Know-how for Horticulture™

Developing and communicating management strategies for controlling carrot virus Y

Dr. Roger Jones
Department of Agriculture
Western Australia

Project Number: VG01016

VG01016

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

The research contained in this report was funded by Horticulture Australia Ltd with the financial support of the vegetable industry.

All expressions of opinion are not to be regarded as expressing the opinion of Horticulture Australia Ltd or any authority of the Australian Government.

The Company and the Australian Government accept no responsibility for any of the opinions or the accuracy of the information contained in this report and readers should rely upon their own enquiries in making decisions concerning their own interests.

ISBN 0 7341 1086 3

Published and distributed by:
Horticultural Australia Ltd
Level 1
50 Carrington Street
Sydney NSW 2000
Telephone: (02) 8295 2300
Fax: (02) 8295 2399
E-Mail: horticulture@horticulture.com.au

© Copyright 2005

FINAL REPORT

HORTICULTURE AUSTRALIA PROJECT VG01016

DEVELOPING AND COMMUNICATING MANAGEMENT STRATEGIES FOR CONTROLLING CARROT VIRUS Y

Dr R.A.C. Jones, *et al*.
Department of Agriculture, Western Australia

March 2005

HORTICULTURE AUSTRALIA PROJECT VG01016

Project Leader

Dr Roger Jones

Principal Plant Virologist

Department of Agriculture

Locked Bag 4, Bentley Delivery Centre

Western Australia 6983

Email: rjones@agric.wa.gov.au

This is the final report for Project VG01016 'Developing and Communicating management strategies for controlling carrot virus Y'.

March 2004

Any recommendations contained in this publication do not necessarily represent current HAL or Department of Agriculture policy. No persons should act on the basis of the contents of this publication, whether as to matters of fact or opinion or other content, without first obtaining specific, independent professional advice in respect of the matters set out in this publication.

KEY RESEARCH PERSONNEL

Name: **Ms Lindrea Latham**
Position: Plant Virologist
Company: Formerly of the Department of Agriculture, Western Australia
Address: Formerly Department of Agriculture, Locked Bag 4, Bentley Delivery Centre, WA, 6983
Ph: 0011 44 1732 848 383
Fax: 0011 44 1732 848 498
Current Email: lindrea.latham@hdc.org.uk

Name: **Ms Lisa Smith**
Position: Technical Officer/ Acting Research Officer
Company: Department of Agriculture, Western Australia
Address: Department of Agriculture, Locked Bag 4, Bentley Delivery Centre, WA, 6983
Ph: 08 9368 3333
Fax: 08 9474 2840
Email: ljsmith@agric.wa.gov.au

Name: **Ms Tracey Smith**
Position: Technical Officer
Company: Department of Agriculture, Western Australia
Address: Department of Agriculture, Locked Bag 4, Bentley Delivery Centre, WA, 6983
Ph: 08 9368 3333
Fax: 08 9474 2840
Email: tnsmith@agric.wa.gov.au

Name: **Ms Eva Gajda**
Position: Technical Officer
Company: Department of Agriculture, Western Australia
Address: Department of Agriculture, Locked Bag 4, Bentley Delivery Centre, WA, 6983
Ph: 08 9368 3333
Fax: 08 9474 2840
Email: egajda@agric.wa.gov.au

Name: **Mr Allan McKay**
Position: Leader, Root Crops Program
Company: Department of Agriculture, Western Australia
Address: Department of Agriculture, Locked Bag 4, Bentley Delivery Centre, WA, 6983
Ph: 08 9368 3333
Fax: 08 9474 2840
Email: amckay@agric.wa.gov.au

Name: **Dr Brendan Rodoni**
Position: Plant Virologist
Company: Horticultural Development Institute, Agriculture Victoria
Address: Institute for Horticultural Development, Agriculture Victoria, Private Bag 15, Ferntree Gully Delivery Centre, VIC 3156
Ph: 03 9210 9222
Fax: 03 9800 3521
Email: Brendan.Rodoni@dpi.vic.gov.au

Name: **Dr Peter Ridland**
Position: Entomologist
Company: Horticultural Development Institute, Agriculture Victoria
Address: Institute for Horticultural Development, Agriculture Victoria, Private Bag 15, Ferntree Gully Delivery Centre, VIC 3156
Ph: 03 9210 9222
Fax: 03 9800 3521
Email: Peter.Ridland@dpi.vic.gov.au

Name: **Ms Violeta Traievski**
Position: Research Officer
Company: Horticultural Development Institute, Agriculture Victoria
Address: Institute for Horticultural Development, Agriculture Victoria, Private Bag 15, Ferntree Gully Delivery Centre, VIC 3156
Ph: 03 9210 9222
Fax: 03 9800 3521
Email: Violeta. Traicevski@dpi.vic.gov.au

Name: **Mr Robin Coles**
Position: Research Officer
Company: South Australian research and Development Institute
Address: Lenswood Research Centre, South Australian Research and Development Institute, Swamp Road, Lenswood, SA 5240
Ph: 08 8389 8817
Fax: 08 8389 8899
Email: Coles.Robin@saugov.sa.gov.au

Name: **Mr Denis Persley**
Position: Plant Virologist
Company: Queensland Department of Primary Industries and Fishersies
Address: Plant Pathology Building, 80 Meiers Rd, Indooroopilly, Qld 4068
Ph: 07 38969375
Fax: 07 38969533
Email: Denis.Persley@dpi.qld.gov.au

Name: **Dr Calum Wilson**
Position: Plant Virologist
Company: Tasmanian Institute of Agriculture
Address: Tasmanian Institute of Agricultural Research, University of Tasmania, New Town Research Laboratories, 13 Saint Johns Avenue, New Town, TAS 7008
Ph: 03 6233-6841
Fax: 03 6233-6145
Email: Calum.Wilson@dpiwe.tas.gov.au

Name: **Mr Len Tesoriero**
Position: Research Officer
Company: New South Wales Agriculture
Address: Elizabeth Macarthur Agricultural Institute, New South Wales Agriculture, PMB 8, Camden, NSW 2570
Ph: 02 4640 6333
Fax: 02 4640 6300
Email: tesoril@agric.nsw.gov.au

CONTENTS

Section Title Page

A

CONTENTS i

MEDIA SUMMARY iii

TECHNICAL SUMMARY iv

FINANCIAL ANALYSIS v

B.

1.0 DISTRIBUTION AND INCIDENCE OF *CARROT VIRUS Y* IN *AUSTRALIA* **1**

By L.J. Latham, V. Traicevski, D.M. Persley, C.R. Wilson, L. Tesoriero, R. Coles, and R.A.C. Jones

2.0 *CARROT VIRUS Y*: SYMPTOMS, LOSSES, INCIDENCE, EPIDEMIOLOGY AND CONTROL **10**

By L. J. Latham and R. A.C. Jones

3.0 FURTHER STUDIES ON *CARROT VIRUS Y:* HOSTS, SYMPTOMATOLOGY, SEARCH FOR RESISTANCE AND TESTS FOR SEED TRANSMISSIBILITY **28**

By R. A. C. Jones, L. J. Smith, B. E. Gajda, T. N. Smith and L. .J Latham

4.0 PATTERNS OF SPREAD OF *CARROT VIRUS Y* IN CARROT PLANTINGS AND VALIDATION OF CONTROL MEASURES **44**

By R. A. C. Jones, L. J. Smith, B. E. Gajda and L. J. Latham

5.0 RELATIVE ABILITIES OF DIFFERENT APHID SPECIES TO ACT AS VECTORS OF *CARROT VIRUS Y* **59**

By R. A. C. Jones, L. J. Smith, T. N. and L. J. Latham

6.0 FIELD EXPERIMENTS WITH INSECTICIDES AND APHID TRAPPING DATA FROM WESTERN AUSTRALIA 69

By L.J. Latham, L. J. Smith, T. N. Smith and R.A.C. Jones

7.0 APHID TRAPPING, VECTOR EFFICIENCY AND VIRUS INCIDENCE DATA FROM VICTORIA 79

By V. Traicevski, B.C. Rodoni and P. Ridland

8.0 VIRUS AND APHID DATA FROM THE NORTHERN ADELAIDE PLAINS, SOUTH AUSTRALIA 94

By R. Coles

9.0 TECHNOLOGY TRANSFER 104

10.0 RECOMMENDATONS 106

11.0 APPENDIX A – DETAILS OF SOUTH AUSTRALIAN APHID DATA 107

By R. Coles

12.0 APPENDIX B - FARMNOTE ON *CARROT VIRUS Y* 122

By L. J. Latham L. J. Smith and R. A. C. Jones

MEDIA SUMMARY

Carrot virus Y (CarVY) reduces carrot yields and seriously impairs quality. Roots from plants infected early are stubby showing severe distortion and knobbliness, while those from plants infected late are thin with little distortion. Yield losses were 37% (early infection) and 32% (late infection). High infection incidences in crops cause their abandonment due to unmarketability of the carrots.

CarVY occurrence was greater where carrot production was continuous (New South Wales, South Australia, Victoria, Western Australia), than where production was restricted mostly to the summer (Tasmania) or winter (Queensland) months. The percentages of carrot farms and crops found infected respectively were: New South Wales (71%, 56%), Queensland (5%, 4%), South Australia (56%, 56%), Tasmania (4%, 4%), Victoria (93%, 74%) and Western Australia (27%, 19%).

Fourteen different aphid species transmitted CarVY in a non-persistent manner, many at high efficiencies (up to 58%), including both carrot colonising and non-colonising species. The main aphid species associated with CarVY epidemics in WA, SA and VIC were determined. The virus was not transmitted via seed. Key infection sources for spread of CarVY by aphid vectors to newly sown crops were infected volunteer carrots and adjacent infected carrot crops. No alternative host reservoirs of any importance were found, including among cultivated relatives of carrot.

All carrot varieties are susceptible. When 50 wild carrot lines were screened for CarVY resistance, no resistance was found. As little as 15m of separation from a small virus source greatly reduced spread. With a larger virus source, spread was concentrated close to it, with virus levels reducing rapidly over a distance of as little as 45m. Spread was greater downwind than upwind. Insecticides were ineffective in reducing CarVY spread and none could be recommended for use in controlling CarVY.

A multi-faceted, robust, cost-effective integrated disease management (IDM) strategy for sustainable management of CarVY in carrot crops was devised and disseminated to the carrot industry nationally. Case histories following CarVY levels on carrot producing properties that used or did not use the IDM strategy showed that it was very effective in reducing infection (from 65% to 0% over as little as 2 years).

TECHNICAL SUMMARY

CarVY was shown to reduce gross carrot yields substantially and seriously impair carrot root quality. Infection sometimes reached very high incidences within individual crops resulting in their being abandoned due to unmarketability of the carrots. Commonly grown carrot varieties were all susceptible. CarVY symptoms in carrot foliage varied widely in intensity with variety but included chlorotic mottle, marginal necrosis or reddening and generalised chlorosis of leaves, increased subdivision of leaflets giving a 'feathery' appearance and plant stunting. Roots from plants infected early were stubby showing severe distortion and knobbliness, while those from plants infected late were thin with little distortion; yield losses were 37% (early infection) and 32% (late infection).

CarVY was found infecting carrot crops in six Australian states. Its occurrence was greater where carrot production was continuous (New South Wales, South Australia, Victoria and Western Australia), than where production was restricted mostly to the summer (Tasmania) or winter (Queensland) months. The percentages of carrot farms and crops infected respectively were: New South Wales (71%, 56%), Queensland (5%, 4%), South Australia (56%, 56%), Tasmania (4%, 4%), Victoria (93%, 74%) and Western Australia (27%, 19%). Infection was detected in 30/36 carrot varieties.

The key infection sources for spread of CarVY by aphid vectors to newly sown crops were infected volunteer carrots and adjacent infected carrot crops. No alternative host reservoirs of any importance were found, including among cultivated relatives of carrot that are hosts when inoculated in the glashouse. Continuous irrigated carrot production in sequential plantings on the same farm all-year-round resulted in massive infection with the virus, while discontinuous production resulted in low levels. Exposure of young carrot plants to peak aphid populations started epidemics off early. Fourteen different aphid species transmitted CarVY in a non-persistent manner, many at high efficiencies (up to 58%), including both carrot colonising and non-colonising species. Large-scale trapping revealed the main aphid species associated with CarVY epidemics in WA, SA and VIC. When caught live near to an infected carrot planting the honeysuckle aphid, which colonises carrots, transmitted CarVY at an 11% efficiency. Exhaustive tests failed to confirm that seed transmission occurs even at low levels in carrot and other Apiaceous hosts of CarVY.

As little as 15m of separation from a small virus source greatly reduced spread of CarVY infection. With a larger virus source, CarVY spread was concentrated close to it, with virus levels reducing rapidly (from 100% to 20%) over a distance of as little as 45m downwind, giving a 'safe planting distance' of 100m. Spread of infection was greater downwind than upwind of CarVY sources. Pyrethroids and other insecticides were ineffective in reducing CarVY spread and none could be recommend for use in controlling CarVY. Commonly grown carrot varieties were all susceptible to CarVY. When 50 wild carrot lines were screened for CarVY resistance, no resistance was found.

A multi-faceted, robust, cost-effective integrated disease management strategy for sustainable management of CarVY in carrot crops was devised (page 27) and disseminated to the carrot industry nationally. Case histories following CarVY levels on carrot producing properties that used or did not use the integrated disease management strategy showed that it was very effective in reducing infection (from 65% to 0% over as little as 2 years).

The major achievements of the project are: delivery to and adoption by the national carrot industry of an effective, user-friendly integrated disease management strategy for CarVY, grower awareness of the problem and how to recognise it enhanced nationally, production and quality losses due to CarVY reduced nationally, enhanced reliability of production of high quality carrots for domestic and export markets achieved, and understanding of the occurrence, economic impact and epidemiology of CarVY increased.

Future studies should investigate the role of native Apiaceous hosts as potential CarVY reservoirs, and attempt to provide an explanation as to how the virus gets introduced to new carrot production areas that are very isolated and located far away from others.

FINANCIAL ANALYSIS OF THE PROJECT

Horticulture Australia Limited
Financial Statement for Life of Project 2001/02 - 2004/05

Project VG01016 'Developing and communicating management strategies for controlling carrot virus Y'

Funding Received	Budget	Actual	Variance
2001/02	$158,750.00	$158,750.00	$0.00
2002/03	$185,000.00	$185,000.00	$0.00
2003/04	$225,849.00	$225,849.00	$0.00
2004/05	$10,000.00	$5,000.00	$5,000.00
Totals	**$579,599.00**	**$574,599.00**	**$5,000.00**

Expenditure Actuals	Operating	Capital	Total
2001/02	$149,591.45	$0.00	$149,591.45
2002/03	$141,989.63	$0.00	$141,989.63
2003/04	$250,843.38	$0.00	$250,843.38
2004/05 *as at 31st January 2005	$39,189.70	$0.00	$39,189.70
Totals	**$581,614.16**	**$0.00**	**$581,614.16**

Funds Surplus/(Deficit)	as at 28th June 2004*		
Budget Revenue vs Actual Exps	100%		-$2,015.16
Actual Revenue vs Actual Exps	101%		-$7,015.16

B. SECTION 1.0

DISTRIBUTION AND INCIDENCE OF *CARROT VIRUS Y* IN AUSTRALIA

L.J. Latham[A], V. Traicevski[B], D.M. Persley[C], C.R. Wilson[D], L. Tesoriero[E], R. Coles[F], and R.A.C. Jones[A]

[A] Department of Agriculture, Locked Bag No. 4,
Bentley Delivery Centre, WA 6983, Australia
[B] Institute for Horticultural Development, Agriculture Victoria, Private Bag 15,
Ferntree Gully Delivery Centre, VIC 3156, Australia
[C] Department of Primary Industries and Fisheries, Queensland Agency for Food and Fibre Sciences (Horticulture),80 Meiers Rd, Indooroopilly, QLD 4068, Australia
[D] Tasmanian Institute of Agricultural Research, University of Tasmania,
New Town Research Laboratories, 13 Saint Johns Avenue, New Town, TAS 7008, Australia
[E] Elizabeth Macarthur Agricultural Institute, New South Wales Agriculture,
PMB 8, Camden, NSW 2570 Australia
[F] Lenswood Research Centre, South Australian Research and Development Institute,
Swamp Road, Lenswood, SA 5240, Australia

[Draft of paper published in 2004]

Abstract

In 2001-2002, Carrot virus Y (CarVY) was found infecting carrot crops in six Australian states. Its occurrence was greater where carrot production was continuous (New South Wales, South Australia, Victoria and Western Australia), than where production was restricted mostly to the summer (Tasmania) or winter (Queensland) months. The percentages of farms and crops infected respectively were: New South Wales (71%, 56%), Queensland (5%, 4%), South Australia (56%, 56%), Tasmania (4%, 4%), Victoria (93%, 74%) and Western Australia (27%, 19%). Infection was detected in 30/36 carrot cultivars. Possible explanations for the widespread distribution and incidence of CarVY in Australian carrots are discussed.

Additional keywords: *Daucus carota*, potyvirus, surveys, occurrence, prevalence.

Introduction

In 2000, Australia produced 283,000 tonnes of carrots (*Daucus carota*). The largest carrot growing state was Victoria, which produced *c.* 122,000 tonnes. Western Australia produced *c.* 52,000 tonnes and accounted for over 90% of all carrot exports, selling them to Japan, countries in south-east Asia and states in the Persian Gulf. In New South Wales, South Australia, Victoria and Western Australia, carrots are produced all year round. In Tasmania, which has a cold winter, and Queensland, which has a hot summer, carrots are produced mainly in the summer and winter months, respectively (Anon. 2002; McKay 2002).

Carrot virus Y (CarVY) is one of three serologically related potyviruses infecting species of Apiaceae in Australia. It has only been reported in this country where it is found only in carrots (Moran *et al.* 2002; Latham and Jones 2000, 2002, 2003a). *Celery mosaic virus* (CeMV) commonly occurs in celery (*Apium graveolens*) in Australia (Latham and Jones

2003b) and has been found once here in a feral carrot. Apium virus Y (ApVY) infects cultivated parsley (*Petroselinum crispum*) and several apiaceous weed species in Australia. Both CeMV and ApVY are also found overseas. These three viruses form a distinct subgenus within the *Potyviridae* (Moran *et al.* 2002).

CarVY is transmitted by aphids in a non-persistent manner and has a narrow natural host range. Foliar symptoms in carrots are a chlorotic mottle, marginal leaf necrosis or reddening and generalised chlorosis, increased subdivision of leaflets giving a 'feathery appearance' and mild plant stunting (Latham and Jones 2002). Carrot roots from plants infected when young are stubby and show severe distortion and knobliness, a symptom combination sometimes known as 'Michelin carrots' (Latham and Jones 2002).

This paper reports the results of surveys to determine the incidence and distribution of CarVY in carrot crops growing in the major carrot producing regions of Australia and suggests possible reasons for its widespread occurrence.

Materials and Methods

Glasshouse grown plants

All plants were grown in insect-proof, air-conditioned glasshouses maintained at 15-20°C. Plants of carrot cv. Stefano, and celery cv. Tendercrisp were grown in a steam-sterilized soil, sand and peat mix (1:1:1).

Virus isolates and inoculations

Isolates used in Western Australia were CarVY WA-1 from a symptomatic carrot collected at Guilderton, Western Australia (Latham and Jones 2000), and *Celery mosaic virus* (CeMV) CeMV WA-1 described by Latham and Jones (2003b). Isolates used in Victoria were CarVY WA-1 and CeMV Vic-1. Those used in New South Wales were freeze-dried CarVY NSW-1 and CeMV NSW-1, in Tasmania they were CarVY WA-1 and CeMV Tas-1, while in Queensland they were CarVY WA-1 and CeMV DPI 972 (Moran *et al.* 2002). CarVY was maintained in carrot by aphid transmission using *Myzus persicae.* CeMV was maintained in celery by manual inoculation. These cultures of CarVY and CeMV were used as positive controls in enzyme-linked immunosorbent assay (ELISA).

Enzyme-linked immunosorbent assay

A generic monoclonal antibody specific to most potyviruses was obtained from Agdia Inc., USA and polyclonal antibodies to CeMV were obtained from DSMZ GmbH, Germany. To test for potyviruses using the generic potyvirus monoclonal antibody, leaf samples were extracted in 0.05 M sodium carbonate buffer pH 9.6 (1-2 g leaf/20 mL) and tested using the antigen-coated indirect ELISA protocol of Torrance and Pead (1986). To test for infection with CeMV, samples were extracted in phosphate-buffered saline (10 mM potassium phosphate, 150 mM sodium chloride), pH 7.4, containing 0.5-5 mL/L of Tween 20 and 20 g/L of polyvinyl pyrrolidone and tested with CeMV specific polyclonal antibodies using double antibody sandwich ELISA (Clark and Adams, 1977). With both types of ELISA, each sample extract and appropriate controls were tested in duplicate wells of a microtitre plate. The substrate used was 0.6 mg/mL of *p*-nitrophenyl phosphate in 100 ml/L of diethanolamine buffer, pH 9.8. Absorbance values (A_{405nm}) were measured in a Multiskan plate reader (Labsystems, Finland) and values more than twice those of healthy leaf sap were considered positive.

Field surveys of carrot crops

During 2000/2002, carrot crops that were close to harvest were surveyed for CarVY (Table 1). For each crop, 100 young shoots were sampled (one shoot per plant) at intervals of *c.* 3m down several crop rows. Initially, samples were always tested in groups of ten using both the potyvirus monoclonal antibody and the CeMV polyclonal antibody. Except with those from Victoria, samples were re-tested in smaller groups or individually when the incidence of infection was high. For this retesting, generic potyvirus and CeMV specific antibodies were both used, except in Queensland and New South Wales, where only the CeMV antibodies were used. Percentage virus incidence was estimated from grouped sample test results using the formula of Gibbs and Gower (1960).

Results

Differentiation of CarVY from CeMV

After one hour of incubation at room temperature, extracts from CarVY-infected carrot leaves (control isolates) gave ELISA absorbance values $_{(A405nm)}$ that were 10–40 times greater than that of the healthy carrot control with the generic potyvirus antibody, but only 5-15 times greater than that of the healthy carrot control with the CeMV antibodies. Extracts from CeMV-infected celery leaves (control isolates) gave absorbance values $_{(A405nm)}$ that were 50–90 times greater than that of the healthy celery control with the generic potyvirus antibody and 40–120 times greater than that of the healthy celery control with the CeMV antibodies. Extracts from naturally infected carrot samples had absorbance values $_{(A405nm)}$ which were 10-90 times greater than that of the healthy carrot control with the generic monoclonal potyvirus antibody, and 9-15 times greater than that of the healthy carrot control with the CeMV polyclonal antibodies. The weakness of the reactions observed with the CeMV antibodies relative to those observed with the generic potyvirus monoclonal antibody suggest that all ELISA positive samples collected in our surveys were CarVY.

Surveys

In Western Australia, CarVY was found in carrot crops in northern and southern metropolitan Perth, and Myalup but not at Augusta (Fig. 1; Table 1). Incidences of infection in most affected crops were 1-2% but on two carrot export farms in the Guilderton region, they exceeded 50% in 11 crops. In Victoria, CarVY was detected in crops in all five carrot growing regions (north-western irrigation, northern irrigation, Port Phillip, south Gippsland and central Gippsland). In South Australia, CarVY was detected in six carrot growing regions (Blanchetown, Kybybolite, Mount Gambier, Nurioopta, Virginia and Waikerie) but not at Parilla. Incidences of infection in production crops were from 1-11%, but were as high as 98% in seed crops at Binnum. In New South Wales, carrot crops were surveyed only in the Murrumbidgee irrigation region where incidences in infected crops were 2-100%. In Queensland, carrot crops were surveyed in the Fassifern, Granite Belt, Lockyer Valley and southern Darling Downs regions. CarVY was only found in one crop in the southern Darling Downs, with an incidence of 1%. In Tasmania, CarVY was found in only one crop at Forth, with an incidence of 3%.

CarVY was detected in 30/36 cultivars (Table 2). In Western Australia, cv. Stefano was the most frequently surveyed and 20/61 crops of this cultivar were found infected. In Victoria, cvs Stefano and Mokum were the most frequently surveyed, with 13/15 and 3/9 crops

infected, respectively. In South Australia, cvs Carissma, Ricardo and Stefano were most frequently surveyed, with 1/2, 1/5 and 2/3 crops of each infected, respectively. In New South Wales, cvs Western Red, All Seasons and Kamaran were the most frequently surveyed, with 5/5, 2/3 and 1/3 crops infected, respectively. All but one of the carrot crops surveyed in Queensland were of cv. Stefano, including the one infected crop. Senior, Coral II and Stefano were the predominant cultivars surveyed in Tasmania but only cv. Senior was infected.

Discussion

In the absence of CarVY-specific antibodies during this study, we used CeMV-specific antibodies to detect CarVY in our surveys, as the two viruses are serologically related. However, we have confidence that we were only detecting CarVY and not CeMV in the carrot crops for several reasons. Firstly, all records to date indicate that cultivated carrots in Australia are naturally infected only with CarVY and not CeMV (Moran *et al.* 2002). Secondly, the ELISA positive carrot samples from diverse origins collected by us always reacted weakly with the CeMV antibodies but strongly with the potyvirus monoclonal antibody. The weak reactions between CeMV antibodies and positive carrot samples contrasted with those observed with the CeMV control isolates. Independent verification that the naturally infected carrot samples from the surveys contained CarVY was obtained in two ways. Firstly, two survey isolates from Western Australia, one from Victoria and four from South Australia gave strong positive values in ELISA with CarVY-specific monoclonal antibodies from DSMZ that became available after the surveys were completed (L. J. Latham, Smith L. J. and R. A. C. Jones, unpublished). Secondly, four virus isolates from our carrot surveys were sequenced by Moran *et al*. (2002), who confirmed them to be CarVY. ApVY, which is reported to infect carrots in Europe (Kusterer *et al.* 2002), does not cross-react with CeMV antibodies in ELISA (Latham and Jones 2003b), thus excluding confusion between ApVY and CarVY in our surveys.

CarVY was detected in carrot crops in all six Australian states surveyed and in 17 different carrot-growing regions. Infection was found in 30/36 cultivars. Incidences of CarVY infection in New South Wales and South Australia, where carrots are grown continuously throughout the year, were often high, sometimes exceeding 90%. Such high incidences are also found in Victoria (Traicevski *et al*. 2001). The highest incidences of infection were on farms where carrot production was intensive with carrot plantings sown close to one another throughout the year. Incidences of infection in Western Australia were generally low, except on two large export carrot farms where they were greater than 50%. These two farms practice continuous production under irrigation while other farms in the state usually rotate carrots with other crops under irrigation and have sufficient space to sow new crops at large distances from old ones. In Queensland and Tasmania, where carrots are only grown for six months of the year, infection did not exceed 3%. Crops for seed production are usually grown for two years, which provides a greatly extended period for additional virus spread. The two carrot seed crops tested in our surveys had very high incidences of CarVY infection.

These findings suggest that a break in carrot production can greatly diminish the extent of virus carryover between carrot crops. Short of such a drastic approach at sites where carrots are produced all year round, the best control strategy is through phytosanitary and cultural control measures using integrated disease management tactics (Latham and Jones 2003a).

The reason for the occurrence of CarVY infection in carrot crops in isolated and climatically diverse production regions throughout Australia is unknown. Seed transmission and contamination of commercial seed stocks of carrots provides one possible explanation.

Preliminary studies suggest that CarVY may be seed-borne in carrot but at very low levels (Latham and Jones 2003a). Such seed transmission might also sometimes occur in alternative apiaceous hosts. Another possible explanation is presence of infection reservoirs with alternative hosts belonging to certain introduced apiaceous weeds, native Australian apiaceous plants or other apiaceous crop plants These could provide sources for spread when carrot crops are first introduced to new areas. Further investigations into the seed transmissibility of CarVY and studies to determine if there are alternative infection reservoirs are underway.

Acknowledgments

We thank Christine Woods, Fiona Bertus, Judith Parry, Andrew Watson, Lisa Whelan and Annabel Wilson for technical support. Horticulture Australia Ltd funded the surveys.

References

Anon. (2002) Australian Bureau of Statistics. www.abs.gov.au

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

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

Kusterer A., Rabenstein F, Gabler J, Kuhne T (2002) Occurrence of apium potyvirus Y and carrot red leaf luteovirus (CRLV) in dill and other umbelliferous plant species. In: VIIIth International Plant Virus Epidemiology Symposium, Aschersleben, Germany, May 12-17 2002, p. 95. (Abstr.)

Latham LJ, Jones RAC (2000) Yield and quality losses in carrots infected with carrot virus Y. In *Proceedings and abstracts of Carrot Conference Australia* (Ed. E Davison, A McKay) Perth, Western Australia, 24-28 October 2000, p. 48. (Abstr.)

Latham LJ, Jones RAC (2002) Carrot Virus Y. In *Compendium of Umbelliferous Diseases* (Ed. M Davis, R Raid), p 53 (Minesota, USA: American Phytopathological Society Press).

Latham LJ, Jones RAC (2003a) Carrot virus Y: symptoms, losses, incidence, epidemiology and control. *Virus Research* (in press).

Latham LJ, and Jones RAC (2003b) Incidence of *Celery mosaic virus* in celery crops in south-west Australia and its management using a celery-free period. *Australasian Plant Pathology* **32**: 527-531.

McKay A (2002) The WA export carrot industry. *Western Australian Grower* **34**:6-12.

Moran J, van Rijswijk B, Traicevski V, Katijima EW, Mackenzie AM, Gibbs AJ (2002) Potyviruses, novel and known in cultivated and wild species of the family Apiaceae in Australia. *Archives of Virology* **147**:1855-1867.

Raid RN, Zitter TA (2002) Celery mosaic. In: Compendium of Umbelliferous Diseases, (Eds M Davis, R Raid) pp. 53-54, (Minnesota, USA: American Phytopathological Society).

Torrance L, Pead MT (1986) The application of monoclonal antibodies to routine tests for two plant viruses. In *Developments in Applied Biology 1: Developments and Applications on Virus Testing* (Eds RAC Jones, L Torrance) pp.103-118 (Wellesborne, UK: Association of Applied Biologists).

Traicevski V, van Rijswijk B, Rowles A, Ziehrl A, Rundle B and Moran J. (2001) HRDC Project VG97103 - Management of celery mosaic virus (Final Report to Horticultural Australia Ltd). Institute for Horticultural Development Agriculture Victoria, Knoxfield, Victoria, 55p.

