HpMV	38	51	20.37	20	2.67	0.05
25	HpMV	12	51	19.35	11	2.52	-3.11*
Along Rows (Contiguous[1])	HpMV	985	1275	449.08	327	12.54	-9.70*

! #$,)#, (Individually)	i!u#,	m	,	E(U)	O(U)	S(U)	Z
1	HpMV	19	25	10.12	9	1.76	-0.35
2	HpMV	23	25	4.68	5	0.64	1.28
3	HpMV	19	25	10.12	11	1.76	0.79
4	HpMV	19	25	10.12	11	1.76	0.79
5	HpMV	14	25	13.32	15	2.41	0.90
6	HpMV	19	25	10.12	11	1.76	0.79
7	HpMV	19	25	10.12	11	1.76	0.79
8	HpMV	16	25	12.52	12	2.25	-0.01
9	HpMV	21	25	7.72	7	1.27	-0.17
10	HpMV	15	25	13.00	11	2.35	-0.64
11	HpMV	20	25	9.00	8	1.53	-0.33
12	HpMV	19	25	10.12	7	1.76	-1.49
13	HpMV	21	25	7.72	7	1.27	-0.17
14	HpMV	18	25	11.08	10	1.95	-0.30
15	HpMV	19	25	10.12	8	1.76	-0.92
16	HpMV	15	25	13.00	10	2.35	-1.07
17	HpMV	21	25	7.72	6	1.27	-0.96
18	HpMV	22	25	6.28	7	0.97	1.26
19	HpMV	18	25	11.08	8	1.95	-1.32
20	HpMV	21	25	7.72	8	1.27	0.62
21	HpMV	18	25	11.08	14	1.95	1.75
22	HpMV	18	25	11.08	12	1.95	0.73
23	HpMV	21	25	7.72	8	1.27	0.62
24	HpMV	20	25	9.00	8	1.53	-0.33
25	HpMV	21	25	7.72	8	1.27	0.62
26	HpMV	21	25	7.72	8	1.27	0.62
27	HpMV	19	25	10.12	12	1.76	1.35
28	HpMV	19	25	10.12	12	1.76	1.35
29	HpMV	21	25	7.72	8	1.27	0.62
30	HpMV	18	25	11.08	10	1.95	-0.30
31	HpMV	20	25	9.00	9	1.53	0.33
32	HpMV	18	25	11.08	10	1.95	-0.30
33	HpMV	17	25	11.88	12	2.12	0.29
34	HpMV	20	25	9.00	8	1.53	-0.33
35	HpMV	18	25	11.08	8	1.95	-1.32
36	HpMV	21	25	7.72	8	1.27	0.62
37	HpMV	20	25	9.00	10	1.53	0.98
38	HpMV	19	25	10.12	12	1.76	1.35
39	HpMV	17	25	11.88	12	2.12	0.29
40	HpMV	20	25	9.00	10	1.53	0.98
41	HpMV	15	25	13.00	10	2.35	-1.0 ,
42	HpMV	19	25	10.12	10	1.76	0.22
43	HpMV	17	25	11.88	15	2.12	1.71
44	HpMV	20	25	9.00	10	1.53	0.98
45	HpMV	20	25	9.00	8	1.53	-0.33
46	HpMV	20	25	9.00	10	1.53	0.98
47	HpMV	21	25	7.72	6	1.27	-0.96
48	HpMV	22	25	6.28	6	0.97	0.23
49	HpMV	22	25	6.28	6	0.97	0.23
50	HpMV	23	25	4.68	2	0.64	-3.40*
51	HpMV	22	25	6.28	7	0.97	1.26
Across Rows (Contiguous[1])	HpMV	985	1275	449.08	429	12.54	-1.56

[1] rows combined by l st of row conti uou s with "% plant of r w *i*+1

* -st ;%ics less than –1.64 indicate $ ni ic nt " (, of infected plants (*P*=0.05)

m = number ,infected plants; , = total number of plants; (U) = expected number runs; O(U) = observed number runs; S(U) = standard deviation ,runs; ,– z-statistic

Appendix 3.6. Ordinary runs analysis of PNRSV (A & I) distribution in block one ('Victoria') in Bushy Park, Tasmania on 15th October 1997.

Along Rows (Individually)	Virus	m	N	E(U)	O(U)	S(U)	Z
1	PNRSV[2]	47	51	8.37	9	0.97	1.16
2	PNRSV	44	51	13.08	9	1.64	-2.19*
3	PNRSV	49	51	4.84	3	0.47	-2.87*
4	PNRSV	50	51	2.96	3	0.19	2.78
5	PNRSV	39	51	19.35	18	2.52	-0.34
6	PNRSV	51	51	NA	1	NA	NA
7	PNRSV	36	51	22.18	20	2.92	-0.57
8	PNRSV	40	51	18.25	9	2.37	-3.70*
9	PNRSV	44	51	13.08	10	1.64	-1.58
10	PNRSV	50	51	2.96	3	0.19	2.78
11	PNRSV	46	51	10.02	8	1.20	-1.26
12	PNRSV	45	51	11.59	10	1.42	-0.76
13	PNRSV	46	51	10.02	11	1.20	1.23
14	PNRSV	45	51	11.59	8	1.42	-2.17*
15	PNRSV	50	51	2.96	3	0.19	2.78
16	PNRSV	50	51	2.96	3	0.19	2.78
17	PNRSV	48	51	6.65	5	0.72	-1.58
18	PNRSV	51	51	NA	1	NA	NA
19	PNRSV	47	51	8.37	5	0.97	-2.96*
20	PNRSV	51	51	NA	1	NA	NA
21	PNRSV	50	51	2.96	3	0.19	2.78
22	PNRSV	47	51	8.37	3	0.97	-5.03*
23	PNRSV	45	51	11.59	11	1.42	-0.06
24	PNRSV	47	51	8.37	9	0.97	1.16
25	PNRSV	51	51	NA	1	NA	NA
Along Rows (Contiguous[1])	PNRSV	1169	1275	195.38	151	5.43	-8.08*

Across Rows (Individually)	Virus	m	N	E(U)	O(U)	S(U)	Z
1	PNRSV	20	25	9.00	7	1.53	-0.98
2	PNRSV	21	25	7.72	5	1.27	-1.75*
3	PNRSV	21	25	7.72	6	1.27	-0.96
4	PNRSV	23	25	4.68	5	0.64	1.28
5	PNRSV	24	25	2.92	3	0.27	2.14
6	PNRSV	23	25	4.68	5	0.64	1.28
7	PNRSV	23	25	4.68	5	0.64	1.28
8	PNRSV	22	25	6.28	3	0.97	-2.86*
9	PNRSV	23	25	4.68	5	0.64	1.28
10	PNRSV	22	25	6.28	7	0.97	1.26
11	PNRSV	20	25	9.00	9	1.53	0.33
12	PNRSV	23	25	4.68	5	0.64	1.28
13	PNRSV	24	25	2.92	3	0.27	2.14
14	PNRSV	25	25	NA	1	NA	NA
15	PNRSV	23	25	4.68	5	0.64	1.28
16	PNRSV	23	25	4.68	5	0.64	1.28
17	PNRSV	24	25	2.92	3	0.27	2.14
18	PNRSV	23	25	4.68	5	0.64	1.28
19	PNRSV	22	25	6.28	7	0.97	1.26
20	PNRSV	24	25	2.92	3	0.27	2.14
21	PNRSV	22	25	6.28	6	0.97	0.23
22	PNRSV	23	25	4.68	5	0.64	1.28
23	PNRSV	23	25	4.68	5	0.64	1.28
24	PNRSV	23	25	4.68	5	0.64	1.28
25	PNRSV	23	25	4.68	5	0.64	1.28
26	PNRSV	24	25	2.92	3	0.27	2.14
27	PNRSV	22	25	6.28	4	0.97	-1.83*
28	PNRSV	21	25	7.72	9	1.27	1.41
29	PNRSV	21	25	7.72	9	1.27	1.41
30	PNRSV	24	25	2.92	3	0.27	2.14
31	PNRSV	24	25	2.92	3	0.27	2.14
32	PNRSV	25	25	NA	1	NA	NA
33	PNRSV	24	25	2.92	3	0.27	2.14
34	PNRSV	25	25	NA	1	NA	NA
35	PNRSV	24	25	2.92	3	0.27	2.14
36	PNRSV	22	25	6.28	5	0.97	-0.80
37	PNRSV	24	25	2.92	3	0.27	2.14
38	PNRSV	25	25	NA	1	NA	NA
39	PNRSV	25	25	NA	1	NA	NA
40	PNRSV	23	25	4.68	4	0.64	-0.28
41	PNRSV	22	25	6.28	7	0.97	1.26
42	PNRSV	25	25	NA	1	NA	NA
43	PNRSV	24	25	2.92	3	0.27	2.14
44	PNRSV	24	25	2.92	3	0.27	2.14
45	PNRSV	21	25	7.72	9	1.27	1.41
46	PNRSV	20	25	9.00	11	1.53	1.64
47	PNRSV	23	25	4.68	5	0.64	1.28
48	PNRSV	23	25	4.68	5	0.64	1.28
49	PNRSV	24	25	2.92	3	0.27	2.14
50	PNRSV	23	25	4.68	3	0.64	-1.84*
51	PNRSV	20	25	9.00	11	1.53	1.64
Across Rows (Contiguous[1])	PNRSV	1169	1275	195.38	191	5.43	-0.71

[1] rows combined by last plant of row *i* contiguous with first plant of row *i*+1
[2] apple and intermediate serotypes not differentiated
* Z-statistics less than –1.64 indicate significant aggregation of infected plants (P=0.05)
NA results from ordinary run analysis invalid due to either low (<5%) or high (>95%) disease incidence
m = number of infected plants; N = total number of plants; E (U) = expected number of runs; O(U) = observed number of runs; S(U) = standard deviation of runs; Z – z-statistic

Appendix 3.7. Ordinary runs analysis of HpLV distribution in block two ('Victoria') in Myrtleford, Victoria on 21st October 1998.

Along Rows (Individually)	Virus	m	N	E(U)[1]	O(U)	S(U)	Z[2]
1	HpLV	3	20	6.10	7	1.05	1.33
2	HpLV	3	20	6.10	5	1.05	-0.57
3	HpLV	2	20	4.60	5	0.70	1.28
4	HpLV	2	20	4.60	5	0.70	1.28
5	HpLV	4	20	7.40	8	1.35	0.82
6	HpLV	5	20	8.50	9	1.60	0.62
7	HpLV	4	20	7.40	9	1.35	1.56
8	HpLV	1	20	2.90	2	0.30	-1.33
9	HpLV	2	20	4.60	3	0.70	-1.57
10	HpLV	3	20	6.10	5	1.05	-0.57
11	HpLV	3	20	6.10	6	1.05	0.38
12	HpLV	2	20	4.60	5	0.70	1.28
13	HpLV	1	20	2.90	3	0.30	2.00
14	HpLV	2	20	4.60	5	0.70	1.28
15	HpLV	5	20	8.50	6	1.60	-1.25
16	HpLV	4	20	7.40	8	1.35	0.82
17	HpLV	3	20	6.10	4	1.05	-1.53
18	HpLV	7	20	10.10	8	1.97	-0.81
19	HpLV	3	20	6.10	7	1.05	1.33
20	HpLV	3	20	6.10	5	1.05	-0.57
21	HpLV	1	20	2.90	2	0.30	-1.33
22	HpLV	5	20	8.50	8	1.60	0.00
23	HpLV	5	20	8.50	7	1.60	-0.62
24	HpLV	2	20	4.60	5	0.70	1.28
25	HpLV	9	20	10.90	13	2.15	1.21
Along Rows (Contiguous[1])	HpLV	84	500	140.78	130	6.23	-1.65*
Across Rows (Individually)	Virus	m	N	E(U)	O(U)	S(U)	Z
1	HpLV	4	25	7.72	6	1.27	-0.96
2	HpLV	1	25	2.92	2	0.27	-1.55
3	HpLV	4	25	7.72	9	1.27	1.41
4	HpLV	3	25	6.28	6	0.97	0.23
5	HpLV	1	25	2.92	2	0.27	-1.55
6	HpLV	2	25	4.68	4	0.64	-0.28
7	HpLV	3	25	6.28	5	0.97	-0.80
8	HpLV	6	25	10.12	10	1.76	0.22
9	HpLV	4	25	7.72	7	1.27	-0.17
10	HpLV	4	25	7.72	7	1.27	-0.17
11	HpLV	3	25	6.28	5	0.97	-0.80
12	HpLV	4	25	7.72	5	1.27	-1.75
13	HpLV	4	25	7.72	9	1.27	1.41
14	HpLV	10	25	13	14	2.35	0.64
15	HpLV	3	25	6.28	7	0.97	1.26
16	HpLV	6	25	10.12	10	1.76	0.22
17	HpLV	5	25	9	7	1.53	-0.98
18	HpLV	5	25	9	8	1.53	-0.33
19	HpLV	3	25	6.28	5	0.97	-0.80
20	HpLV	9	25	12.52	10	2.25	-0.90
Across Rows – (Contiguous[1])	HpLV	84	500	140.776	129	6.23	-1.81*

[1] rows combined by last plant of row i contiguous with first plant of row $i+1$

[2] Z-statistics less than −1.64 indicate significant aggregation of infected plants (P=0.05)

m = number of infected plants; N = total number of plants; E (U) = expected number of runs, O(U) = observed number of runs, S(U) = standard deviation of runs, Z = z-statistic

Appendix 3.8. Ordinary run analysis of HpMV distribution in block two ('Victoria') in Myrtleford, Victoria on 21st October 1998.