Figure legend

Fig. 1. Locations of Australian carrot growing regions sampled in this survey (● = regions where CarVY found; ○ = no CarVY found)

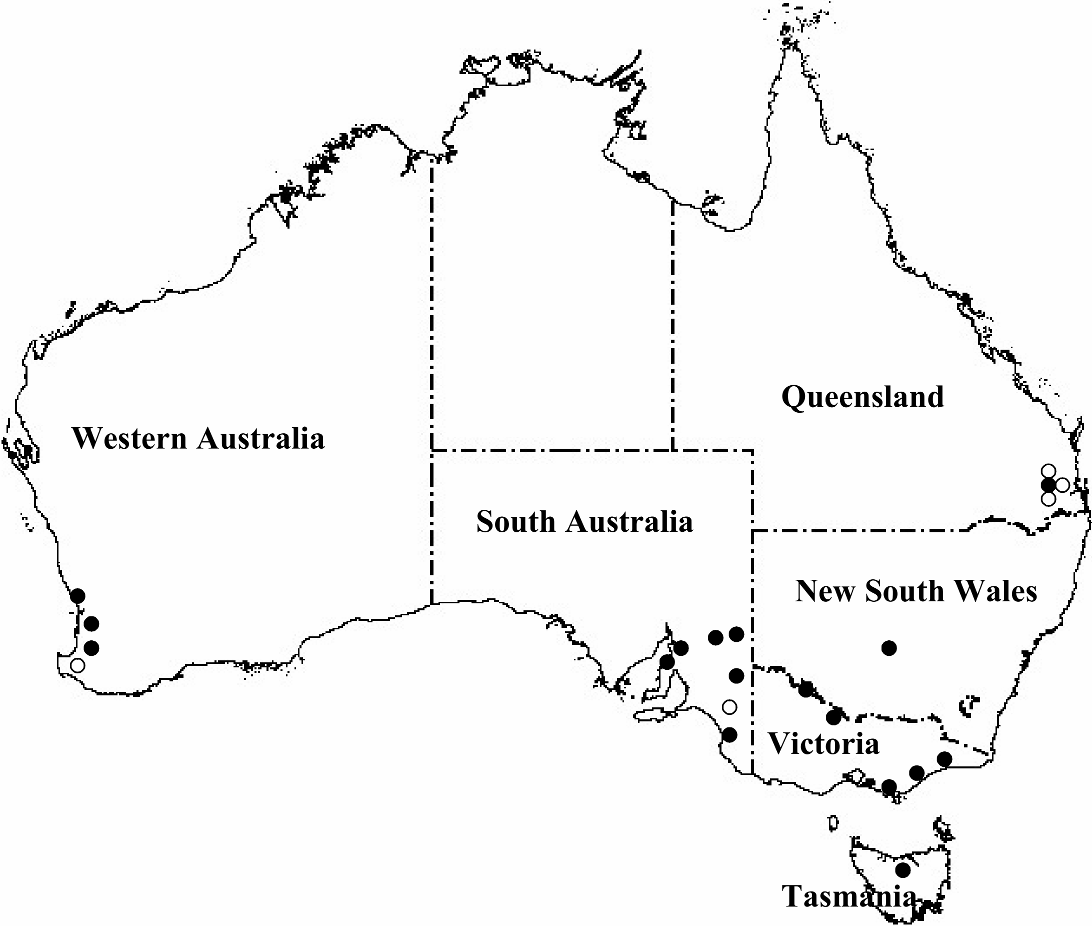


Table 1. Occurrence of CarVY in carrots in six Australian states

State	Seasons crops sampled	Number of farms surveyed	% farms where infection found	Number of crops sampled	% crops infected	Range of infection in infected crops (%)
New South Wales	Autumn, winter, spring	7	71	25	56	2-100
South Australia	Autumn, winter, spring, summer	25	56	25	56	1-98
Victoria	Winter, spring, summer	16	93	54	74	ND
Western Australia	Spring, summer, autumn	18	27	67	19	1-95
Queensland	Winter, early spring	20	5	27	4	1
Tasmania	Summer, early autumn	25	4	25	4	3

ND = not determined

Table 2. Detection of CarVY in different carrot cultivars [A]

Cultivar	Numbers of crops with positive samples/numbers tested	Incidences of infection (%)
All Seasons	2/3	0-85
Bangor	1/1	47
Bastille	4/4	2-12
Cameron	1/1	100
Carissma	½	0-2
CLX3161	1/1	1
Coral II	0/12	0
Crusader	13/15	0-76
Havana	3/3	1-7
Ivor	0/4	0
Jarit	1/1	1
Kamaran	1/3	0-1
Kendo	¼	0-9
Koyo II	2/2	25-40
Leonore	1/1	ND[B]
Mojo	4/5	ND
Mokum	3/9	0-2
Murdoch	4/8	0-52
Nigel	1/1	1
Nairobi	0/3	0
Omeros	2/2	3-5
Ostende	1/1	ND
Paris	2/4	0-32
Red Cloud	0/2	0
Red Count	0/1	0
Red Hot	1/3	0-11
Red Sabre	1/1	ND
Red Victor	1/1	ND
Ricardo	3/9	0-4
Senator	0/2	0
Senior	4/11	ND
Stefano	38/109	0-95
Sun Star	½	0-11
Victor	1/1	2
Viking	1/1	1
Western Red	5/5	1-5
Kuroda type	1/3	0-98
Nantes type	1/1	0-45
Unknown	2/8	0-10

[A]100 samples were collected per crop and tested for CarVY by ELISA to determine % infection, [B]ND = not determined

SECTION 2.0

CARROT VIRUS Y: SYMPTOMS, LOSSES, INCIDENCE, EPIDEMIOLOGY AND CONTROL

L. J. Latham and R. A.C. Jones

Plant Pathology Section, Department of Agriculture, Locked Bag No. 4, Bentley Delivery Centre, WA 6983, Australia.

[Draft of paper published in 2004]

Abstract

Carrot virus Y (CarVY) is a newly described potyvirus that causes a foliar and root disease in carrots which seriously diminishes yield and quality. It infects crops in most commercial carrot producing areas of Australia. Infection sometimes reaches very high incidences within individual crops resulting in their being abandoned due to unmarketability of the roots. A range of commonly grown carrot cultivars were all susceptible. CarVY symptoms in carrot foliage are chlorotic mottle, marginal necrosis or reddening and generalised chlorosis of leaves, increased subdivision of leaflets giving a ‘feathery’ appearance and plant stunting. Roots from plants infected early are stubby showing severe distortion and knobliness, while those from plants infected late are thin with little distortion. The known host range of CarVY is narrow and the key infection sources for spread by aphid vectors to newly sown crops are infected volunteer carrots and adjacent infected carrot crops. Continuous irrigated carrot production in sequential plantings on the same farm all-year-round results in massive infection with the virus, while discontinuous production results in low incidences. Exposure of young carrot plants to peak aphid populations starts epidemics off early. Case histories showing how control measures affected CarVY incidence are described for one farm that deployed them compared with one that did not. An integrated control strategy devised for sustainable management of CarVY in carrot crops is described. Preliminary tests indicate that seed transmission of CarRVY may occur at low levels in carrot, so its introduction to isolated sites may be from inadvertent sowings of contaminated carrot seed stocks.

Keywords: *Carrot virus Y*, potyvirus, carrot, *Daucus carota*, Apiaceae, virus reservoirs, symptoms, losses, surveys, incidence, aphid transmission, seed transmission, epidemiology, control, integrated disease management.

1. Introduction

Carrots are produced all-year-round across the southern part of the Australian continent. On the southern island of Tasmania, which has cold winters, and in the northern state of Queensland, which has hot summers, they are produced in the summer or winter months respectively. They are an integral part of vegetable consumption in Australia where *c*.10.4 kg are consumed annually *per capita*. In the year 2000, Australia produced 283,000 tonnes of carrots and approximately one quarter of this was exported (Anonymous, 2002). The state of Western Australia accounts for 90% of all exports which go to Japan, and countries in South-East Asia and the Arabian gulf (McKay, 2002).

In 1997, plants with symptoms of leaf mottle and a 'feathery' type appearance to their foliage were seen within carrot crops growing at Guilderton, located 75 km north of Perth on the Swan Coastal Plain in south-west Australia. The affected crops were on a farm where carrots are grown for export under large irrigation pivots. In 1998 on this farm, a 65% incidence of the disease was found in spring-planted crops and, when plants with these same foliage symptoms were harvested, they often had symptoms of severe knobliness and distortion in their roots, a condition referred to locally as 'Michelin carrots'. By 1999, another farm nearby with similar cropping practices had disease incidences up to 95% and reported heavy losses. Entire carrot crops were sometimes destroyed with herbicide sprays and ploughed in due to presence of the root symptoms, resulting in major financial losses (Latham and Jones, 2001a). In 2000, several more carrot farms were found infected. In eastern Australia, similar foliage symptoms were first recorded in carrot crops in Victoria and Queensland in 1998 and, later, in New South Wales and South Australia. The same root symptoms were subsequently associated with diseased plants in these states.

Virus diseases of carrots have been little studied in Australia. The only viruses reported to infect the crop are the two components of the carrot motley dwarf disease complex, *Carrot mottle virus* and *Carrot red-leaf virus*, and a potyvirus (Price and McLean, 1984; Buchen-Osmond, 1988). However, carrot motley dwarf disease does not cause the severe root symptoms described above and its foliage symptoms are more severe. In a study of potyviruses infecting wild and cultivated Apiaceae in Australia, Moran et al. (1999, 2002) isolated a novel potyvirus, which they named *Carrot virus Y* (CarVY) (family *Potyviridae,* genus *Potyvirus*), from carrot leaf samples with foliage symptoms of mottle and a 'feathery' appearance from different Australian states, including Western Australia. This was the only potyvirus they found in carrot crops, but one isolated feral carrot sample was infected with *Celery mosaic virus* (CeMV) (family *Potyviridae,* genus *Potyvirus*). Whether the old report of a potyvirus from carrot in Victoria (Buchen-Osmond, 1988) represents an early record of CarVY is unknown, but an early carrot isolate collected in Queensland in 1986 was shown later to be CarVY (Moran et al., 2002). This article reviews the knowledge so far obtained on symptomatology, losses, incidence, epidemiology and control of CarVY in Australia and outlines future research needs. Brief reports of this work were published by Latham and Jones (2000, 2001a, 2002 a, b, c).

2. Detection and relationships

A number of methods are available for detection of CarVY in diseased carrot leaf samples including sap and aphid transmission to indicator plants, electron microscopy, enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction assay (PCR). For inoculation tests to Apiaceous species in general, we found aphid transmission using *Myzus persicae* (green peach aphid) to be the most reliable. It is easier than sap transmission because leaves of Apiaceae tend to consist of many small leaflets that are difficult to infect mechanically. However, sap transmission is suitable to use with *Chenopodium quinoa,* which eventually develops expanding chlorotic blotches in inoculated leaves and Bishop's weed (*Ammi majus*), which develops systemic palor, mottle, leaf distortion and leaf curling. In addition to carrot, *C. quinoa,* and Bishop's weed, other useful hosts include cummin (*Cuminum cyminum*) and coriander (*Coriandrum sativum*), both of which are readily infected. They develop characteristic symptoms consisting of reddening and yellowing of young leaves, upright habit, leaf and stem necrosis and severe plant stunting followed by early death (cummin) or vein clearing, cupping of leaves, decreased leaf size, and plant stunting (coriander) (L.J. Latham, L.J. Smith and R.A.C. Jones, unpubl.).

Flexuous filamentous virions typical of potyviruses can be identified readily in extracts of CarVY-infected carrot leaf samples using electron microscopy. The virions are, on average, 11nm wide and 770nm long (Latham and Jones, 2002b). In the absence of CarVY-specific antiserum, a general potyvirus monoclonal antibody is used to detect the virus by immunological means (ELISA). Extracts from infected leaf samples give only a weak reaction in ELISA with CeMV-specific polyclonal antiserum, which is therefore not very suitable for routine tests. Although Moran et al. (2002) reported that some CarVY isolates react strongly with CeMV antiserum in ELISA, in exhaustive tests with CarVY-isolates from throughout Australia we obtained only weak absorbance values with it (Latham et al., 2004). PCR assays can also be used to detect the virus in leaf samples (Moran et al., 2002). However, because it is less labour intensive, using general potyvirus monoclonal antibody in ELISA has proved more suitable for large-scale routine tests on leaf samples than using indicator plants, electron microscopy or PCR. When different parts of infected carrot plants were tested by ELISA, CarVY was readily detected in new and old leaves, petioles, stems, non-storage roots and crown or shoulder tissue from storage roots (L.J. Latham, L.J. Smith and R.A.C. Jones unpubl.). However, procedures for reliable, large-scale, sensitive detection of CarVY in storage roots are needed. Moreover, exploitation of the latest technologies that become available, e.g. diagnostic microchips (Mumford et al., 2003), to ensure cost effective, accurate diagnosis of CarVY in carrot samples is desirable.

Moran et al. (2002) studied nucleotide sequences from 39 different potyvirus isolates from Apiaceous plant species and used the results obtained to produce a tree showing that these sequences represented five distinct virus species (Fig.1). In their study, an alignment of 429 shared nucleotide sequences from the 3' end of the NIb gene was compared. The five distinct virus species were CarVY, CeMV, *Apium virus Y* (ApVY), *Celery yellow mosaic virus* (CYMV) from Brazil, and *Carrot thin leaf virus* (CTLV)/*Carrot virus B* (CVB) from North America. CarVY was most similar to CeMV, with only a 28-30% sequence difference from it. ApVY and CYMV were closer to each other than to the other viruses as they only differed in sequence by 18-20%. CTLV and CVB formed a distinct cluster with a 35-40% sequence difference from those of CarVY and the other viruses. Moran et al. (2002) concluded that CTLV and CVB are basal outliers, ApVY and CYMV are intermediate, while CeMV and CarVY are related sister species. ApVY is now known to occur in Europe (Kusterer et al*.*, 2002) and Australia, whereas CeMV occurs in many countries.

To provide more information on its relationships to other potyviruses, research is needed to provide missing knowledge on the properties of CarVY, including its stability in sap, relationships with cells and tissues and physico-chemical properties.

3. Symptoms and losses

Symptoms of CarVY in carrot foliage include chlorotic mottle, marginal leaflet necrosis or reddening and generalised chlorosis of leaves, increased subdivision of leaflets giving a 'feathery' appearance and plant stunting (Fig. 2). The severity of leaf symptoms and plant stunting differs greatly between different carrot cultivars. Some (e.g. cv. Bangor) develop obvious symptoms seen easily when viewing a crop from a distance, while others (e.g. cv. Bastille) have symptoms that are so mild they are difficult to see even on close inspection of affected plants. Foliage symptoms of CarVY in actively growing carrot crops could easily be confused with those of nutritional deficiencies. Moreover, at the end of the growing season, growers routinely stop adding fertilisers to reduce post-harvest carrot disorders. Yellowing leaf symptoms then develop which resemble those caused by CarVY, so the two are often confused. Carrot roots from plants that become infected with CarVY when young are stubby showing severe distortion and knobliness (the 'Michelin carrot' syndrome), but later infected

carrots are thinner and longer than early infected ones (Figs 3 and 4). The shoulders of roots of plants infected early are often green as they tend to emerge from the soil exposing them to the sun. Internally, with early infection, the vascular cambium becomes severely distorted and its normal uniform circular shape becomes star-like with multiple contortions.

Experiments were done in the glasshouse to attempt to satisfy Koch's postulates by determining whether the foliage and root symptoms seen in diseased carrots in the field could be reproduced by inoculation of healthy carrot plants with CarVY. To achieve this, wingless *M. persicae* were starved for six hours, placed on CarVY-infected carrot leaves for 10 minute acquisition access feeds and transferred to healthy carrot plants (10 aphids/plant) of cv. Stefano (= cv. Maestro) growing in pots for 1hour inoculation access feeds. Aphids were then killed by spraying with a pyrethroid insecticide. In experiment 1, 15 young plants were inoculated 28 days after sowing and 15 plants were left uninoculated. In experiment 2, the same CarVY inoculation treatment was applied to the same number of plants but at 56 days after sowing so that the plants were older when infected. The plants were observed for characteristic foliage symptoms and tip leaf samples from all plants were tested for CarVY six weeks after inoculation by ELISA using general potyvirus monoclonal antibody. In both experiments, virus-like symptoms became apparent in leaves of infected plants three weeks after inoculation. All 15 inoculated plants were infected in experiment 1 and nine of 15 in experiment 2. The virus was detected in each affected plant by ELISA. In both experiments, the foliage symptoms were chlorotic mottle, generalised chlorosis, necrosis and reddening of leaflet margins, increased subdivision of leaflets giving a feathery appearance and stunted plant growth. However, no symptoms developed in uninoculated plants and no virus was detected in them by ELISA. When the roots were examined after early inoculation, the symptoms were green 'shoulders', severe distortion, knobliness and decreased length (experiment 1), but later infection resulted in thin, narrow carrots with very mild distortion and knobliness and no green 'shoulders' or shortening (experiment 2) (Fig. 4). These results, and many other similar observations on symptoms that developed in CARVY-inoculated plants growing in the glasshouse, confirm that the damaging foliar and root disease found in carrot crops is caused by CarVY.

Quantitative information on the impact of CarVY on yield and quality of carrots was obtained from experiments 1 and 2. The effect of time of infection on yield of individual plants was assessed within each experiment by harvesting 15 pairs of plants from experiment 1 and nine pairs from experiment 2, each pair consisting of one plant with infection and one without. The shoots were cut off and the roots then washed. For each plant, data were obtained on shoot fresh weight, crown width, root length and root fresh weight. Then, roots were rated for distortion on a 1-10 scale where 1 = perfectly formed carrots and 10 = severe knobliness and distortion. Data for the pairs of infected and healthy plants from each experiment were subjected to *t*-tests for each parameter.

In experiment 1, early CarVY infection caused statistically significant decreases ($P<0.05$) in shoot weight, root length and root weight (Table 1) with yield losses of 20%, 24% and 37% for these parameters, respectively. All carrot roots from infected plants had misshapen rankings of 10 (the maximum). In experiment 2, late CarVY infection caused significant decreases in crown width and root weight ($P<0.02$), but there were no significant differences in root length or shoot weight. The root yield loss was 32%. Overall misshapen rankings were much less than in experiment 1, although they were still significantly greater than those of healthy roots. Thus, late-infected carrots were confirmed to be longer and thinner than early-infected ones but lacked their severe malformation. They are still marketable domestically but likely to fetch low prices, while early infected carrots are unmarketable.

Because, CeMV has been reported to infect carrots crops occasionally outside Australia (e.g. Raid and Zitter, 2002), its symptoms in carrot were examined to see if they resemble those caused by CarVY. When carrot cv. Stefano plants were aphid-inoculated with CeMV four weeks after sowing, very severe symptoms developed in their foliage consisting of severe mottle, bunching of leaves, greatly increased subdivision of leaflets giving a marked 'feathery' appearance and very severe plant stunting. Roots from infected plants were short, thin and small, but without the distortion and knobliness observed in carrots from plants infected with CarVY at the same growth stage. Also, the vascular cambium was normal looking (L.J. Latham, L.J. Smith and R.A.C. Jones unpubl.). Thus foliage symptoms of CeMV were far more severe than those of CarVY while its root symptoms were much milder so symptoms of the two are unlikely to be confused.

4. Incidence, distribution and susceptibility

During 2000-2002, Latham et al. (2004) surveyed crops in 22 carrot growing districts in six Australian states (number of crops/state): New South Wales (25), Queensland (27), South Australia (25), Tasmania (25), Victoria (54) and Western Australia (67). For each crop, 100 leaf samples were collected at random. The carrot leaf extracts from each sample were tested for CarVY presence by 1) the antigen-coated indirect ELISA protocol of Torrance and Pead (1986) using general potyvirus monoclonal antibody, and 2) double-antibody-sandwich ELISA as described by Clark and Adams (1977) using polyclonal antiserum to CeMV. As mentioned previously, the former antibody gives a strong absorbance value with CarVY in ELISA, whereas the later antiserum gives only a weak one. Together the results excluded CeMV from being present as no samples gave strong absorbancy values with the CeMV antiserum.

CarVY was detected in carrot crops in all six states and in 17 of 22 different carrot growing regions (Fig. 5). Incidences in the four states where carrots are often grown continuously (New South Wales, South Australia, Victoria and Western Australia) sometimes exceeded 90%. In general, CarVY incidences were high on farms that practised continuous carrot production but low where there was rotation with non-host crops or fallow. When two carrot seed crops growing in South Australia were sampled, CarVY incidences were 98% and 45%. Such crops remain in the ground for up to two years. In Queensland and Tasmania, where carrots are only grown for six months of the year, incidences were always low, not exceeding 3%. These findings indicate that an annual break of up to six months in carrot production can greatly diminish the CarVY inoculum source resulting in low incidences within crops (Latham et al., 2004).

Thirty six different carrot cultivars were sampled during the survey for CarVY and 30 of these were infected with CarVY (Latham et al., 2004). In 2001, 19 carrot cultivars commonly grown in Australia were exposed to extreme natural CarVY inoculum pressure in a field experiment. The different cultivars were sown in plots consisting of paired rows 2.5m long in an irrigated bay. Each cultivar was replicated four times in a randomised block design. At sowing time two CarVY-infector plants of carrot were transplanted one at either end of each plot. Naturally occurring aphids spread CarVY rapidly into all cultivars and, when infection incidences were determined by ELISA tests on leaf samples, final infection incidences of 82-100% were detected (Table 2). Thus all cultivars were highly susceptible. At harvest, roots were assessed for their sensitivity (extent of root deformation) to CarVY. The sensitivities recorded differed significantly with cultivar: Mojo had the mildest root symptoms whereas Havana, Joan and Bolero had the most severe knobliness and distortion. These results suggest that breeding carrots for tolerance to CarVY is possible. Breeding for resistance to CarVY

will not be possible unless resistant genotypes are first identified so screening of a wide selection of land races and wild Daucus sp. for resistance has started to address this need.

5. Epidemiology

5.1. Host Range

In ongoing host range studies in the glasshouse, CarVY has infected so far only carrot, Bishop's weed, chervil (*Anthriscus cerefolium*), cummin, coriander, dill (*Antheum graveolens*) and *C. quinoa* (L.J. Latham, L.J. Smith and R.A.C. Jones unpubl.). Moreover, carrot is still the only host known to become infected in the field. Wild fennel (*Foeniculum vulgare*) commonly grows on track and roadside verges and in ditches near carrot growing farms in south-west Australia and is the only abundant Apiaceous weed found in association with carrots. From 28 sites, 1185 samples of fennel were collected and tested for CarVY by ELISA but none was found infected. Also, fennel did not become infected following aphid inoculation with CarVY in the glasshouse. To obtain a more complete picture, additional Apiaceous species consisting of vegetables, herbs, weeds and Australian native plants are being challenged to see whether they are hosts of CarVY. Also, further surveys of alternative hosts are underway to see if any that become infected in the glasshouse become infected naturally and play roles as virus reservoirs for infection of carrot crops.

5.2 Aphid vectors

The relative efficiencies of different aphid species as CarVY vectors were determined in a series of glasshouse experiments. For this, 10 minute acquisition access periods on CarVY-infected carrot leaves were used, followed by 1 hour inoculation access periods on four-week-old carrot cv. Stefano test plants. There were 10 plants inoculated for each treatment, each of which involved a different number of aphids (0, 1, 2, 4 or 8 aphids/plant). *M. persicae* transmitted at an efficiency of 58%. Other aphid species that colonise Apiaceous hosts and transmitted CarVY were [transmission efficiencies in brackets, greatest to least efficient]: *Aphis spiraecola* (green citrus aphid) [44%], *Dysaphis foeniculus* [19%], *D. apiifolia* (hawthorn parsley aphid) [15%], *Hyadaphis foeniculi* (honeysuckle aphid) [4%], and *H. coriandri* (coriander aphid) [2%]. The non-colonising aphid species *Lipahis erysimi* (turnip aphid) [34%], *Acyrthosiphon kondoi* (bluegreen aphid) [15%], *Hysteroneura setariae* (rusty plum aphid) [14%], *Rhopalosiphum maidis* (corn leaf aphid) [4%] and *R. padi* (bird-cherry oat aphid) [0.3%] were also vectors (L.J. Latham, L.J. Smith and R.A.C. Jones unpubl.). Other colonising and non-colonising aphid species are being tested to determine whether they can transmit CarVY. Studies to examine which species are playing significant roles in transmission to carrots in the field, like those of Berlandier et al. (1997), who caught live aphids on nets to determine which species were transmitting CMV to lupins, are also required with CarVY.

5.3 Seed transmission

Seeds harvested from glasshouse and field-grown CarVY-infected plants of carrot cv. Stefano, were germinated in moist paper towels and, after their seed coats were removed, their radicles were grouped in 10's and tested for CarVY by ELISA. Over 29,000 radicles have been tested, but, so far, only 1 gave a clear positive result. Further tests are required with the same and other cultivars, but, if seed transmission is confirmed, it seems likely that it will only be at a low level. However, even this might suffice to distribute infection to new locations in carrot seed stocks and initiate epidemics there. As CarVY is readily transmitted to carrots by aphid vectors and with the high plant densities at which carrots are grown (70 plants/m2), sowing such seed stocks, could still generate sufficient primary infection sources

to initiate the disease cycle. Following further sowings in continuous production systems, it could eventually increase to damaging levels. Another vegetable potyvirus, Lettuce mosaic virus (LMV), behaves in this way in lettuce such that the threshold level of seed infection recommended for sowing commercial seed stocks in the USA and Australia is <1 in 30,000 (Grogan, 1980; Jones, 2000). Unless seed transmission of CarVY is disproved, it would seem prudent to take precautionary measures (as outlined in the Control section below) to prevent carrot seed crops from becoming infected. Also, a similar process of exhaustive testing of commercial seed stocks may be required like that already employed commercially for LMV in lettuce. To test representative samples, the development of a rapid PCR test for sensitive bulk detection of CarVY in carrot seed stocks would be appropriate, as used commercially already to detect *Cucumber mosaic virus* (CMV) in lupin seed (Wylie et al., 1993; Jones, 2000; 2001).

5.4 Factors favouring epidemics and case histories

The magnitude of epidemics of non-persistently aphid-borne viruses in crops is dependent on two principal factors: 1) the size of the local reservoir of virus-infected host plants, which acts as the major infection source, and its proximity to the crop, and 2) the time of arrival of vector aphids, their abundance and the duration of their activity. In general, spread of such viruses decreases rapidly with increasing distance from the source (see Thresh, 1983; Jones, 2001). As mentioned previously, substantial CarVY epidemics are associated with carrot-growing farms where the crops are sown in sequential plantings all-year-round. Continuous plantings of crops side by side in close proximity, one after the other, results in a steady build up of infection as CarVY cycles from older crops to nearby young ones with infection incidence increasing on each successive cycle. In addition, volunteer plants infected with CarVY growing from infected carrots left over from previous plantings play a key role in providing within-crop virus inoculum sources for infection of newly sown crops. Leaving old CarVY-infected carrot crops in the ground unharvested also provides a potent external source of infection for nearby newly sown crops. In contrast, if a non-host crop (e.g. vegetable brassica or lettuce) is grown in rotation with carrots, then the ability of the virus to cycle from crop to crop is greatly diminished. Sowings that expose young carrot seedlings to peak aphid populations in spring and autumn maximise spread by initiating epidemics early such that high final incidences are reached. Warm temperatures (25/15°C day/night) favour aphid population increase within the crop, which results in increased CarVY spread. Continued irrigation of old crops that prolongs their life permits aphid infestations to continue longer than would otherwise occur resulting in greater final virus incidences and larger reservoirs for further spread (e.g. Jones, 2001).

In Washington State, USA, the initial spread of CTLV is from regenerating volunteer carrots left in the ground at harvest in the previous year. Aphids readily spread CTLV from them to nearby carrot crops. Moreover, because of their growth all-year-round, carrot seed crops, if present, pose a particular hazard for spread of CTLV to nearby ordinary crops (Howell and Mink, 1977). The very high incidence of CarVY in the two carrot seed crops sampled in the national survey (see above) suggests that similar considerations apply in Australia with CarVY. Because of their prolonged growing period and the resulting prolonged aphid activity, CarVY is likely to build up to very high levels within them. Conditions in Washington State differ, however, from those in most of southern Australia in that ordinary carrot crops are only grown for six months of the year, so cycling of CTLV from one crop to the next does not occur in the absence of seed crops. CTLV epidemics are, therefore, largely driven by proximity to infected volunteer carrots.

In south-west Australia, carrot crops on two large farms (A and B) growing export carrots were monitored to determine CarVY incidences over three years (1999-2001). Monitoring involved taking leaf samples at random from crops and testing them for CarVY by ELISA and detailed observations for foliage and root symptoms. Initially both farms grew carrots all-year-round and this crop comprised >90% of their total production. Initial sampling in 1999 determined overall crop infection incidences of up to 65% at A and 85% at B. At A, control measures were then introduced to decrease CarVY incidence which included planting new carrot crops in isolation from old ones, rigorous removal and destruction of volunteer carrots, prompt harvesting of mature carrot crops and sowing intervening non-host crops of vegetable brassicas. In contrast, at B, management practices remained unchanged with no control of volunteer carrots, new carrot crops still sown next to old infected ones, carrot production continued all-year-round and no non-host rotational crops introduced. In 2000, overall infection incidences dropped to trace levels at A but at B they rose to 95%. In 2001, CarVY was not detected at A while at B the overall incidence was 46%. These experiences paralleled the general observations made during the national survey for CarVY that continuous carrot production favoured high CarVY infection incidences while rotation with non-host crops or fallow was associated with low incidences.

6. Control

As with other non-persistently transmitted, aphid-vectored viruses, the primary strategy for control involves removal, minimisation and avoidance of sources of virus infection (e.g. Bawden, 1964). Our specific case studies, survey findings and general understanding of factors driving CarVY epidemics in carrot crops allowed us to devise an integrated disease management strategy that is tailored to suit the special circumstances of CarVY epidemics in carrot crops in Australia. Table 3 summarises the different control measures, how they are achieved, their modes of action and their relative ease of adoption. The key measures are avoiding spread from nearby infected crops including mature crops (and seed crops if present), minimising spread from volunteer carrots and introducing non-host rotational crops. Such an approach was very effective at farm A, as described above. Other strategies included are manipulating planting date to avoid exposure of vulnerable young carrot plants at annual peak aphid population times and employing non-host barrier crops. Although herbicides are still needed to control volunteer carrots, this is the only requirement for chemical control so the strategy is ecologically responsible provided that herbicides are used sparingly. If used correctly, all of these control measures acting in different ways, have complimentary effects and provide a robust, inexpensive yet effective, CarVY management 'package' that is relatively easy to adopt.

To improve the CarVY management 'package' further, research is needed to determine what constitute safe planting distances from CarVY-infected crops when new crops are sown on affected farms. Also, pending further research, an additional more costly measure that might be added specifically for severe epidemic situations is regular application of insecticides, such as pyrethroids that give a rapid aphid knockdown and anti-feeding activity (Perring et al., 1999, Thackray et al., 2000; Jones and Ferris, 2001). However, unless chemicals with minimal side effects and human toxicity are used judiciously, this approach may prove undesirable on environmental grounds. Application of oil sprays to limit CarVY spread by aphids (Simons and Zitter, 1980) is another option yet to be tested with CarVY but unlikely to be cost effective because of the repeated applications likely to be required. Should seed transmission of CarVY be confirmed, provision of tested seed stocks for sowing will be important with threshold seed-infection levels likely to be similar to those currently used with LMV in lettuce (Grogan, 1980; Jones, 2000). Sowing alternate rows of cereal rye in between

those of carrots as a cover crop is currently practised in south-west Australia to help combat wind erosion on sandy soils. This is unlikely to help much by decreasing aphid landing rates because the additional groundcover provided is only present for 2-4 weeks before the cover crop is sprayed out with selective herbicide. As an ultimate control measure, if all else fails, instigating a carrot ‘crop-free’ period, e.g. as used to control CeMV in celery (Apium graveolens) crops in south-west Australia (Latham and Jones, 2001b, 2003), is a feasible but costly option. Finally, development is warranted of a predictive model and decision support system for farmers and horticultural consultants to forecast when and where severe epidemics of CarVY are likely and whether extra control measures are needed.