Along Rows (Individually)	Virus	m	N	E(U)	O(U)	S(U)	Z
1	HpMV	2	20	4.60	5	0.70	1.28
2	HpMV	3	20	6.10	5	1.05	-0.57
3	HpMV	2	20	4.60	5	0.70	1.28
4	HpMV	2	20	4.60	5	0.70	1.28
5	HpMV	3	20	6.10	6	1.05	0.38
6	HpMV	5	20	8.50	9	1.60	0.62
7	HpMV	2	20	4.60	5	0.70	1.28
8	HpMV	1	20	2.90	2	0.30	-1.33
9	HpMV	2	20	4.60	3	0.70	-1.57
10	HpMV	3	20	6.10	5	1.05	-0.57
11	HpMV	2	20	4.60	5	0.70	1.28
12	HpMV	2	20	4.60	4	0.70	-0.14
13	HpMV	1	20	2.90	3	0.30	2.00
14	HpMV	1	20	2.90	3	0.30	2.00
15	HpMV	1	20	2.90	2	0.30	-1.33
16	HpMV	5	20	8.50	8	1.60	0.00
17	HpMV	3	20	6.10	4	1.05	-1.53
18	HpMV	5	20	8.50	10	1.60	1.25
19	HpMV	3	20	6.10	7	1.05	1.33
20	HpMV	2	20	4.60	5	0.70	1.28
21	HpMV	0	20	NA	1	NA	NA
22	HpMV	2	20	4.60	5	0.70	1.28
23	HpMV	4	20	7.40	7	1.35	0.07
24	HpMV	1	20	2.90	3	0.30	2.00
25	HpMV	1	20	2.90	3	0.30	2.00
Along Rows (Contiguous[1])	HpMV	58	500	103.54	101	4.57	-0.45
Across Rows (Individually)	Virus	m	N	E(U)	O(U)	S(U)	Z
1	HpMV	2	25	4.68	5	0.64	1.28
2	HpMV	0	25	NA	1	NA	NA
3	HpMV	3	25	6.28	7	0.97	1.26
4	HpMV	1	25	2.92	3	0.27	2.14
5	HpMV	0	25	NA	1	NA	NA
6	HpMV	2	25	4.68	4	0.64	-0.28
7	HpMV	2	25	4.68	3	0.64	-1.84*
8	HpMV	4	25	7.72	8	1.27	0.62
9	HpMV	3	25	6.28	5	0.97	-0.80
10	HpMV	4	25	7.72	7	1.27	-0.17
11	HpMV	3	25	6.28	5	0.97	-0.80
12	HpMV	3	25	6.28	4	0.97	-1.83*
13	HpMV	3	25	6.28	7	0.97	1.26
14	HpMV	8	25	11.88	13	2.12	0.77
15	HpMV	3	25	6.28	7	0.97	1.26
16	HpMV	3	25	6.28	7	0.97	1.26
17	HpMV	3	25	6.28	7	0.97	1.26
18	HpMV	2	25	4.68	5	0.64	1.28
19	HpMV	2	25	4.68	5	0.64	1.28
20	HpMV	7	25	11.08	9	1.95	-0.81
Across Rows (Contiguous[1])	HpMV	58	500	103.544	97	4.57	-1.32

[1] rows combined by last plant of row *i* contiguous with first plant of row *i*+1

* Z-statistics less than –1.64 indicate significant aggregation of infected plants (P=0.05)

NA results from ordinary run analysis invalid due to either low (<5%) or high (>95%) disease incidence

m = number of infected plants; N = total number of plants; E (U) = expected number of runs; O(U) = observed number of runs; S(U) = standard deviation of runs; Z – z-statistic

Appendix 3.9. Ordinary run analysis of PNRSV (apple, 'A' and intermediate, 'I' serotypes) in block two ('Victoria') in Myrtleford, Victoria on 21st October 1998.

Along Rows (Individually)	Virus	m	N	E(U)	O(U)	S(U)	Z
1	PNRSV[2]	10	20	11.00	12	2.18	0.69
2	PNRSV	11	20	10.90	8	2.15	-1.11
3	PNRSV	7	20	10.10	7	1.97	-1.32
4	PNRSV	6	20	9.40	10	1.81	0.61
5	PNRSV	9	20	10.90	11	2.15	0.28
6	PNRSV	7	20	10.10	8	1.97	-0.81
7	PNRSV	7	20	10.10	4	1.97	-2.84*
8	PNRSV	10	20	11.00	5	2.18	-2.53*
9	PNRSV	10	20	11.00	5	2.18	-2.53*
10	PNRSV	8	20	10.60	3	2.08	-3.41*
11	PNRSV	7	20	10.10	9	1.97	-0.30
12	PNRSV	6	20	9.40	7	1.81	-1.05
13	PNRSV	3	20	6.10	4	1.05	-1.53
14	PNRSV	7	20	10.10	6	1.97	-1.83*
15	PNRSV	5	20	8.50	3	1.60	-3.12*
16	PNRSV	3	20	6.10	7	1.05	1.33
17	PNRSV	5	20	8.50	6	1.60	-1.25
18	PNRSV	1	20	2.90	2	0.30	-1.33
19	PNRSV	8	20	10.60	4	2.08	-2.93*
20	PNRSV	5	20	8.50	6	1.60	-1.25
21	PNRSV	2	20	4.60	4	0.70	-0.14
22	PNRSV	8	20	10.60	4	2.08	-2.93*
23	PNRSV	2	20	4.60	2	0.70	-2.99*
24	PNRSV	3	20	6.10	5	1.05	-0.57
25	PNRSV	7	20	10.10	9	1.97	-0.30
Along Rows (Contiguous[1])	PNRSV	157	500	216.40	136	9.62	-8.31*
Across Rows (Individually)	Virus	m	N	E(U)	O(U)	S(U)	Z
1	PNRSV	18	25	11.08	8	1.95	-1.32
2	PNRSV	12	25	13.48	7	2.44	-2.45*
3	PNRSV	12	25	13.48	9	2.44	-1.63
4	PNRSV	15	25	13	11	2.35	-0.64
5	PNRSV	9	25	12.52	4	2.25	-3.57*
6	PNRSV	4	25	7.72	6	1.27	-0.96
7	PNRSV	2	25	4.68	4	0.64	-0.28
8	PNRSV	4	25	7.72	5	1.27	-1.75*
9	PNRSV	4	25	7.72	3	1.27	-3.33*
10	PNRSV	7	25	11.08	6	1.95	-2.35*
11	PNRSV	7	25	11.08	10	1.95	-0.30
12	PNRSV	2	25	4.68	3	0.64	-1.84*
13	PNRSV	5	25	9	9	1.53	0.33
14	PNRSV	8	25	11.88	10	2.12	-0.65
15	PNRSV	9	25	12.52	12	2.25	-0.01
16	PNRSV	8	25	11.88	14	2.12	1.24
17	PNRSV	5	25	9	11	1.53	1.64
18	PNRSV	10	25	13	6	2.35	-2.77*
19	PNRSV	6	25	10.12	6	1.76	-2.06*
20	PNRSV	10	25	13	11	2.35	-0.64
Across Rows (Contiguous[1])	PNRSV	157	500	216.404	144	9.62	-7.47*

[1] rows combined by last plant of row *i* contiguous with first plant of row *i*+1

[2] apple and intermediate serotypes not differentiated

Z-statistics less than −1.64 indicate significant aggregation of infected plants (P=0.05)

NA results from ordinary run analysis invalid due to either low (<5%) or high (>95%) disease incidence

m = number of infected plants; N = total number of plants; E (U) = expected number of runs; O(U) = observed number of runs; S(U) = standard deviation of runs; Z – z-statistic

Appendix 3.10. Ordinary run analysis of HpLV distributions in block three (cultivar x row spacing trial) in Bushy Park, Tasmania on 1st November 1996 and 4th November 1997.

1996 Along Rows (Replicate 1) – Contiguous[1]										
Row Spacing	Cultivar	Virus	m	N	(N-m)	2*m* (N-m)	E(U)	O(U)	S(U)	Z
3x2	Opal	HpLV	5	102	97	970	10.51	9	0.90	-1.69*
3x2	T11	HpLV	28	102	74	4144	41.63	28	3.99	-3.41*
3x2	T25	HpLV	28	90	62	3472	39.58	28	4.04	-2.87*
3x2	Victoria	HpLV	39	90	51	3978	45.20	31	4.63	-3.07*
6	Opal	HpLV	6	90	84	1008	12.20	11	1.13	-1.06
6	T11	HpLV	66	102	36	4752	47.59	39	4.59	-1.87*
6	T25	HpLV	33	90	57	3762	42.80	26	4.38	-3.84*
6	Victoria	HpLV	88	102	14	2464	25.16	16	2.35	-3.89*
5	Opal	HpLV	0	85	85	0	1.00	0	0.00	NA
5	T11	HpLV	15	75	60	1800	25.00	25	2.73	0.00
5	T25	HpLV	31	85	54	3348	40.39	18	4.24	-5.28*
5	Victoria	HpLV	16	75	59	1888	26.17	27	2.87	0.29
4	Opal	HpLV	5	68	63	630	10.26	11	1.07	0.69
4	T11	HpLV	17	60	43	1462	25.37	19	3.11	-2.05*
4	T25	HpLV	9	68	59	1062	16.62	7	1.85	-5.21*
4	Victoria	HpLV	16	60	44	1408	24.47	21	2.99	-1.16
3	Opal	HpLV	0	45	45	0	1.00	0	0.00	NA
3	T11	HpLV	18	45	27	972	22.60	21	3.18	-0.50
3	T25	HpLV	20	51	31	1240	25.31	18	3.37	-2.17*
3	Victoria	HpLV	12	51	39	936	19.35	17	2.52	-0.93
1996 Across Rows (Replicate 1) – Contiguous[1]										
3x2	Opal	HpLV	5	102	97	970	10.51	7	0.90	-3.92*
3x2	T11	HpLV	28	102	74	4144	41.63	30	3.99	-2.91*
3x2	T25	HpLV	28	90	62	3472	39.58	24	4.04	-3.86*
3x2	Victoria	HpLV	39	90	51	3978	45.20	31	4.63	-3.07*
6	Opal	HpLV	6	90	84	1008	12.20	11	1.13	-1.06
6	T11	HpLV	66	102	36	4752	47.59	45	4.59	-0.56
6	T25	HpLV	33	90	57	3762	42.80	30	4.38	-2.92*
6	Victoria	HpLV	88	102	14	2464	25.16	19	2.35	-2.62*
5	Opal	HpLV	0	85	85	0	1.00	0	0.00	NA
5	T11	HpLV	15	75	60	1800	25.00	23	2.73	-0.73
5	T25	HpLV	31	85	54	3348	40.39	27	4.24	-3.16*
5	Victoria	HpLV	16	75	59	1888	26.17	25	2.87	-0.41
4	Opal	HpLV	5	68	63	630	10.26	9	1.07	-1.18
4	T11	HpLV	17	60	43	1462	25.37	23	3.11	-0.76
4	T25	HpLV	9	68	59	1062	16.62	15	1.85	-0.88
4	Victoria	HpLV	16	60	44	1408	24.47	25	2.99	0.18
3	Opal	HpLV	0	45	45	0	1.00	0	0.00	NA
3	T11	HpLV	18	45	27	972	22.60	21	3.18	-0.50
3	T25	HpLV	20	51	31	1240	25.31	20	3.37	-1.58
3	Victoria	HpLV	12	51	39	936	19.35	23	2.52	1.45

1997 Along Rows (Replicate 1) – Contiguous[1]										
Row Spacing	Cultivar	Virus	m	N	(N-m)	2*m* (N-m)	E(U)	O(U)	S(U)	Z
3x2	Opal	HpLV	12	102	90	2160	22.18	19	2.06	-1.54
3x2	T11	HpLV	39	102	63	4914	49.18	38	4.74	-2.36*
3x2	T25	HpLV	39	90	51	3978	45.20	34	4.63	-2.42*
3x2	Victoria	HpLV	45	90	45	4050	46.00	31	4.72	-3.18*
6	Opal	HpLV	10	90	80	1600	18.78	15	1.83	-2.06*
6	T11	HpLV	86	102	16	2752	27.98	25	2.63	-1.13
6	T25	HpLV	41	90	49	4018	45.64	29	4.68	-3.56*
6	Victoria	HpLV	90	102	12	2160	22.18	10	2.06	-5.92*
5	Opal	HpLV	1	85	84	168	2.98	3	0.15	NA
5	T11	HpLV	24	75	51	2448	33.64	29	3.74	-1.24
5	T25	HpLV	43	85	42	3612	43.49	24	4.58	-4.25*
5	Victoria	HpLV	29	75	46	2668	36.57	41	4.08	1.09
4	Opal	HpLV	6	68	62	744	11.94	11	1.27	-0.74
4	T11	HpLV	24	60	36	1728	29.80	20	3.68	-2.66*
4	T25	HpLV	18	68	50	1800	27.47	19	3.17	-2.67*
4	Victoria	HpLV	27	60	33	1782	30.70	16	3.80	-3.87*
3	Opal	HpLV	0	45	45	0	1.00	0	0.00	NA
3	T11	HpLV	28	45	17	952	22.16	20	3.11	-0.69
3	T25	HpLV	33	51	18	1188	24.29	14	3.22	-3.19*
3	Victoria	HpLV	16	51	35	1120	22.96	22	3.03	-0.32
1997 Across Rows (Replicate 1) – Contiguous[1]										
3x2	Opal	HpLV	12	102	90	2160	22.18	21	2.06	-0.57
3x2	T11	HpLV	39	102	63	4914	49.18	42	4.74	-1.51
3x2	T25	HpLV	39	90	51	3978	45.20	27	4.63	-3.93*
3x2	Victoria	HpLV	45	90	45	4050	46.00	35	4.72	-2.33*
6	Opal	HpLV	10	90	80	1600	18.78	17	1.83	-0.97
6	T11	HpLV	86	102	16	2752	27.98	21	2.63	-2.65*
6	T25	HpLV	41	90	49	4018	45.64	28	4.68	-3.77*
6	Victoria	HpLV	90	102	12	2160	22.18	16	2.06	-3.00*
5	Opal	HpLV	1	85	84	168	2.98	3	0.15	NA
5	T11	HpLV	24	75	51	2448	33.64	37	3.74	0.90
5	T25	HpLV	43	85	42	3612	43.49	28	4.58	-3.38*
5	Victoria	HpLV	29	75	46	2668	36.57	31	4.08	-1.37
4	Opal	HpLV	6	68	62	744	11.94	11	1.27	-0.74
4	T11	HpLV	24	60	36	1728	29.80	24	3.68	-1.57
4	T25	HpLV	18	68	50	1800	27.47	29	3.17	0.48
4	Victoria	HpLV	27	60	33	1782	30.70	30	3.80	-0.18
3	Opal	HpLV	0	45	45	0	1.00	0	0.00	NA
3	T11	HpLV	28	45	17	952	22.16	23	3.11	0.27
3	T25	HpLV	33	51	18	1188	24.29	27	3.22	0.84
3	Victoria	HpLV	16	51	35	1120	22.96	25	3.03	0.67

1996 Along Rows (Replicate Contiguous[1]										
Row Spacing	Cultivar	Virus	L X	6 X	6 L	X L X 6 L X	E(U)	O(U)	S(U)	Z
3x2	Opal	HpLV	1	90	89	178	2.98	3	0.15	NA
3x2	T11	HpLV	11	102	91	2002	20.63	19	1.90	-0.86
3x2	T25	HpLV	8	102	94	1504	15.75	11	1.42	-3.35*
3x2	Victoria	HpLV	22	90	68	2992	34.24	25	3.47	-2.66*
6	Opal	HpLV	6	90	84	1008	12.20	6	1.13	-5.47*
6	T11	HpLV	49	102	53	5194	51.92	36	5.02	-3.17*
6	T25	HpLV	17	90	73	2482	28.58	21	2.87	-2.64*
6	Victoria	HpLV	58	102	44	5104	51.04	47	4.93	-0.82
5	Opal	HpLV	2	75	73	292	4.89	3	0.39	NA
5	T11	HpLV	7	75	68	952	13.69	9	1.42	-3.31*
5	T25	HpLV	13	85	72	1872	23.02	16	2.35	-2.99*
5	Victoria	HpLV	13	85	72	1872	23.02	19	2.35	-1.71*
4	Opal	HpLV	7	68	61	854	13.56	10	1.47	-2.42*
4	T11	HpLV	8	60	52	832	14.87	13	1.74	-1.07
4	T25	HpLV	21	68	47	1974	30.03	15	3.48	-4.31*
4	Victoria	HpLV	22	60	38	1672	28.87	15	3.56	-3.89*
3	Opal	HpLV	9	51	42	756	15.82	9	2.02	-3.37*
3	T11	HpLV	32	51	19	1216	24.84	11	3.30	-4.19*
3	T25	HpLV	23	45	22	1012	23.49	13	3.31	-3.16*
3	Victoria	HpLV	22	45	23	1012	23.49	14	3.31	-2.86*
1996 Across Rows (Replicate Contiguous[1]										
3x2	Opal	HpLV	1	90	89	178	2.98	3	0.15	0.15
3x2	T11	HpLV	11	102	91	2002	20.63	17	1.90	-1.91*
3x2	T25	HpLV	8	102	94	1504	15.75	15	1.42	-0.53
3x2	Victoria	HpLV	22	90	68	2992	34.24	29	3.47	-1.51
6	Opal	HpLV	6	90	84	1008	12.20	12	1.13	-0.18
6	T11	HpLV	49	102	53	5194	51.92	46	5.02	-1.18
6	T25	HpLV	17	90	73	2482	28.58	21	2.87	-2.64*
6	Victoria	HpLV	58	102	44	5104	51.04	50	4.93	-0.21
5	Opal	HpLV	2	75	73	292	4.89	5	0.39	NA
5	T11	HpLV	7	75	68	952	13.69	13	1.42	-0.49
5	T25	HpLV	13	85	72	1872	23.02	24	2.35	0.42
5	Victoria	HpLV	13	85	72	1872	23.02	27	2.35	1.69
4	Opal	HpLV	7	68	61	854	13.56	13	1.47	-0.38
4	T11	HpLV	8	60	52	832	14.87	9	1.74	-3.37*
4	T25	HpLV	21	68	47	1974	30.03	21	3.48	-2.59*
4	Victoria	HpLV	22	60	38	1672	28.87	26	3.56	-0.80
3	Opal	HpLV	9	51	42	756	15.82	15	2.02	-0.41
3	T11	HpLV	32	51	19	1216	24.84	19	3.30	-1.77*
3	T25	HpLV	23	45	22	1012	23.49	23	3.31	-0.15
3	Victoria	HpLV	22	45	23	1012	23.49	18	3.31	-1.66*

1997 Along Rows (Replicate 2) – Contiguous[1]										
Row Spacing	Cultivar	Virus	m	N	(N-m)	2*m* (N-m)	E(U)	O(U)	S(U)	Z
3x2	Opal	HpLV	3	90	87	522	6.80	9	0.56	NA
3x2	T11	HpLV	18	102	84	3024	30.65	23	2.90	-2.64*
3x2	T25	HpLV	8	102	94	1504	15.75	11	1.42	-3.35*
3x2	Victoria	HpLV	26	90	64	3328	37.98	27	3.87	-2.84*
6	Opal	HpLV	8	90	82	1312	15.58	10	1.49	-3.74*
6	T11	HpLV	60	102	42	5040	50.41	42	4.87	-1.73*
6	T25	HpLV	30	90	60	3600	41.00	34	4.19	-1.67*
6	Victoria	HpLV	75	102	27	4050	40.71	32	3.90	-2.23*
5	Opal	HpLV	2	75	73	292	4.89	3	0.39	NA
5	T11	HpLV	10	75	65	1300	18.33	15	1.96	-1.70*
5	T25	HpLV	24	85	61	2928	35.45	24	3.70	-3.09*
5	Victoria	HpLV	21	85	64	2688	32.62	29	3.40	-1.07
4	Opal	HpLV	9	68	59	1062	16.62	12	1.85	-2.50*
4	T11	HpLV	15	60	45	1350	23.50	18	2.86	-1.92*
4	T25	HpLV	23	68	45	2070	31.44	15	3.66	-4.50*
4	Victoria	HpLV	26	60	34	1768	30.47	14	3.77	-4.37*
3	Opal	HpLV	11	51	40	880	18.25	11	2.37	-3.06*
3	T11	HpLV	38	51	13	988	20.37	13	2.67	-2.76*
3	T25	HpLV	26	45	19	988	22.96	15	3.23	-2.46*
3	Victoria	HpLV	27	45	18	972	22.60	16	3.18	-2.08*
1997 Across Rows (Replicate 2) – Contiguous[1]										
3x2	Opal	HpLV	3	90	87	522	6.80	9	0.56	NA
3x2	T11	HpLV	18	102	84	3024	30.65	29	2.90	-0.57
3x2	T25	HpLV	8	102	94	1504	15.75	15	1.42	-0.53
3x2	Victoria	HpLV	26	90	64	3328	37.98	31	3.87	-1.80*
6	Opal	HpLV	8	90	82	1312	15.58	14	1.49	-1.06
6	T11	HpLV	60	102	42	5040	50.41	38	4.87	-2.55*
6	T25	HpLV	30	90	60	3600	41.00	35	4.19	-1.43
6	Victoria	HpLV	75	102	27	4050	40.71	30	3.90	-2.74*
5	Opal	HpLV	2	75	73	292	4.89	5	0.39	NA
5	T11	HpLV	10	75	65	1300	18.33	21	1.96	1.36
5	T25	HpLV	24	85	61	2928	35.45	34	3.70	-0.39
5	Victoria	HpLV	21	85	64	2688	32.62	33	3.40	0.11
4	Opal	HpLV	9	68	59	1062	16.62	11	1.85	-3.04*
4	T11	HpLV	15	60	45	1350	23.50	20	2.86	-1.22
4	T25	HpLV	23	68	45	2070	31.44	21	3.66	-2.85*
4	Victoria	HpLV	26	60	34	1768	30.47	26	3.77	-1.18
3	Opal	HpLV	11	51	40	880	18.25	16	2.37	-0.95
3	T11	HpLV	38	51	13	988	20.37	15	2.67	-2.01*
3	T25	HpLV	26	45	19	988	22.96	26	3.23	0.94
3	Victoria	HpLV	27	45	18	972	22.60	17	3.18	-1.76*

[1] rows combined by last plant of row *i* contiguous with first plant of row *i*+1

* Z-statistics less than –1.64 indicate significant aggregation of infected plants (P=0.05)

NA results from ordinary run analysis invalid due to either low (<5%) or high (>95%) disease incidence

Appendix 3.11. Ordinary run analysis of HpMV distributions in block three (cultivar x row spacing trial) in Bushy Park, Tasmania on 1st November 1996 and 4th November 1997.

1996 Along Rows (Replicate 1) – Contiguous[1]										
Row Spacing	Cultivar	Virus	m	N	(N-m)	2*m*(N-m)	E(U)	O(U)	S(U)	Z
3x2	Opal	HpMV	21	102	81	3402	34.35	28	3.27	-1.94*
3x2	T11	HpMV	39	102	63	4914	49.18	34	4.74	-3.20*
3x2	T25	HpMV	29	90	61	3538	40.31	34	4.11	-1.53
3x2	Victoria	HpMV	51	90	39	3978	45.20	31	4.63	-3.07*
6	Opal	HpMV	22	90	68	2992	34.24	35	3.47	0.22
6	T11	HpMV	45	102	57	5130	51.29	33	4.95	-3.69*
6	T25	HpMV	22	90	68	2992	34.24	30	3.47	-1.22
6	Victoria	HpMV	70	102	32	4480	44.92	30	4.32	-3.45*
5	Opal	HpMV	1	85	84	168	2.98	3	0.15	NA
5	T11	HpMV	7	75	68	952	13.69	13	1.42	-0.49
5	T25	HpMV	32	85	53	3392	40.91	22	4.30	-4.40*
5	Victoria	HpMV	9	75	66	1188	16.84	15	1.78	-1.03
4	Opal	HpMV	6	68	62	744	11.94	11	1.27	-0.74
4	T11	HpMV	10	60	50	1000	17.67	13	2.10	-2.22*
4	T25	HpMV	7	68	61	854	13.56	9	1.47	-3.10*
4	Victoria	HpMV	6	60	54	648	11.80	9	1.34	-2.09*
3	Opal	HpMV	15	45	30	900	21.00	15	2.94	-2.04*
3	T11	HpMV	2	45	43	172	4.82	5	0.50	NA
3	T25	HpMV	12	51	39	936	19.35	20	2.52	0.26
3	Victoria	HpMV	17	51	34	1156	23.67	19	3.13	-1.49
1996 Across Rows (Replicate 1) – Contiguous[1]										
3x2	Opal	HpMV	21	102	81	3402	34.35	28	3.27	-1.94*
3x2	T11	HpMV	39	102	63	4914	49.18	38	4.74	-2.36*
3x2	T25	HpMV	29	90	61	3538	40.31	32	4.11	-2.02*
3x2	Victoria	HpMV	51	90	39	3978	45.20	39	4.63	-1.34
6	Opal	HpMV	22	90	68	2992	34.24	33	3.47	-0.36
6	T11	HpMV	45	102	57	5130	51.29	44	4.95	-1.47
6	T25	HpMV	22	90	68	2992	34.24	28	3.47	-1.80*
6	Victoria	HpMV	70	102	32	4480	44.92	33	4.32	-2.76*
5	Opal	HpMV	1	85	84	168	2.98	3	0.15	NA
5	T11	HpMV	7	75	68	952	13.69	13	1.42	-0.49
5	T25	HpMV	32	85	53	3392	40.91	29	4.30	-2.77*
5	Victoria	HpMV	9	75	66	1188	16.84	15	1.78	-1.03
4	Opal	HpMV	6	68	62	744	11.94	9	1.27	-2.31*
4	T11	HpMV	10	60	50	1000	17.67	15	2.10	-1.27
4	T25	HpMV	7	68	61	854	13.56	11	1.47	-1.74
4	Victoria	HpMV	6	60	54	648	11.80	13	1.34	0.90
3	Opal	HpMV	15	45	30	900	21.00	19	2.94	-0.68
3	T11	HpMV	2	45	43	172	4.82	5	0.50	NA
3	T25	HpMV	12	51	39	936	19.35	16	2.52	-1.33
3	Victoria	HpMV	17	51	34	1156	23.67	27	3.13	1.06