Carrot seed crops present a special situation where additional control measures against CarVY are likely to be needed because such crops grow for up to two years. Also, because of their exceptionally high value, the costs of extra control measures are likely to be more than offset by the returns they provide. Stringent application of all the standard measures recommended, especially isolation from other crops and destruction of volunteer plants is needed but may be insufficient without additional measures such as application of insecticides or oil sprays. Also, the seed crops should be monitored regularly for CarVY occurrence and, unless seed transmission is disproved, it would seem wise to test representative samples for infection following seed harvest.

There is an urgent need to increase awareness of the disease CarVY causes in carrots and of the integrated disease management strategy devised to control it. An extension campaign is currently underway throughout Australia to maximise adoption of the strategy.

7. Conclusions

The wide geographical distribution of CarVY throughout carrot growing areas in Australia can be explained in three ways. Firstly, an unknown major alternative infection source (Apiaceous weed, native plant or crop) may exist throughout the continent from which CarVY spreads into carrot crops. Secondly, seed transmission may occur albeit at a low level and lead to CarVY-infected carrot seed being widely distributed, inadvertently, to many sites that were previously free from it. Thirdly, a combination of the two is also possible. However the experience at farm B in the case histories tends to support the seed infection scenario. Here, employing simple control measures, such as avoidance of carrot infection sources and introduction of non-host rotation crops, decreased the incidence to 0% in three years indicating that alternative hosts were not involved. Intensive, continuous production of carrots in the absence of rotational crops is a relatively recent development that favours high CarVY incidences, but these can only result after an initial introduction of the virus to a site.

Carrot producers overseas need to be on their guard against possible outbreaks of CarVY and surveys are needed to establish whether the virus occurs elsewhere. Indeed, it may already be present in other countries: Moran et al. (2002) suggested that CarVY was introduced to Australia from overseas because of lack of sequence diversity in isolates from different states.

This work emphasises how, even with much studied virus groups like the potyviruses within well known crops like carrots, when changes in agricultural practices, such as the move to intensive continuous carrot production, take place, there is a need for vigilance over the potential for damaging new virus diseases to develop or for previously unknown or unimportant viruses present at low levels to become damaging. It also emphasises the importance of maintaining plant virus epidemiological skills such that they are available to

respond quickly in developing effective control strategies when important agricultural or horticultural industries are threatened.

8. Acknowledgments

We thank Lisa Smith, Tracey Blanchard and Christine Woods for technical assistance, Allan McKay for advice on carrot production, and Robin Coles, Denis Persley, Len Tesoriero, Violeta Traicevski and Calum Wilson who permitted us to summarise their states results from the national survey of Australian carrot crops for CarVY. Horticulture Australia Limited provided financial support.

9. References

Anonymous 2002. Australian Bureau of Statistics. http://www.abs.gov.au/

Bawden, F.C. 1964. Plant Viruses and Virus Diseases, Fourth Edition. The Ronald Press Company, New York. 361p.

Berlandier, F.A., Thackray, D.J., Jones, R.A.C., Latham, L.J., Cartwright, L. 1997. Determining the relative roles of different aphid species as vectors of cucumber mosaic and bean yellow mosaic viruses in lupins. Ann. Appl. Biol. 131,297-314.

Buchen-Osmond, C., Crabtree, K., Gibbs, A., McLean, G. (Eds) 1988. Viruses of plants in Australia. Australian National University Printing Service: Canberra. 590 p.

Clark, M.F., Adams, A.N. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34,475-483.

Grogan, R.G. 1980. Lettuce mosaic virus control by use of virus-free seed. Plant Dis. 64,446-449.

Howell, W.E., Mink, G.I. 1977. The role of weed hosts, volunteer carrots and overlapping growing seasons in the epidemiology of carrot thin leaf and carrot motley dwarf viruses in central Washington. Pl. Dis. Reporter 61,217-222.

Jones, R.A.C. 2000. Determining 'threshold' levels for seed-borne virus infection in seed stocks. Virus Res. 71,171-183.

Jones, R.A.C. 2001. Developing integrated disease management strategies against non-persistently aphid-borne viruses: a model programme. Integr. Pest Man. Rev. 6:15-46.

Jones, R.A.C., Ferris, D.J. 2000. Suppressing spread of alfalfa mosaic virus in grazed legume pasture swards using insecticides and admixture with grass, and effects of insecticides on numbers of aphids and three other pasture pests. Ann. Appl. Biol. 137,259-271.

Kusterer, A., Rabenstein, F., Gabler, J., Kuhne, T. 2002. Occurrence of apium potyvirus Y and carrot red leaf luteovirus (CRLV) in dill and other umbelliferous plant species. In: VIIIth International Plant Virus Epidemiology Symposium, Aschersleben, Germany, May 12-17, 2002. p. 95. (Abstr.)

Latham, L.J., Jones, R.A.C. 2000. Yield and quality losses in carrots infected with carrot virus Y. In: Proceedings of Carrot Conference Australia, E. Davison, A. McKay (Eds). Perth, Western Australia. pp. 48-49 (Abstr.)

Latham, L.J., Jones, R.A.C. 2001a. Causal agent of devastating carrot disease is identified. Good Fruit and Vegetables 11(10) p. 21.

Latham, L.J., Jones, R.A.C. 2001b. Virus-free celery brings returns. Good Fruit and Vegetables 12(5), p.43.

Latham, L.J., Jones, R.AC. 2002a. Carrot virus Y, a new and devastating disease of carrots. In: 29th International Carrot Conference, Bakersfield, California, February 10-12th 2002. (Abstr.)

Latham, L.J., Jones, R.A.C. 2002b. Carrot Virus Y. In: Compendium of Umbelliferous Diseases, M. Davis, R. Raid (Eds). American Phytopathological Society Press, Minnesota, USA p. 53.

Latham, L.J., Jones, R.A.C. 2002c. Epidemiology and control of carrot virus Y. In: VIIIth International Plant Virus Epidemiology Symposium, Aschersleben, Germany, May 12-17, 2002. p. 64. (Abstr.)

Latham, L.J. and Jones, R.A.C. (2003). Incidence of celery mosaic virus in celery crops in south-west Australia, and its management using a “celery-free period”. Australasian Plant Pathology 32: 527-531.

Latham, L.J., Traicevski, V., Persley, D., Wilson, C., Tesoriero, L., Coles, R., and Jones, R.A.C. (2004). Distribution and incidence of Carrot virus Y in Australia. Australasian Plant Pathology 33: 83-86.

McKay, A. 2002. The Western Australian export carrot industry. Western Australian Grower 34,6-12.

Moran, J., Gibbs, A., van Rijswijk, B., Mackenzie, A., Gibbs, M., Traicevski, V. 1999. Potyviruses in the cultivated and wild Apiaceae in Australia and the implications for disease control. In: ‘12th Biennial Conference of the Australasian Plant Pathological Society’, Canberra, September 27-30, 1999, p. 132. (Abstr.)

Moran, J., van Rijswijk, B., Traicevski, V., Kitajima, E., Mackenzie, A.M., Gibbs, A.J. 2002. Potyviruses, novel and known, in cultivated and wild species of the family Apiaceae in Australia. Arch. Virol. 147:1855-1867.

Mumford, R.A., Blockley, A., Flint, J., Smith, P., Walsh, K., Barker, I., Danks, C., Boonham, N. 2003. Advances in the diagnosis of vegetable viruses: from on-site detection to micro-arrays. J. Plant Dis. and Prot. 110:76.

Perring, T.M., Gruenhagen, N.M., Farrar, C.A. 1999. Management of plant viral diseases through chemical control of insect vectors. Ann. Rev. Ent. 44,457-481.

Price, G.D., McLean, L.K. 1984. Virus, viroid, mycoplasma and rickettsial diseases of plants in Western Australia. Johnston DAW (Ed.). Western Australian Department of Agriculture Technical Bulletin 68. 22p.

Raid, R.N., Zitter, T.A. 2002. Celery mosaic virus. In: Compendium of Umbelliferous Diseases. M Davis, R Raid (Eds). American Phytopathological Society Press, Minnesota, USA p. 53-54.

Simmons, J.N. and Zitter, T.A. 1980. Use of oils to control aphid-borne viruses. Plant Dis. 64,542-546.

Thackray, D.J., Jones, R.A.C., Bwye, A.M., Coutts, B.A. 2000. Further studies on the effects of insecticides on aphid vector numbers and spread of cucumber mosaic virus in narrow-leafed lupins (Lupinus angustifolius). Crop Prot. 19,121-139.

Thresh, M. 1983. Progress curves of plant virus disease. Adv. Appl. Biol. 8,1-85.

Torrance L, Pead M.T. 1986. The application of monoclonal antibodies to routine tests for two plant viruses. In: Developments in Applied Biology 1: Developments and Applications on Virus Testing Eds R.A.C. Jones and L. Torrance. Association of Applied Biologists, Wellesborne, UK. pp.103-118.

Wylie, S., Wilson, C.R., Jones, R.A.C., Jones, M.G.K. 1993. A polymerase chain reaction assay for cucumber mosaic virus in lupin seeds. Aust. J. Agric. Res. 44, 41-51.

Figure Legends

Fig. 1 A tree calculated by the maximum likelihood method to show the relationships between potyviruses found in Apiaceae. Except for Vic #86 from feral carrot (*D.c*), all CeMV and CYMV isolates were from celery; all CarVY isolates, and CTLV and CVB, were from carrot; and the ApVY isolates were from sea celery (*Apium prostratum; A.p*), poison hemlock (*Conium maculatum; C.m*) or parsley (*Petroselinum crispum*; *P.c*).

(for Fig. 1 please refer to the original figure in Moran *et al.,* 2002 or Fig. 1 in Latham and Jones, 2004)).

Fig. 2 CarVY-infected carrot cv. Stefano leaves showing mottle and chlorosis (right), healthy (left).

Fig. 3 CarVY-infected carrot cv. Stefano roots showing severe distortion and knobliness.

Fig. 4 Healthy carrot cv. Stefano root (left), root from plant inoculated with CarVY at 8 weeks after sowing (middle) and root from plant inoculated 4 weeks after sowing (right). Note severe symptoms of distortion and knobliness (right) versus thin, narrow appearance (middle) and normal appearance (left).

Fig. 5. Distribution and incidence of CarVY in carrot crops in Australia in 2000-2002. Percentage CarVY infection values given for each state are for 1) farms found infected (below) and 2) range of CarVY infection within individual crops (above). • = carrot growing districts where CarVY found. o = carrot growing districts where no CarVY found. ND = % crop infection not determined. Data from Latham et al. (2004).

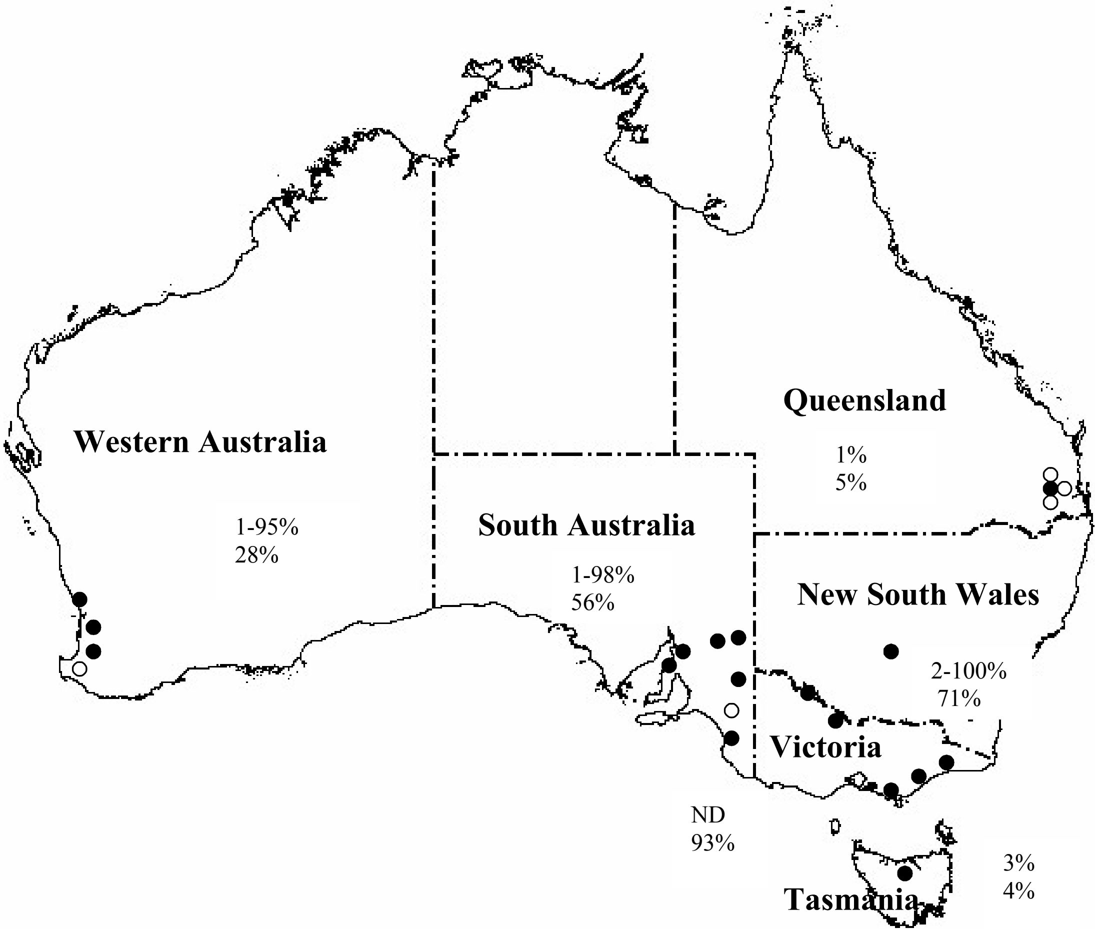


Table 1. Effect of time of inoculation with CarVY on growth, yield and appearance of carrot cv. Stefano shoots and roots

	Shoot fresh weight (g)	Crown width (mm)	Root length (mm)	Root fresh weight (g)	Misshapen ranking[A] (1-10 scale)
Experiment 1: inoculated 28 days after sowing					
Healthy	35	45	146	175	2
Infected	28	40	111	110	10
% change	-20	-11	-24	-37	-
P	0.040	0.056 (ns)	<0.001	<0.001	<0.001
df	24	24	24	24	24
t	2.17	2.01	5.30	4.71	30.52
Experiment 2: inoculated 56 days after sowing					
Healthy	35	45	146	170	2
Infected	36	40	150	116	3
% change	-	-11	-	-32	-
P	(ns)	0.010	(ns)	0.001	<0.001
df	16	16	16	16	16
t	0.21	2.93	0.52	3.84	4.11

Plants inoculated individually with CarVY using aphids. Data based on 15 and nine paired plant comparisons in experiments 1 and 2 respectively.

[A]1 = perfectly formed and 10 = severe knobbliness and distortion

(ns) = not significant

Table 2. Susceptibilities and sensitivities of 19 carrot cultivars exposed to a high field inoculum pressure of CarVY.

Cultivar	Susceptibility (final % infection)[A]	Sensitivity [B]	
Mojo	96	2.41	a
Kendo	82	2.62	b
Sunstar	89	2.66	b
Primo	89	2.82	bc
Stefano (=Maestro)	93	2.87	c
Red Hot (=Apache)	96	2.88	c
Navarre	96	3.14	d
Tempo	86	3.22	de
Senator	96	3.25	def
Nigel	93	3.39	efg
Ivor	100	3.40	fg
Murdoch	96	3.43	fg
Omerus	89	3.43	fg
Bastille	100	3.43	fg
Nairobi	93	3.53	gh
Crusader (=Tino)	100	3.58	gh
Havana	93	3.67	h
Joan	100	3.68	h
Bolero	96	3.69	h
P		<0.001	
d.f.		3768	
L.S.D.		0.204	

[A] Based on field experiment with four replications/ treatment; 50 leaf samples collected from one replicate/ cultivar and tested for CarVY by ELISA.
[B] 1 = carrot roots without symptoms, 5 = very severe root knobliness and distortion. Letters indicate significance classes separated using the L.S.D..

Table 3. Integrated disease management strategy to minimise infection with CarVY and consequent losses in carrot crops

Measure	**How achieved**	**Mode of action**	Adoption
Avoid spread from finished crops	Promptly harvest and remove finished carrot crops or, if not to be harvested, destroy with herbicide, burn or plough deeply underground. Take special care not to leave behind unharvested carrots.	Removes major virus infection source for spread to nearby crops.	Easy
Avoid spread from nearby crops	No overlapping sowings in close proximity or sequential plantings side-by-side. Plant at safe planting distances from potentially infected carrot crops. Plant upwind of potential sources.	Minimises major external source of virus infection for spread to nearby crops.	Easy, if sufficient land available
Minimise spread from volunteer carrots	Control volunteer carrots rigorously by spraying with herbicide or deep ploughing before re-sowing land with carrots.	Removes potent internal or external virus infection source for spread to new plantings.	Easy
Sow non-host barrier crops	Surround carrot crop with non-host barrier crop. Sow tall non-host crop, e.g. cereal or vegetable brassica around target crop.	Aphids probing non-host crop lose the virus before reaching carrot crop.	Moderate
Manipulate planting date	Select planting dates to avoid exposure of young carrot plants to peak aphid populations when these plants are at their most vulnerable, young growth stage.	Diminishes infection occurring at the most vulnerable early growth stage of plants. Plants infected later produce less damaged carrots.	Easy
Institute carrot ‘crop free’ period	Large individual or smaller neighbouring carrot producing farms together ensure carrot ‘crop free’ period of 3 months for entire area. Other non-host crops can be planted during this time. An ultimate measure if all else fails.	Breaks infection cycle over entire area by removing all herbaceous growing plant virus sources.	Difficult
Tentative measure that requires further research before incorporation into the main strategy.			
Apply oil sprays	Apply oil sprays regularly.	Protects crop by preventing aphids probing plants and so introducing to them.	Moderate

SECTION 3.0

Further studies on *Carrot virus Y:* hosts, symptomatology, search for resistance and tests for seed transmissibility

R A C Jones[A], L J Smith[A], B E Gadja[A], T N Smith[A] and L J Latham[A,B]

[A] Department of Agriculture, Locked Bag No. 4, Bentley Delivery Centre, Bentley, WA 6983, Australia.
[B] Current address: Horticultural Development Council, Bradbourne House, East Malling, Kent ME19 6DZ, UK

[Draft of paper submitted for publication]

Abstract

Glasshouse and field studies were done with *Carrot virus Y* (CarVY) to provide information on its host range and symptoms, identify any alternative natural hosts or sources of host resistance in carrot germplasm, and determine whether it is seed-borne. Twenty two species belonging to the Apiaceae were challenged with CarVY by inoculation with viruliferous aphids in the glasshouse. Systemic infection with CarVY developed in carrot itself, 4 other *Daucus* species, 5 herbs, 1 naturalised weed and 2 Australian native plants. When 7 of these host species were exposed to infection in the field, all became infected systemically. In both glasshouse and field, the different types of symptoms that developed in infected plants and the overall severity of host reactions varied widely from host to host. Following inoculation with infective sap, the virus was detected in inoculated leaves of 2 species of Chenopodiaceae. A field survey did not reveal any alternative hosts likely to be important as CarVY infection reservoirs. When 34 accessions of wild carrot germplasm and 16 of other *Daucus spp.* were inoculated with infective aphids, symptom severity varied widely between accessions but no source of extreme resistance to CarVY was found. Tests on seedlings grown from seed collected from infected plantings or individual plants of cultivated carrot (34,135 seeds), wild carrot (20,978 seeds), *Anethum graveolens* (22,921 seeds), and 3 other host species (3,304 seeds) did not detect any seed transmission of CarVY. The implications of these results for control of the virus in carrot crops, minimising the losses it causes and avoiding its introduction to new locations are discussed.

Additional key words: CarVY, potyvirus, aphids, root crops, herbs, weeds, virus reservoirs, germplasm, control.

Introduction

Moran *et al.* (2002) described a new virus from carrot (*Daucus carota*) in Australia, which they named *Carrot virus Y* (CarVY) (family *Potyviridae,* genus *Potyvirus*). The virus infects carrot crops in most commercial carrot producing areas of the country, infection sometimes reaching very high incidences (Latham *et al.* 2004). It is transmitted non-persistently by at least 14 different aphid species, and the

main sources for spread by aphid vectors to newly sown crops are infected volunteer carrot plants and carrot crops. Continuous irrigated carrot production in sequential plantings all-year-round on the same farm results in heavy infection with the virus, while discontinuous production results in low incidences (Latham *et al.* 2004; Latham and Jones 2004; Jones *et al.* 2005a,b). The symptoms that CarVY causes in carrot foliage are chlorotic mottle, marginal necrosis or reddening and generalised chlorosis of leaves, increased subdivision of leaflets giving a 'feathery' appearance and plant stunting. Roots from plants infected early are stubby showing severe distortion and knobbliness, while those from plants infected late are thin with little distortion. Epidemics that start in crops at the vulnerable early growth stage can cause their abandonment due to un-marketability of the produce (Latham and Jones 2002, 2004).

CarVY infects a wide range of carrot cultivars causing similar types of symptoms in each but symptoms vary considerably in severity depending on the sensitivity of the cultivar concerned (Latham *et al.* 2004; Latham and Jones 2004). Information is lacking on the host range of CarVY, its symptomatology in hosts other than carrot, and whether there are any alternative hosts that act as sources of infection for spread to carrot crops in the field. A long term approach toward controlling CarVY is to breed new carrot cultivars resistant to it, but there is no known source of host resistance. Preliminary tests indicated that the virus might be seed-borne at low levels in carrot (Latham and Jones 2004). It is important to confirm this as, if correct, sowing infected seed stocks of carrot inadvertently would introduce the virus to new locations. Also, it would provide potent within-crop infection foci capable of initiating severe epidemics in carrot production areas where CarVY already occurs. This paper describes studies on the host range and symptomatology of CarVY, a small-scale survey for naturally-infected alternative hosts and a search for sources of host resistance in carrot germplasm. It also reports exhaustive tests with carrot and other Apiaceous hosts which provided no supporting evidence that seed transmission of CarVY occurs.

Materials and Methods

Culture, test and 'infector' plants, virus isolates, inoculations and antiserum

Virus culture, test and 'infector' plants of carrot, and the various hosts used in host range and germplasm studies, were grown from seed in a steam sterilised potting mix containing soil, sand and peat in air-conditioned, insect-proofed glasshouses kept at 18-20°C. Colonies of *Myzus persicae* (green peach aphid) were maintained on canola (*Brassica napus*) plants kept inside cages with mesh sides at 15-20°C in a controlled environment room. For aphid inoculations, aphids were starved for 2 h, fed on infected leaves for 10 min and then transferred to healthy plants for 1 h using a fine tipped paint brush before being killed with insecticide. For sap inoculations, sap extracts in 0.02 M phosphate buffer, pH 7.2, were mixed with celite and rubbed onto leaves. The isolates of CarVY and *Celery mosaic virus* (CeMV; family *Potyviridae,* genus *Potyvirus*) used, both coded as WA-1, were from previous research in south-west Australia (Latham and Jones 2003; Latham *et al*. 2004). Cultures of CarVY and CeMV were maintained by aphid-inoculation to plants of carrot cv. Stefano or sap-inoculation to celery (*Apium graveolens*) cv. Tendercrisp respectively. To produce 'infector' plants, young carrot cv. Stefano plants were inoculated with CarVY. Before transplanting outside, tip leaf samples from each potential 'infector' plant were tested by enzyme-linked immunosorbent assay (ELISA) to confirm presence of

CarVY. Generic monoclonal antibody specific to potyviruses was obtained from Agdia Inc., USA and polyclonal antisera specific to CarVY and CeMV from DSMZ GmbH, Germany. The generic antibody was used in initial ELISA tests for CarVY, but, once it became available, the CarVY polyclonal antiserum was always used instead. CarVY isolate WA-1 was always used as a positive control in ELISA tests with the generic antibody or the CarVY antiserum; in tests with CeMV antiserum the positive control was CeMV isolate WA-1.

Enzyme-linked immunosorbent assay

In early tests with generic potyvirus monoclonal antibody, plant tissue samples were extracted (1g/20 mL) in 0.05 M sodium carbonate buffer, pH 9.6, and tested using the antigen-coated indirect ELISA protocol of Torrance and Pead (1986). In later tests using CarVY or CeMV specific antibodies, the samples were extracted (1g/20ml) in phosphate buffered saline (10 mM potassium phosphate, 150 mM sodium chloride), pH 7.4, containing 5 ml/Litre of Tween 20 and 20 g/Litre of polyvinyl pyrrolidone and tested by direct ELISA as described by Clark and Adams (1977). With both types of ELISA, the sample extracts and appropriate control extracts were collected in labelled, plastic sample tubes and tested in paired wells in immunoplates using 0.6 mg/mL of *p*-nitrophenyl phosphate in 100ml/Litre of diethanolamine, pH 9.8, as substrate. Absorbance values (A_{405}nm) from sample extracts were measured in a Titertek Multiskan immunoplate reader (Flow Laboratories, Finland). Absorbance values of more than twice those for healthy sap were considered to represent infected plants.

Host range and symptomatology

Seed of Apiaceous hosts came from commercial sources or, for native species, from the Western Australian Department of Conservation and Land Management. For each Apiaceous host, 20 pots were sown and the seedlings then thinned to 1/pot. When the seedlings had at least 4 true leaves, 10 plants were inoculated with aphids from CarVY-infected carrot leaves (20/plant). The other 10 plants ('negative control') were mock-inoculated with aphids from healthy carrot leaves (20/plant). In addition, 5 carrot cv. Stefano plants ('positive control') were inoculated at the same time with aphids from CarVY-infected carrot leaves (20/plant). The 'negative control' also served as a comparison for symptom records, and the 'positive control' served as a check that the aphids were viruliferous. As mentioned above, aphids were starved for 2 h, fed on isolate CarVY-infected carrot leaves (or healthy carrot leaves) for 10 min, and then transferred to the test plants. After 1 h, they were killed with insecticide. The plants were observed weekly for virus symptoms. After 3 weeks and again after 6-7 weeks, a tip leaf sample from each plant was tested for CarVY presence by ELISA.

In addition to the aphid inoculations, test plants of carrot cv. Stefano, bishops' weed (*Ammi majus*), native pennywort (*Centella cordifolia*), parsnip (*Pastinaca sativa*), and several virus indicator hosts were inoculated by rubbing extracts from carrot leaves infected with CarVY isolate WA-1 mixed with 'celite' onto their leaves. Samples were taken from inoculated leaves after 2 weeks and again after 4-5 weeks, and from tip leaves after 4-5 weeks and tested for CarVY presence by ELISA.

Survey for alternative naturally-infected hosts

All samples were collected from vegetable growing districts within the overall Perth Metropolitan area. Symptomatic whole plant samples of celery and parsley (*Petroselinum crispum*) were collected at Wattleup in 2000 and Waneroo in 2002 respectively. In 2002, leaf samples from 890 wild fennel (*Foeniculum vulgare*) plants were collected at random from 21 different sites at diverse locations north and south of the Swan River where populations of this species were growing. In 2003, 100 leaf samples/crop were taken at random from 12 crops belonging to 6 different types of Apiaceous herbs growing on 3 farms in different locations: (i) at Baldivis the crops sampled were 1 each of angelica (*Angelica archangelica*), chervil (*Anthriscus cerefolium*), coriander (*Coriandrum sativum*), dill (*Anethum graveolens*), Florence fennel (*F. vulgare* var. *azoricum*) and parsley; (ii) at Kenwick they were 2 crops each of coriander and parsley and 1 of dill, all growing in tunnel houses; and (iii) at Waneroo 1 crop of coriander was sampled. At Kenwick, 3 whole symptomatic plant samples were also collected from a coriander crop. In addition to use of the generic potyvirus antibody, all survey samples apart from the wild fennel samples were also tested by ELISA with antibodies to CarVY and CeMV. The random samples were grouped in 10s beforehand but if a grouped sample gave a positive result the individual samples were then retested separately. Leaf tissue samples from each symptomatic whole plant were always tested individually.

Germplasm evaluation

Seeds of wild carrot germplasm accessions were supplied by the Plant Genetic Resources Laboratory, Research Institute for Vegetable Crops, Skierniewice, Poland, and the Genetic Resources Unit, Horticulture Research International, Wellesbourne, UK. The Polish Gene Bank supplied accessions of wild carrot, *D. muricatus, D. bicolor* and unidentified *Daucus* spp; the original countries of origin were Greece, Moldova, Poland, Slovakia, Turkey, Ukraine and Syria. The UK Gene Bank supplied accessions of wild carrot, *D. littoralis* and *D. hispidifolius*; information on their countries of origin was not supplied, but the 7 carrot subspecies tested were *commutatus, gadecaei, gummifer, hispanicus, maritimus, maximus* and *sativus.*

For each of the 50 different wild carrot germplasm accessions tested, 20 pots were sown and the seedlings then thinned to 1/pot. When the seedlings had at least 4 true leaves, 10 plants were inoculated with aphids from CarVY-infected carrot leaves (10/plant), while the other 10 plants ('negative control') were mock-inoculated with aphids from healthy leaves (10/plant). The inoculations were as for the host range tests. The plants were observed weekly for virus symptoms. After 6 weeks, a tip leaf sample from each plant was tested for CarVY by ELISA. If any plants within an accession were not found infected, the individual un-infected plants were then re-inoculated with CarVY by viruliferous aphids and retested by ELISA as before.

Seed transmission tests and field symptom records

Tests for seed transmission of CarVY involved a total of 81,338 seeds harvested from CarVY-infected field plantings or glasshouse grown plants, 55,113 from carrot itself and 26,225 from 4 other Apiaceous hosts. Table 1 lists the infected carrot cultivars or accessions and other Apiaceous species that provided seed, and shows the origins of the seed samples and the types of virus sources involved. The

glasshouse-derived seed was harvested from plants infected with CarVY in the host range tests or germplasm evaluations. Once these studies were completed, the infected plants were transferred into large-sized pots and moved outside to continue growing over a longer period and produce seed. However, this procedure was often relatively unsuccessful as seed yields from infected plants growing in pots tended to be poor, the seed produced was often not viable, and, sometimes, none formed. Infection prevented seed production completely with those species that became infected in the host range studies but are not represented in Table 1. The field-derived seeds came from infected plots of several different carrot field experiments with CarVY growing in 2001-2004 at the Department of Agriculture Research Station at Medina (32°14'S, 115°48'E). They also came from a field exposure block at this Research Station in 2003/04 planted specifically to produce seed from infected plants of carrot and other Apiaceous hosts. In the former, the plots harvested for seed were mostly >90% infected with CarVY. In the latter, the plots from which seed was tested also had >90% CarVY incidences, except for dill with 39%. In addition, seed was also obtained from a commercial carrot seed crop with 98% infection. The incidence values for the infected plantings came from ELISA tests on leaf samples collected at random within them.

The field block planted to produce seed from infected plants measured 7 x 17.5 m. On 26 September 2003, seed was sown by hand in single row plots arranged along raised beds each 1.5 m wide. Two rows of plots were sown along each bed 50 cm apart with 1 m spaces between their ends and each plot was 2.5 m long. On the same day, 48 cv. Stefano 'infector' plants were transplanted into the block. One was positioned near each end of every plot, 50 cm away from it, providing a uniform exposure to virus inoculum throughout the block. There were 2 replications of 10 treatments consisting of anise (*Pimpinella anisum*), bishops' weed, chervil, coriander, cumin (*Cuminum cyminum*), dill, carrot cvv. Mojo and Navarre, and wild carrot accessions 13:009,259 and 13:010,491. Each plot was thinned to about 30 plants. Irrigation was daily by overhead sprinklers and the plants were fertilised according to standard commercial practice; no insecticide was applied and weeding was by hand. Naturally occurring aphids spread the virus within the block. Observation for virus symptoms was combined with ELISA tests on leaf samples which confirmed presence of infection. This provided information on CarVY symptoms induced as a result of natural spread in the field that complemented the symptom records from the host range studies under glasshouse conditions. Recording of symptoms continued until 81 days from sowing (early summer). At 116 days (mid summer), a tip leaf sample was taken from each plant (normally 30/row) and tested individually by ELISA to obtain a percentage CarVY incidence value for each plot. To compare incidence data between species and carrot genotypes, the percentage infection incidence data obtained were transformed to angles and the transformed data subjected analysis of variance. After harvest, seed from each plot was combined within genotypes, cleaned and stored at room temperature for later testing. For this, the seeds were germinated in rolled moist paper towels maintained at *c.*20°C as described by Jones and Cowling (1995). The radicle was separated from the seed coat and shoot of each seedling. Then, the different radicle samples were grouped in 10's, the combined samples ground using a sap extractor machine and their extracts tested for CarVY presence by ELISA.

Results

Host range and symptomatology

When cultivated carrot and 21 other species belonging to the Apiaceae were challenged with CarVY by inoculation with viruliferous aphids, systemic infection was detected in 13 of them (Table 2) but not in the other 9 despite ready infection of carrot plants inoculated at the same time. The infected hosts were carrot itself, 4 wild carrot species from overseas, *D. bicolor*, *D. hispidifolius*, *D. muricatus* and *D. littoralis*, 5 herbs (anise, chervil, coriander, cumin and dill), 1 weed (bishops' weed) and 2 Australian native plants, Australian carrot (*D. glochidiatus*) and native parsnip (*Trachymene pilosa*). Infection in dill was asymptomatic. Among the other hosts, the different types of leaflet symptoms induced were vein clearing, mottle, vein banding, pallor, reddening, deformation, curling, marginal necrosis and generalized necrosis, while the whole shoot symptom types were a feathery appearance, upright growth habit, stunting, weak stems, wilting and death (Figs 1-3). The combination of symptom types that each species exhibited varied widely, as did symptom severity. At one extreme was *D. bicolor* with mild symptoms of pallor and leaf curling in young leaves while at the other was cumin which had 8 different types of symptoms, including eventual death of the infected plant. Within carrot itself, but not in the 6 *D. muricatus* accessions tested, the combination of symptom types found and degree of severity varied widely depending on the genotype inoculated. In most hosts, all plants became infected readily with CarVY when aphid-inoculated but this was not so with anise, chervil and dill, which required further inoculations for this to occur (anise) or in which it was not achieved (chervil and, especially, dill).

Although systemic infection was not detected in its tip leaves following aphid or sap inoculation, parsnip became infected with CarVY in sap-inoculated leaves, some of which developed a mild chlorotic 'oak leaf' pattern (Table 2). Sap inoculation did not infect inoculated or un-inoculated leaves of native pennywort but induced systemic infection in carrot and bishops' weed, both of which are suitable diagnostic and propagation hosts for studies on the virus. However, when using carrot, aphid-inoculation is preferable because its subdivided leaflets make it difficult to infect using infective sap. When aphid-inoculation is used, coriander and cumin are other suitable diagnostic indicator hosts for CarVY.

In sap inoculations to several common indicator hosts outside the Apiaceae, CarVY did not cause any systemic infection. However, it was detected by ELISA in symptomatic inoculated leaf samples from *Chenopodium quinoa* and *C. amanticolor* (Chenopodiaceae). *C. quinoa* and *C. amaranticolor* developed spreading chlorotic blotch local lesions in inoculated leaves (Fig. 3); the former is suitable as an assay host for CarVY.

Survey for alternative naturally-infected hosts

No CarVY was detected by ELISA in the symptomatic field samples of celery, coriander or parsley, the 890 random samples of wild fennel, or in the 100 random samples/crop of the following herbs (numbers of crops tested in parentheses): angelica (1), chervil (1), coriander (3), dill (2), Florence fennel (1) and parsley (3). One of the asymptomatic random samples of coriander gave a positive reaction with CeMV antibodies. Although the virus infecting symptomatic parsley and celery gave a negative reaction with CarVY and CeMV antibodies, it gave a positive one with

generic potyirus antibody. It was later identified as the related *Apium virus Y* (ApVY; family *Potyviridae,* genus *Potyvirus*) following decoration of its flexuous filamentous particles with ApVY antibodies at the Plant Virology Laboratory, Biologische Bundesanstalt fur Land und Forstwirtshaft, Braunschweig, Germany. Similarly, the virus infecting symptomatic coriander reacted only with generic potyvirus antibody in our tests so it may also have been ApVY.

Germplasm evaluation

Plants within all of the following wild germplasm accessions became infected systemically when challenged with CarVY using viruliferous aphids: a) 21 from the Polish germplasm collection, 7 of wild carrot and 6 of *D. muricatus,* 2 of *D. bicolor,* and 6 of unidentified *Daucus* spp.; and b) 29 from the UK collection, 27 of wild carrot, and 1 each of *D. hispidifolius* and *D. littoralis.* However, several accessions required more than 1 inoculation to infect all inoculated plants, and within 3 of them (*D. bicolor* 254,099, *Daucus sp*. 176,670 and carrot 13:007,193) some inoculated plants still remained un-infected after the second inoculation. The difficulty in infecting all plants of these 3 accessions indicates that they may posses 'infection resistance' (i.e. partial resistance to infection by aphid inoculation). In addition, the infected plants of carrot accession 13:007,194 developed systemic necrosis and eventually died, indicating that it may have a slow acting systemic hypersensitive type of resistance. None of the 50 accessions tested exhibited extreme resistance to CarVY.

The range of different types of symptoms that developed in CarVY-infected plants of each named *Daucus* species tested is shown in Table 2. Within carrot itself, there were 7 principal symptom types, which were present in different combinations depending on the accession, more types being present in sensitive than in tolerant ones. No carrot accession showed asymptomatic infection but in some the symptoms were very subtle indicating tolerance to infection, e.g. 13:008,706 just had very mild leaf curling while 13:009,246 had only slight pallor. Apart from the 1 accession that developed systemic necrosis and died (the most severe reaction), a few others were also very sensitive to infection, e.g. 13:009,226 and 13:009,715 were severely stunted with a feathery appearance and small, curled and bunched up leaves showing obvious pallor or reddening (Fig. 4). However, most carrot accessions were intermediate or mild in their reactions. The 6 different carrot subspecies tested varied widely in sensitivity.

Field symptom records

The principal types of symptoms that developed in each host species and carrot genotype included within the field exposure block in 2003/04 are shown in Table 3. All of the different types of symptoms that appeared in the field were seen previously in the glasshouse studies (Table 2) but fewer types were recorded in the field. Also, the spectrum of different symptom types observed within individual species and genotypes tended to be smaller in the field. In bishops' weed, cumin and dill, the severity of host reactions resembled that recorded previously in the glasshouse. In anise and chervil, the recorded symptoms were more severe in the field, but in coriander they were more severe in the glasshouse. In the 2 wild carrot accessions, symptom severity resembled that obtained in them in the germplasm evaluations in the glasshouse. At day 116, the incidence of infection in field plots

ranged from 39% in dill and 70% in anise to 83-98% in the other 6 host species and carrot genotypes still alive then. The percentage incidence in dill was significantly smaller than those in all other hosts, while that in anise was significantly smaller than those in bishop's weed, coriander and carrot accession 13:009,259. As mentioned above, dill and anise (especially dill) had also been difficult to infect using aphids during the host range studies. These results revealed considerable differences in susceptibility to aphid inoculation with CarVY between the different hosts used.

No viable seed was produced by infected plants of carrot cvv. Mojo or Navarre, while the infected plants of anise, coriander and cumin provided only 3, 12 and 6 viable seeds respectively. Infected plants of bishops' weed and chervil produced few seeds but reasonable seed yields were obtained from the infected dill, 2 wild carrot genotypes and the carrot cv. Stefano 'infector' plants. Such large differences in seed production suggest that CarVY-induced losses in seed yield vary widely between host species and carrot genotypes.

Tests for seed-transmission

Regardless of where the seed originated and how the plants became infected, no CarVY was detected in any of the tests on radicles from germinated seeds harvested from CarVY-infected plantings or individual plants of (numbers of seeds tested in parentheses): cultivated carrot (34,135), wild carrot (20,978), anise (330), bishops' weed (2,638), chervil (336) or dill (22,921) (Table 1).

Discussion

These results suggest that CarVY infects relatively few hosts systemically, all of which are members of the Apiaceae. A slightly wider range of hosts became infected in sap-inoculated leaves only, including 2 species in one other plant family. Depending on the host infected, CarVY induced a range of different types of symptoms ranging in severity from very mild to plant death, while infection of one host (dill) was asymptomatic. The reactions of the 50 genotypes of wild and cultivated carrot inoculated also ranged from very mild to very severe. In general, the reactions of different host species in the field resembled those in the glasshouse but symptom severity sometimes differed between the two situations and fewer different types developed in the field. Some hosts were more susceptible to CarVY infection by aphids than others, with dill the most difficult to infect.

Despite exhaustive tests involving numerous seeds from infected plants or plantings, no evidence that seed transmission of CarVY occurs in carrot or other Apiaceous hosts was obtained. In preliminary tests for transmission of CarVY *via* seed of carrot cv. Stefano, Latham and Jones (2004) reported detecting infection once using generic potyvirus antibody in ELISA. We undertook similar but more extensive tests involving 81,338 seedlings germinated from seeds collected from infected plants or plantings of carrot and other Apiaceous hosts from the glasshouse or field respectively. Regardless of where the seed originated, how the mother plants became infected or whether the CarVY source was isolate WA-1 or natural field infection, no virus was detected in any of the seedlings tested. Thus, if seed transmission occurs at all, it must be at very low levels. When sowing seed from infected carrot growing areas, very low CarVY levels in seed could still be important for introducing it to new districts for carrot production. Whether such sowings would generate major epidemics in carrot crops sown with the affected seed seems less likely, although the

recommended ‘threshold’ level for seed infection with another potyvirus, *Lettuce mosaic virus* (family *Potyviridae,* genus *Potyvirus*) in lettuce is as low as <1 in 30,000 (Grogan 1980; Jones 2000). Further studies on this topic are likely to be difficult because, except with dill and wild carrots, viable seed proved hard to obtain from cultivated carrot or Apiaceous herb hosts infected with CarVY.

Our research did not identify any alternative reservoir host that might play a significant role in spread of CarVY to carrot crops in the field. Wild fennel was the only naturalised Apiaceous weed that was common in carrot growing areas. Many samples of it were collected from diverse locations but no CarVY or other potyvirus was detected in them. Also, no virus was detected when this host was inoculated with CarVY using aphids. The 5 Apiaceous herb hosts that became infected systemically in CarVY host range tests (anise, chervil, coriander, cumin and dill) are sometimes grown near to carrot crops on small farms with diverse cropping but no CarVY was detected in any samples collected of the hosts chervil, coriander or dill. In contrast, the related viruses ApVY and CeMV were both detected in coriander. Bishops' weed, a naturalised Apiaceous weed that occurs occasionally in these areas (Marchant *et al.* 1987), proved to be a CarVY host in glasshouse inoculations. It would be worth testing field samples of it for CarVY. Remnant native vegetation, which can include native Apiaceous species, sometimes occurs within carrot growing areas and so might act as a CarVY reservoir. Unfortunately, however, we were unable to survey native plants for presence of CarVY. Also, although seed of 13 different native Apiaceous species was obtained to test as hosts, most of this seed did not germinate. Of the 4 native species for which plants were obtained, Australian carrot and native parsnip became infected systemically with CarVY but neither sea celery (*Apium prostratum*) nor native pennywort were infected. Both Australian carrot and native parsnip occur in native vegetation within carrot growing areas (Marchant *et. al.* 1987). Thus, although the possibility remains that Apiaceous hosts among naturalised weeds, herb crops or native vegetation, might occasionally act as reservoirs of infection for spread to carrots, our findings agree with previous observations that infected volunteer carrots and adjacent infected carrot crops constitute the main infection reservoirs from which the virus spreads to new carrot crops on infected farms (Latham and Jones 2004).

Latham and Jones (2004) described an integrated disease management strategy against CarVY in carrot which was based mainly on phytosanitary and agronomic measures. They also described a case history where this strategy proved effective when deployed. The absence of any significant alternative host reservoirs in this study serves to reinforce the importance of including control measures involving removal or avoidance of potential virus sources involving other carrot crops or volunteer carrots within the strategy. Host resistance was not included as there are no known CarVY resistant cultivars. Testing of carrot germplasm (Rubatzky *et al.* 1999) did not reveal a source of ‘extreme resistance’ suitable for use in breeding such cultivars. However, potential sources of ‘infection resistance’ and ‘systemic hypersensitive’ resistance were detected. Following appropriate confirmation, they might be suitable for use in breeding carrots for CarVY resistance.

This study did not explain why CarVY is found widely dispersed through carrot growing areas in 6 different Australian states (Latham *et al.* 2004). Many of these growing areas are geographically isolated and located far apart. As mentioned above, we were unable to find any evidence of seed transmission that might explain its introduction to new locations through sowing contaminated seed of carrot or other Apiaceous hosts, and no naturally-infected alternative weed or Apiaceous crop hosts

were demonstrated in carrot growing areas from which the virus might spread to carrots. Infection reservoirs may sometimes occur in Apiaceous species present in native vegetation from which the virus can spread to crops sown in new areas for carrots. However, proof that such reservoirs exist is lacking. Moreover, based on the lack of sequence diversity amongst different CarVY isolates, Moran *et al.* (2002) suggested that CarVY might have been introduced recently to Australia rather than it being an indigenous virus that co-evolved here with native plants. Widespread infection of native Apiaceae would seem more likely with an indigenous virus than with one that arrived less than 200 years ago after crops were first introduced to the continent by European colonisers.

Acknowledgements

We thank Rohan Prince for technical support, staff of the Medina Research station for field support, Brenda Coutts and Allan McKay for helpful discussion, Teresa Kotlinska and David Astley for supplying seed of carrot germplasm, and Dietrich Lessemam and Annette Kusterer for the ApVY diagnoses.

References

Clark MF, Adams AN 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *Journal of General Virology* **34,** 475-483.

Grogan RG 1980. Control of lettuce mosaic virus by use of virus-free seed. Plant *Disease* **64**, 446-449.

Jones RAC 2000. Determining 'threshold' levels for seed-borne virus infection in seed stocks. *Virus Research* **71**, 171-183.

Jones RAC, Cowling WA 1995. Resistance to seed transmission of cucumber mosaic virus in narrow-leafed lupins (*Lupinus angustifolius*). *Australian Journal of Agricultural Research* **46,** 1339-1352.

Jones RAC, Smith LJ, Gajda BE, Latham LJ 2005a. Patterns of spread of *Carrot virus Y* in carrot plantings and validation of control measures. *Annals of Applied Biology* (submitted)

Jones RAC, Smith LJ, Smith TN, Latham LJ 2005b. Relative abilities of different aphid species to act as vectors of *Carrot virus Y*. *Annals of Applied Biology* (submitted)

Latham LJ, Jones RAC 2002. *Carrot Virus Y*. In 'Compendium of Umbelliferous Diseases' (Eds M Davis, R Raid) p. 53 (American Phytopathological Society Press: Minnesota, USA)

Latham LJ, Jones RAC 2003. Incidence of *Celery mosaic virus* in celery crops in south-west Australia and its management using a 'celery-free' period' *Australasian Plant Pathology* **32,** 527-531.

Latham LJ, Jones RAC 2004. *Carrot virus Y*: symptoms, losses, incidence, epidemiology and control. *Virus Research* **100,** 89-99.

Latham LJ, Traicevski V, Persley DM, Wilson CR, Tesoriero L, Coles R, Jones RAC 2004**.** Distribution and incidence of *Carrot virus Y* in Australia. *Australasian Plant Pathology* **33,** 83-86.

Marchant NG, Wheeler JR, Rye BL, Bennett, EM, Lander NS, Macfarlane TD 1987. *Flora of the Perth region.* Western Australian Hebarium: Perth, WA.

Moran J, van Rijswijk B, Traicevski V, Kitajima E, Mackenzie AM, Gibbs AJ 2002. Potyviruses, novel and known, in cultivated and wild species of the family Apiaceae in Australia. *Archives of Virology* **147,** 1855-1867.

Rubatzky VE, Quiros CF, Simon PW 1999. Carrots and related vegetable *Umbelliferae*. CAB1 Publishing: Wallingford, UK.

Torrance L, Pead MT 1986. The application of monoclonal antibodies to routine tests for two plant viruses. In ‘Developments in Applied Biology 1: Developments and Applications in Virus Testing’ (Eds RAC Jones, L Torrance) pp.103-118 (Association of Applied Biologists: Wellesbourne, UK)

Legends to Figures

Fig. 1. Old leaf of anise (*Pimpinella anisum*) showing vein banding symptoms caused by systemic infection with CarVY (5 weeks after inoculation).

Fig. 2. Plants of cumin (*Cuminum cymium*) systemically infected with CarVY (right; 4 weeks after inoculation) and healthy (left). Note severe stunting, pallor and weak stem symptoms.

Fig. 3. Mature plants of coriander (*Coriandrum sativum*) systemically infected with CarVY (right) and healthy (left). Note severe stunting, pallor and poor growth caused by CarVY.

Fig. 4. Plants of wild carrot germplasm accession 13:009,226 showing pallor, reddening, feathery appearance and stunting caused by systemic infection with CarVY (left) and healthy (right).

Table 1. Species, origins and numbers of seeds used in CarVY seed transmission tests

Species	Cultivar or accession no.	Origin of seed sample	No. of seeds tested	CarVY source	Notes
Ammi majus (bishops' weed)	-	Glasshouse	1,753	Inoculated with isolate WA-1	Seed from infected plants
Ammi majus (bishops' weed)	-	Field exposure block, 2003/04	885	'Infector' plants with isolate WA-1	Rows with 98% infection produced little viable seed
Anethum graveolens (dill)	-	Field exposure block, 2003/04	20,681	'Infector' plants with isolate WA-1	Seed from 39%-infected rows
Anethum graveolens (dill)	-	Glasshouse	2,240	Inoculated with isolate WA-1	Seed from infected plants
Anthriscus cerefolium (chervil)	-	Mostly from field exposure block, 2003/04	336	'Infector' plants with isolate WA-1	Surviving plants produced little seed
Daucus carota (carrot)	Stefano	Field trials, 2001-04	25,550	'Infector' plants with isolate WA-1	Seed from >90%-infected plots
Daucus carota (carrot)	Stefano	From 'infector' plants in field exposure block, 2003/04	3,299	'Infector' plants with isolate WA-1	Seed from infected plants only
Daucus carota (carrot)	unknown	Commercial crop, South Australia, 2002	5,000	No virus source introduced	Crop naturally 98%-infected
Daucus carota (carrot)	various	Glasshouse	286	Inoculated with isolate WA-1	Infected plants produced little viable seed
Daucus carota ssp. *gummifer* (carrot)	13:007,161	Glasshouse	4,167	Inoculated with isolate WA-1	Seed from infected plants
Daucus carota (carrot)	13:009,259	Field exposure block, 2003/04	10,090	'Infector' plants with isolate WA-1	Seed from 94%-infected rows
Daucus carota (carrot)	13:010,491	Field exposure block, 2003/04	6,721	'Infector' plants with isolate WA-1	Seed from 92%-infected rows
Pimpinella anisum (anise)		Glasshouse	330	Inoculated with isolate WA-1	Infected plants produced little viable seed
Total	-	-	81,338	-	-

Table 2. Host range and symptomatology of CarVY isolate WA-1 in aphid-inoculated Apiaceous hosts in the glasshouse

Host	Common name	Symptoms	Detection in tip leaves
Ammi majus	Bishops' weed	Mo, P, LD, Cu[A]	Yes
Anethum graveolens	Dill	AS	Yes
Angelica archangelica	Angelica	NS	No
Anthriscus cerefolium	Chervil	Mo, P, MSt	Yes
Apium graveolens var. *dulce*	Celery	NS	No
Apium graveolens var. *rapaceum*	Celeriac	NS	No
Apium prostratum	Sea celery	NS	No
Centella cordifolia	Native pennywort	NS	No
Coriandrum sativum	Coriander	VC, Mo, U, LN, SSt, WS	Yes
Carum carvi	Caraway	NS	No
Cuminum cyminum	Cumin	R, P, U, LN, SSt, WS, W, D	Yes
Daucus bicolor	-	Cu, P	Yes
Daucus carota	Carrot	Mo, P, R, MN, F, LD, St[B]	Yes
Daucus glochidiatus	Australian carrot	R, N, SSt, D	Yes
Daucus hispidifolius	-	Mo, MSt	Yes
Daucus littoralis	-	P, R, Cu, F, LN	Yes
Daucus muricatus	-	Mo, Cu, P, R, LD, F, St	Yes
Foeniculum vulgare var. *azoricum*	Florence fennel	NS	No
Foeniculum vulgare var. *vulgare*	Wild fennel	NS	No
Levisticum officinale	Lovage	NS	No
Pastinaca sativa	Parsnip	Co[C]	No
Petroselinum crispum	Parsley	NS	No

Pimpinella anisum	Anise	VB	Yes
Trachymene pilosa	Native parsnip	R, U, Cu, St, WS	Yes

[A] Coded symptom descriptions: AS, asymptomatic infection; VC, vein clearing; Mo, mosaic or mottle; Co, chlorotic 'oak-leaf' pattern in sap inoculated leaves only; Cu, leaf curling; VB, vein banding in older leaves; R, reddening; P, pallor; LD, leaf deformation; F, feathery appearance; MN, marginal leaflet necrosis; LN, leaf necrosis; U, upright habit; MSt, mild stunting; St, stunting, SSt, severe stunting; WS, weak stem; W, wilting; D, death of plant; NS, no symptoms.
[B] Shows the principal symptom types observed within different genotypes of carrot; range of types and symptom severity varied with genotype.
[C] Parsnip was infected by sap inoculation but not by aphids and virus was detected in symptomatic, old inoculated leaves only.

Table 3. Symptoms and incidence of CarVY in wild and cultivated carrot, and 6 other Apiaceous hosts in the field exposure block

Host	Common name, cultivar and accession	Symptoms[A]	% CarVY incidence at 116 days[B]
Ammi majus	Bishops' weed	Mo, F	82.6 (98)
Anethum graveolens	Dill	AS	38.6 (39)
Anthriscus cerefolium	Chervil	R, SSt, D	NT
Coriandrum sativum	Coriander	Mo, R, P	75.8 (94)
Cuminum cyminum	Cumin	SSt, D	NT
Daucus carota	Carrot, cv. Mojo	Mo, MN	65.3 (83)
Daucus carota	Carrot, cv. Navarre	MMo	73.1 (92)
Daucus carota	Carrot, 13:010,491	Mo, R, Cu, F, VB, U, St	73.1 (92)
Daucus carota	Carrot, 13:009,259	MMo, R, F, VB, U,	76.7 (95)
Pimpinella anisum	Anise	MMo, LD, F	57.0 (70)
Significance			*P*=0.008
Lsd			17.96
Df			8

[A] Coded symptom descriptions: AS, asymptomatic infection; MMo, mild mosaic or mottle; Mo, mosaic or mottle; Cu, leaf curling; LD, leaf deformation; VB, vein banding in older leaves; R, reddening; P, pallor; F, feathery appearance; MN, marginal leaflet necrosis; U, upright habit; MSt, mild stunting; St, stunting, SSt, severe stunting; D, death of plant.
NT = Died from CarVY infection before samples taken.
[B] Figures are angular transformations upon which the statistical analysis is based. Values in parentheses are de-transformed % incidences.

SECTION 4.0

Patterns of spread of *Carrot virus Y* in carrot plantings and validation of control measures

By R A C JONES[1], L J SMITH[1], B E GAJDA[1] and L J LATHAM [1,2]

[1] Department of Agriculture, Locked Bag No. 4, Bentley Delivery Centre, WA 6983, Australia

[2] *Current address: Horticultural Development Council, Bradbourne House, East Malling, Kent ME19 6DZ, UK*

[Draft of paper submitted for publication]

Summary

Spatial patterns of spread of *Carrot virus Y* (CarVY) were examined in carrot plantings into which naturally occurring aphid vectors spread the virus from external infection sources. Within three field trials, CarVY 'infector' plants were introduced in between or at different distances from carrot plantings. There was a marked decline in CarVY incidence over distance from adjacent introduced infection sources. Clusters of infected plants that enlarged and coalesced were concentrated next to such sources but, later, isolated, expanding clusters formed further away. With a small external virus source, initial spread into the edge of a planting was less extensive than with a larger source. When 15m wide fallow areas separated a CarVY source from carrot plots, spread was much slower than when the separation was only 1m; it was also slower upwind than downwind of this source. The data collected help validate inclusion of isolation and 'safe' planting distances, intervening fallow, planting upwind, prompt removal of virus sources, avoidance of side-by-side plantings and manipulation of planting date within an integrated disease management strategy for CarVY in carrots.

Key words: CarVY, carrots, spread, pattern, clustering, gradients, wind direction, spatial analysis, control measures, fallow barriers, 'safe' planting distances, integrated disease management.

Introduction

In a study of potyviruses infecting wild and cultivated Apiaceae, Moran *et al.* (2002) described a novel virus which they named *Carrot virus Y* (CarVY) (family *Potyviridae,* genus *Potyvirus*). They found the virus in symptomatic leaf samples of carrot from different parts of Australia. The virus is transmitted non-persistently by at least 14 different aphid species but no evidence of seed transmission was obtained despite exhaustive tests (Latham and Jones, 2002, 2004; Jones *et al*. 2005a,b). Carrot crops become infected with CarVY in most commercial carrot producing areas of Australia, infection sometimes reaching very high incidences (Latham *et al.*, 2004). In carrot foliage, the symptoms consist mostly of chlorotic mottle, marginal necrosis or reddening and chlorosis of leaves, increased subdivision of leaflets giving a 'feathery' appearance and plant stunting. Roots from plants infected early are stubby showing severe distortion and knobbliness, while those from plants infected late are thin with little distortion. An early start to epidemics combined with high final CarVY incidences within crops result in their being abandoned due to unmarketability of the carrots harvested (Latham & Jones, 2002, 2004).

The principal reservoirs from which aphid vectors acquire CarVY and spread it to newly sown crops are infected volunteer carrot plants and nearby infected carrot crops. Continuous irrigated carrot production in sequential plantings all-year-round on the same farm results in heavy infection with the virus, while discontinuous production results in small incidences (Latham *et al.,* 2004; Latham & Jones, 2004; Jones *et al.,* 2005a). An integrated disease management (IDM) strategy devised against CarVY places a major emphasis on phytosanitary and agronomic measures that minimise the source of virus infection for spread to susceptible plantings (Latham & Jones, 2004). To help validate this IDM strategy, studies on spatial patterns of CarVY spread are required that provide information on the effectiveness of control measures such as isolation between susceptible crops, 'safe' planting distances, fallow barriers, planting upwind, prompt removal of virus sources and avoiding side-by-side plantings. However, no such spatial data are available.

This paper describes spatial patterns of CarVY spread in carrot plantings in a) two field trials in which CarVY 'infector' plants were introduced in between different plantings, and b) a field trial in which bare earth fallow separated CarVY 'infector' plants from plots planted at different distances from them. Each trial was oriented parallel to the prevailing wind direction. Spatial Analysis by Distance IndicEs (SADIE) (Perry *et al*., 1996, 1999) was used to assess cumulative infection data. Contour maps based on clustering indices from SADIE, and pathogen progress curves helped with interpretation of the data. The information obtained assists in validating inclusion of cultural control measures within the IDM strategy for CarVY.

Materials and Methods

Virus isolates, inoculations and antiserum

The isolate of CarVY used was WA-1 from south-west Australia (Latham *et al*., 2004). CarVY cultures kept in plants of carrot cv. Stefano were used to produce 'infector' plants of carrots and as positive controls in enzyme-linked immunosorbent assay (ELISA). Aphid inoculations were used to infect culture and 'infector' plants. For these, *Myzus persicae* (green peach aphid) were starved for 2 h, placed on infected leaves for 5-10 min and then transferred to healthy plants (10 aphids/plant) for 1 h before being killed with insecticide. Generic monoclonal antibody specific to potyviruses was obtained from Agdia Inc., USA and polyclonal antiserum specific to CarVY from DSMZ GmbH, Germany. The former was used initially. Once it became available commercially, the latter was used instead.

Plants

Virus culture plants, healthy transplants and 'infector' plants of carrot cv. Stefano were grown from seed in a steam sterilised potting mix containing soil, sand and peat in air-conditioned, insect-proofed glasshouses kept at 18-20°C. To produce 'infector' plants, carrot plants were aphid-inoculated with isolate WA-1 at an early growth stage. Before transplanting outside, tip leaf samples from each potential 'infector' plant were tested by ELISA to confirm presence of CarVY.

Enzyme-linked immunosorbent assay

In early tests with generic potyvirus monoclonal antibody, leaf samples were extracted (1g 20ml^{-1}) in 0.05 M sodium carbonate buffer, pH 9.6, using a leaf press (Pollahne, Germany)

and tested using the antigen-coated indirect ELISA protocol of Torrance & Pead (1986). In later tests using CarVY specific antibodies, leaf samples were extracted (1g $20ml^{-1}$) in phosphate buffered saline (10 mM potassium phosphate, 150 mM sodium chloride), pH 7.4, containing 5ml litre-1 of Tween 20 and 20 g $litre^{-1}$ of polyvinyl pyrrolidone and tested by direct ELISA as described by Clark & Adams (1977). With both types of ELISA, the sample extracts and appropriate control extracts were collected in labelled, plastic sample tubes and tested in paired wells in immunoplates using 0.6 mg ml-1 of p-nitrophenyl phosphate in 100 ml $litre^{-1}$ of diethanolamine, pH 9.8, as substrate. Absorbance values (A_{405}nm) from sample extracts were measured in a Titertek Multiskan immunoplate reader (Flow Laboratories, Finland) and values more than twice those of healthy leaf sap were considered positive.