1997 Along Rows (Replicate 1) – Contiguous[1]										
Row Spacing	Cultivar	Virus	m	N	(N-m)	2*m* (N-m)	E(U)	O(U)	S(U)	Z
3x2	Opal	HpMV	28	102	74	4144	41.63	34	3.99	-1.91*
3x2	T11	HpMV	52	102	50	5200	51.98	36	5.02	-3.18*
3x2	T25	HpMV	43	90	47	4042	45.91	35	4.71	-2.32*
3x2	Victoria	HpMV	60	90	30	3600	41.00	31	4.19	-2.39*
6	Opal	HpMV	26	90	64	3328	37.98	39	3.87	0.26
6	T11	HpMV	80	102	22	3520	35.51	31	3.38	-1.33
6	T25	HpMV	35	90	55	3850	43.78	33	4.48	-2.41*
6	Victoria	HpMV	87	102	15	2610	26.59	21	2.50	-2.24*
5	Opal	HpMV	4	85	81	648	8.62	9	0.78	0.49
5	T11	HpMV	24	75	51	2448	33.64	30	3.74	-0.97
5	T25	HpMV	42	85	43	3612	43.49	26	4.58	-3.82*
5	Victoria	HpMV	30	75	45	2700	37.00	37	4.13	0.00
4	Opal	HpMV	14	68	54	1512	23.24	11	2.65	-4.61*
4	T11	HpMV	22	60	38	1672	28.87	16	3.56	-3.61*
4	T25	HpMV	18	68	50	1800	27.47	17	3.17	-3.30*
4	Victoria	HpMV	26	60	34	1768	30.47	24	3.77	-1.72*
3	Opal	HpMV	15	45	30	900	21.00	15	2.94	-2.04*
3	T11	HpMV	20	45	25	1000	23.22	18	3.27	-1.60*
3	T25	HpMV	30	51	21	1260	25.71	20	3.42	-1.67*
3	Victoria	HpMV	23	51	28	1288	26.25	17	3.50	-2.64*
1997 Across Rows (Replicate 1) – Contiguous[1]										
3x2	Opal	HpMV	28	102	74	4144	41.63	40	3.99	-0.41
3x2	T11	HpMV	52	102	50	5200	51.98	48	5.02	-0.79
3x2	T25	HpMV	43	90	47	4042	45.91	37	4.71	-1.89*
3x2	Victoria	HpMV	60	90	30	3600	41.00	34	4.19	-1.67*
6	Opal	HpMV	26	90	64	3328	37.98	33	3.87	-1.29
6	T11	HpMV	80	102	22	3520	35.51	31	3.38	-1.33
6	T25	HpMV	35	90	55	3850	43.78	34	4.48	-2.18*
6	Victoria	HpMV	87	102	15	2610	26.59	23	2.50	-1.44
5	Opal	HpMV	4	85	81	648	8.62	9	0.78	0.49
5	T11	HpMV	24	75	51	2448	33.64	36	3.74	0.63
5	T25	HpMV	42	85	43	3612	43.49	29	4.58	-3.16*
5	Victoria	HpMV	30	75	45	2700	37.00	29	4.13	-1.94*
4	Opal	HpMV	14	68	54	1512	23.24	25	2.65	0.66
4	T11	HpMV	22	60	38	1672	28.87	26	3.56	-0.80
4	T25	HpMV	18	68	50	1800	27.47	27	3.17	-0.15
4	Victoria	HpMV	26	60	34	1768	30.47	34	3.77	0.94
3	Opal	HpMV	15	45	30	900	21.00	19	2.94	-0.68
3	T11	HpMV	20	45	25	1000	23.22	27	3.27	1.15
3	T25	HpMV	30	51	21	1260	25.71	28	3.42	0.67
3	Victoria	HpMV	23	51	28	1288	26.25	27	3.50	0.21

1996 Along Rows (Replicate 2) – Contiguous[1]										
Row Spacing	Cultivar	Virus	m	N	(N-m)	2*m* (N-m)	E(U)	O(U)	S(U)	Z
3x2	Opal	HpMV	2	90	88	352	4.91	5	0.36	NA
3x2	T11	HpMV	9	102	93	1674	17.41	17	1.58	-0.26
3x2	T25	HpMV	6	102	96	1152	12.29	11	1.07	-1.21
3x2	Victoria	HpMV	9	90	81	1458	17.20	19	1.66	1.08
6	Opal	HpMV	6	90	84	1008	12.20	8	1.13	-3.71*
6	T11	HpMV	29	102	73	4234	42.51	23	4.08	-4.78*
6	T25	HpMV	14	90	76	2128	24.64	17	2.45	-3.12*
6	Victoria	HpMV	19	102	83	3154	31.92	40	3.03	2.67
5	Opal	HpMV	4	75	71	568	8.57	7	0.82	-1.92*
5	T11	HpMV	8	75	67	1072	15.29	13	1.60	-1.43
5	T25	HpMV	4	85	81	648	8.62	8	0.78	NA
5	Victoria	HpMV	20	85	65	2600	31.59	27	3.28	-1.40
4	Opal	HpMV	7	68	61	854	13.56	10	1.47	-2.42*
4	T11	HpMV	4	60	56	448	8.47	9	0.90	0.59
4	T25	HpMV	11	68	57	1254	19.44	9	2.19	-4.77*
4	Victoria	HpMV	22	60	38	1672	28.87	14	3.56	-4.17*
3	Opal	HpMV	16	51	35	1120	22.96	18	3.03	-1.63
3	T11	HpMV	30	51	21	1260	25.71	10	3.42	-4.59*
3	T25	HpMV	20	45	25	1000	23.22	17	3.27	-1.90*
3	Victoria	HpMV	24	45	21	1008	23.40	18	3.30	-1.64
1996 Across Rows (Replicate 2) – Contiguous[1]										
3x2	Opal	HpMV	2	90	88	352	4.91	3	0.36	NA
3x2	T11	HpMV	9	102	93	1674	17.41	11	1.58	-4.05*
3x2	T25	HpMV	6	102	96	1152	12.29	9	1.07	-3.07*
3x2	Victoria	HpMV	9	90	81	1458	17.20	13	1.66	-2.53*
6	Opal	HpMV	6	90	84	1008	12.20	12	1.13	-0.18
6	T11	HpMV	29	102	73	4234	42.51	43	4.08	0.12
6	T25	HpMV	14	90	76	2128	24.64	23	2.45	-0.67
6	Victoria	HpMV	19	102	83	3154	31.92	38	3.03	2.01
5	Opal	HpMV	4	75	71	68	8.57	9	0.82	0.52
5	T11	HpMV	8	75	67	1072	15.29	15	1.60	-0.18
5	T25	HpMV	4	85	81	648	8.62	8	0.78	NA
5	Victoria	HpMV	20	85	65	2600	31.59	29	3.28	-0.79
4	Opal	HpMV	7	68	61	854	13.56	12	1.47	-1.06
4	T11	HpMV	4	60	56	448	8.47	9	0.90	0.59
4	T25	HpMV	11	68	57	1254	19.44	13	2.19	-2.94*
4	Victoria	HpMV	22	60	38	1672	28.87	29	3.56	0.04
3	Opal	HpMV	16	51	35	1120	22.96	24	3.03	0.34
3	T11	HpMV	30	51	21	1260	25.71	12	3.42	-4.00*
3	T25	HpMV	20	45	25	1000	23.22	22	3.27	-0.37
3	Victoria	HpMV	24	45	21	1008	23.40	18	3.30	-1.64

Row Spacing	Cultivar	Virus	m	N	(N-m)	2*m* (N-m)	1 > X	8 X	: = X	Z
1997 Along Rows (Replicate 2) – Contiguous[1]										
3x2	Opal	HpMV	23	90	67	3082	35.24	36	3.58	0.21
3x2	T11	HpMV	36	102	66	4752	47.59	45	4.59	-0.56
3x2	T25	HpMV	31	102	71	4402	44.16	34	4.24	-2.39*
3x2	Victoria	HpMV	49	90	41	4018	45.64	50	4.68	0.93
6	Opal	HpMV	20	90	70	2800	32.11	26	3.24	-1.88*
6	T11	HpMV	43	102	59	5074	50.75	26	4.90	-5.05*
6	T25	HpMV	31	90	59	3658	41.64	33	4.25	-2.03*
6	Victoria	HpMV	67	102	35	4690	46.98	39	4.53	-1.76*
5	Opal	HpMV	16	75	59	1888	26.17	19	2.87	-2.50*
5	T11	HpMV	14	75	61	1708	23.77	23	2.59	-0.30
5	T25	HpMV	30	85	55	3300	39.82	28	4.18	-2.83*
5	Victoria	HpMV	35	85	50	3500	42.18	45	4.44	0.64
4	Opal	HpMV	13	68	55	1430	22.03	16	2.51	-2.40*
4	T11	HpMV	8	60	52	832	14.87	16	1.74	0.65
4	T25	HpMV	23	68	45	2070	31.44	15	3.66	-4.50*
4	Victoria	HpMV	25	60	35	1750	30.17	12	3.73	-4.87*
3	Opal	HpMV	18	51	33	1188	24.29	14	3.22	-3.19*
3	T11	HpMV	32	51	19	1216	24.84	13	3.30	-3.59*
3	T25	HpMV	23	45	22	1012	23.49	17	3.31	-1.96*
3	Victoria	HpMV	25	45	20	1000	23.22	18	3.27	-1.60
1997 Across Rows (Replicate 2) – Contiguous[1]										
3x2	Opal	HpMV	23	90	67	4 X3082	35.24	33	3.58	-0.63
3x2	T11	HpMV	36	102	66	4752	47.59	40	4.59	-1.65*
3x2	T25	HpMV	31	102	71	4402	44.16	46	4.24	0.43
3x2	Victoria	HpMV	49	90	41	4018	45.64	45	4.68	-0.14
6	Opal	HpMV	20	90	70	2800	32.11	30	3.24	-0.65
6	T11	HpMV	43	102	59	5074	50.75	54	4.90	0.66
6	T25	HpMV	31	90	59	3658	41.64	35	4.25	-1.56
6	Victoria	HpMV	67	102	35	4690	46.98	45	4.53	-0.44
5	Opal	HpMV	16	75	59	1888	26.17	27	2.87	0.29
5	T11	HpMV	14	75	61	1708	23.77	27	2.59	1.25
5	T25	HpMV	30	85	55	4 X3300	39.82	42	4.18	0.52
5	Victoria	HpMV	35	85	50	3500	42.18	43	4.44	0.19
4	Opal	HpMV	13	68	55	1430	22.03	16	2.51	-2.40*
4	T11	HpMV	8	60	52	832	14.87	14	1.74	-0.50
4	T25	HpMV	23	68	45	2070	31.44	21	3.66	-2.85*
4	Victoria	HpMV	25	60	35	1750	30.17	30	3.73	-0.04
3	Opal	HpMV	18	51	33	1188	24.29	24	3.22	-0.09
3	T11	HpMV	32	51	19	1216	24.84	13	3.30	-3.59*
3	T25	HpMV	23	45	22	1012	23.49	22	3.31	-0.45
3	Victoria	HpMV	25	45	20	1000	23.22	16	3.27	-2.21*

[1] rows combined by last plant of row *i* contiguous with first plant of row *i*+1

* Z-statistics less than –1.64 indicate significant aggregation of infected plants (P=0.05)

NA results from ordinary run analysis invalid due to either low (<5%) or high (>95%) disease incidence

Appendix 3.12. Ordinary run analysis of PNRSV (apple, 'A' and intermediate, 'I' serotypes) distributions in block three (cultivar x row spacing trial) in Bushy Park, Tasmania on 1st November 1996 and 4th November 1997.