Details of field trials

The two locations used were Department of Agriculture irrigated field plots at South Perth (31°59'S, 115°52'E) and the nearby Research Station at Medina (32°14'S, 115°48'E), both of which have sandy soils. All trial blocks were rectangular and arranged west-east. At Medina, the prevailing wind normally comes from the west, but at South Perth, where city buildings influence its direction, it came from the east during the growing period used. Irrigation was daily by overhead sprinklers and carrot plants were fertilised according to standard commercial practice; no insecticide was applied. The carrot sown was cv. Stefano. Introduced 'infector' plants acted as the primary CarVY source and naturally occurring aphids spread the virus to healthy plants.

Concurrent trials 1 and 2 were each within different 12 x 100 m irrigated bays spaced 100 m apart in the same field at Medina. Each trial consisted of two 10.5 x 45 m blocks arranged end-to-end and 5 m apart. Seven raised beds each 1.5 m wide ran lengthwise along each block. On 10 October 2002, standard commercial sowing practice was followed to produce a plant density of about 66 carrot plants/m^2. For this, seed was sown in four paired rows 37 cm apart along each raised bed using a cone seeder; within row spacing was set at 7.9 cm. On the same day, 315 (trial 1) and 16 (trial 2) carrot 'infector' plants were transplanted into the 5 m wide source band in between each block. The numbers of 'infector' plants introduced were selected so as to mimic CarVY sources with infection incidences of 8% (trial 1) and 0.4% (trial 2). They were transplanted within the source bands at regularly spaced intervals. Weed control using herbicide followed standard commercial practice in both trials.

Trial 3 was at South Perth and the irrigated bay used measured 10.5 x 50 m. On 14 October 2003, 11 CarVY-'infector' plants were transplanted into each of three rows running across the middle of the bay (north to south) forming a 1.5 x 2.2 m plot; the rows were spaced 50 cm apart with 20 cm plant spacing within rows. On 13 November 2003, 14 healthy 5-wk-old carrot plants were transplanted into each of 12 further plots. Each of these plots measured 0.8 x 2.1 m and consisted of paired rows 40 cm apart with 30 cm plant spacing within rows. Six of these plots were arranged around the CarVY 'source plot' such that they all started 1 m away from it and radiated outwards, three each to the east and west, with a 40 cm space between them at their closest point to the source plot. Another six identical plots started 15 m away from the 'source plot' and radiated outwards, three each to the east and west with a 2.5 m space between each of them at their closest point to the source. The block was rigorously hand weeded to maintain a bare earth fallow. The rest of this site was left without irrigation over the dry late spring to early autumn growing period, so it remained fallow and barren.

Assessment of CarVY spread

In trials 1 and 2, at the time of sowing each block was sub-divided into 315 quadrats 1 x 1.5 m in size, arranged 45 deep and seven across. The left hand corner of each quadrat was identified with a numbered large wooden stake. Three wks later, five plants within each quadrat were selected from regularly spaced positions as on the face of a 'five dice' and each plant chosen was tagged individually with a numbered plastic stake. At days 34, 62, 89, 132, 160 and 194 after sowing in trial 1, and days 41, 69, 96, 138, 165 and 201 after sowing in trial 2, a sample was collected from a young leaf from each of these tagged plants, stored in a labelled plastic bag at $4^{O}C$ and then tested for CarVY by ELISA. Initially all of the samples from two adjacent quadrats were combined together and tested as a grouped sample of ten. When a positive result was obtained for a grouped sample, however, each sample was retested individually to identify which plant(s) was infected. Thereafter, the subsequent samples from within an infected quadrat were always tested individually. In trial 3, at days 22, 34, 54, 82, 112, 125, 138, 152, 167 and 180 after sowing a young leaf sample was collected from each plant as before and tested by ELISA for presence of CarVY. All the samples from each row were combined together as a grouped sample of seven until a positive result identified infection within a row. The samples from the infected row were then retested individually, and subsequent samples from it were always tested thus. The date when each carrot plant first became infected was recorded on a map.

Analysis of spatial pattern

For trials 1-3, infection data for individual plants were used to plot pathogen progress curves. With data from trials 1-2, the counts for CarVY infection in the five plants tested within each quadrat on each assessment date provided a sample unit figure between 0 and 5/quadrat. Spatial pattern of infected plants was quantified using Spatial Analysis by Distance IndicEs (SADIE) and contour maps based on clustering indices (v). For a random arrangement of the counts amongst the sample units, the expected value for the index of aggregation (I_a), an index of the extent of clustering over the whole area sampled, is one, while $I_a > 1$ indicates aggregation of counts into clusters (Perry *et al*., 1996). The values for v for cumulative infections were contoured using 'Surfer' (Anonymous, 1997) to provide maps of spatial pattern as described previously (Thackray *et al.,* 2002; Coutts *et al*., 2004a,b). Contours indicate where estimated indices are half as great again as expected by chance (v =1.5 for infection patches and v=-1.5 for infection gaps). The resulting maps indicate the spatial location and extent of patches and gaps of infection. Spots represent individual quadrat sample units denoting infection patches with $v>0$ (red) and infection gaps with $v<0$ (blue). Small spots represent clustering indices of 0 to +/-0.99 (clustering below expectation), intermediate spots +/- 1 to +/- 1.49 (clustering slightly exceeds expectation) and large spots >1.5 or <-1.5 (clustering more than half as much again as expectation). Red lines enclosing patch clusters are contours of v=1.5 and blue lines enclosing gap clusters are of v=-1.5. Black lines are zero-value contours, representing boundaries between patch and gap regions where the count is close to the sample mean. The units on the contour map axes are distances in metres.

Aphid occurrence

In trials 1 and 2, cylindrical traps consisting of plastic jars covered with yellow sticky paper ('Contact', Nylex Corporation, Australia) mounted 1.5 m above the ground on stakes were used to monitor alate aphids flying above the plantings as described by Bwye *et al.* (1997). On 11 October 2002, one such trap was positioned at opposite ends of each trial. The sticky paper (14 x 43 cm) used in each trap was changed weekly, labeled and the aphids caught counted. The alatae were identified by CSIRO Entomology, Centre for Mediterranean Agriculture, Wembley, Western Australia by referral to Blackman & Eastop (1985). In addition, on each sampling visit, small numbers of carrot shoot tips were examined for presence of colonising aphids.

Results

CarVY spread

Trial 1. The first CarVY infections were detected on day 34 in four quadrats all within 1 m of the source band, one upwind and three downwind. Infection then spread slowly reaching 26 quadrats within 8 m of the source band by day 62, eight (12 infected plants) upwind and 18 (23 infected plants) downwind. By day 89, when the overall incidence of infection was still only 2.4% over the two blocks, there were 16 infected quadrats upwind and 27 downwind, with only three of these >8 m away from the source. However, spread then accelerated and by final assessment at day 194, the overall incidence in both blocks reached 43% (Fig. 1a), 50% upwind and 36% downwind (Table 1).

Ia values for the assessments at days 89, 132, 160 and 194 revealed that, over the entire area of each block, clustering of infected plants was always significant at $P<0.005$ (Table 1). Clusters of infected plants that enlarged and coalesced were concentrated on either side of the source band. On days 89 and 132 the main patch cluster zone adjacent to the source band extended further downwind than upwind, as indicated by the red contours and the distribution of red spots near to the source band on each of the maps (Fig. 2). However, by days 160 and 194, when the upwind block contained more infected plants (Table 1), this was no longer so. On day 194, the large concentrated cluster zones on either side of the source band reached about 20 m upwind and 15 m downwind, as indicated by the positions of the red contours. The portion of each block beyond the main cluster zone was mostly occupied by gap clusters, as shown by the large blue spots and blue contours. There were very few patch clusters outside the main cluster zone until day 132 when some appeared further away in both blocks. However, by day 194, in both of the maps several large expanding patch clusters were present among the gap clusters beyond 20 m from the source band.

Trial 2. The first CarVY infections were detected at day 41 in three quadrats all within 3 m of the source band. Infection spread more slowly than in trial 1 reaching only 17 quadrats (21 infected plants, 0.7% overall incidence) by day 96. Although these infected quadrats were in similar numbers in both blocks rather than more appearing downwind as occurred at this stage in trial 1 (day 89), they were more dispersed downwind, occurring within 6 m (upwind) and 13 m (downwind) of the source band. Spread then accelerated fast approaching the levels seen in trial 1, overall incidence eventually reaching 30% (Fig. 1b), 29% upwind and 32% downwind. *Ia* values for the assessments at days 96, 138, 165 and 201 again showed that, over the entire area of each block, clustering of infected plants was always significant at $P<0.005$ (Table 1). Also, patch clusters that enlarged and coalesced again appeared around

the source band (Fig. 3). On the contour maps, patch clustering on either side of this band was first evident one month later than in trial 1, not appearing until day 96 (day 62 in trial 1). For days 138, 165 and 201, in general, the patch and gap clustering in the contour maps resembled that in the maps for days 132, 160 and 194 respectively in trial 1 (Fig. 2). However, on days 138 and 165 the main patch cluster zone on either side of the source band still extended further downwind than upwind, which was no longer so by day 160 in trial 1. By day 201, both zones were of similar proportions reaching about 15m in both directions, rather than extending out to 20m as in trial 1. The portion of each block beyond the main cluster zone was mostly occupied by gap clusters, as shown by the large blue spots and blue contours, but considerably fewer patch clusters developed outside the main patch cluster zone than in trial 1.

Trial 3. Infection with CarVY was first detected at 22 days in a plot sited 1m from the virus source but not until 125 days (103 days later) in any of the plots 15m away (Fig. 4). In the plots 1m away, spread was slow initially in both upwind and downwind plots but accelerated rapidly after 82 days reaching 100% (upwind) and 96% (downwind) of plants by day 138. In the plots 15m away, once spread started it accelerated rapidly in the downwind plots reaching 100% of plants by 167 days. In contrast, spread was considerably slower in the upwind plots only accelerating after day 138 and still not infecting all plants at final assessment on day 180.

Aphid counts

Examination of carrot shoot tips during sampling visits to trials 1 and 2, did not reveal any aphid colonisation. The only exception was when one unidentified apterous aphid was seen on day 160 in trial 1. Very few aphids were caught in the yellow sticky traps in either trial. Combining the weekly data from all for four traps together revealed that on each trapping date the average number caught/trap mostly fluctuated between 0.5 and 2 alatae. It reached 3.5 alatae once (trapping days 47-54) but none whatsoever were caught over another trapping period (days 74-83). Thus, the numbers caught were always low over the dry hot period up until day 201 when both trials finished. The alatae caught that were identified were predominantly *Brevicoryne brassicae* (cabbage aphid) and *Hyadaphis foeniculi* (honeysuckle aphid) but over the entire tapping period over all four traps 1-3 individuals each of *M. persicae*, *Aphis gossypii* (melon aphid), *Acyrthosiphon kondoi* (bluegreen aphid) and *Hyperomyzus lactucae* (sowthistle aphid) were also caught. Of the aphid species trapped, only *H. foeniculi* and *M. persicae* colonise carrots. *H. foeniculi* was caught mainly between days 13 and 46 in late spring (mid October and November), and days 103 and 159 in late summer and early autumn (late January to mid March). The CarVY vector status of *A. gossypii* and *H. lactucae* is unknown but *A. kondoi*, *B. brassicae, H. foeniculi* and *M. persicae* can all transmit it (Jones *et al.,* 2005b).

Discussion

When CarVY spread from adjacent virus sources into carrot plantings, clusters of infected plants that enlarged and coalesced were concentrated next to such sources. Later, isolated, expanding clusters also formed further away. There was a pronounced decline in incidence of CarVY-infected plants with increasing distance from the original source, resembling previous studies when such declines occurred with other potyviruses spreading into crops from external infection sources (e.g. Hampton, 1967; Jones, 1991, 2005). Such a pronounced effect of external source proximity upon the pattern of virus spread is typical of non-persistently aphid-

borne viruses because most incoming migrant aphids alight at the crop margin, where they probe plants, before moving deeper into a crop. When those that are viruliferous first alight, they infect the plants probed but lose the virus in the process so that plants probed later remain healthy. The plants first infected then provide internal infection sources for further cycles of acquisition and spread by aphids (polycyclic pattern). As occurred with CarVY in our trials, this scenario results in expanding clusters around the plants infected first and initiation of new clusters dispersed deeper into the stand as infection foci gradually arise further way, the pronounced 'edge effect' diminishing as epidemics progress (Thresh, 1974, 1976, 1983; Thackray *et al*., 2002; Jones, 2005).

Thackray *et al.* (2002) and Jones (2005) used maps based on clustering indices to examine the clusters associated with spread of two non-persistently aphid-borne viruses from adjacent external sources into lupin stands. The viruses concerned were *Bean yellow mosaic virus* (BYMV, family *Potyviridae*, genus *Potyvirus*) and *Cucumber mosaic virus* (CMV, family *Bromoviridae*, genus *Cucumovirus*). When the maps for BYMV and CMV were compared with those for CarVY, they closely resembled eachother, with large areas of patch clustering close to the external virus sources and large areas of gap clustering further away. The only dissimilarity was a pattern of intermingling small patch and gap clustered areas that resulted when a 'non-host barrier' separated a BYMV source from the lupins (Jones, 2005). The explanation for this 'non-host barrier' effect is that when incoming migrant aphids probe non-host plants whilst in search of their preferred hosts, they lose a non-persistently aphid-borne virus from their mouthparts, thereby diminishing the amount of virus introduced once they arrive at the crop margin, which removes most of the 'edge effect' (Jones, 1993, 2001, 2005; Fereres, 2000). Whether such barriers will produce similar effects with CarVY in carrot plantings warrants future investigation.

Our studies demonstrated the effects of magnitude of initial virus source and prevailing wind direction on the incidence of CarVY. When 0.4% or 8% infection sources were present in bands of plants that separated upwind and downwind blocks of carrots, the initial spread from the less potent source into the edge of the planting was slower and less extensive. Also, at least initially, the rate of decline in CarVY incidence was faster upwind than downwind. Similarly, when small plots of carrots separated by fallow were planted 1 m and 15 m from a source plot, CarVY not only spread faster to the plots next to this source than to those further away (not detected until 103 days later) but also spread to the latter was considerably slower upwind than downwind. These results help illustrate the potential benefits if isolation, intervening fallow, planting upwind, prompt virus source removal and avoiding successive side-by-side plantings are used together as control measures (Jones, 2004; Latham & Jones, 2004). Isolation is effective because when fallow separates localised virus sources from susceptible crops, dispersal of aphid vectors by wind and flight increases with increasing distance from the source, vector numbers reaching the crop declining as distance from source increases (Thresh, 1974, 1983).

Our carrot trials were present over the dry and hot late spring to early autumn period. Only one colonising aphid was ever seen in trials 1 and 2 and very few aphids were caught flying overhead, weekly catches never exceeding 3.5 aphids/trap, while no aphid colonisation was noted in trial 3. Such low aphid numbers meant that the CarVY epidemics were slow to develop. In other carrot trials sown in early spring over 2001-2003 on the same Research Station as trials 1 and 2 and with similar introduced virus sources, plant emergence coincided with the peak aphid flight period in early spring. CarVY epidemics developed much faster in such trials (unpubl.) than in trials 1-3, which all missed the peak aphid population and flight times that typically occur in the cooler late winter and early spring periods at these sites (Jones, 1991, 2001; McKirdy and Jones, 1994, 1995; Berlandier *et al.,* 1997; Thackray *et al.,*

2004). Thus, our trials all represent a prolonged slow epidemic scenario rather than a rapidly developing one. Latham & Jones (2004) included the control measure 'select planting dates to avoid exposure of young carrot plants to peak aphid populations at their most vulnerable, young growth stage' within their IDM strategy for CarVY. Such experiences over sowing times and aphid flights help justify this recommendation.

H. foeniculi, which colonises carrots, and *B. brassicae*, which does not, were the predominant aphid species caught on sticky traps placed at either ends of trials 1 and 2. Although information on the actual aphid species associated with virus spread in carrots was not collected here, such data were obtained at the Research Station used for trials 1 and 2 during another field study with CarVY done over the same dry period in the following year (2003-2004). Then, the principal CarVY vector caught on nets placed downwind of an infected block of carrots was *H. foeniculi* which colonised the carrots, but small numbers of *A. gossypii*, *B. brassicae* and *H. lactucae* were also caught (Jones *et al.,* 2005b) However, aphids that do not colonise carrots are also likely to be important as vectors of CarVY, as several transmit it (Jones *et al.,* 2005b), and non-colonising aphids often contribute to spread of other non-persistently aphid-borne viruses in other crops the region, e.g. in previous studies with BYMV and CMV in lupins at the location used for trial 3 (McKirdy and Jones, 1994, 1995; Berlandier *et al.,* 1997; Jones, 2001; Thackray *et al.,* 2004).

Although their effectiveness depends on factors like vector numbers and flights, prevailing wind direction and magnitude of virus source, the need to establish 'safe' planting distances to help diminish CarVY spread to carrot crops was illustrated here. Our results permit tentative estimates to be made for such distances in situations where prompt removal of virus sources or deployment of non-host barriers may be impossible. Thus, with a small nearby, upwind virus source when aphid vector numbers were small, as little as 15 m of separation by fallow contributed greatly towards diminishing CarVY incidence. A suitable 'safe' planting distance recommendation for this scenario that errs on the side of safety would be 25 m. In contrast, with a massive source, e.g. as would occur when a crop is sown near to an established carrot crop heavily infected with CarVY, or with a smaller nearby virus source in a crop emerging at peak vector flight times, a 'safe' planting distance of >100 m would probably be more suitable. Deployment of intervening non-host barriers instead of fallow is likely to diminish such 'safe' planting distances, as they not only provide physical separation but also act as 'virus cleansing barriers' with non-persistently aphid-borne viruses (Jones, 2005).

As mentioned in the Introduction, the IDM strategy for CarVY in carrot field crops proposed by Latham & Jones (2004) includes a wide range of phytosanitary and agronomic control measures. Its design was based partly on generic information over control measures used previously with similar potyvirus-crop pathosystems (Jones, 2001, 2004; Jones *et al.,* 2004). However, epidemiological information on the CarVY-carrot pathosystem was also used to construct it, e.g. knowledge that i) the known host range of CarVY is narrow and the key infection sources for spread by aphid vectors to newly sown crops are infected volunteer carrots and adjacent infected carrot crops; (ii) continuous irrigated carrot production in sequential side-by-side plantings on the same farm all-year-round results in massive infection with the virus, while discontinuous production results in low incidences; and iii) exposure of young carrot crops to peak aphid populations start epidemics off early leading to high final incidences and crop produce rejections (Latham *et al.,* 2003; Latham & Jones, 2004). Case histories showing that the IDM approach recommended was effective when deployed on commercial carrot farms with high incidences of CarVY infection helped validate it (Latham & Jones, 2004). Our studies involving spatial analysis of CarVY epidemics in carrots under different infection scenarios complement these case histories by providing additional

validation for many of the control measures recommended, including isolation, 'safe' planting distances, intervening fallow, planting upwind, prompt removal of virus sources, avoiding successive side-by-side plantings and manipulation of planting date to avoid peak aphid vector flight periods.

Acknowledgements

We thank Brenda Coutts for help with data analysis, Rohan Prince and Tracey Smith for technical assistance, Owain Edwards and Rick Horbury for aphid identifications, Allan McKay for helpful discussion over carrots, and staff at Medina Research Station for help with the field experiments. Horticulture Australia Ltd provided financial support.

References

Anonymous 1997. *Surfer for Windows v. 6.04, Surface Mapping System.* Colorado, USA: Golden Software Inc.

Bwye AM, Proudlove W, Berlandier FA, Jones, RAC. 1997. Effects of applying insecticides to control aphid vectors and cucumber mosaic virus in narrow-leafed lupins *(Lupinus angustifolius*). *Australian Journal of Experimental Agriculture* **37:**93-102.

Berlandier F A, Thackray D J, Jones R A C, Latham L J, Cartwright L. 1997. Determining the relative roles of different aphid species as vectors of cucumber mosaic and bean yellow mosaic viruses in lupins. *Annals of Applied Biology* **131:**297-314.

Blackman R L, Eastop V F. 1985. *Aphids on the world' crops – an identification guide*. Brisbane, Australia: John Wiley & Sons.

Coutts B A, Thomas-Carroll M L, Jones R A C. 2004a. Patterns of spread of *Tomato spotted wilt virus* in field crops of lettuce and pepper: spatial dynamics and validation of control measures. *Annals of Applied Biology* **145**:231-245.

Coutts B A, Thomas-Carroll M L, Jones R A C. 2004b. Analysing spatial patterns of spread of *Lettuce necrotic yellows virus* and lettuce big-vein disease in lettuce field plantings. *Annals of Applied Biology* **145:**339-343.

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

Fereres A. 2000. Barrier crops as a cultural control measure of non-persistently transmitted aphid-borne viruses. *Virus Research* **71**:221-231.

Hampton R O. 1967. Natural spread of viruses infectious to beans. *Phytopathology* **57**:476-481.

Jones R A C. 1991. Reflective mulch decreases the spread of two non-persistently aphid-transmitted viruses to narrow-leafed lupin (*Lupinus angustifolius*). *Annals of Applied Biology* **118**:79-85.

Jones R A C. 1993. Effects of cereal borders, admixture with cereals, and plant density on the spread of bean yellow mosaic potyvirus into narrow-leafed lupins (*Lupinus angustifolius*). *Annals of Applied Biology* **122**:501-518.

Jones R A C. 2001. Developing integrated disease management strategies against non-persistently aphid-borne viruses: A model programme. *Integrated Pest Management Reviews* **6**:15-46.

Jones R A C. 2004. Using epidemiological information to develop effective integrated virus disease management strategies. *Virus Research* **100:**5-30.

Jones R A C. 2005. Patterns of spread of two non-persistently aphid-borne viruses in lupin stands under four different infection scenarios. *Annals of Applied Biology* **156**: (in press)

Jones R A C, Latham L J, Coutts B A. 2004. Devising integrated disease management tactics against plant viruses from 'generic' information on control measures. *Agricultural Science, Australia* **17**:10-18.

Jones R A C, Smith L J, Gajda B E, Smith T N, Latham L J. 2005a. Further studies on *Carrot virus Y*: hosts, symptomatology, search for resistance and tests for seed transmissibility. *Australian Journal of Agricultural Research* (submitted)

Jones R A C, Smith L J, Smith T N, Latham L J. 2005b. Relative abilities of different aphid species to act as vectors of *Carrot virus Y*. *Annals of Applied Biology* (submitted)

Latham L J, Jones R A C. 2002. *Carrot Virus Y*. In: *Compendium of Umbelliferous Diseases*, p. 53, Eds M Davis, R Raid. Minnesota, USA: American Phytopathological Society Press.

Latham L J, Jones R A C. 2004. *Carrot virus Y*: symptoms, losses, incidence, epidemiology and control. *Virus Research* **100:**89-99.

Latham L J, Traicevski V, Persley D M, Wilson C R, Tesoriero L, Coles R, Jones R A C. 2004. Distribution and incidence of *Carrot virus Y* in Australia. *Australasian Plant Pathology* **33:**83-86.

McKirdy S J, Jones R A C. 1994. Infection of alternative hosts associated with narrow-leafed lupins (*Lupinus angustifolius*) and subterranean clover (*Trifolium subterraneum*) by cucumber mosaic virus and its persistence between growing seasons. *Australian Journal of Agricultural Research* **45**:1035-1049.

McKirdy S J, Jones R A C. 1995. Bean yellow mosaic virus infection of alternative hosts associated with subterranean clover (*Trifolium subterraneum*) and narrow-leafed lupin (*Lupinus angustifolius*): field screening procedure, relative susceptibility/resistance rankings, seed transmission and persistence between growing seasons. *Australian Journal of Agricultural Research* **46**:135-152.

Moran J, van Rijswijk B, Traicevski V, Kitajima E W, Mackenzie A M, Gibbs A J. 2002. Potyviruses, novel and known, in cultivated and wild species of the family Apiaceae in Australia. *Archives of Virology* **147**:1855-1867.

Perry J N, Bell E D, Smith R H, Woiwod I P. 1996. SADIE: software to measure and model spatial pattern. *Aspects of Applied Biology* **46**:95-102.

Perry J N, Winder L, Holland J M, Alston R D. 1999. Red-blue plots for detecting clusters in count data. *Ecology Letters* **2**:106-113.

Thackray D J, Smith L J, Cheng Y, Perry J N, Jones R A C. 2002. Effect of strain-specific hypersensitive resistance on spatial patterns of virus spread. *Annals of Applied Biology* **141**:45-59.

Thackray D J, Diggle A J, Berlandier F A, Jones R A C. 2004. Forecasting aphid outbreaks and *Cucumber mosaic virus* epidemics in lupin crops in a Mediterranean-type environment. *Virus Research* **100:**67-82.

Thresh J M. 1974. Temporal patterns of virus spread. *Annual Review of Phytopathology* **12:**111-128.

Thresh J M. 1976. Gradients of plant virus diseases. *Annals of Applied Biology* **82:**381-406.

Thresh J M. 1983. Progress curves of plant virus diseases. *Advances in Applied Biology* **8**:1-85.

Torrance L, Pead M T. 1986. The application of monoclonal antibodies to routine tests for two plant viruses. In *Developments in Applied Biology 1: Developments and Applications on Virus Testing,* pp. 103-118, Eds R A C Jones and L Torrance. Wellesborne, UK: Association of Applied Biologists.

Legends to Figures

Fig. 1. Pathogen progress curves for carrot plants infected with CarVY in a) trial 1 with an 8% initial infection source (■, solid square) and b) trial 2 with a 0.4% initial infection source (●, solid spot).

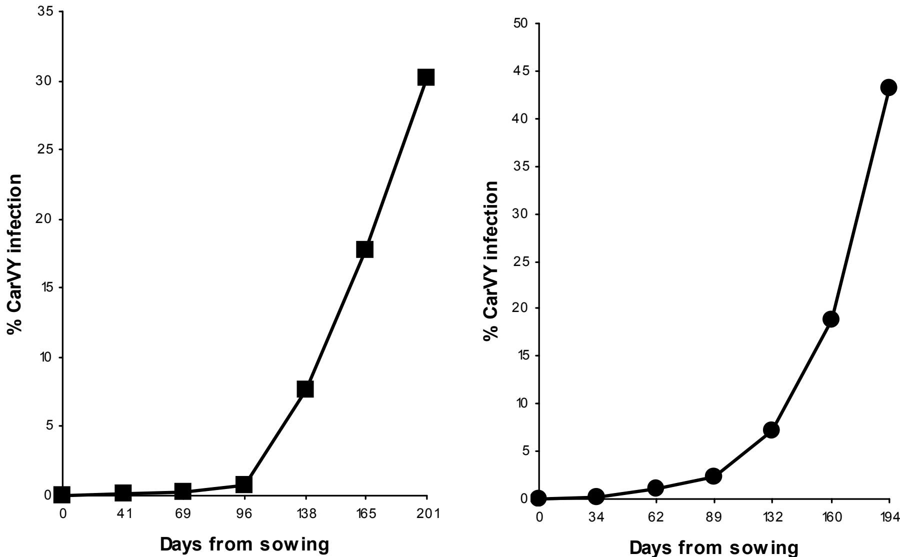


Fig. 2. Maps of clustering indices for cumulative numbers of CarVY infected carrot plants on four different days after sowing (das) in trial 1. Axes show distances in metres. Spots represent units denoting infection patches with v>0 (red) and infection gaps with v<0 (blue). Small spots represent clustering indices of 0 to +/-0.99 (clustering below expectation), intermediate sized spots +/-1 to +/-1.49 (clustering exceeds expectation) and large spots >1.5 or <-1.5 (half as much again as expectation). Red lines enclosing patch clusters are contours of v=1.5 and blue lines enclosing gap clusters are of v=-1.5. Black lines are zero-value contours, representing boundaries between patch and gap regions where the count is close to the overall sample mean.

West

East

CarVY source

89 das | 132 das | 160 das | 194 das

Fig. 3. Maps of clustering indices for cumulative numbers of CarVY-infected carrot plants on four different days after sowing (das) in trial 2. Symbols, contours and axes are as for Fig. 2.

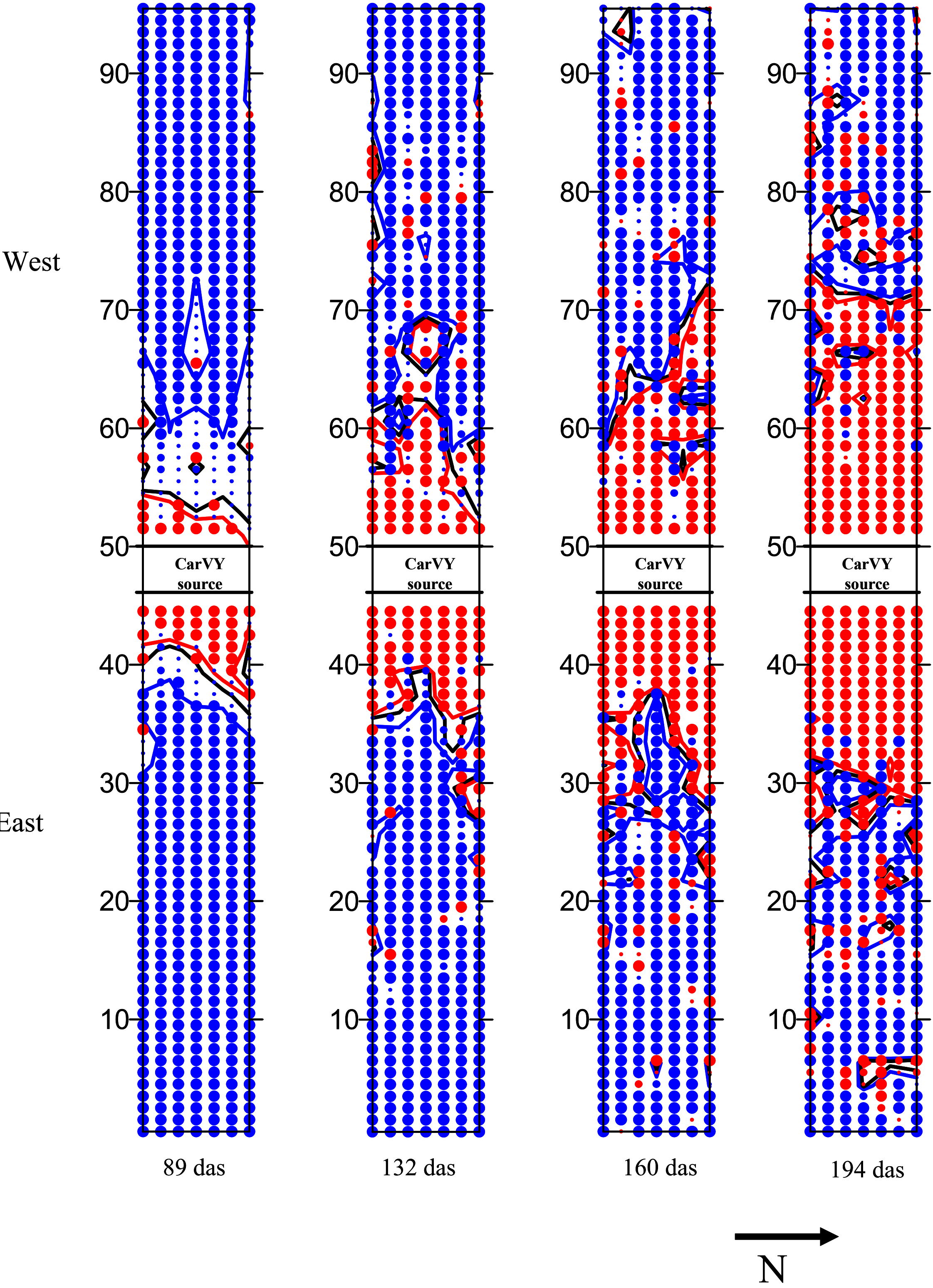


Fig. 4. Pathogen progress curves for carrot plants infected with CarVY in plots at 1m and 15m distances from a 100%-infected virus source plot in trial 3; ●, solid spot = 1 m upwind; ■, solid square = 1 m downwind; ▲, solid triangle = 15 m upwind; ♦, =15 m downwind.