Row Spacing	Cultivar	Virus	m	N	(N-m)	2*m* (N-m)	E(U)	O(U)	S(U)	Z
1996 Along Rows (Replicate 1) – Contiguous[1]										
3x2	Opal	PNRSV[2]	9	102	93	1674	17.41	11	1.58	-4.05*
3x2	T11	PNRSV	3	102	99	594	6.82	5	0.53	NA
3x2	T25	PNRSV	83	90	7	1162	13.91	13	1.31	-0.69
3x2	Victoria	PNRSV	84	90	6	1008	12.20	6	1.13	-5.47*
6	Opal	PNRSV	3	90	87	522	6.80	7	0.56	NA
6	T11	PNRSV	6	102	96	1152	12.29	11	1.07	-1.21
6	T25	PNRSV	57	90	33	3762	42.80	27	4.38	-3.61*
6	Victoria	PNRSV	81	102	21	3402	34.35	12	3.27	-6.84*
5	Opal	PNRSV	0	85	85	0	1.00	0	0.00	NA
5	T11	PNRSV	1	75	74	148	2.97	3	0.16	NA
5	T25	PNRSV	85	85	0	0	1.00	1	0.00	NA
5	Victoria	PNRSV	70	75	5	700	10.33	8	1.03	-2.28*
4	Opal	PNRSV	2	68	66	264	4.88	5	0.41	NA
4	T11	PNRSV	5	60	55	550	10.17	5	1.13	-4.59*
4	T25	PNRSV	67	68	1	134	2.97	3	0.17	NA
4	Victoria	PNRSV	43	60	17	1462	25.37	9	3.11	-5.27*
3	Opal	PNRSV	0	45	45	0	1.00	0	0.00	NA
3	T11	PNRSV	11	45	34	748	17.62	14	2.43	-1.49
3	T25	PNRSV	30	51	21	1260	25.71	15	3.42	-3.13*
3	Victoria	PNRSV	37	51	14	1036	21.31	23	2.80	0.60
1996 Across Rows (Replicate 1) – Contiguous[1]										
3x2	Opal	PNRSV	9	102	93	1674	17.41	19	1.58	1.00
3x2	T11	PNRSV	3	102	99	594	6.82	9	0.53	NA
3x2	T25	PNRSV	83	90	7	1162	13.91	11	1.31	-2.21*
3x2	Victoria	PNRSV	84	90	6	1008	12.20	7	1.13	-4.59*
6	Opal	PNRSV	3	90	87	522	6.80	7	0.56	NA
6	T11	PNRSV	6	102	96	1152	12.29	11	1.07	-1.21
6	T25	PNRSV	57	90	33	3762	42.80	50	4.38	1.64
6	Victoria	PNRSV	81	102	21	3402	34.35	29	3.27	-1.64
5	Opal	PNRSV	0	85	85	0	1.00	0	0.00	NA
5	T11	PNRSV	1	75	74	148	2.97	3	0.16	NA
5	T25	PNRSV	85	85	0	0	1.00	1	0.00	NA
5	Victoria	PNRSV	70	75	5	700	10.33	11	1.03	0.65
4	Opal	PNRSV	2	68	66	264	4.88	5	0.41	NA
4	T11	PNRSV	5	60	55	550	10.17	11	1.13	0.74
4	T25	PNRSV	67	68	1	134	2.97	3	0.17	NA
4	Victoria	PNRSV	43	60	17	1462	25.37	21	3.11	-1.41
3	Opal	PNRSV	0	45	45	0	1.00	0	0.00	NA
3	T11	PNRSV	11	45	34	748	17.62	18	2.43	0.16
3	T25	PNRSV	30	51	21	1260	25.71	30	3.42	1.25
3	Victoria	PNRSV	37	51	14	1036	21.31	19	2.80	-0.83

1997 Along Rows (Replicate 1) – Contiguous[1]										
Row Spacing	Cultivar	Virus	m	N	(N-m)	2*m* (N-m)	E(U)	O(U)	S(U)	Z
3x2	Opal	PNRSV[2]	27	102	75	4050	40.71	25	3.90	-4.03*
3x2	T11	PNRSV	7	102	95	1330	14.04	11	1.25	-2.44*
3x2	T25	PNRSV	89	90	1	178	2.98	3	0.15	NA
3x2	Victoria	PNRSV	90	90	0	0	1.00	1	0.00	NA
6	Opal	PNRSV	7	90	83	1162	13.91	13	1.31	-0.69
6	T11	PNRSV	19	102	83	3154	31.92	17	3.03	-4.93*
6	T25	PNRSV	89	90	1	178	2.98	3	0.15	NA
6	Victoria	PNRSV	88	102	14	2464	25.16	12	2.35	-5.59*
5	Opal	PNRSV	0	85	85	0	1.00	0	0.00	NA
5	T11	PNRSV	1	75	74	148	2.97	3	0.16	NA
5	T25	PNRSV	85	85	0	0	1.00	1	0.00	NA
5	Victoria	PNRSV	74	75	1	148	2.97	3	0.16	NA
4	Opal	PNRSV	7	68	61	854	13.56	13	1.47	-0.38
4	T11	PNRSV	6	60	54	648	11.80	7	1.34	-3.58*
4	T25	PNRSV	68	68	0	0	1.00	1	0.00	NA
4	Victoria	PNRSV	59	60	1	118	2.97	3	0.18	NA
3	Opal	PNRSV	0	45	45	0	1.00	0	0.00	NA
3	T11	PNRSV	21	45	24	1008	23.40	6	3.30	-5.27*
3	T25	PNRSV	51	51	0	0	1.00	1	0.00	NA
3	Victoria	PNRSV	50	51	1	100	2.96	3	0.19	NA
1997 Across Rows (Replicate 1) – Contiguous[1]										
3x2	Opal	PNRSV	27	102	75	4050	40.71	47	3.90	1.61
3x2	T11	PNRSV	7	102	95	1330	14.04	13	1.25	-0.83
3x2	T25	PNRSV	89	90	1	178	2.98	3	0.15	NA
3x2	Victoria	PNRSV	90	90	0	0	1.00	1	0.00	NA
6	Opal	PNRSV	7	90	83	1162	13.91	15	1.31	0.83
6	T11	PNRSV	19	102	83	3154	31.92	27	3.03	-1.63
6	T25	PNRSV	89	90	1	178	2.98	3	0.15	NA
6	Victoria	PNRSV	88	102	14	2464	25.16	23	2.35	-0.92
5	Opal	PNRSV	0	85	85	0	1.00	0	0.00	NA
5	T11	PNRSV	1	75	74	148	2.97	3	0.16	NA
5	T25	PNRSV	85	85	0	0	1.00	1	0.00	NA
5	Victoria	PNRSV	74	75	1	148	2.97	3	0.16	NA
4	Opal	PNRSV	7	68	61	854	13.56	13	1.47	-0.38
4	T11	PNRSV	6	60	54	648	11.80	13	1.34	0.90
4	T25	PNRSV	68	68	0	0	1.00	1	0.00	NA
4	Victoria	PNRSV	59	60	1	118	2.97	3	0.18	NA
3	Opal	PNRSV	0	45	45	0	1.00	0	0.00	NA
3	T11	PNRSV	21	45	24	1008	23.40	20	3.30	-1.03
3	T25	PNRSV	51	51	0	0	1.00	1	0.00	NA
3	Victoria	PNRSV	50	51	1	100	2.96	3	0.19	NA

1996 Along Rows (Replicate Contiguous[1]										
Row Spacing	Cultivar	Virus	L X	6 X	6 L	X L X 0 < 6 L X		X7 <	X S(U)	Z
3x2	Opal	PNRSV	0	90	90	0	1.00	0	0.00	NA
3x2	T11	PNRSV	0	102	102	0	1.00	0	0.00	NA
3x2	T25	PNRSV	101	102	1	202	2.98	3	0.14	NA
3x2	Victoria	PNRSV	21	90	69	2898	33.20	23	3.36	-3.04*
6	Opal	PNRSV	3	90	87	522	6.80	7	0.56	NA
6	T11	PNRSV	21	102	81	3402	34.35	13	3.27	-6.53*
6	T25	PNRSV	46	90	44	4048	45.98	22	4.71	-5.09*
6	Victoria	PNRSV	44	102	58	5104	51.04	25	4.93	-5.28*
5	Opal	PNRSV	2	75	73	292	4.89	4	0.39	NA
5	T11	PNRSV	0	75	75	0	1.00	0	0.00	NA
5	T25	PNRSV	81	85	4	648	8.62	9	0.78	NA
5	Victoria	PNRSV	68	85	17	2312	28.20	17	2.91	-3.85*
4	Opal	PNRSV	8	68	60	960	15.12	6	1.66	-5.48*
4	T11	PNRSV	4	60	56	448	8.47	3	0.90	-6.04*
4	T25	PNRSV	68	68	0	0	1.00	1	0.00	NA
4	Victoria	PNRSV	26	60	34	1768	30.47	18	3.77	-3.31*
3	Opal	PNRSV	11	51	40	880	18.25	13	2.37	-2.22*
3	T11	PNRSV	0	51	51	0	1.00	0	0.00	NA
3	T25	PNRSV	43	45	2	172	4.82	5	0.50	NA
3	Victoria	PNRSV	32	45	13	832	19.49	8	2.71	-4.24*
1996 Across Rows (Replicate Contiguous[1]										
3x2	Opal	PNRSV	0	90	90	0	1.00	0	0.00	NA
3x2	T11	PNRSV	0	102	102	0	1.00	0	0.00	NA
3x2	T25	PNRSV	101	102	1	202	2.98	3	0.14	NA
3x2	Victoria	PNRSV	21	90	69	2898	33.20	27	3.36	-1.85*
6	Opal	PNRSV	3	90	87	522	6.80	7	0.56	NA
6	T11	PNRSV	21	102	81	3402	34.35	21	3.27	-4.09*
6	T25	PNRSV	46	90	44	4048	45.98	40	4.71	-1.27
6	Victoria	PNRSV	44	102	58	5104	51.04	49	4.93	-0.41
5	Opal	PNRSV	2	75	73	292	4.89	4	0.39	-NA
3 X 5	T11	PNRSV	0	75	75	0	1.00	0	0.00	NA
5	T25	PNRSV	81	85	4	648	8.62	7	0.78	NA
5	Victoria	PNRSV	68	85	17	2312	28.20	25	2.91	-1.10
4	Opal	PNRSV	8	68	60	960	15.12	17	1.66	1.13
4	T11	PNRSV	4	60	56	448	8.47	9	0.90	0.59
4	T25	PNRSV	68	68	0	0	1.00	1	0.00	NA
4	Victoria	PNRSV	26	60	34	1768	30.47	39	3.77	2.26
3	Opal	PNRSV	11	51	40	880	18.25	21	2.37	1.16
3	T11	PNRSV	0	51	51	0	1.00	0	0.00	NA
3	T25	PNRSV	43	45	2	172	4.82	5	0.50	NA
3	Victoria	PNRSV	32	45	13	832	19.49	26	2.71	2.40

Row Spacing	Cultivar	Virus	m	N	(N-m)	2*m* (N-m)	E(U)	O(U)	S(U)	Z
1997 Along Rows (Replicate 1) – Contiguous[1]										
3x2	Opal	PNRSV[2]	27	102	75	4050	40.71	25	3.90	-4.03*
3x2	T11	PNRSV	7	102	95	1330	14.04	11	1.25	-2.44*
3x2	T25	PNRSV	89	90	1	178	2.98	3	0.15	NA
3x2	Victoria	PNRSV	90	90	0	0	1.00	1	0.00	NA
6	Opal	PNRSV	7	90	83	1162	13.91	13	1.31	-0.69
6	T11	PNRSV	19	102	83	3154	31.92	17	3.03	-4.93*
6	T25	PNRSV	89	90	1	178	2.98	3	0.15	NA
6	Victoria	PNRSV	88	102	14	2464	25.16	12	2.35	-5.59*
5	Opal	PNRSV	0	85	85	0	1.00	0	0.00	NA
5	T11	PNRSV	1	75	74	148	2.97	3	0.16	NA
5	T25	PNRSV	85	85	0	0	1.00	1	0.00	NA
5	Victoria	PNRSV	74	75	1	148	2.97	3	0.16	NA
4	Opal	PNRSV	7	68	61	854	13.56	13	1.47	-0.38
4	T11	PNRSV	6	60	54	648	11.80	7	1.34	-3.58*
4	T25	PNRSV	68	68	0	0	1.00	1	0.00	NA
4	Victoria	PNRSV	59	60	1	118	2.97	3	0.18	NA
3	Opal	PNRSV	0	45	45	0	1.00	0	0.00	NA
3	T11	PNRSV	21	45	24	1008	23.40	6	3.30	-5.27*
3	T25	PNRSV	51	51	0	0	1.00	1	0.00	NA
3	Victoria	PNRSV	50	51	1	100	2.96	3	0.19	NA
1997 Across Rows (Replicate 1) – Contiguous[1]										
3x2	Opal	PNRSV	27	102	75	4050	40.71	47	3.90	1.61
3x2	T11	PNRSV	7	102	95	1330	14.04	13	1.25	-0.83
3x2	T25	PNRSV	89	90	1	178	2.98	3	0.15	NA
3x2	Victoria	PNRSV	90	90	0	0	1.00	1	0.00	NA
6	Opal	PNRSV	7	90	83	1162	13.91	15	1.31	0.83
6	T11	PNRSV	19	102	83	3154	31.92	27	3.03	-1.63
6	T25	PNRSV	89	90	1	178	2.98	3	0.15	NA
6	Victoria	PNRSV	88	102	14	2464	25.16	23	2.35	-0.92
5	Opal	PNRSV	0	85	85	0	1.00	0	0.00	NA
5	T11	PNRSV	1	75	74	148	2.97	3	0.16	NA
5	T25	PNRSV	85	85	0	0	1.00	1	0.00	NA
5	Victoria	PNRSV	74	75	1	148	2.97	3	0.16	NA
4	Opal	PNRSV	7	68	61	854	13.56	13	1.47	-0.38
4	T11	PNRSV	6	60	54	648	11.80	13	1.34	0.90
4	T25	PNRSV	68	68	0	0	1.00	1	0.00	NA
4	Victoria	PNRSV	59	60	1	118	2.97	3	0.18	NA
3	Opal	PNRSV	0	45	45	0	1.00	0	0.00	NA
3	T11	PNRSV	21	45	24	1008	23.40	20	3.30	-1.03
3	T25	PNRSV	51	51	0	0	1.00	1	0.00	NA
3	Victoria	PNRSV	50	51	1	100	2.96	3	0.19	NA

[1] rows combined by last plant of row *i* contiguous with first plant of row *i*+1

[2] apple, 'A' and intermediate, 'I' serotypes of PNRSV were not differentiated

* Z-statistics less than –1.64 indicate significant aggregation of infected plants (P=0.05)

NA results from ordinary run analysis invalid due to either low (<5%) or high (>95%) disease incidence

APPENDIX 4: PROPAGATION AND MECHANICAL INOCULATION

APPENDIX 4.1: SOFTWOOD CUTTING PROPAGATION

Mother plants in commercial gardens were selected from field surveys. Virus tested mother plants had no detectable infection by HpLV, HpMV, and PNRSV (A & I) after ELISA testing twice early in the season, and were surrounded by two virus tested plants in all directions. Virus-infected mother plants had detectable infection by the specific virus in a number of shoots early in the season.