Fig. 4

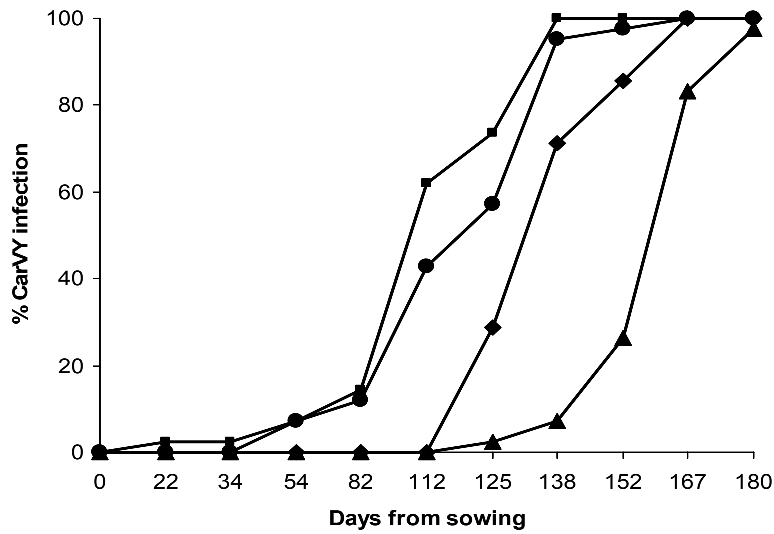


Table 1. *Analyses of spatial spread data for CarVY in trials 1 and 2.*

Block assessed	Assessment day	Cumulative no. of infected plants (%)*	*I*a**
		Trial 1	
Upwind	89	28(1.8)	3.66
	132	106(6.7)	5.47
	160	341(21.7)	6.58
	194	792(50.3)	7.62
Downwind	89	47(3.0)	5.30
	132	117(7.4)	6.85
	160	255(16.2)	7.80
	194	563(35.7)	7.95
		Trial 2	
Upwind	96	12(0.8)	3.53
	138	139(8.8)	6.70
	165	264(16.8)	8.15
	201	453(28.8)	9.03
Downwind	96	9(0.6)	2.77
	138	104(6.6)	5.68
	165	286(18.2)	8.40
	201	497(31.6)	9.19

* Total number of plants tested per assessment day within each block is 1575

** *Ia* = SADIE Overall Mean Index of Aggregation for cumulative numbers of infected plants, where *Ia* = 1 indicates randomly arranged infected plants and *Ia* >1 indicates clustering of affected plants. All *Ia* values shown indicate significant clustering at $P < 0.005$.

SECTION 5.0

Relative abilities of different aphid species to act as vectors of *Carrot virus Y*

R A C JONES[1], L J SMITH[1], T N SMITH[1] and L J LATHAM [1,2]

[1] Department of Agriculture, Locked Bag No. 4, Bentley Delivery Centre, WA 6983, Australia

[2] *Current address: Horticultural Development Council, Bradbourne House, East Malling, Kent ME19 6DZ, UK*

[Draft of paper submitted for publication]

Summary

Glasshouse and field studies provided information on the abilities of different aphid species to act as vectors of *Carrot virus Y* (CarVY) in carrots. Their effectiveness at transmitting the virus from infected to healthy carrot plants was compared in the glasshouse using 5-10 min acquisition access feeds. With species that colonise Apiaceous hosts, the percentage transmission efficiencies found were: *Myzus persicae* (56), *Dysaphis foeniculus* (19), *Aphis spiraecola* (17), *D. apiifolia* (13), *Hyadaphis foeniculi* (7), *Cavariella aegopodii* (4) and *H. coriandri* (3). With non-colonising species the respective transmission efficiencies were: *Lipaphis erysimi* (34), *Hysteroneura setariae* (14), *Brevicoryne brassicae* (12), *Acyrthosiphon kondoi* (10), *Sitobion miscanthi* (7), *Rhopalosiphum maidis* (2) and *R. padi* (0.5). When flying aphids were trapped on vertical nets near to a CarVY-infected carrot planting, 11 out of 101 *H. foeniculi* caught transmitted the virus to carrot seedlings. The 13 other aphids caught, which belonged to *B. brassicae* and two other non-colonising species, did not transmit it. If present in sufficient numbers, all 14 aphid species that transmitted the virus have the potential to be important CarVY vectors in carrot crops.
Key words: CarVY, carrots, aphids, vectors, trapping, transmission, epidemiology.

Introduction

Moran *et al.* (2002) described a new virus from carrots (*Daucus carota*) in Australia, which they named *Carrot virus Y* (CarVY) (family *Potyviridae,* genus *Potyvirus*). The virus infects carrot crops in most commercial carrot producing areas of the country (Latham *et al.*, 2004). It is transmitted non-persistently by aphids and has a narrow host range. The main infection sources for spread by aphid vectors to newly sown crops are infected volunteer carrot plants and nearby infected carrot crops. Continuous irrigated carrot production in sequential plantings on the same farm all-year-round often results in heavy infection with the virus, but infection incidences remain low when production is discontinuous and volunteer carrots are controlled (Latham & Jones, 2002, 2004; Latham *et al.,* 2004). The main symptoms that CarVY infection causes in carrot foliage are chlorotic mottle, marginal necrosis or reddening and generalised chlorosis of leaves, increased subdivision of leaflets giving a 'feathery' appearance and plant stunting. Roots from plants infected early are stubby showing severe distortion and knobbliness, while those from plants infected late are

thin with little distortion. When epidemics start at the vulnerable early growth stage in carrot crops, they can cause their abandonment due to un-marketability of the produce (Latham & Jones, 2002, 2004). The magnitude and availability of the virus infection source, climatic and cultural factors, and the time of aphid vector arrival, their abundance and activity, and the species involved, and are all important features determining the extent of CarVY epidemics and consequent damage to harvested carrots (Latham & Jones, 2004; Jones *et al.,* 2005).

To help build a clearer understanding of the factors driving CarVY epidemics and how to control them effectively, studies are needed to determine which aphid species are playing significant roles in its transmission to carrots. In particular, knowledge of their vector efficiencies and propensities is required. Vector propensity is the probability that a single vector, having had the opportunity to acquire a virus by landing on an infected plant, will then transmit the virus provided it lands on a healthy host plant. Vector efficiency, a component of vector propensity, provides a measure of a species' inherent capacity to transmit the virus under controlled conditions, but does not necessarily reflect what occurs in the field (Irwin & Ruesinck, 1986). Berlandier *et al.* (1997) provided vector efficiency and propensity information for transmission of two non-persistently aphid-borne viruses, *Bean yellow mosaic virus* (BYMV, family *Potyviridae,* genus *Potyvirus*) and *Cucumber mosaic virus* (CMV; family *Bromoviridae*, genus *Cucumovirus*), to lupin (*Lupinus angustifolius*) in south-west Australia. They did this by determining the vector efficiencies of different colonising and non-colonising aphid species in glasshouse tests, and their vector propensities by catching them live on nets downwind of virus-infected lupin stands in the field. This paper describes similar studies with CarVY in carrots in the same region of Australia.

Materials and Methods

Culture, test and 'infector' plants, virus isolate, inoculation and antibodies

Virus culture, test and 'infector' plants of carrot cv. Stefano, and the various hosts used to culture aphids, were grown from seed in a steam sterilised potting mix containing soil, sand and peat in air-conditioned, insect-proofed glasshouses kept at 18-20°C. The isolate of CarVY used was WA-1 (Latham *et al*., 2004) and its cultures were maintained by aphid inoculation to carrot plants. To produce 'infector' plants, young carrot plants were inoculated with WA-1 using aphids. Before transplanting outside, tip leaf samples from each potential 'infector' plant were tested by enzyme-linked immunosorbent assay (ELISA) to confirm presence of CarVY. For aphid inoculations to culture and 'infector' plants, *Myzus persicae* (green peach aphid) were starved for 2 h, placed on infected leaves for 5-10 min and then transferred to healthy plants (10 aphids/plant) for 1 h before being killed with insecticide. Isolate WA-1 was always used as a positive control in ELISA. Generic monoclonal antibody specific to potyviruses was obtained from Agdia Inc., USA and polyclonal antiserum specific to CarVY from DSMZ GmbH, Germany. The former was used in the initial ELISA tests but, once it became available commercially, the latter was used instead.

Enzyme-linked immunosorbent assay

In early tests with generic potyvirus monoclonal antibody, leaf samples were extracted (1g $20ml^{-1}$) in 0.05 M sodium carbonate buffer, pH 9.6, and tested using the

antigen-coated indirect ELISA protocol of Torrance & Pead (1986). In later tests using CarVY specific antibodies, leaf samples were extracted (1g $20ml^{-1}$) in phosphate buffered saline (10 mM potassium phosphate, 150 mM sodium chloride), pH 7.4, containing 5ml $litre^{-1}$ of Tween 20 and 20 g $litre^{-1}$ of polyvinyl pyrrolidone and tested by direct ELISA as described by Clark & Adams (1977). With both types of ELISA, the sample extracts and appropriate control extracts were collected in labeled, plastic sample tubes and tested in paired wells in immunoplates using 0.6 mg ml^{-1} of p-nitrophenyl phosphate in 100 ml $litre^{-1}$ of diethanolamine, pH 9.8, as substrate. Absorbance values (A_{405nm}) from sample extracts were measured in a Titertek Multiskan immunoplate reader (Flow Laboratories, Finland). Samples with absorbance values greater than twice that of the negative control sample were considered positive.

Aphid colonies

Aphid colonies were maintained on plants kept inside cages with mesh sides in controlled environment cabinets or rooms at 15-20°C. Carrot cv. Stefano was used to culture *Cavariella aegopodii* (willow carrot aphid) and *Dysaphis foeniculus.* Both carrot and celery (*Apium graveolens*) were used to culture *Aphis spiraecola* (green citrus aphid) and *Hyadaphis foeniculi* (honeysuckle aphid). For the other aphid species, parsley (*Petroselinum crispum*) was used for *D. apiifolia* (hawthorn parsley aphid); coriander (*Coriandrum sativum*) for *H. coriandri* (coriander aphid); canola (*Brassica napus*) for *Brevicoryne brassicae* (cabbage aphid), *M. persicae* and *Lipaphis erysimi* (turnip aphid); burr medic (*Medicago polymorpha*) for *Acyrthosiphon kondoi* (bluegreen aphid); barley (*Hordeum vulgare*) for *Rhopalosiphum maidis* (maize or corn aphid); and wheat (*Triticum aestivum*) for *Hysteroneura setariae* (rusty plum aphid), *R. padi* (oat aphid) and *Sitobium miscanthi* (grain aphid). One clone of each aphid species was used. The cultures of *A. kondoi*, *M. persicae, R. maidis* and *R. padi* came from previous work (Berlandier *et al*., 1997). *L. erysimi* was supplied by CSIRO Entomology, Centre for Mediterranean Agriculture, Wembley, Western Australia. All the other aphid species were new collections in 2001-2003 from Apiaceous or other hosts growing at field sites in south-west Australia: *A. spiraecola, D. foeniculus* and *H. coriandri* were collected from wild fennel (*Foeniculum vulgare*); *D. apiifolia* from parsley; *C. aegopodii* and *H. foeniculi* from carrot; *B. brassicae* from canola; *H. setariae* from *Cynodon dactylon* (couch grass); and *S. miscanthi* from wheat. These aphid species were identified using an insect reference collection and by referral to Blackman & Eastop (1985).

Determining aphid transmission efficiencies

The relative efficiencies of different aphid species as CarVY vectors were determined in a series of glasshouse experiments. For this, apterae were starved for 2-3 h (or 12 h with *D. apiifolia*) prior to acquisition access feeds of 5-10 min on carrot plants infected with isolate WA-1. Inoculation access feeds were for *c*.1 h on carrot test plants at the 4-6 leaf stage. Except with *M. persicae*, the transmission experiments consisted of different treatments each with 10 plants: a negative control treatment on which no aphids were placed, and treatments to which 1, 2, 4 or 8 aphids/plant were transferred individually from infected plants using a fine tipped paint brush. With *M. persicae*, there were 140 plants without aphids and 139 to which one aphid/plant was

transferred from infected plants. All carrot test plants were sprayed with insecticide after 1-2 h to terminate the inoculation access feeds. Numbers of test plants that became infected with CarVY were determined by taking a tip leaf sample from each plant after 6-7 weeks and testing the sample by ELISA. Where there was more than one aphid/plant, percentage virus transmission was calculated for each group of 10 plants using the formula of Gibbs & Gower (1960). Overall transmission efficiencies for each aphid species were the average values obtained by combining the percentage values for each number of aphids used to inoculate the plants.

Determining transmission by field caught aphids

A 20 x 20 m block of carrot cv. Stefano was planted on 26 September 2003 at the Department of Agriculture Research Station at Medina (32° 14' S., 115 ^{o} 48' E.). The carrot seed was sown in raised beds each 1.5m wide using a cone seeder: there were four paired rows 37 cm apart along each raised bed, within row spacing was set at 7.9 cm, and there were 82 plants/m^{2}. On the same day, 200 'infector' plants were introduced at regularly spaced intervals within the plot to act as the primary CarVY inoculum source. Naturally occurring aphids then spread the virus within the block. Irrigation was daily by overhead sprinklers and plants were fertilised according to standard commercial practice. No insecticide was applied and weeding was by hand.

To trap flying aphids live, a white rectangular 1.5 x 5 m nylon net (125 µm mesh) supported on steel posts was deployed as described by Berlandier *et al.* (1997). Nets were first used to trap aphids in early December. However, since aphid numbers were low over the hot, dry summer period, further trapping was delayed until autumn (April to May) and the first two weeks of winter (June). The net was placed downwind of the block (eight times) or across its middle (three times) on the following days after sowing: 80 in early summer; 194, 202, 209, 216, 220, 222 and 230 in autumn; and 248, 250 and 256 in early winter. Trapping was done in the middle of the day for *c.*2 h/day. On each occasion, young test plants of carrot cv. Stefano were taken to the trapping site in a vehicle, shaded from sunshine and a fine cloth (125 µm mesh) placed over them to prevent stray aphids from landing. Within 5 min of becoming trapped, individual winged aphids were collected live from the windward side of the net using a fine paint brush. Caught aphids were transferred immediately to one carrot test plant each to allow them to probe. A transparent Perspex cylindrical cage with a fine mesh top was placed over the plant to confine the aphid. Labelling of test plants, removal and preservation of the aphids after 1 h and insecticide application to the test plants were as described by Berlandier *et al.* (1997). Test plants were kept in a controlled environment chamber at 18-20°C and sprayed at 2 wk intervals with insecticide. After 6 wk and again after 8 wks, tip leaf samples from all test plants were tested by ELISA. The alatae caught were identified by CSIRO Entomology, Centre for Mediterranean Agriculture, Wembley, Western Australia by referral to Blackman & Eastop (1985).

Cylindrical traps consisting of plastic jars covered with yellow sticky paper ('Contact', Nylex Corporation, Australia) mounted 1.5m above the ground on stakes were used to monitor alatae flying above the plantings as described by Bwye *et al.* (1997). Between 17 and 153 days after sowing, one such trap was positioned at opposite ends of the block. The sticky paper (14 x 43 cm) used in each trap was changed fortnightly, labeled and the alatae caught counted.

Results

Transmission by aphids in glasshouse tests

With *M. persicae*, 78/139 aphid-inoculated and 0/140 un-inoculated carrot test plants became infected with CarVY, giving a transmission efficiency of 56%. Table 1 shows the results of the CarVY transmission experiments with the 13 other aphid species. All of them transmitted CarVY from carrot to carrot at differing efficiencies, though none were as efficient as *M. persicae*. The transmission efficiencies for the species that colonise Apiaceous hosts were (transmission rates in parentheses, greatest to least): *M. persicae* (49%), *D. foeniculus* (19%), *A. spiraecola* (17%), *D. apiifolia* (13%), *H. foeniculi* (7%), *C. aegopodii* (4%), and *H. coriandri* (3%). Of these, only *D. apiifolia* does not colonise carrot. The range of transmission efficiencies found with the non-colonising species resembled that obtained with the colonising species. The results were: *L. erysimi* (34%), *H. setariae* (14%), *B. brassicae* (12%), *A. kondoi* (10%), *S. miscanthi* (7%), *R. maidis* (2%) and *R. padi* (0.5%).

Transmission by field caught aphids

When 100 tip leaf samples were taken at random from within the test block 72 days after sowing and tested individually by ELISA, 90% of them were infected with CarVY. The carrot plants were colonised by *H. foeniculi* from April 2004 (about day 186) onwards. Over the entire trapping period, a total of 114 aphids belonging to four different species were caught live, identified and tested to see if they were transmitting CarVY. The number of alatae caught on each trapping date varied from 0 to 35, with 12 the largest number caught/trapping date before early June, numbers caught then increasing to 15-35 on days 248-256. *H. foeniculi* comprised 89% of the catch (101 aphids). In order of abundance, the other species were: *Hyperomyzus lactucae* (sowthistle aphid) (7 aphids), *Aphis gossypii* (melon aphid) (5 aphids), and *B. brassicae* (1 aphid). *H. foeniculi* was caught from day 216 onwards, while none of the other species were caught after day 220. Eleven of the aphids transmitted CarVY to carrot test plants, all of which were *H. foeniculi*.

Sticky trap catches

For each fortnightly trapping count up to day 153, combining the data from the two traps together showed that the average number caught/trap fluctuated between 0 and 5 unidentified alatae. It reached 5 alatae only once (8th fortnight) but on two other trapping dates none whatsoever were caught (3rd and 10th fortnights). Thus, the numbers caught were always low over the dry hot period when these traps were present. The alatae caught on similar sticky traps sited next to a carrot insecticide field trial with CarVY that was run over the same period the year previpously (see chapter 6iii) were identified; these traps were left in place until day 184 and located only 500 m away. *A. gossypii, B. brassicae, H. foeniculi* and *H. lactucae* were again present but two *A. kondoi* was also caught, both before day 35.

Discussion

All 14 aphid species included in our glasshouse assays transmitted CarVY from carrot to carrot but transmission efficiencies varied greatly within both carrot

colonising and non-carrot colonising aphid species groupings. *M. persicae* and *L. erysimi*, a coloniser and a non-coloniser respectively, were the most efficient vectors (34-56% efficiencies), while the least efficient were two colonisers, *C. aegopodii* and *H. coriandri* (3-4%) and two non colonisers, *R. maidis* and *R. padi* (0.5-2%). The 14 species tested are all common in cropping areas in south-west Australia (McKirdy & Jones, 1993, 1994, 1996; Jones, 1993, 2001; Berlandier *et al.,* 1997; Bwye *et al.,* 1997; Thackray *et al.,* 2000, 2004), so, assuming that alatae and apterae of the same species have the same transmission abilities, all have the potential to be important CarVY vectors in carrot fields if present in sufficient numbers. Such transmissions can occur while the aphids are moving through the crop and probing it regardless of whether colonisation takes place. Migrants of non-colonising species are more likely than those of colonising species to move on after landing, probing further plants as they go, thereby increasing their transmission potential. Moreover, species with very low transmission efficiencies might still sometimes be important if very abundant, e.g. *R. padi* with only 0.5% efficiency because of its great abundance in flights in late winter and early spring in the region. *R. padi*, despite its relatively low transmission efficiency with the non-persistently aphid-borne viruses BYMV and CMV (5%), acts as key non-colonising vector in lupins (Berlandier *et al*., 1997; Thackray *et al.,* 2004). The large number of aphid species that can act as vectors when an adequate CarVY source is present help explain why crop infection incidences in carrot crops are often so high (Latham *et al*., 2004).

In live trapping close to a heavily CarVY-infected carrot stand, 10% of the 114 alatae caught transmitted the virus. This correlates with previous live trapping studies at a nearby site were 6% of 186 and 2% of 727 alatae for CMV and BYMV respectively were caught downwind of infected lupins (Berlandier *et al*., 1997). In North America and Europe, similar live trapping studies with other non-persistently aphid-borne viruses obtained 2-4% transmission figures (Halbert *et al*., 1981; Harrington *et al.,* 1986; Irwin & Ruesinck, 1986). The higher values we obtained with CarVY were presumably because the carrot colonising species *H. foeniculi,* which made up 89% of the catch, was flying directly from CarVY-infected carrot plants within the infected block. The transmission efficiency of 7% for this species in glasshouse tests is similar to our 11% value from live trapping, suggesting all came from infected plants within the block. The rest of the Research Station was left without irrigation and fallow over much of the trapping period which was predominantly hot and dry rendering it baron. This explains why, overall, so few alatae were caught on nets or sticky traps. Two of the three other species that were caught live in low numbers, *A. gossypii* and *H. lactucae*, were not among those tested in our glasshouse transmission tests so it would be worthwhile including them in future CarVY transmission studies. Future studies should also include (i) tile traps to provide information on the landing rates of different aphid species in carrot crops, and (ii) live trapping studies with CarVY at other times of year, especially at the late winter and early spring peak aphid population time, to examine scenarios when as wide a range of aphid species as possible are flying. Such information is critical for development of models predicting CarVY epidemics in carrots and in validating components of an integrated disease management approach now in use against this virus (Latham & Jones, 2004; Jones, 2004; Jones *et al.,* 2004; Jones *et al.* 2005).

]

Acknowledgements

We thank Rohan Prince and Eva Gajda for technical assistance, Lynette Cartwright, Owain Edwards and Rick Horbury for aphid identifications, Brenda Coutts and Allan McKay for helpful discussion, and staff at Medina Research Station for help with the field block. Horticulture Australia Ltd provided financial support.

References

Blackman R L, Eastop V F. 1985. *Aphids on the world' crops – an identification guide*. Brisbane, Australia: John Wiley & Sons.

Bwye A M, Proudlove W, Berlandier F A, Jones, R A C. 1997. Effects of applying insecticides to control aphid vectors and cucumber mosaic virus in narrow-leafed lupins *(Lupinus angustifolius*). *Australian Journal of Experimental Agriculture* **37:**93-102.

Berlandier F A, Thackray D J, Jones R A C, Latham L J, Cartwright L. 1997. Determining the relative roles of different aphid species as vectors of cucumber mosaic and bean yellow mosaic viruses in lupins. *Annals of Applied Biology* **131:** 297-314.

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

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

Halbert S E, Irwin M E, Goodman R M. 1981. Alate aphid (Homoptera: Aphididae) species and their relative importance as field vectors of soybean mosaic virus. *Annals of Applied Biology* **97**:1-9.

Harrington R, Katis N, Gibson R W. 1986. Field assessment of the relative importance of different aphid species in the transmission of potato virus Y. *Potato Research* **29**:67-76.

Irwin M E, Ruesinck W G. 1986. Vector intensity: a product of propensity and activity. In *Plant Virus Epidemics: Monitoring, Modelling and Predicting Outbreaks*, pp. 13-33, Eds McLean G D, Garret R G and Ruesinck W G. Sydney, Australia: Academic Press.

Jones R A C. 1993. Effects of cereal borders, admixture with cereals and plant density on the spread of bean yellow mosaic potyvirus into narrow-leafed lupins *(Lupinus angustifolius*). *Annals of Applied Biology* **122**:501-518.

Jones R A C. 2001. Developing integrated disease management strategies against non-persistently aphid-borne viruses: A model programme. *Integrated Pest Management Reviews* **6**:15-46.

Jones R A C. 2004. Using epidemiological information to develop effective integrated virus disease management strategies. *Virus Research* **100:**5-30.

Jones R A C, Latham L J, Coutts B A. 2004. Devising integrated disease management tactics against plant viruses from 'generic' information on control measures. *Agricultural Science, Australia* **17**:10-18.

Jones R A C, Smith L J, Gajda E, Latham L J. 2005. Patterns of spread of *Carrot virus Y* in carrot plantings and validation of control measures. *Annals of Applied Biology* (to be submitted)

Latham L J, Jones R A C. 2002. *Carrot Virus Y*. In *Compendium of Umbelliferous Diseases*, p. 53, Eds M Davis and R Raid. Minnesota, USA: American Phytopathological Society Press,

Latham L J, Jones R A C. 2004. *Carrot virus Y*: symptoms, losses, incidence, epidemiology and control. *Virus Research* **100:**89-99.

Latham L J, Traicevski V, Persley D M, Wilson C R, Tesoriero L, Coles R, Jones R A C. 2004. Distribution and incidence of *Carrot virus Y* in Australia. *Australasian Plant Pathology* **33:**83-86.

McKirdy S J, Jones R A C. 1993. Occurrence of barley yellow dwarf virus serotypes MAV and RMV in over-summering grasses. *Australian Journal of Agricultural Research* **44:**1195-1209.

McKirdy S J, Jones R A C. 1994. Infection of alternative hosts associated with narrow-leafed lupins (*Lupinus angustifolius*) and subterranean clover (*Trifolium subterraneum*) by cucumber mosaic virus and its persistence between growing seasons. *Australian Journal of Agricultural Research* **45**:1035-1049.

McKirdy S J, Jones R A C. 1996. Use of imidacloprid and newer generation synthetic pyrethroids to control the spread of barley yellow dwarf luteovirus in cereals. *Plant Disease* **80:**895-901.

Moran J, van Rijswijk B, Traicevski V, Kitajima E W, Mackenzie A M, Gibbs A J. 2002. Potyviruses, novel and known, in cultivated and wild species of the family Apiaceae in Australia. *Archives of Virology* **147**:1855-1867.

Thackray D J, Jones R A C, Bwye A M, Coutts B A. 2000. Further studies on the effects of insecticides on aphid vector numbers and spread of cucumber mosaic virus in narrow-leafed lupins (*Lupinus angustifolius*). *Crop Protection* **19**:121-139.

Thackray D J, Diggle A J, Berlandier F A, Jones R A C. 2004. Forecasting aphid outbreaks and *Cucumber mosaic virus* epidemics in lupin crops in a Mediterranean-type environment. *Virus Research* **100:**67-82.

Torrance L, Pead M T. 1986. The application of monoclonal antibodies to routine tests for two plant viruses. In *Developments in Applied Biology 1: Developments and Applications on Virus Testing,* pp. 103-118, Eds R A C Jones and L Torrance. Wellesborne, UK: Association of Applied Biologists.

Table 1. ***Efficiencies of transmission of CarVY from carrot to carrot by 13 aphid species***

Aphid species (common name)		No. aphids/plant 0	1	2	4	8	Mean transmission efficiencies
Acyrthosiphon kondoi		0/10**	-	1/10	5/10	5/10	
(bluegreen aphid)	% efficiency	-	-	5	16	8	10
*Aphis spiraecola**		0/10	-	4/10	5/10	6/10	
(green citrus aphid)	% efficiency	-	-	23	16	11	17
Brevicoryne brassicae		0/10	1/10	2/10	4/10	7/10	
(cabbage aphid)	% efficiency	-	10	11	12	14	12
*Cavariella aegopodii**		0/10		-	1/10	3/10	
(willow carrot aphid)	% efficiency	-	-	-	3	4	4
Dysaphis apiifolia		0/10	1/10	2/10	6/10	5/10	
(hawthorn parsley aphid)	% efficiency	-	10	11	21	8	13
*Dysaphis foeniculus**		0/10	2/10	2/10	7/10	9/10	
	% efficiency	-	20	11	26	+	19
*Hyadaphis coriandri**		0/10	-	-	1/10	2/10	
(coriander aphid)	% efficiency	-	-	-	3	3	3
*Hyadaphis foeniculi**		0/10	-	-	1/10	6/10	
(honeysuckle aphid)	% efficiency	-	-	-	3	11	7
Hysteroneura setariae		0/10	2/10	4/10	2/10	5/10	
(rusty plum aphid)	% efficiency	-	20	23	5	8	14
Lipaphis erysimi		0/10	3/10	6/10	8/10	10/10	
(turnip aphid)	% efficiency	-	30	37	+	+	34
Rhopalosiphum maidis		0/10	-	-	1/10	1/10	
(maize or corn aphid)	% efficiency	-	-	-	3	1	2
Rhopalosiphum padi		0/10	-	-	0/10	1/10	
(oat aphid)	% efficiency	-	-	-	0	1	0.5
Sitobion miscanthi		0/10	-	1/10	2/10	6/10	

(grain aphid)	% efficiency	-	-	5	5	11	7

* An aphid species that colonises carrots
** Figures are numbers of carrot test plants infected/total number tested.
\+ = level of infection too high (>70%) to calculate transmission efficiency.

SECTION 6.0

Field experiments with insecticides and aphid trapping data from Western Australia

By LJ Latham, LJ Smith, TN Smith and RAC Jones

(i) Insecticide field experiment in 2002-03

Introduction

Carrot virus Y (CarVY) is non-persistently transmitted by aphids. Newer generation synthetic pyrethroid insecticides (eg. alpha-cypermethrin) have been somewhat more successful than other pyrethroids and than carbamate or organophosphate insecticides in controlling viruses transmitted non-persistently by aphids. This is because they have a faster knockdown effect and more prolonged anti-feedant activity than earlier insecticide types. Imidacloprid belongs to the new chemistry neonicotinoid insecticide group to which aphids have as yet not developed insecticide resistance. Both alpha-cypermethrin and imidacloprid have the potential to provide better control of CarVY in carrots than older insecticides in common use. Alpha-cypermethrin might be expected to achieve this by minimising probing by colonising and non-colonising aphid species at time of spraying and for three subsequent weeks on sprayed leaves. Imidacloprid should help by preventing colonisation of carrots by insecticide-resistant green peach aphids (*Myzus persicae)*, in addition to killing other aphids. A field experiment was set up at Medina Research Station to investigate the effectiveness of applying these two insecticides to control the spread of CarVY in carrots and thereby minimise subsequent yield and quality losses.