Basal shoots were cut from selected plants using sterile scalpel blades soon after the second ELISA testing and kept in cool storage, regularly sprayed with water, for no longer than an hour for transport from the field. Basal shoots were separated into individual cuttings consisting of approximately 5 cm of stem below one node. Cuttings were dipped in rooting powder (Rootex^{R}), and placed in individual cells containing propagation soil mixture containing equal quantities of sand and peat, under mist. Cuttings remained under mist until sufficient roots had developed (usually six to eight weeks), after which the mist was gradually reduced. After plants were hardened off (approximately two weeks) they were transferred to larger pots for approximately three weeks before use in glasshouse experiments, or hardened off further outside for another two months before planting in the field (chapter six).

APPENDIX 4.2:

SOIL AND PASTEURISATION

Soil mixture

Peat	2 parts
Pine bark	5 parts
Sand	3 parts
Dolomite	415 g

Fertiliser mixture

Ferrous sulphate	0.3 kg
Micromax	0.3 kg
Nutricote (4-5 month)	1.5 kg
Osmocote (8-9 month)	2.5 kg
Nutricote (12-14 month)	2.5 kg
Dolomite	7.0 kg

Soil was exposed to 60°C for one hour and allowed to cool prior to use in pots.

APPENDIX 4.3:

MECHANICAL INOCULATION BUFFER

Di-potassium hydrogen orthophosphate	2.916 g
Potassium di-hydrogen orthophosphate	0.444 g
Cysteine	0.316 g
Distilled water	200 ml

Celite was sprinkled over the plants as an abrasive.

Appendix 5.1. Standardised absorbance reactions to PNRSV-A and RMV-3 antisera from PNRSV infected 'Victoria' plants in block one in Bushy Park, Tasmania.

Serotype	Absorbance Values (A_{405}) [1]		Serotype	Absorbance Values (A_{405}) [1]	
	PNRSV-A	RMV-3		PNRSV-A	RMV-3
PNRSV-A	0.575	0.209	PNRSV-I	0.344	0.691
PNRSV-A	0.645	0.196	PNRSV-I	0.373	0.704
PNRSV-A	0.683	0.186	PNRSV-I	0.368	0.697
PNRSV-A	0.582	0.198	PNRSV-I	0.382	0.703
PNRSV-A	0.549	0.200	PNRSV-I	0.342	0.680
PNRSV-A	0.595	0.208	PNRSV-I	0.413	0.681
PNRSV-A	0.615	0.205	PNRSV-I	0.317	0.692
PNRSV-A	0.805	0.195	PNRSV-I	0.412	0.704
PNRSV-A	0.603	0.197	PNRSV-I	0.358	0.725
PNRSV-A	0.674	0.204	PNRSV-I	0.345	0.759
PNRSV-A	0.580	0.199	PNRSV-I	0.382	0.713
PNRSV-A	0.772	0.196	PNRSV-I	0.275	0.725
PNRSV-A	0.579	0.186	PNRSV-I	0.382	0.698
PNRSV-A	0.574	0.198	PNRSV-I	0.407	0.776
PNRSV-A	0.568	0.208	PNRSV-I	0.419	0.668
PNRSV-A	0.498	0.203	PNRSV-I	0.341	0.744
PNRSV-A	0.579	0.197	PNRSV-I	0.401	0.682
PNRSV-A	0.598	0.188	PNRSV-I	0.378	0.670
PNRSV-A	0.770	0.200	PNRSV-I	0.407	0.684
PNRSV-A	0.666	0.201	PNRSV-I	0.385	0.714
PNRSV-A	0.647	0.208	PNRSV-I	0.415	0.713
PNRSV-A	0.618	0.195	PNRSV-I	0.375	0.723
PNRSV-A	0.588	0.197	PNRSV-I	0.325	0.701
PNRSV-A	0.575	0.198	PNRSV-I	0.408	0.709
PNRSV-A	0.516	0.203	PNRSV-I	0.367	0.704
PNRSV-A	0.564	0.205	PNRSV-I	0.348	0.714
PNRSV-A	0.556	0.211	PNRSV-I	0.333	0.735
PNRSV-A	0.562	0.240	PNRSV-I	0.331	0.747
PNRSV-A	0.486	0.234	PNRSV-I	0.330	0.733
PNRSV-A	0.556	0.242	PNRSV-I	0.349	0.712
PNRSV-A	0.642	0.196	PNRSV-I	0.374	0.729
PNRSV-A	0.549	0.199	PNRSV-I	0.353	0.736
PNRSV-A	0.554	0.219	PNRSV-I	0.371	0.719
PNRSV-A	0.589	0.222	PNRSV-I	0.351	0.710
PNRSV-A	0.582	0.188	PNRSV-I	0.328	0.699
PNRSV-A	0.614	0.209	PNRSV-I	0.359	0.696
PNRSV-A	0.498	0.193	PNRSV-I	0.375	0.702
PNRSV-A	0.565	0.195	PNRSV-I	0.362	0.753
PNRSV-A	0.563	0.203	PNRSV-I	0.332	0.701
PNRSV-A	0.603	0.198	PNRSV-I	0.385	0.690
PNRSV-A	0.700	0.212	PNRSV-I	0.356	0.691
PNRSV-A	0.582	0.217	PNRSV-I	0.400	0.692
PNRSV-A	0.667	0.207	PNRSV-I	0.362	0.702
PNRSV-A	0.528	0.201	PNRSV-I	0.506	0.679
PNRSV-A	0.609	0.208	PNRSV-I	0.438	0.681
PNRSV-A	0.681	0.216	PNRSV-I	0.354	0.691
PNRSV-A	0.721	0.221	PNRSV-I	0.358	0.705
PNRSV-A	0.635	0.195	PNRSV-I	0.329	0.670

PNRSV-A	0.612	0.211	PNRSV-I	0.337	0.680
PNRSV-A	0.648	0.222	PNRSV-I	0.366	0.671
PNRSV-A	0.664	0.225	PNRSV-I	0.359	0.682
PNRSV-A	0.655	0.194	PNRSV-I	0.374	0.706
PNRSV-A	0.659	0.197	PNRSV-I	0.514	0.697
PNRSV-A	0.644	0.211	PNRSV-I	0.375	0.696
PNRSV-A	0.575	0.186	PNRSV-I	0.350	0.674
PNRSV-A	0.556	0.214	PNRSV-I	0.335	0.665
PNRSV-A	0.587	0.189	PNRSV-I	0.376	0.684
PNRSV-A	0.615	0.186	PNRSV-I	0.356	0.682
PNRSV-A	0.590	0.190	PNRSV-I	0.365	0.692
PNRSV-A	0.605	0.177	PNRSV-I	0.356	0.702
PNRSV-A	0.636	0.178	PNRSV-I	0.357	0.713
PNRSV-A	0.645	0.212	PNRSV-I	0.338	0.716
PNRSV-A	0.658	0.222	PNRSV-I	0.343	0.735
PNRSV-A	0.590	0.187	PNRSV-I	0.365	0.693
PNRSV-A	0.559	0.188	PNRSV-I	0.358	0.690
PNRSV-A	0.949	0.200	PNRSV-I	0.356	0.687
PNRSV-A	0.513	0.205	PNRSV-I	0.352	0.662
PNRSV-A	0.529	0.215	PNRSV-I	0.340	0.690
PNRSV-A	1.092	0.207	PNRSV-I	0.359	0.691
PNRSV-A	1.075	0.227	PNRSV-I	0.390	0.692
PNRSV-A	0.625	0.231	PNRSV-I	0.363	0.695
PNRSV-A	0.570	0.199	PNRSV-I	0.387	0.702
PNRSV-A	0.544	0.206	PNRSV-I	0.359	0.704
PNRSV-A	0.568	0.214	PNRSV-I	0.382	0.698
PNRSV-A	0.590	0.216	PNRSV-I	0.372	0.699
PNRSV-A	0.494	0.210	PNRSV-I	0.347	0.674
PNRSV-A	0.482	0.224	PNRSV-I	0.348	0.689
PNRSV-A	0.586	0.226	PNRSV-I	0.365	0.691
PNRSV-A	0.513	0.195	PNRSV-I	0.337	0.704
PNRSV-A	0.494	0.223	PNRSV-I	0.402	0.705
PNRSV-A	0.507	0.185	PNRSV-I	0.345	0.735
PNRSV-A	0.559	0.182	PNRSV-I	0.355	0.723
PNRSV-A	0.529	0.188	PNRSV-I	0.366	0.734
PNRSV-A	0.550	0.212	PNRSV-I	0.370	0.668
PNRSV-A	0.598	0.192	PNRSV-I	0.350	0.690
PNRSV-A	0.594	0.190	PNRSV-I	0.346	0.676
PNRSV-A	0.668	0.210	PNRSV-I	0.372	0.684
PNRSV-A	1.051	0.221	PNRSV-I	0.347	0.693
PNRSV-A	1.127	0.236	PNRSV-I	0.387	0.699
PNRSV-A	0.577	0.184	PNRSV-I	0.298	0.708
PNRSV-A	0.565	0.174	PNRSV-I	0.338	0.708
PNRSV-A	0.603	0.206	PNRSV-I	0.378	0.706
PNRSV-A	0.603	0.228	PNRSV-I	0.353	0.687
PNRSV-A	0.670	0.217	PNRSV-I	0.371	0.684
PNRSV-A	0.638	0.207	PNRSV-I	0.310	0.675
PNRSV-A	0.608	0.202	PNRSV-I	0.296	0.672
PNRSV-A	0.638	0.199	PNRSV-I	0.342	0.684
PNRSV-A	0.591	0.194	PNRSV-I	0.251	0.679
PNRSV-A	0.634	0.197	PNRSV-I	0.358	0.687

[1] absorbance values standardised to a common positive control

Appendix 5.2. Standardised absorbance reactions to PNRSV-A and RMV-3 antisera from PNRSV infected 'Victoria' plants in block two in Myrtleford, Victoria.

Serotype	Absorbance Values (A_{405}) [1]		Serotype	Absorbance Values (A_{405}) [1]	
	PNRSV-A	RMV-3		PNRSV-A	RMV-3
PNRSV-A	0.932	0.095	PNRSV-I	0.343	0.756
PNRSV-A	0.935	0.091	PNRSV-I	0.394	0.768
PNRSV-A	1.043	0.090	PNRSV-I	0.418	0.780
PNRSV-A	1.060	0.094	PNRSV-I	0.436	0.792
PNRSV-A	0.780	0.100	PNRSV-I	0.463	0.793
PNRSV-A	0.781	0.102	PNRSV-I	0.472	0.803
PNRSV-A	0.922	0.092	PNRSV-I	0.494	0.814
PNRSV-A	0.906	0.097	PNRSV-I	0.433	0.813
PNRSV-A	0.895	0.098	PNRSV-I	0.453	0.818
PNRSV-A	0.927	0.100	PNRSV-I	0.454	0.809
PNRSV-A	0.996	0.103	PNRSV-I	0.383	0.794
PNRSV-A	0.761	0.123	PNRSV-I	0.377	0.798
PNRSV-A	0.749	0.118	PNRSV-I	0.381	0.770
PNRSV-A	0.688	0.114	PNRSV-I	0.374	0.752
PNRSV-A	0.783	0.125	PNRSV-I	0.395	0.792
PNRSV-A	0.775	0.126	PNRSV-I	0.373	0.795
PNRSV-A	0.782	0.127	PNRSV-I	0.403	0.785
PNRSV-A	0.688	0.089	PNRSV-I	0.407	0.783
PNRSV-A	0.702	0.102	PNRSV-I	0.373	0.776
PNRSV-A	0.749	0.101	PNRSV-I	0.371	0.796
PNRSV-A	0.720	0.108	PNRSV-I	0.378	0.784
PNRSV-A	0.695	0.117	PNRSV-I	0.375	0.786
PNRSV-A	0.747	0.125	PNRSV-I	0.381	0.775
PNRSV-A	0.756	0.096	PNRSV-I	0.395	0.771
PNRSV-A	0.762	0.093	PNRSV-I	0.432	0.791
PNRSV-A	0.784	0.097	PNRSV-I	0.417	0.792
PNRSV-A	0.780	0.111	PNRSV-I	0.389	0.794
PNRSV-A	0.918	0.114	PNRSV-I	0.385	0.784
PNRSV-A	0.866	0.118	PNRSV-I	0.392	0.776
PNRSV-A	0.875	0.119	PNRSV-I	0.398	0.782

[1] absorbance values standardised to a common positive control

Appendix 5.3. Standardised absorbance reactions to PNRSV-A and RMV-3 antisera from PNRSV infected 'Victoria' plants in block three (cultivar x row spacing trial) in Bushy Park, Tasmania.