Materials and Methods

Carrot cv. Stefano seed was sown to generate 66 plants/m^2 in rows within 2 raised beds/plot. There were 42 plots in total each 3m wide x 5m long. A randomised block design was used with 6 replications. For plots with the seed dressing treatment, carrot seed was treated with imidacloprid (Gaucho 350® at 0.7L/100kg) prior to sowing. Carrot plants previously infected with isolate WA-1 of CarVY by aphid inoculation in the glasshouse (= CarVY infector plants), were transplanted into plots of 5 of the 7 treatments 1 week after sowing. Two infector plants were transplanted into the centre of each plot (5 treatments x 6 replications x 2 infectors/plot = 60 infector plants). No infector plants were placed into the plots of two control treatments. Oat cv. Swan buffers at 1m wide were sown around each plot on the same day that the carrots were sown, which was 9th October 2002. In some treatments, two foliar sprays were used Fastac® (alpha-cypermethrin) and Confidor® (imidicloprid). These were used either mixed together or alone depending on the experimental treatment.

Treatments:-

A. Gaucho seed dressing followed by combined Fastac and Confidor foliar sprays at 2, 4, 6, 8, 10, 12, 14, 16 weeks after emergence (- CarVY infector plants) [-ve control]
B. Gaucho seed dressing followed by combined Fastac and Confidor foliar sprays at 2, 4, 6, 8, 10, 12, 14, 16 weeks after emergence (+ CarVY infector plants)
C. Gaucho seed dressing only (+ CarVY infector plants)
D. Foliar sprays with Fastac alone at 2, 4, 6, 8, 10, 12, 14, 16 weeks after emergence (+ CarVY infector plants)
E. Foliar sprays with Confidor alone at 2, 4, 6, 8, 10, 12, 14, 16 weeks after emergence (+ CarVY infector plants)
F. No seed dressing or foliar sprays (- CarVY infector plants) [-ve control without insecticide]
G. No seed dressing or foliar sprays (+ CarVY infector plants) [+ve control]

Foliar applied insecticides were: 500ml/ha of Fastac and 170ml/ha of Confidor both in 100l/ha water. Seed dressing was Gaucho 350 at 0.7L/100kg carrot seed.

Results

CarVY spread was very slow to take off in this field experiment because the peak autumn flight of aphids occurred before it started, and hot summer conditions then followed. Spread did not take off until the end of summer/ early autumn (Fig. 1). As shown in Table 1 and Fig. 1, the least amount of spread of CarVY in carrots was in treatment A (Gaucho seed treatment and both foliar insecticides applied together but no CarVY infector plants present). At 215 days after sowing, the incidence value for this treatment was not significantly different from that of treatment B which had exactly the same insecticide regime but with CarVY infector plants introduced to its plots (Table 1). The values for treatments E (foliar applied Confidor only) and F (no insecticides applied or infector plants present) were not significantly different from each other. The value for treatment C (Gaucho seed dressing alone) was not significantly different from that of positive control treatment G (no seed dressing or foliar sprays applied). While treatment D, Fastac (foliar applied alpha-cypermethrin) applied alone gave an incidence value that was significantly greater than those of all other treatments. Thus, the foliar Confidor sprays decreased spread and Gaucho seed dressing had no effect, while Fastac foliar spray actually made matters worse.

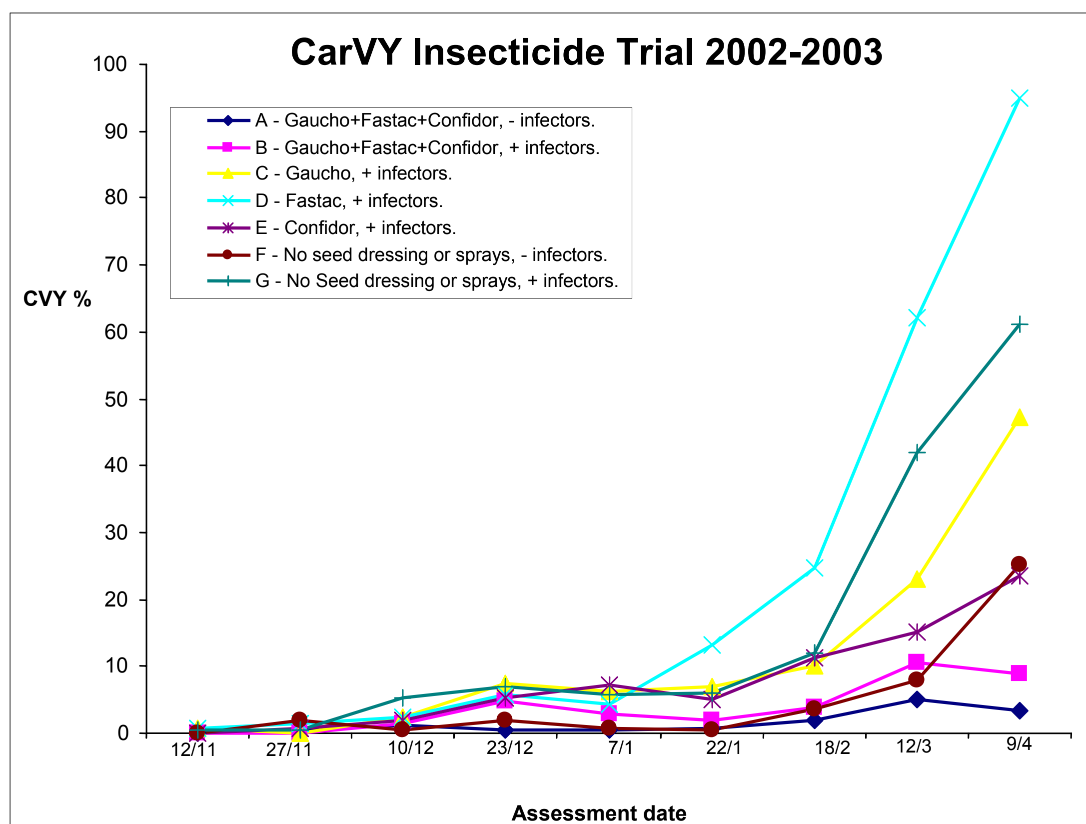
Counts for colonizing aphids were done on each visit between week 2 after sowing (23 October 2002) and wek 22 (12 March 2003) but none whatsoever were found colonizing the carrot plants.

Table 1. Statistical analysis of CarVY incidence data from the insecticide field experiment

Treatment code	Treatment	% of plants with CarVY 215 days after sowing*
A	Gaucho + Fastac + Confidor (- infector plants)	10.1 (3)
B	Gaucho + Fastac + Confidor (+ infector plants)	16.5 (9)
E	Confidor (+ infector plants)	28.1 (23)
F	No seed dressing or sprays (- infector plants)	29.6 (25)
C	Gaucho (+ infector plants)	43.2 (47)
G	No Seed dressing or sprays (+ infector plants)	52.2 (61)
D	Fastac (+ infector plants)	80.0 (95)
	lsd	9.53
	P (df =30)	<0.001

*All percentage CarVY incidence data were angular transformed before analysis. Figures in parentheses are detransformed final percentages.

Fig. 1. Pathogen progress curves for CarVY incidence in the 2002-2003 insecticide experiment



Conclusions

When CarVY infector plants were present in carrot plots, neither Fastac foliar sprays nor Gaucho seed dressing diminished final incidences of CarVY when these incidences were compared to those in control plots with no seed treatment or insecticide sprays. However, application of Confidor foliar sprays on a fortnightly basis to plots with infector plants decreased the incidence of CarVY (to 23%) when this incidence was compared with the incidences in all treatments that lacked Confidor sprays but in which infector plants were present. In contrast, Fastac application increased CarVY incidence to a level above that in all other treatments (to 95%). Presumably this increase was from increased aphid movement with the aphids probing more plants thereby increasing the virus spread. The Gaucho seed dressing was ineffective when applied at the low rate used despite having the same active ingredient as Confidor. Presumably the effect of the seed dressing had worn off by the time spread started. Also, after further discussions with the chemical company that market Gaucho, it was suggested that the application rate to the seed that they had recommended should have been higher. As expected, the most effective treatment at decreasing CarVY spread when infector plants were present was application of Gaucho seed dressing followed by combined sprays of Fastac and Confidor (to 9%). Here, there was an 85% reduction in CarVY incidence compared to its incidence in the plots with no seed dressing or foliar sprays but with infector plants present (61% incidence).

Key points:

- The trial examined a later CarVY spread scenario where all transmission was by migrant aphids from outside the trial area.
- These results suggest that foliar sprays of Confidor on a fortnightly basis are worth investigating further for control of CarVY. Seed dressing with Gaucho is also worth investigating further at a higher rate as this product contains the same active ingredient (Imidacloprid) as Confidor.
- Fortnightly foliar applications with the pyrethroid Fastac made things worse, presumably because the aphids were agitated by its presence and so moved around more, causing more CarVY infections. Its application should be avoided, and the same may well apply to other pyrethroids.

(ii) Insecticide field experiment 2003-04

Introduction

In 2003-2004, a follow up insecticide trial was done also at the Medina Research Station. This trial further investigated use of imidacloprid applied as Gaucho® seed dressing or Confidor® as fortnightly foliar sprays in controlling spread of CarVY. These two products were used either together or alone depending on the experimental treatment. On advice from the chemical company, the application rate of Gaucho ® used was not increased.

Methods

Carrot cv. Stefano seed with or without a dressing with Gaucho insecticide was seeded to generate 66 plants/m2 in rows in 3 x 5m plots in the week starting the 22nd

of September. A similar trial design to that in the previous year's experiment was used: randomised block design; plot size was 3m x 5m; oat buffers were 3m wide around all plots; there were 6 treatments x 6 replications = 36 plots; total trial area was 0.24ha (two irrigated bays 15m x 100m); there were raised beds 1.5 m wide with 4 double rows/raised bed; plant spacing was 7.9cm within rows. Oat buffers were sown on the same day as the carrots. Carrot plants infected with CarVY in the glasshouse (= CarVY 'infector plants') were transplanted into all plots of 4 of the treatments 1 week after seeding. Two infector plants were transplanted into the centre of each plot (4 treatments x 6 replications x 2 'infectors'/plot = 48 infector plants). No 'infector plants' were placed into two of the three control treatments.

The treatments were:-

A. Gaucho seed dressing + Confidor sprays at 2, 4, 6, and 8 weeks after emergence (- CVY infector plants), [-ve control 1]
B. Gaucho seed dressing + Confidor sprays at 2, 4, 6, and 8 weeks after emergence (+ CVY infector plants)
C. Gaucho seed dressing only (+ CVY infector plants)
D. Sprays with Confidor at 2, 4, 6 and 8 weeks after emergence (+ CVY infector plants)
E. No seed dressing or sprays (- CVY infector plants) [-ve control 2]
F. No seed dressing or sprays (+ CVY infector plants) [+ve control]

170ml/ha Confidor in 100l/ha water. Seed dressing with Gaucho 350 at 0.7L/100kg carrot seed

Results

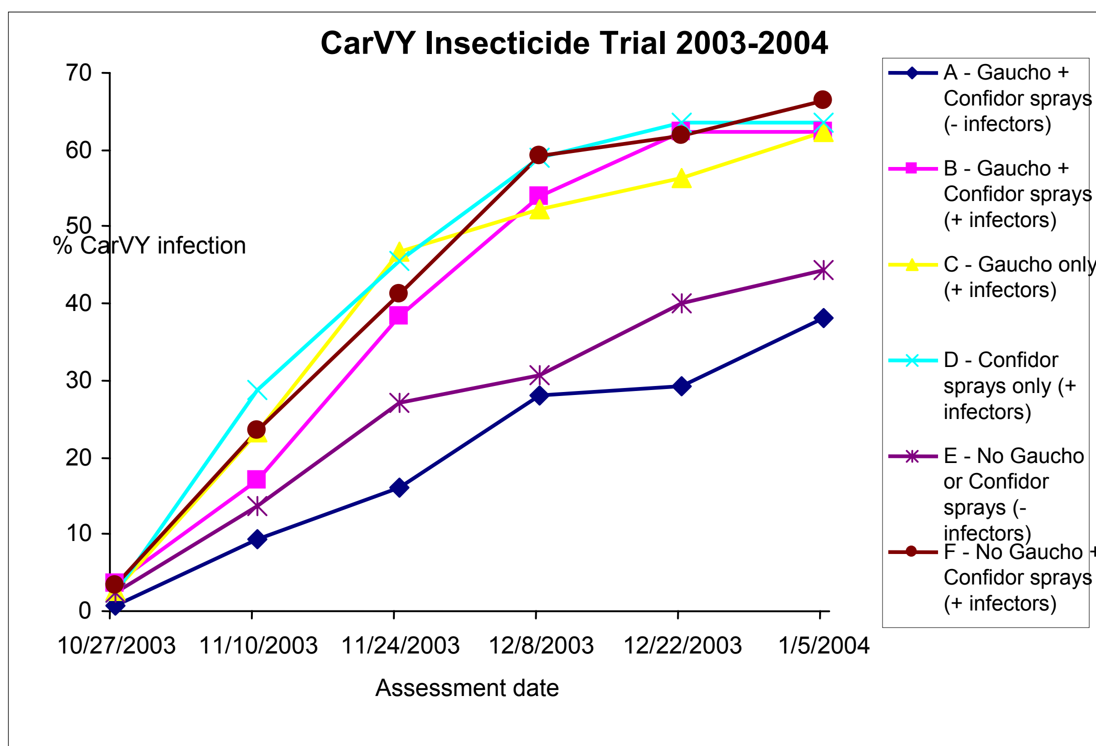
In this field trial, spread of CarVY strated much earlier than in the 2002-2003 field trial (Figs 1 and 2). Under this early spread scenario, the trial did not confirm any beneficial effect of either type of application of imidacloprid in suppressing spread of CarVY (Table 2; Fig. 2). The only significant differences in the extent of spread were between those plots that had infector plants inserted and those that did not. Thus, presence of an internal virus source proved the overriding consideration.

Table 2. Statistical analysis of CarVY incidence data from the insecticide field experiment

Treatment code	Treatment	% of plants with CarVY on 22/12/03*	% of plants with CarVY on 5/1/04*
A	Gaucho + Confidor – infectors	32.8 (29.3)	38.1 (38.1)
B	Gaucho + Confidor + infectors	52.2 (62.4)	52.2 (62.4)
C	Gaucho + infectors	48.6 (56.2)	52.2 (62.4)
D	Confidor + infectors	52.8 (63.4)	52.8 (63.4)
E	No Gaucho or Confidor – infectors	39.2 (39.9)	41.7 (44.2)
F	No Gaucho or Confidor + infectors	51.9 (61.9)	54.6 (66.4)
	P, Df=25	0.003	0.008
	lsd	11.04	9.97

*All percentage CarVY incidence data were angular transformed before analysis. Figures in parentheses are detransformed final percentages.

Fig. 2. Pathogen progress curves for CarVY incidence in the 2003-2004 insecticide experiment



Between weeks 3 and 17 from sowing, fortnightly aphid counts on 10 plants within each of the plots within the insecticide experiment detected non-winged aphids (ie. colonizing aphids) only on week 3 (13 October 2003). By week 5, they were all gone and did not return thereafter. In week 3, there were none on treatments A or B, 5 on treatment C, 217 on treatment D, 419 on treatment E, and 274 on treatment F. They were virtually all nymphs. Thus, at this stage Gaucho was supressing them effectively while the first Confidor spray had not yet been applied. Presumably conditions then became too hot for the colonization of carrots to continue so subsequent virus spread was all bY winged migrants from elsewhere.

Key points:

- The two field trials differed in that they examined early (2003-2004) and late 2002-2003) CarVY spread scenarios.
- These results did not confirm those in the previous trial (2002-2003) which suggested that foliar sprays of Confidor on a fortnightly basis may control CarVY.
- Althoigh seed dressing with Gaucho prevented aphid colonization early on, it was also ineffective at suppressing CarVY spread at the rate used.
- The overriding importance of proximity to virus source in determining the rate of virus was the clear message of the experiment

- Except at the very beginning, all spread was by winged migrants from outside the carrot trial.

Overall Conclusions:

Based on these 2 field trials, in the early CarVY spread scenario that is critical for damage to carrot apprearance and quality, insecticides seem unlikely to be of any benefit in contolling spread of CarVY, and cannot be recommended for this purpose. This is consistent with many previous studies with non-persistently aphid-borne viruses where insecticides fail to act fast enough to limit virus spread by aphids and may actually make things worse by causing the aphid vectors to move around more and infect more plants.

(iii) 2002 - 2003 aphid trapping data

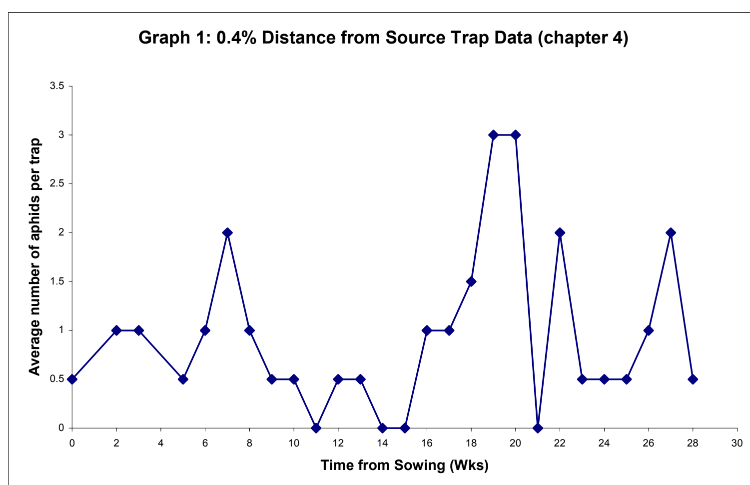
Introduction

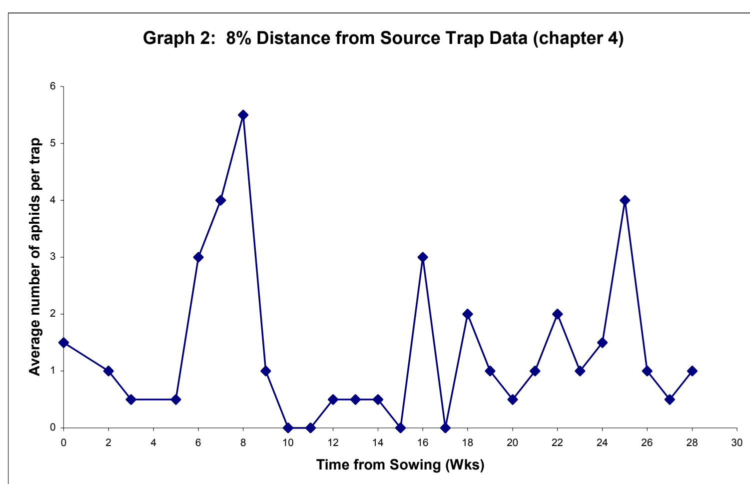
In the 2002-2003 growing season, three field trials were done at Medina Research Station, near Perth. One examined the effectiveness of the new generation synthetic pyrethroid, alpha-cypermethrin, and the 'new chemistry' neonicotinoid insecticide Imidacloprid in controlling CarVY spread (see previous section), while the other two studied the effect of distance from source and magnitude of source on CarVY spread to carrots (see chapter 4). At each of the three trials, two yellow aphid sticky traps were erected at sowing time and monitored weekly over a 6 month period to record the numbers of winged aphids flying over the carrot field trial sites.

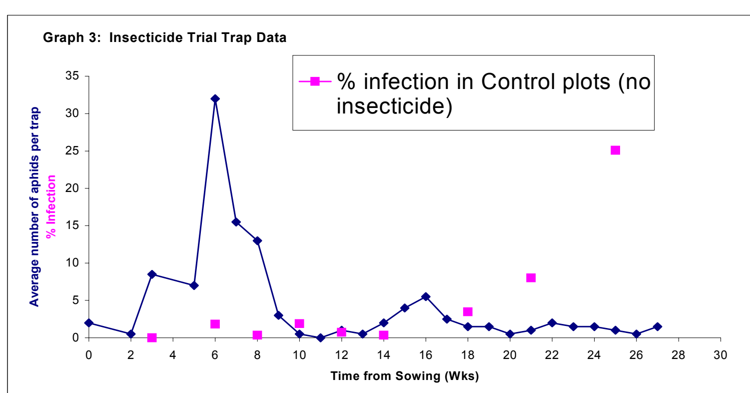
Material and Methods

In placing the yellow sticky traps, star pickets were placed in the ground to a height of approximately 1.5m, at opposite ends of each trial. A plastic bottle (18cm height x 10cm diameter) was placed upside down onto the star picket. A scalpel was used to cut the shape of the top of the star picket into the lid of the plastic bottle. The lid was then slid onto the star picket so that it was upside down. The plastic bottle was placed over the top of the star picket and screwed into the lid. The bottom of the plastic bottle rested on the top of the star picket. Yellow sticky paper ("contact") was cut to 43cm long by 14cm high. This was the same size for each aphid trap. With the paper backing side facing outwards, the yellow sticky paper was attached to the plastic bottle with a small piece of sticky tape. The paper was then wrapped around the bottle and the backing paper removed. Using a waterproof marker pen, the date the trap was put out, the trial or paddock number, and the North direction on top of the trap was recorded. These traps were changed weekly, with a record made of the number of winged aphids present each time. This record gives an indication of what aphids were flying over the plots and what numbers were involved.

Results







For Distance from Source Trials (Graphs 1 and 2) sowing date was 10 October 2002; for Insecticide Trial (Graph 3) it was 9 October 2002.

Graphs 1, 2 and 3 show the average number of aphids (all species) caught per trap each week on each pair of traps at each field trial at Medina Research Station. The two distance from source trial sites were in relatively close proximity to each other (300 metres apart) and the figures (Graphs 1 and 2) illustrate that the patterns of aphid flights into the vicinity were somewhat similar. There were quite large numbers of

aphids flying into the area in week 7 (13/11/02) but few between weeks 10 and 14, afterwards numbers varied more between trials. Combining the weekly data from all for four traps together revealed that on each trapping date the average number caught/trap mostly fluctuated between 0.5 and 2 alatae. It reached 3.5 alatae once (trapping days 47-54) but none whatsoever were caught over another trapping period (days 74-83). Thus, the numbers caught were always low over the dry hot period up until day 201 when both trials finished. The alatae caught that were identified by CSIRO Entomology, Floreat Park, WA were predominantly *Brevicoryne brassicae* (cabbage aphid) and *Hyadaphis foeniculi* (honeysuckle aphid) but over the entire tapping period over all four traps 1-3 individuals each of *M. persicae*, *Aphis gossypii* (melon aphid)*, Acyrthosiphon kondoi* (bluegreen aphid) and *Hyperomyzus lactucae* (sowthistle aphid) were also caught. Of the aphid species trapped, only *H. foeniculi* and *M. persicae* colonise carrots. *H. foeniculi* was caught mainly between days 13 and 46 in late spring (mid October and November), and days 103 and 159 in late summer and early autumn (late January to mid March). The CarVY vector status of *A. gossypii* and *H. lactucae* is unknown but *A. kondoi*, *B. brassicae, H. foeniculi* and *M. persicae* can all transmit it (see Chapter 4).

The insecticide trial was more than 1km away from the other two trials but had a similar peak of incoming aphids around week 7 (Graph 3). Also shown for the insecticide trial is the percent infection in the control plots (no sprays, without infectors). CarVY incidence did not start to increase rapidly until 2 weeks after the second peak numbers of aphids caught on the traps, and by the time incidence increased, aphid numbers had fallen. This was because the aphids were probing and spreading CarVY while at their peak, but virus did not move systemically for 2 weeks, so that it could not be detected in the young leaves of the carrots until 2-3 weeks afterwards by which time aphid numbers had diminished. The alatae caught were identified by CSIRO Entomology, Floreat Park, WA. *A. gossypii, B. brassicae H. foeniculi* and *H. lactucae* were all trapped, and two *A. kondoi* were also caught, both before day 35.

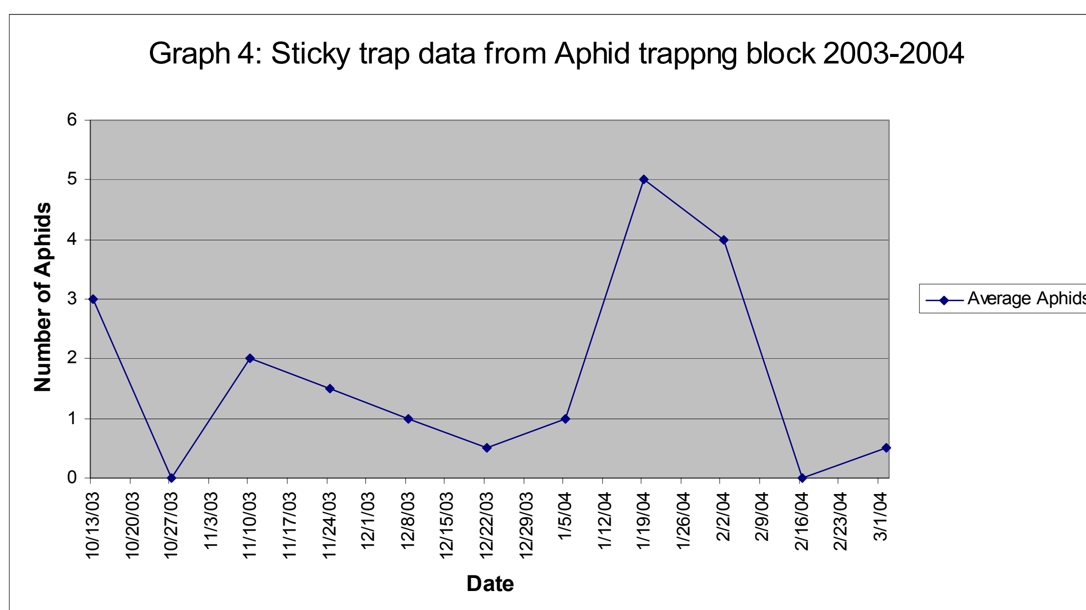
(iv) 2003 -2004 aphid trapping data

In the 2003-2004 growing season, three field trials were done at Medina Research Station, near Perth. One examined the effectiveness of the 'new chemistry' neonicotinoid insecticide Imidacloprid in controlling CarVY spread (see section ii, this chapter) while the other two studied whether seed transmission of CarVY might occur through seed of diverse apiaceous hosts (described in chapter 3) or what proportiuon of aphids caught live on nets downwind of a heavily CarVY-infected block of carrots transmitted the viris to carrot plants (described in chapter 5). At the second of these three trials, two yellow aphid sticky traps were erected at sowing time and monitored weekly over a 6 month period to record the numbers of winged aphids flying over the carrot field trial sites. The sticky trap data obtained are shown in graph 4. The relevant excerpts from the results already described in chapter 5 for net- and sticky-trapped aphids are as follows:

Net-trapped catches. "Over the entire trapping period, a total of only 114 aphids belonging to four different species were caught live, identified and tested to see if they

were transmitting CarVY. The number of alatae caught on each trapping date varied from 0 to 35, with 12 the largest number caught/trapping date before early June, numbers caught then increasing to 15-35 on days 248-256. *H. foeniculi* comprised 89% of the catch (101 aphids). In order of abundance, the other species were: *H. lactucae* (7 aphids), *A. gossypii* (5 aphids), and *B. brassicae* (1 aphid). *H. foeniculi* was caught from day 216 onwards, while none of the other species were caught after day 220. Eleven of the aphids transmitted CarVY to carrot test plants, all of which were *H. foeniculi*."

Sticky trap catches. "For each fortnightly trapping count up to day 153, combining the data from the two traps together showed that the average number caught/trap fluctuated between 0 and 5 unidentified alatae. It reached 5 alatae only once (8th fortnight) but on two other trapping dates none whatsoever were caught (3rd and 10th fortnights). Thus, the numbers caught were always low over the dry hot period when these traps were present. The aphid species involved were not identified."



For the aphid trapping block, the sowing date was 26 September 2003.

Overall conclusions:

Based on these two years of aphid trapping data from Western Australia:

- Numbers of aphids caught were generally low over both of the hot, dry late spring to early autumn periods.
- CarVY spread was very slow to develop when the planting date missed exposing young carrot seedlings to the spring peak aphid flight (as in 2002 but not 2003). Manipulation of planting date to avoid such exposure of young carrot plants to CarVY infection at the critcal early growth stage is critical to avoid excessive root disfiguration (see Table on page 27).
- *H. foeniculi* is clearly a key vector of CarVY, but *A. gossipyii, A. kondoi, B. brassicae, H. lactucae* and *M. persicae* may also play important roles as vectors when their numbers are abundant.

SECTION 7.0

Aphid trapping, vector efficiency and virus incidence data from Victoria

By V. Traicevski, B.C. Rodoni and P. Ridland

1. Aphid numbers and levels of virus infection in the field in Victorian carrot crops

1.1 Introduction

Here we present results of winged aphid numbers and the incidence of CarVY in carrot crops in SE Victoria and one carrot crop in NW Victoria. The study was undertaken to assess the level of CarVY and to determine which winged aphid species are prevalent in Victorian carrot crops.

1.2 Material and Methods

1.2.1 Crop details

Table 1.1 Details of the carrot crops monitored

	Crop 1	Crop 2	Crop 3	Crop 4
Location	SE Victoria	SE Victoria	SE Victoria	NW Victoria
Variety	Kendo	Stefano	Stefano	Kendo
Size	25 lands (6 rows of carrots planted across each land) at 100 metres long (150 carrots/metre)	25 lands (6 rows of carrots planted across each land) at 70 metres long (150 carrots/metre)	25 lands (6 rows of carrots planted across each land) at 70 metres long (150 carrots/metre)	0.2 ha
Sowing date	18 July 2002	30 December 2002	15 February 2003	23 April 2003
Harvest date	21 November 2002	26 March 2003	4 June 2003	
Previous crop	Virgin ground	Broccoli	Broccoli	fallow
#Average mean temp (°C)	13.2 (81%)#	18.8 (63%)	14.1 (49%)	10.2 (40%)
#Average min. temp (°C)	9.1 (43%)	14.7 (67%)	10.2 (30%)	4.9 (47%)
#Average max. temp (°C)	17.3 (88%)	22.8 (51%)	18.0 (72%)	15.6 ((47%)
#Average rainfall (mm)	155.4 (2%)	93 (44%)	88.5 (12%)	176.9 (88%)

	Crop 5	Crop 6	Crop 7
Location	SE Victoria	NW Victoria	SE Victoria
Variety	Kendo	Kendo	Stefano
Size	25 lands (6 rows of carrots planted across each land) at 100 metres long (150 carrots/metre)	0.2 ha	25 lands (6 rows of carrots planted across each land) at 70 metres long (150 carrots/metre)
Sowing date	13/8/03	12/11/03	14/1/04
Harvest date	17/12/03	31/3/04	7/4/04

Average weather parameter during the monitoring period for a given crop. Figure in parentheses refers to relative rank in last 43 years (highest reading =100%)

1.2.2 Aphid numbers

Yellow water pan traps were placed in all the carrot crops to monitor winged aphid numbers during the cropping season. Each pan trap was 38 cm in length, 30 cm in width and 15 cm deep. The traps had an overflow hole drilled near to the rim of the container. This hole was covered with wire gauze. Each week, the traps were filled to capacity with water containing a detergent (Pyroneg Powder™) to reduce the surface tension of the water and copper sulphate ($CuSO_4$) to prevent build up of algae in the traps. The water in these traps was changed weekly.

Trapped winged aphids were collected weekly and taken back to the laboratory for identification to species level.