Serotype	Absorbance Values (A_{405}) [1]		Serotype	Absorbance Values (A_{405}) [1]	
	PNRSV-A	RMV-3		PNRSV-A	RMV-3
PNRSV-A	0.635	0.197	PNRSV-I	0.268	0.769
PNRSV-A	0.695	0.201	PNRSV-I	0.273	0.763
PNRSV-A	0.698	0.203	PNRSV-I	0.277	0.781
PNRSV-A	0.676	0.205	PNRSV-I	0.348	0.751
PNRSV-A	1.262	0.209	PNRSV-I	0.322	0.754
PNRSV-A	1.168	0.196	PNRSV-I	0.319	0.784
PNRSV-A	1.019	0.188	PNRSV-I	0.292	0.783
PNRSV-A	1.112	0.184	PNRSV-I	0.273	0.774
PNRSV-A	1.233	0.182	PNRSV-I	0.281	0.781
PNRSV-A	1.322	0.187	PNRSV-I	0.292	0.792
PNRSV-A	0.993	0.180	PNRSV-I	0.326	0.792
PNRSV-A	1.018	0.192	PNRSV-I	0.327	0.797
PNRSV-A	0.621	0.191	PNRSV-I	0.311	0.791
PNRSV-A	0.626	0.199	PNRSV-I	0.337	0.777
PNRSV-A	0.654	0.209	PNRSV-I	0.309	0.783
PNRSV-A	0.658	0.213	PNRSV-I	0.290	0.784
PNRSV-A	0.562	0.216	PNRSV-I	0.298	0.790
PNRSV-A	0.651	0.210	PNRSV-I	0.321	0.782
PNRSV-A	0.714	0.193	PNRSV-I	0.296	0.774
PNRSV-A	0.916	0.197	PNRSV-I	0.302	0.775
PNRSV-A	0.818	0.188	PNRSV-I	0.309	0.772
PNRSV-A	0.641	0.191	PNRSV-I	0.294	0.770
PNRSV-A	0.618	0.193	PNRSV-I	0.329	0.792
PNRSV-A	0.612	0.182	PNRSV-I	0.347	0.798
PNRSV-A	0.708	0.180	PNRSV-I	0.275	0.774
PNRSV-A	0.761	0.178	PNRSV-I	0.247	0.789
PNRSV-A	0.752	0.195	PNRSV-I	0.314	0.788
PNRSV-A	0.768	0.189	PNRSV-I	0.343	0.797
PNRSV-A	0.778	0.199	PNRSV-I	0.362	0.785
PNRSV-A	0.780	0.203	PNRSV-I	0.328	0.783

[1] absorbance values standardised to a common positive control

Appendix 5.4. Standardised absorbance reactions to PNRSV-A and RMV-3 antisera from PNRSV infected 'T11' plants in block three (cultivar x row spacing trial) in Bushy Park, Tasmania.

Serotype	Absorbance Values (A_{405}) [1]		Serotype	Absorbance Values (A_{405}) [1]	
	PNRSV-A	RMV-3		PNRSV-A	RMV-3
PNRSV-A	0.953	0.199	PNRSV-I	0.375	0.763
PNRSV-A	0.905	0.200	PNRSV-I	0.397	0.770
PNRSV-A	0.904	0.208	PNRSV-I	0.400	0.772
PNRSV-A	0.918	0.198	PNRSV-I	0.405	0.795
PNRSV-A	0.768	0.197	PNRSV-I	0.408	0.791
PNRSV-A	0.832	0.188	PNRSV-I	0.410	0.785
PNRSV-A	0.692	0.186	PNRSV-I	0.395	0.784
PNRSV-A	0.670	0.210	PNRSV-I	0.438	0.787
PNRSV-A	0.780	0.206	PNRSV-I	0.422	0.774
PNRSV-A	0.803	0.199	PNRSV-I	0.442	0.772
PNRSV-A	0.808	0.202	PNRSV-I	0.431	0.771
PNRSV-A	0.767	0.210	PNRSV-I	0.446	0.770
PNRSV-A	0.768	0.208	PNRSV-I	0.519	0.784
PNRSV-A	0.803	0.195	PNRSV-I	0.471	0.793
PNRSV-A	0.665	0.192	PNRSV-I	0.472	0.792
PNRSV-A	0.737	0.191	PNRSV-I	0.402	0.801
PNRSV-A	0.761	0.185	PNRSV-I	0.425	0.797
PNRSV-A	0.702	0.184	PNRSV-I	0.463	0.792
PNRSV-A	0.711	0.191	PNRSV-I	0.521	0.794
PNRSV-A	0.628	0.188	PNRSV-I	0.395	0.795
PNRSV-A	0.615	0.202	PNRSV-I	0.409	0.785
PNRSV-A	0.703	0.199	PNRSV-I	0.442	0.783
PNRSV-A	0.735	0.207	PNRSV-I	0.414	0.775
PNRSV-A	0.760	0.205	PNRSV-I	0.419	0.765
PNRSV-A	0.759	0.201	PNRSV-I	0.395	0.792
PNRSV-A	0.679	0.208	PNRSV-I	0.402	0.770
PNRSV-A	0.795	0.210	PNRSV-I	0.408	0.793
PNRSV-A	0.756	0.193	PNRSV-I	0.412	0.800
PNRSV-A	0.737	0.195	PNRSV-I	0.396	0.775
PNRSV-A	0.782	0.199	PNRSV-I	0.392	0.774

[1] absorbance values standardised to a common positive control

Appendix 5.5. Standardised absorbance reactions to PNRSV-A and RMV-3 antisera from PNRSV infected 'Opal' plants in block three (cultivar * ,row spacing trial) in Bushy Park, Tasmania.

Serotype	Absorbance Values (A_{405}) [1]		Serotype	Absorbance Values (A_{405}) [1]	
	PNRSV-A	RMV-3		PNRSV-A	RMV-3
PNRSV-A	0.607	0.251	PNRSV-I	0.292	0687
PNRSV-A	0.655	0.209	PNRSV-I	0.239	0.693
PNRSV-A	0.668	0.208	PNRSV-I	0.262	0.691
PNRSV-A	0.657	0.231	PNRSV-I	0.242	0680
PNRSV-A	0.702	0.228	PNRSV-I	0.255	0650
PNRSV-A	0.756	0.209	PNRSV-I	0.250	0689
PNRSV-A	0.700	0.222	PNRSV-I	0.317	0.679
PNRSV-A	0.654	0.200	PNRSV-I	0.327	0.779
PNRSV-A	0.663	0.196	PNRSV-I	0.331	0.713
PNRSV-A	0.672	0.197	PNRSV-I	0.362	0.702
PNRSV-A	0.688	0.204	PNRSV-I	0.307	0680
PNRSV-A	0.682	0.187	PNRSV-I	0.274	0647
PNRSV-A	0.633	0.193	PNRSV-I	0.290	0624
PNRSV-A	0.655	0.198	PNRSV-I	0.306	0.634
PNRSV-A	0.668	0.204	PNRSV-I	0.307	0.632
PNRSV-A	0.681	0.199	PNRSV-I	0.308	0.644
PNRSV-A	0.692	0.198	PNRSV-I	0.315	0.593
PNRSV-A	0.686	0.201	PNRSV-I	0.363	0.595
PNRSV-A	0.698	0.190	PNRSV-I	0.358	0.592
PNRSV-A	0.700	0.192	PNRSV-I	0.306	0.677
PNRSV-A	0.702	0.194	PNRSV-I	0.281	0.666
PNRSV-A	0.659	0.196	PNRSV-I	0.298	0.716
PNRSV-A	0.669	0.199	PNRSV-I	0.286	0.755
PNRSV-A	0.680	0.190	PNRSV-I	0.288	0.691
PNRSV-A	0.692	0.188	PNRSV-I	0.298	0.705
PNRSV-A	0.697	0.202	PNRSV-I	0.265	0.735
PNRSV-A	0.681	0.207	PNRSV-I	0.299	0.772
PNRSV-A	0.692	0.201	PNRSV-I	0.301	0.761
PNRSV-A	0.699	0.204	PNRSV-I	0.305	0.735
PNRSV-A	0.682	0.205	PNRSV-I	0.258	0.677

[1] absorbance values standardised to a common positive control

Appendix 5.6. Standardised absorbance reactions to PNRSV-A, PNRSV-I, and RMV-3 antisera from PNRSV infected plants in the Museum block.

Cultivar	Absorbance Values (A_{405}) [1]			Serotype
	PNRSV-A	PNRSV-I	RMV-3	
Southern Brewer	0.387	0.925	0.796	PNRSV-I
K56	0.343	0.949	0.593	PNRSV-I
Fuggle	0.252	0.810	0.675	PNRSV-I
Hallertau MF (Tas)	0.345	0.827	0.693	PNRSV-I
30-74-32	0.350	0.825	0.687	PNRSV-I
96-76-44	0.427	0.954	0.942	PNRSV-I
BH 2/72	0.360	0.918	0.675	PNRSV-I
D6	0.336	0.849	0.678	PNRSV-I
N-81-43	0.315	0.875	0.626	PNRSV-I
81-76-2	0.353	0.879	0.666	PNRSV-I
N-81-79	0.279	0.875	0.518	PNRSV-I
30-74-58	0.362	1.007	0.672	PNRSV-I
AH 14/1	0.284	0.760	0.337	PNRSV-I
OM26	0.298	0.816	0.492	PNRSV-I
AF 3/26	0.378	0.915	0.713	PNRSV-I
E2 EX FRF	0.264	0.431	0.190	PNRSV-I
L1 EX FRF	0.184	1.297	0.447	PNRSV-I
N-81-21	0.335	0.967	0.712	PNRSV-I
V-85-9	0.385	0.946	0.791	PNRSV-I
E-85-16	0.347	0.910	0.579	PNRSV-I
Cascade	0.383	1.147	0.544	PNRSV-I
Huller Bitterer	0.482	1.021	0.766	PNRSV-I
TI-84-20	0.395	0.900	0.587	PNRSV-I
G-85-9	0.238	0.903	0.595	PNRSV-I
4 x Ringwood Special	0.224	0.641	0.235	PNRSV-I
SK30	0.375	0.803	0.737	PNRSV-I
4 x Pride of Ringwood	0.689	0.594	0.174	PNRSV-A
T-86-21	0.662	0.655	0.197	PNRSV-A
E-85-20	0.626	0.681	0.234	PNRSV-A
Eastern Gold	0.518	0.491	0.162	PNRSV-A
Brewers Gold	0.663	0.613	0.219	PNRSV-A
E2	0.715	0.667	0.211	PNRSV-A
Yeoman	0.591	0.645	0.179	PNRSV-A
Zenith	0.548	0.626	0.193	PNRSV-A
4 x Southern Brewer	0.802	0.695	0.205	PNRSV-A
Wye Target	0.584	0.620	0.184	PNRSV-A
Wye Northdown	0.527	0.569	0.196	PNRSV-A
Williamette	0.455	0.538	0.181	PNRSV-A
Shinshuwase	0.559	0.598	0.212	PNRSV-A
LA-85-70	0.686	0.477	0.150	PNRSV-A
WS	0.578	0.595	0.189	PNRSV-A
Styrian	0.621	0.755	0.264	PNRSV-A

[1] absorbance values standardised to a common positive control

Appendix 5.7. Standardised absorbance reactions to PNRSV-A, PNRSV-I, and RMV-3 antisera from PNRSV infected 'Victoria', 'Tettnang', 'Nugget', and 'Pride of Ringwood' plants in gardens in north east Tasmania.

Cultivar	Absorbance Values (A_{405}) [1]			Serotype	Farm
	PNRSV-A	PNRSV-I	RMV-3		
Victoria	0.328	0.893	0.721	PNRSV-I	FRF
Victoria	0.515	0.976	0.784	PNRSV-I	FRF
Victoria	0.211	0.560	0.798	PNRSV-I	FRF
Victoria	0.480	0.918	0.802	PNRSV-I	FRF
Victoria	0.430	0.920	0.765	PNRSV-I	FRF
Victoria	0.471	0.857	0.777	PNRSV-I	FRF
Victoria	0.338	0.881	0.784	PNRSV-I	FRF
Victoria	0.467	0.963	0.709	PNRSV-I	FRF
Victoria	0.465	0.925	0.809	PNRSV-I	FRF
Victoria	0.463	0.907	0.803	PNRSV-I	FRF
Victoria	0.398	0.839	0.812	PNRSV-I	FRF
Victoria	0.460	0.978	0.800	PNRSV-I	FRF
Victoria	0.424	0.872	0.799	PNRSV-I	FRF
Victoria	0.435	0.889	0.768	PNRSV-I	FRF
Tettnang	0.437	0.863	0.754	PNRSV-I	FRF
Tettnang	0.424	0.842	0.783	PNRSV-I	FRF
Tettnang	0.366	0.857	0.769	PNRSV-I	FRF
Tettnang	0.455	0.872	0.802	PNRSV-I	FRF
Nugget	0.242	0.488	0.804	PNRSV-I	FRF
Nugget	0.230	0.393	0.809	PNRSV-I	FRF
Nugget	0.248	0.467	0.793	PNRSV-I	FRF
Nugget	0.333	0.276	0.794	PNRSV-A	FRF
Nugget	0.263	0.262	0.798	PNRSV-A	FRF
Nugget	0.342	0.179	0.809	PNRSV-A	FRF
Pride of Ringwood	0.698	0.655	0.216	PNRSV-A	X
Pride of Ringwood	0.836	0.617	0.281	PNRSV-A	Y
Pride of Ringwood	0.747	0.563	0.219	PNRSV-A	Y
Pride of Ringwood	0.839	0.505	0.198	PNRSV-A	Y
Pride of Ringwood	0.698	0.637	0.276	PNRSV-A	Y
Pride of Ringwood	0.922	0.653	0.289	PNRSV-A	Y
Pride of Ringwood	0.657	0.574	0.236	PNRSV-A	Y
Pride of Ringwood	0.701	0.560	0.225	PNRSV-A	Y
Pride of Ringwood	0.730	0.574	0.235	PNRSV-A	Y
Pride of Ringwood	0.681	0.533	0.194	PNRSV-A	Y
Pride of Ringwood	0.678	0.591	0.249	PNRSV-A	Y
Pride of Ringwood	0.684	0.516	0.203	PNRSV-A	Y
Pride of Ringwood	0.787	0.563	0.220	PNRSV-A	Y
Pride of Ringwood	0.981	0.438	0.182	PNRSV-A	Y
Pride of Ringwood	0.772	0.503	0.193	PNRSV-A	Y
Victoria	0.664	0.609	0.208	PNRSV-A	X
Victoria	0.726	0.552	0.201	PNRSV-A	X

[1] absorbance values standardised to a common positive control

Appendix 5.8. Standardised absorbance values (A_{405})[1] for 'Victoria' plants infected by PNRSV-A or PNRSV-I from August 1996 to July 1997.

	Aug-96		Sep-96		Oct-96		Nov-96		Dec-96		Jan-97	
PNRSV Serotype	PNRSV-A	RMV-3	PNRSV-A	RMV-3	PNRSV-A	RMV-3	PNRSV-A	RMV-3	PNRSV-A	RMV-3	PNRSV-A	RMV-3
PNRSV-A	1.16	0.201	0.759	0.204	0.455	0.203	0.317	0.204	0.644	0.201	0.724	0.212
PNRSV-A	1.211	0.205	0.824	0.203	0.988	0.221	0.912	0.207	0.904	0.206	0.821	0.213
PNRSV-A	0.966	0.208	0.824	0.209	1.312	0.224	0.907	0.206	0.639	0.205	0.721	0.218
PNRSV-A	1.278	0.209	0.951	0.213	1.161	0.217	0.987	0.21	0.776	0.21	0.701	0.216
PNRSV-A	1.088	0.211	0.890	0.198	1.006	0.223	0.925	0.221	0.744	0.214	0.761	0.205
PNRSV-A	1.093	0.209	1.101	0.199	1.093	0.215	0.903	0.219	0.819	0.243	0.817	0.203
PNRSV-A	1.164	0.213	1.019	0.19	1.131	0.239	0.989	0.215	0.702	0.235	0.854	0.199
PNRSV-A	1.112	0.224	0.915	0.191	1.215	0.214	0.945	0.206	0.869	0.213	0.849	0.197
PNRSV-A	1.291	0.202	0.988	0.188	0.832	0.219	0.932	0.208	0.839	0.219	0.963	0.199
PNRSV-A	1.15	0.209	0.797	0.201	1.082	0.198	0.919	0.21	0.866	0.217	0.857	0.194
PNRSV-A	0.812	0.207	0.855	0.2	0.825	0.196	0.854	0.199	0.994	0.199	0.804	0.196
PNRSV-A	1.081	0.195	0.871	0.21	1.056	0.207	0.808	0.195	0.864	0.195	0.904	0.196
PNRSV-A	0.989	0.194	0.741	0.212	0.927	0.205	0.889	0.21	0.839	0.196	0.977	0.197
PNRSV-A	1.142	0.199	0.785	0.211	1.113	0.203	0.805	0.215	0.811	0.193	0.869	0.194
PNRSV-A	0.943	0.198	0.845	0.219	0.674	0.201	0.802	0.215	0.904	0.211	0.838	0.201
PNRSV-A	1.084	0.202	0.832	0.217	1.144	0.206	0.991	0.219	0.889	0.214	0.862	0.205
PNRSV-A	0.983	0.202	0.929	0.215	0.907	0.216	0.927	0.221	0.889	0.215	0.837	0.197
PNRSV-A	1.143	0.206	0.779	0.217	1.103	0.197	0.939	0.213	0.92	0.218	0.835	0.195
PNRSV-A	1.512	0.214	0.830	0.207	1.194	0.194	0.853	0.215	0.718	0.216	0.841	0.216
PNRSV-A	1.654	0.232	0.831	0.208	1.162	0.199	0.849	0.222	0.84	0.223	0.991	0.224
PNRSV-A	0.987	0.209	0.778	0.201	1.04	0.195	0.807	0.229	0.702	0.227	0.941	0.228
PNRSV-A	1.239	0.217	0.879	0.209	0.963	0.205	0.801	0.209	0.867	0.198	0.851	0.195
PNRSV-A	1.139	0.219	0.804	0.215	0.939	0.213	0.917	0.205	0.987	0.194	0.879	0.223
PNRSV-A	1.286	0.22	0.756	0.213	1.145	0.218	0.847	0.199	0.719	0.2	0.958	0.227
PNRSV-A	0.989	0.235	0.827	0.219	1.036	0.211	0.878	0.195	0.799	0.201	0.947	0.229
PNRSV-A	1.006	0.209	0.502	0.221	1.017	0.204	0.848	0.217	0.798	0.203	0.969	0.23
PNRSV-A	1.099	0.196	0.365	0.226	0.962	0.206	0.924	0.219	0.894	0.197	0.873	0.198
PNRSV-I	0.625	0.789	0.236	0.777	0.226	0.756	0.337	0.759	0.258	0.785	0.266	0.745
PNRSV-I	0.686	0.79	0.308	0.79	0.239	0.789	0.207	0.791	0.301	0.769	0.233	0.768
PNRSV-I	0.722	0.781	0.366	0.784	0.235	0.79	0.296	0.81	0.383	0.745	0.255	0.723
PNRSV-I	0.757	0.765	0.302	0.779	0.3	0.802	0.266	0.792	0.369	0.821	0.341	0.801
PNRSV-I	0.697	0.743	0.337	0.801	0.216	0.794	0.302	0.769	0.404	0.823	0.304	0.721
PNRSV-I	0.639	0.766	0.402	0.743	0.304	0.747	0.322	0.75	0.321	0.763	0.37	0.746

[1] absorbance values standardised to a common positive control

Feb-97		Mar-97		Apr-97		May-97		Jun-97		Jul-97	
PNRSV-A	RMV-3	PNRSV-A	RMV-3	PNRSV-A	RMV-3	PNRSV-A	RMV-3	PNRSV-A	RMV-3	PNRSV-A	RMV-3
0.937	0.207	0.514	0.205	0864	0.201	1.54	0.205	0.768	0.202	0808	0203
0.895	0.203	0.507	0.215	1.599	0.2	1.38	0.206	0.906	0 204	0 897	0 206
0.868	0.205	0.512	0.222	1.024	0.221	1.659	0.207	0.841	0 201	1.079	0 208
0.804	0.212	0559	0.236	0.887	0.236	0539	0.212	1.025	0 21	0.903	0.214
0.934	0.206	0.547	0.205	0.272	0.204	1.519	0.205	0 85	0.205	0.977	0.205
0.872	0.203	0.498	0.202	0.374	0.206	1.619	0 203	0 641	0205	0.925	0.203
0.874	0.207	0.519	0.2	1.146	0.206	1.487	0 199	0.54	0.206	0.807	0 2
0.919	0.205	0.537	0.203	0.492	0.207	1.484	0.197	0.874	0.209	0.899	0.199
0.964	0.222	0 578	0.214	0 543	0.198	1.504	0.195	0.887	0.21	0.941	0.196
0.962	0.221	0.544	0 231	1.127	0.199	0.937	0.199	0.918	0.211	1.035	0.197
0.575	0.202	0.264	0.212	1.16	0 199	1.544	0.196	0.492	0.212	0.469	0.215
0.953	0.206	0.597	0.218	1.612	0.193	1.498	0.197	0.872	0.199	0.969	0 226
0.832	0.204	0.424	0.228	0.802	0.194	1.776	0.232	1.027	0.198	093	0.227
0761	0.194	0.389	0.226	1.478	0.216	1.558	0.225	0.86	0.187	0964	0 197
0.866	0.196	0.515	0.198	1.104	0.218	1.797	0.226	1.087	0.188	1.013	0.229
0.741	0.193	0.518	0.215	1.268	0.214	0.72	0.224	0.859	0.19	1.047	0.203
0.841	0.205	0.309	0.204	1.545	0.212	1.376	0.204	0.838	0.201	0611	0 225
0.971	0.222	0.51	0.203	0.266	0.2	1.183	0.194	0.955	0.205	0.903	0 206
0.9	0.223	0 404	0.196	0.301	0.212	1.158	0.196	0.799	0.203	0889	0 211
0.945	0.229	0.484	0.199	0.283	0.203	1.551	0.19	0 818	0202	0.868	0.212
0.969	0.199	0.536	0.197	0.952	0 205	1.569	0.214	0 871	0.192	0.965	0 222
0.958	0 197	0.555	0.205	0.712	0.209	1.759	0 21	0943	0 193	0 877	0 2
0.956	0.225	0.509	0.206	1.162	0.21	1.682	0 203	0.936	0.196	0986	0 215
0.749	0.215	0.399	0.214	1.427	0.207	1.696	0.208	0 858	0.194	0.802	0 226
0.859	0223	0.308	0.218	1.087	0 212	1.418	0202	0.93	0.199	1.106	0 229
0.827	0.214	0.449	0.217	1.097	0 213	1.385	0.201	0.974	0 2	0.928	0.225
0.826	0.195	0.558	0.225	0.198	0 218	1.212	0.207	1.084	0201	0.855	0223
0.407	0.769	0.267	0.742	0.221	0.756	0.494	0.789	0.953	0786	0.446	0.766
0.417	0.788	0.26	0.762	1.375	0.778	0797	0.801	0.401	0.798	0.359	0.801
0.466	0.798	0.378	0.665	0.392	0.79	0.749	0.809	0.413	0.799	0.547	0805
0.533	0.805	0 287	0.663	0.367	0.796	0.725	0.811	0875	0765	0.631	0798
0.559	0.809	0273	0.661	0.456	0.8	0.475	0.812	0.459	0.752	0561	0.799
0.531	0755	0.322	0.623	1.279	0.8	0.701	0.802	0.472	0.801	0561	0.764

Appendix 5.9. Standardised absorbance values (A_{405})[1] for 'Victoria' plants infected by PNRSV-A or PNRSV-I from August 1997 to Februaty 1998.

	Aug-97		Sep-97		Oct-97		Nov-97		Dec-97		Jan-98		Feburary 1998	
Sample Number	PNRSV-A	RMV-3	PNRSV-A	RMV-3	PNRSV-A	RMV-3	PNRSV-A	RMV-3	PNRSV-A	RMV-3	PNRSV-A	RMV-3	PNRSV-A	RMV-3
PNRSV-A	0.789	0.211	0.798	0.215	0.882	0.213	0.824	0.201	1.452	0.201	0.797	0.201	1.129	0.205
PNRSV-A	0.745	0.213	0.801	0.212	0.884	0.205	0.826	0.203	1.513	0.232	0.681	0.207	1.049	0.206
PNRSV-A	1.665	0.216	0.756	0.209	0.981	0.2	0.976	0.204	1.057	0.222	0.85	0.214	1.169	0.199
PNRSV-A	1.588	0.214	0.792	0.203	0.965	0.199	0.965	0.206	1.055	0.236	0.699	0.225	1.159	0.197
PNRSV-A	1.611	0.199	0.701	0.201	0.932	0.194	1.012	0.21	0.946	0.214	0.912	0.243	1.107	0.198
PNRSV-I	0.413	0.748	0.401	0.746	0.456	0.765	0.501	0.754	0.504	0.756	0.445	0.509	0.557	0.752
PNRSV-I	0.509	0.801	0.398	0.769	0.421	0.789	0.476	0.694	0.541	0.789	0.359	0.556	0.627	0.777
PNRSV-I	0.657	0.8	0.375	0.785	0.426	0.801	0.586	0.698	0.544	0.801	0.352	0.685	0.592	0.774
PNRSV-I	0.652	0.809	0.385	0.763	0.436	0.809	0.596	0.697	0.561	0.802	0.432	0.742	0.661	0.746
PNRSV-I	0.746	0.798	0.368	0.741	0.425	0.805	0.601	0.685	0.578	0.695	0.435	0.723	0.545	0.687

[1] absorbance values standardised to a common positive control