1.2.3 Incidence of CarVY *in the field.*

One hundred leaf samples were collected at random each week in crops 1 and 2 in SE Victoria. Leaves were collected by walking through the crop and sampling, every five paces, the nearest shoot to the tip of the boot. The methodology was altered slightly for Crop 3 in SE Victoria and Crop 4 in NW Victoria in that leaf samples were collected every 3 weeks.

Leaves from Crop 1 were tested for virus by the enzyme-linked immunosorbent assay (ELISA) using a general potyvirus monoclonal antiserum (Agdia) and Celery mosaic virus (CeMV) specific antisera supplied by DSZM, GmbH, Germany. Leaves collected from Crops 2, 3 & 4 were tested for CarVY by using ELISA with CarVY polyclonal specific antisera supplied by DSZM, GmbH, Germany.

The estimated levels of virus incidence for all the crops were calculated using the formula given by Burrows (1987).

1.3 Results

1.3.1 Aphid numbers and estimated incidence of CarVY in the field.

Crop 1

Virus incidence within the crop monitored in SE Victoria from August- November ranged from 0- 17.9% (Table 1.2). Aphid numbers were also low in spring, with the highest number of aphids caught in the 4 traps recorded in early October (92 aphids) and mid-November (107 aphids). The most abundant aphid species trapped were *Rhopalosiphum padi* and *Brachycaudus rumexicolens.* There was a small peak of *Myzus persicae* trapped in the week ending 9 October, 2 weeks before the peak incidence of virus was measured (23 October).

Table 1.2 Numbers of aphids (5 most abundant species and all aphids) trapped in carrot crop 1 in SE Victoria and the percentage incidence of CarVY in the field (August - November 2002).

Collection date	***Myzus persicae***	***Brachycaudus rumexicolens***	***Rhopalosiphum padi***	***Lipaphis erysimi***	***Capitophorus elaegnai***	**Total**	**% CarVY incidence (95% Confidence Limits) (CeMV antiserum)**	**% Potyvirus incidence (95% Confidence Limits)**
14-Aug-02			2			4		
21-Aug-02	2	2				4		
28-Aug-02	2	1			1	8		
4-Sep-02	1		1			3		
11-Sep-02			3			3		
18-Sep-02	1	5	6		2	18		
2-Oct-02	2	2	7	1	2	20	3.3 (0.9, 9.2)	4.7 (1.5, 11.6)
9-Oct-02	9	3	20	41	5	92	0 (0-3.0)	0 (0-3.0)
16-Oct-02	2	10	9	4		27	0 (0-3.0)	0 (0-3.0)
23-Oct-02	1	2	9		3	31	17.9 (8.7, 41.1)	6.3 (2.4, 14.4)
30-Oct-02	2	1	3		1	20	0 (0-3.0)	0 (0-3.0)
13-Nov-02	12	24	1	2	11	107	2.1 (0.4, 7.1)	2.1 (0.4, 7.1)
20-Nov-02	3	11			13	51	0 (0-3.0)	0 (0-3.0)
TOTAL	**37**	**61**	**61**	**48**	**38**	**388**		

Crop 2

CarVY was only detected on one occasion (2%) just before harvest (Table 1.3). Aphid numbers were relatively high with the dominant species being *Rhopalosiphum maidis* (207 aphids in total). The peak catch of *Myzus persicae* was in early March, just before harvest. As the crop was near harvest, this flight of aphids would have been too late to cause damage by transmitting CarVY.

Table 1.3. Numbers of aphids (5 most abundant species and all aphids) trapped in carrot crop 2 and percentage incidence of CarVY in the crop (January-March 2003).

Collection date	***Myzus persicae***	***Rhopalosiphum maidis***	***Brevicoryne brassicae***	***Brachycaudus rumexicolens***	***Lipaphis erysimi***	**Total**	**% CarVY incidence (95% Confidence Limits)**
22-Jan-03		1		5	1	19	0 (0-3.0)
29-Jan-03		1	4	4	1	20	0 (0-3.0)
05-Feb-03		20	6	1	1	29	0 (0-3.0)
12-Feb-03		85		1	1	94	0 (0-3.0)
19-Feb-03	4	1				9	0 (0-3.0)
26-Feb-03	12	67	70	8	41	219	0 (0-3.0)
12-Mar-03	38	9	3	1	2	60	0 (0-3.0)
19-Mar-03	13	23		2	2	49	2.1 (0.4, 7.1)
Total aphids	**67**	**207**	**83**	**22**	**49**	**499**	
26-Mar-03	**harvest**						

Crop 3

Virus incidence within crop 3 ranged from 0- 10.5% (Table 1.4). Aphid numbers were consistent through autumn with the dominant species being *Myzus persicae* (139 aphids in total). CarVY incidence remained low throughout the growing season of Crop 3 with no statistical difference between the virus incidence being observed during the season. There seemed to be no correlation between the recorded virus incidence and the corresponding aphid numbers (3-4 weeks before virus incidence assay).

Table 1.4. Numbers of aphids (5 most abundant species and all aphids) trapped in carrot crop 3 and percentage incidence of CarVY in the crop (April - May 2003)

Collection date	*Myzus persicae*	*Dysaphis aucupariae*	*Tetraneura nigriabdominalis*	*Capitophorus elaegnai*	*Brachycaudus rumexicolens*	Total	% CarVY incidence (95% Confidence Limits)
02- Apr -03							4.7 (1.5, 11.6)
09- Apr -03	26	6	5	1	4	51	
16- Apr -03	31	18	7		4	71	
23- Apr -03	13	8	5	1	2	38	10.5 (4.7, 22.1)
30- Apr -03	29	9	5	1	2	50	
06- May -03	13	7	6	4		35	
14- May -03	14	14	9	3		55	4.7 (1.5, 11.6)
21- May -03	8	11	7	5	1	52	
28- May -03	5		3	8		21	
Total	**139**	**73**	**47**	**23**	**13**	**373**	
04-Jun -03	**harvest**						

Crop 4

CarVY incidence within crop 4 ranged from 0 – 1% (Table 1.5). There were very high numbers of aphids trapped in autumn at carrot crop 4 in NW Victoria (Table 1.5). Despite the high numbers of aphids, CarVY was only first detected (1%) in this crop at the end of August. The dominant aphid species trapped was *Brachycaudus rumexicolens* (47% of all aphids) while *Myzus persicae* only made up 4% of the catch. High numbers of *Uroleucon sonchi* and *Hyperomyzus lactucae* caught in the early part of the season would have been derived from sowthistle (*Sonchus oleraceous*).

Table 1.5 Numbers of aphids (*Myzus persicae*, the 5 most abundant species and all aphids) trapped in carrot crop 4 (NW Victoria) and the percentage incidence of CarVY between 21 May 2003 - 3 September 2003.

Collection date	*Myzus persicae*	*Brachycaudus rumexicolens*	*Uroleucon sonchi*	*Dysaphis aucupariae*	*Hyperomyzus lactucae*	*Aphis gossypi*	Total	%CarVY incidence (95% confidence limits)
21-May-03	24	595	123	98	161	73	1339	
28-May-03	18	173	38	9	12	12	284	
4-Jun-03	17	351	87	67	26	27	605	
11-Jun-03	7	187	28	35	7	2	268	
18-Jun-03	2	18	7	13	4	0	48	
25-Jun-03	7	10	14	4	5	1	46	
2-Jul-03	1	41	3	6	4	0	61	
9-Jul-03	0	10	8	2	1	1	24	
16-Jul-03	1	1	2	1	0	0	6	0 (0-3.0)
23-Jul-03	1	0	3	0	0	0	4	
6-Aug-03	0	0	2	0	0	0	2	0 (0-3.0)
13-Aug-03	0	1	0	0	0	0	1	
20-Aug-03	0	0	0	0	0	0	1	
27-Aug-03	2	0	1	0	1	0	5	1 (0.1, 5.0)
3-Sep-03	0	0	0	0	0	0	0	
10-Sep-03	0	0	0	0	0	0	0	
17-Sep-03	0	1	0	0	0	0	3	
24-Sep-03	0	0	0	0	0	0	5	
1-Oct-03	2	0	0	1	0	0	13	1 (0.1, 5.0)
8-Oct-03	0	0	0	0	0	0	2	
15-Oct-03	0	0	1	1	1	0	65	
22-Oct-03	5	0	5	1	2	0	76	
29-Oct-03	17	5	5	3	2	0	79	
TOTAL	**104**	**1393**	**327**	**241**	**226**	**116**	**2937**	

Crop 5

Virus incidence within the crop ranged from 3.3 – 10% with the incidence of CarVY estimated at 3.3% just prior to harvest (Table 1.6). Aphid numbers were very low during the first 3 months of crop 5 (southern Victoria crop 4). This reflected the

drought conditions experienced at that time. In November, aphid numbers did increase with *Myzus persicae* (23%) being the most abundant species trapped.

Table 1.6 Number of aphids (5 most abundant species and all aphids) trapped in carrot crop 5 (Southern Victoria Crop 4) and the percentage incidence of CarVY in the crop (20 August 2003 - 17 December 2003)

Collection date	***Myzus persicae***	***Rhopalosiphum rufiabdominalis***	***Capitophorus elaegnai***	***Brevicoryne brassicae***	***Therioaphis trifolii f maculata***	**Total**	**%CarVY incidence (95% confidence limits)**
20-Aug-03	1	0	1	0	0	2	
27-Aug-03	0	0	0	0	0	1	
3-Sep-03	1	0	0	0	0	1	
10-Sep-03	3	0	0	0	0	3	
17-Sep-03	0	0	0	0	0	0	
24-Sep-03	0	0	0	0	0	0	
1-Oct-03	2	0	1	0	0	3	
8-Oct-03	0	3	0	0	0	4	
15-Oct-03	1	1	0	0	0	3	4.7 (1.5, 11.6)
22-Oct-03	0	0	4	0	0	11	
29-Oct-03	0	0	1	0	1	5	
5-Nov-03	1	6	11	3	0	28	3.3 (0.9, 9.2)
13-Nov-03	3	1	0	1	4	19	
20-Nov-03	10	3	4	1	3	24	
27-Nov-03	11	5	0	0	6	25	10.5 (4.7, 22.1)
3-Dec-03	6	3	0	5	3	17	
10-Dec-03	0	2	1	10	2	17	
17-Dec-03	0	1	0	1	0	3	3.3 (0.9, 9.2)
TOTAL	**39**	**25**	**23**	**21**	**19**	**166**	

Crop 6

CarVY incidence within Crop 6 ranged from 1 – 8.2% (Table 1.7). There were very few aphids trapped in carrot crop 6 (NW Victoria Crop 2) (November 2003 - March 2004). The dominant aphid species trapped was *Rhopalosiphum rufiabdominalis* in the early stages of the crop. *Myzus persicae* and *Hyadaphis* coriandri were not common until the end of the crop.

Table 1.7 Numbers of aphids (*Myzus persicae,* five most abundant species and all aphids) trapped in carrot crop 6 (Northern Victoria Crop 2) and percentage incidence of CarVY in the crop (November 2003 - March 2004)

Collection date	*Myzus persicae*	*Rhopalosiphum rufiabdominalis*	*Brachycaudus rumexicolens*	*Lipaphis erysimi*	*Tetraneura nigriabdominalis*	*Hyadaphis coriandri*	Total	%CarVY incidence (95% confidence limits)
26-Nov-03		20	1				25	
03-Dec-03		51	2				56	
24-Dec-03		1		2			3	4.7 (1.5, 11.6)
30-Dec-03							0	
07-Jan-04	1						1	
14-Jan-04				1			1	6.3 (2.4, 14.4)
21-Jan-04							0	
28-Jan-04				3			3	1 (0.0, 3.0)
05-Feb-04			1	1			2	
11-Feb-04	1		1	3			6	
18-Feb-04	2						2	1 (0.0, 3.0)
03-Mar-04				1	2	1	5	
17-Mar-04	4		4	1	8	1	24	
24-Mar-04			5			7	12	8.2 (3.4, 17.7)
TOTAL	**8**	**72**	**14**	**12**	**10**	**9**	**140**	

Crop 7

CarVY incidence within Crop 7 ranged from 4.7% mid way through the crop cycle and increased to 27% just prior to harvest (Table 1.8). The dominant aphid species trapped in carrot crop 7 (SE Victoria Crop 5) was *Myzus persicae* (59% of total catch). There were also high numbers of *M. persicae* apterae trapped in the pan traps in mid-March indicating that the crop was colonised by *M. persicae* at that time.

Table 1.8 Numbers of alate aphids (5 most abundant species and all aphids) and *Myzus persicae* apterae trapped in carrot crop 7 (SE Victoria Crop 5) and the percentage incidence of CarVY in the crop (21 January 2004 - 7 April 2004)

Collection date	*Myzus persicae alatae*	*Myzus persicae apterae*	*Brevicoryne brassicae*	*Tetraneura nigriabdominalis*	*Brachycaudus rumexicolens*	*Rhopalosiphum maidis*	Total	%CarVY incidence (95% confidence limits)
21-Jan-04	1		36	2	1	0	44	
28-Jan-04	0		13	0	1	0	15	
02-Feb-04	0		2	0	1	0	4	
11-Feb-04	1		0	0	2	0	4	
18-Feb-04	2		2	0	2	0	7	
03-Mar-04	8	1	7	5	6	2	41	4.7 (1.5, 11.6)
10-Mar-04	87	1	8	15	9	7	132	
18-Mar-04	263	28	35	5	2	0	311	
24-Mar-04	61	3	26	8	0	0	99	27 (12.6, 100)
31-Mar-04	10		18	7	5	2	55	
07-Apr-04	25		14	2	9	6	66	
TOTAL	**458**	**33**	**161**	**44**	**38**	**17**	**778**	

Aphid species trapped

Alate aphid catches for all sites are shown in Table 1.9. While more aphids were caught in NW Victoria than in southern Victoria, Table 1.10 shows that the geometric mean trapping rate was less in the NW indicating much greater variability in trapping rates in the NW than in the southern area. There were also substantial differences in species composition as can be seen in the abundance ranks for each region.

In southern Victoria, the four most abundant aphid species trapped were *M. persicae* (34%), *Brevicoryne brassicae* (14%), *R. maidis* (18%) and *Brachycaudus rumexicolens* (7%). In NW Victoria, *Brachycaudus rumexicolens* (46%), *Uroleucon sonchi* (11%), *Dysaphis aucupariae* (8%) and *Hyperomyzus lactucae* (7%) made up 72% of the total catch while *M. persicae* only comprised 3% of the catch.

Table 1.9 Numbers and relative abundance for all aphids trapped between August 2002 and April 2004 in yellow water pan traps in carrot fields in Southern Victoria, North Western Victoria and for all traps in Victoria.

Aphid species	Overall			Sthn Vic.			NE Vic.		
	No.	%	Rank	No.	%	Rank	No.	%	Rank
Brachycaudus rumexicolens	1549	29.44%	1	142	6.50%	4	1407	45.73%	1
Myzus persicae	852	16.19%	2	740	33.87%	1	112	3.64%	8
Rhopalosiphum maidis	357	6.78%	3	242	11.08%	3	115	3.74%	7
Uroleucon sonchi	331	6.29%	4	2	0.09%	30	329	10.69%	2
Dysaphis aucupariae	329	6.25%	5	87	3.98%	8	242	7.86%	3
Brevicoryne brassicae	328	6.23%	6	297	13.59%	2	31	1.01%	12
Hyperomyzus lactucae	237	4.50%	7	11	0.50%	17	226	7.34%	4
Rhopalosiphum rufiabdominalis	205	3.90%	8	49	2.24%	10	156	5.07%	5
Lipaphis erysimi	160	3.04%	9	106	4.85%	6	54	1.75%	10
Aphis gossypi	129	2.45%	10	13	0.59%	14.5	116	3.77%	6
Tetraneura nigriabdominalis	118	2.24%	11	108	4.94%	5	10	0.32%	18
Rhopalosiphum padi	105	2.00%	12	76	3.48%	9	29	0.94%	13.5
Acyrthosiphon kondoi	101	1.92%	13	13	0.59%	14.5	88	2.86%	9
Capitophorus elaegnai	98	1.86%	14	93	4.26%	7	5	0.16%	20
Therioaphis trifolii f maculata	85	1.62%	15	44	2.01%	11	41	1.33%	11
Acyrthosiphon pisum	72	1.37%	16	43	1.97%	12	29	0.94%	13.5
Dysaphis radicola	30	0.57%	17	5	0.23%	25	25	0.81%	16
Hyadaphis coriandri	26	0.49%	18	0	0.00%		26	0.84%	15
Aphis spiraecola	20	0.38%	19	19	0.87%	13	1	0.03%	25.5
Capitophorus hippophaes	19	0.36%	20	3	0.14%	28	16	0.52%	17
Brachycaudus helichrysum	15	0.29%	21	8	0.37%	20	7	0.23%	19
Brachycaudus persicae	12	0.23%	22	12	0.55%	16	0	0.00%	
Cavariella aegopodii	10	0.19%	23.5	10	0.46%	18.5	0	0.00%	
Essigella californica	10	0.19%	23.5	10	0.46%	18.5	0	0.00%	
Hyalopterus pruni	9	0.17%	25	6	0.27%	23	3	0.10%	22
Hyadaphis foeniculi	7	0.13%	26.5	7	0.32%	21.5	0	0.00%	
Unidentified A	7	0.13%	26.5	7	0.32%	21.5	0	0.00%	
Aploneura lentisci	6	0.11%	28	2	0.09%	33	4	0.13%	21
Macrosiphum euphorbiae	6	0.11%	29	5	0.23%	25	1	0.03%	25.5
Aphis craccivora	5	0.10%	30	5	0.23%	25	0	0.00%	
Rhopalosiphoninus staphyleae	3	0.06%	33	1	0.05%	36	2	0.06%	23
Aulacorthum solani	3	0.06%	31	3	0.14%	28	0	0.00%	
Dysaphis apiifolia	3	0.06%	32	3	0.14%	28	0	0.00%	
Myzus cerasi	2	0.04%	34	2	0.09%	33	0	0.00%	
Myzus ornatus	2	0.04%	35	2	0.09%	33	0	0.00%	
Pemphigus bursarius	2	0.04%	36	2	0.09%	33	0	0.00%	
Unidentified B	2	0.04%	37	2	0.09%	33	0	0.00%	

Aphid species	Overall			Sthn Vic.			NE Vic.		
	No.	%	Rank	No.	%	Rank	No.	%	Rank
Aphis nerii	1	0.02%	38	0	0.00%		1	0.03%	25.5
Schizaphis rotundiventris	1	0.02%	43	0	0.00%		1	0.03%	25.5
Aphis sp.	1	0.02%	39	1	0.05%	39.5	0	0.00%	
Dysaphis foeniculus	1	0.02%	40	1	0.05%	39.5	0	0.00%	
Neotoxoptera oliveri	1	0.02%	41	1	0.05%	39.5	0	0.00%	
Rhopalosiphum insertum	1	0.02%	42	1	0.05%	39.5	0	0.00%	
Smynthurodes betae	1	0.02%	44	1	0.05%	39.5	0	0.00%	
Total	**5262**			**2185**			**3077**		

Table 1.10 Arithmetic mean and geometric mean (after log (x+1) transformation) trapping rates for each crop (number per trap per week).

Trapping Period	Crop No.	Arithmetic Mean	Geometric mean*
Southern Victoria			
Aug. 2002 - Nov. 2002	1	7.12	0.65
Jan. 2003 - Mar. 2003	2	15.59	1.01
Apr. 2003 - May 2003	3	11.63	1.04
Aug. 2003 - Dec. 2003	4	2.31	0.37
Jan. 2003 - Apr. 2004	5	17.68	0.96
NW Victoria			
May 2003 - Oct. 2003	6	31.92	0.78
Nov. 2003 - Mar. 2004	7	2.50	0.31
Pooled data			
Southern Victoria		9.42	0.73
NW Victoria		20.79	0.61
Overall		13.85	0.68

*$\log_{10}$ (x+1) transformation

1.4. Discussion

CarVY incidence within carrot crops in SE Victoria ranged from 0-18% in the crops tested. The highest incidence of CarVY was recorded in mid-Spring (October) in SE Victoria in 2002. Overall, aphid numbers were low in Spring and Autumn in SE Victoria, with the highest number of aphids caught in mid-February. The flight in mid-February was too late to cause any damage on this particular crop because:

1. the crop was harvested before the virus could affect the growth of the carrots
2. the low incidence of virus in the crop suggests that the likelihood of winged aphids acquiring the virus from the crop would also have been low.

The decrease in virus incidence observed in Victoria compared to previous years may be a direct result of growers implementing production breaks, as well as the effects of the drought conditions currently being seen affecting aphid populations. In SE Victoria, growers have taken up to an eight-week break in carrot production as a direct result of the project and drought conditions.

In NW Victoria, another large carrot growing district in Victoria, a single crop has been monitored since May. The crop size in NW Victoria is comparable to those in SE Victoria. The unusually warm and dry conditions in 2002-2003 may explain the apparent decline in incidence of CarVY in NW Victoria. Aphid numbers in the NW Victoria were very high in autumn but only a very low level of virus has been detected in the crop to date. Monitoring is continuing.

Reference

Burrows P.M. (1987). Improved estimation of pathogen transmission rates by group testing. Phytopathology 77, 363-365.

2. Transmission efficiencies of different aphid vectors of CarVY in Victoria

2.1 Introduction

CarVY was first described by Moran *et al.* (1999). Little was then known about the epidemiology of CarVY, particularly, about which aphid species were vectors of the virus and responsible for its spread in the field. There was no data from Victoria about the transmission efficiencies of various aphid species.

This study was undertaken to assess the efficiency of several aphid species found near to carrot crops in Victoria to transmit a Victorian isolate of CarVY. The information gathered during this study will assist in validating the integrated management strategy produced for the control of CarVY in carrots.

2.2 Materials and Methods

2.2.1 Host plant material

All plants used to rear the aphid colonies were grown in an air-conditioned glasshouse maintained at 15-20°C. Carrot (*Daucus carota* cv. Chantenay Long) and Chinese cabbage (*Brassica rapa* cv. Matilda) plants were grown in steam-sterilised potting mix containing soil, sand and peat (1:1:1). Plants of the 4-6 leaf stage were used for the transmission experiments. CarVY isolate, Vic-1, was maintained in a separate glasshouse.

2.2.2 Aphid colonies

A method developed by Curtis (1998) was used to rear the aphid colonies and was modified from the method described by Blackman (1988) as follows.

- The bases of 30 plastic Petri dishes (5.5 cm diam.) were surface sterilised with 70% ethanol.
- Cabbage leaves and celery leaves were harvested and cut to fit into the petri dish base.
- Petri dish bases were half-filled with 1% water agar (Grade J3, Leiner Davis Gelatin, Australia)
- The adaxial side of the cabbage or celery leaf was placed onto the setting agar.
- The prepared plates were inverted over a base assembly which comprised two perspex sheets (60 × 48 cm). The upper sheet had 80 drilled holes (2 cm diam.) and these were covered with 0.8 mm^2 mesh (Ultra white, Lincraft Australia) to prevent aphid escapes and allow ventilation. The bottom sheet was lined with 4 adhesive (one-sided) foam strips 10 cm apart. The foam strips provided a gap between the sheets, which enable ventilation without desiccating the agar.
- Each day 10-15 adults were moved onto detached leaf plates, using a camelhair paintbrush, and incubated in a controlled temperature room at 20±2°C (12 h photoperiod).
- After a 24 h period, newly-born first instar nymphs were collected and reared to maturity.
- The adults were transferred onto fresh plates to produce more nymphs.
- The plates were moved daily over different holes on the base assembly in order to reduce honeydew deposits on the mesh and the subsequent development of sooty mould. Condensation under the plates was also wiped off daily.
- To prevent overcrowding and any reduction in fecundity, excess aphids were killed, leaving 15-20 aphids per plate.

The four aphid species that were collected from around field sites in SE Victoria were:

1. *Myzus persicae* (green peach aphid) reared on Chinese cabbage (*Brassica rapa* cv. Matilda)
2. *Lipaphis erysimi* (turnip aphid) reared on cabbage (*Brassica rapa* cv. Matilda)
3. *Aphis spiraecola* (apple aphid) reared on celery (*Apium graveolens*)
4. *Caveriella aegopodii* (carrot aphid) reared on celery *(Apium graveolens*)

2.2.3 Transmission Procedure

Aphids were starved for two hours before initiation of transmission experiments. They were then allowed to feed on CarVY-infected plant tissue for 2-3 minutes. They were then placed on healthy carrots for 2 h, at which time Confidor® was applied to kill the aphids.

For each experiment, 150 newly moulted alates were used. There were five treatments in each transmission experiment. The treatments (10 plants) were: a control treatment where no aphids were placed, and four treatments where 1, 2, 4 or 8 aphids/plant were transferred individually from infected CarVY carrots using a fine camel-hair paint brush. Another positive control treatment was used where *Myzus persicae* was transferred 1 aphid/plant. ELISA was used to test plants for CarVY presence 6 weeks later. The antiserum used was supplied by DSZM, GmbH, Germany and was a polyclonal antiserum specific to CarVY.

Except where there was only 1 aphid/plant, percentage transmission efficiency was calculated at each aphid level (i.e. 2/plant, 4/plant, 8/plant) using the formula given by Burrows (1987). The overall efficiency of transmission for each species was estimated by combining the information from each treatment, and fitting a generalized linear model with a complementary log-log link function, as described by Farrington (1992). The models were fitted using the statistical package GenStat, and employed the method of maximum likelihood. The estimates and asymptotic standard errors produced by the models were used to calculate 95% confidence intervals on the transformed scale, which were then back-transformed to the original scale.

2.3 Results

All four species transmitted CarVY but at differing efficiencies (Table 2.1, 2.2). Within individual species, varying the number of aphids/test plant used for the inoculation access feeds sometimes made large differences to the efficiency of virus transmission.

Table 2.1 Overall transmission efficiencies of the four aphid species tested for their ability to transmit a Victorian isolate of CarVY.

Aphid species	**Transmission efficiency (%)**	**95% confidence intervals**
Myzus persicae	20	(13.7, 28.2)
Lipaphis erysimi	11	(6.6, 18.6)
Cavariella aegopodii	14	(8.6, 22.3)
Aphis spiraecola	3	(1.1, 7.5)

Table 2.2. CarVY transmission efficiencies from carrot to carrot by four aphid species

Aphid species	**Experiment no.**	**No. aphids/plant**				
		0	1	2	4	8
Myzus persicae	1	0*/10	1/10	2/10	6/10	8/10
	2		3/10			
	3		4/10			
	4		2/10			
(green peach aphid)	%transmission efficiency	**0**	**25**	**10.3**	**19.4**	**16.6**
Lipaphis erysimi	5	0/10	1/10	3/10	2/10	7/10
(turnip aphid)	%transmission efficiency	**0**	**10**	**15.9**	**5.2**	**12.9**
Aphis spiraecola	6	0/10	0/10	0/10	1/10	3/10
(apple aphid)	%transmission efficiency	**0**	**0**	**0**	**2.5**	**4.1**
Caveriella aegopodii	7	0/10	2/10	3/10	5/10	6/10
(carrot aphid)	% transmission efficiency	**0**	**20**	**15.9**	**15.1**	**10.1**

* number of plants that tested positive for CarVY

2.4 Discussion

Four aphid species collected from SE Victoria were able to transmit a Victorian isolate of CarVY. Except for *Lipaphis erysimi*, they are carrot colonising aphids, suggesting that CarVY transmission can occur by both colonising and non-colonising aphids in Victoria. The results from this study confirm the West Australian study that these species are vectors of CarVY.

References

Blackman R.L. (1988). Rearing and handling aphids. In 'World Crop Pests. Aphids: their biology, natural enemies and control Vol 2B.' (eds Minks, A.K. and Harrewijn, P.), pp59-68. (Elsevier Science Publishers, Netherlands).

Burrows P.M. (1987). Improved estimation of pathogen transmission rates by group testing. Phytopathology 77, 363-365.

Curtis J.E (1998). Enhancing the effectiveness of *Verticuillium lecanii* against the green peach aphid, *Myzus persicae*. PhD thesis, La Trobe University, Melbourne, Australia.

Farrington, C.P. (1992). Estimating prevalence by group testing using generalized linear models. Statistics in Medicine 11, 1591-1597.

Moran J., van Rijswijk B., Traicevski V., Kitajima E.W., Mackenzie A.M. and A.J. Gibbs (2002). Potyviruses, novel and known, in cultivated and wild species of the family Apiaceae in Australia. *Archives of Virology* **147**, 1855-1867

SECTION 8.0

Virus and aphid data from the northern Adelaide Plains, South Australia

By R. Coles

Description:
Activities undertaken by SA researchers:

- *Peak aphid flights determined over 8 months in six locations on the Northern Adelaide Plains*
- *Aphid vectors identified in carrot crops at Virginia*
- *Continuation testing to identify alternative hosts of CarVY*
- *Systemic insecticide imidacloprid evaluated as a seed dressing and foliar application*

Summary:

Aphid flights in carrot plantings were monitored weekly in three different regions of the Northern Adelaide Plains. Samples were taken from 4/9/03 to 2/5/04 using both basin and sticky traps. Flight peaks were recorded during September 2003 and February to March 2004. Warnings were issued to carrot growers following the flights to take precautionary actions to minimise the spread of CarVY to new plantings. Most aphid flights occurred when weekly weather averages ranged from 13^oC-29^oC, wind speeds 8-15km/hr, low rainfall periods and wind directions from the N and S. Twenty-two aphid species were found in association with carrot crops in the Northern Adelaide Plains with species incidence varying over the sampling period.

The incidence of CarVY in harvest carrots (Stefano and Nelix) was between 2%-21% in February 2004, 12% to 41% in April 2004 (Ricardo, Stefano and Nelix) and 5%-6% in mature plantings of Stefano during May 2004. It was probable that carrots planted between December 2003 and January 2004 were infected with CarVY when peak aphid flights were recorded. The reservoir for the virus was likely to be feral carrots of Stefano and Ricardo remaining from 2003 plantings.

At harvest, symptoms of CarVY were found at 13% on carrots grown from seed treated with Imidacloprid (40ml Gaucho 600 FS /kg of seed) compared to 37% grown from untreated seed. A further eight plant species growing adjacent to carrot crops were tested for CarVY but all proved negative. In a shade house study, the “Lesser Water Parsnip” *Berula erecta* was infected with CarVY by aphids transferred after feeding on infected carrots.

Aphid monitoring

Six carrot crops in the Northern Adelaide Plains were monitored weekly for aphid numbers and flight directions. For each crop, sampling commenced at emergence and

ended at harvest. Yellow basin traps gave an estimate of aphid numbers that landed in crops, while cylindrical sticky traps indicated vertical movement and flight direction.

Basin trap catches indicated high numbers of aphids landing in carrot crops in early spring 2003 (September to October) and also in late summer to early autumn 2004 (January to March 2004) (Fig.1, appendix Table 1). These periods corresponded with times when aphids flew from one feeding site to another. Sticky trap results indicated that major flights occurred in early to mid January 2004 and later in February to April 2004, (Fig. 2, appendix Table 2) with flight directions from the North, South, West and West.

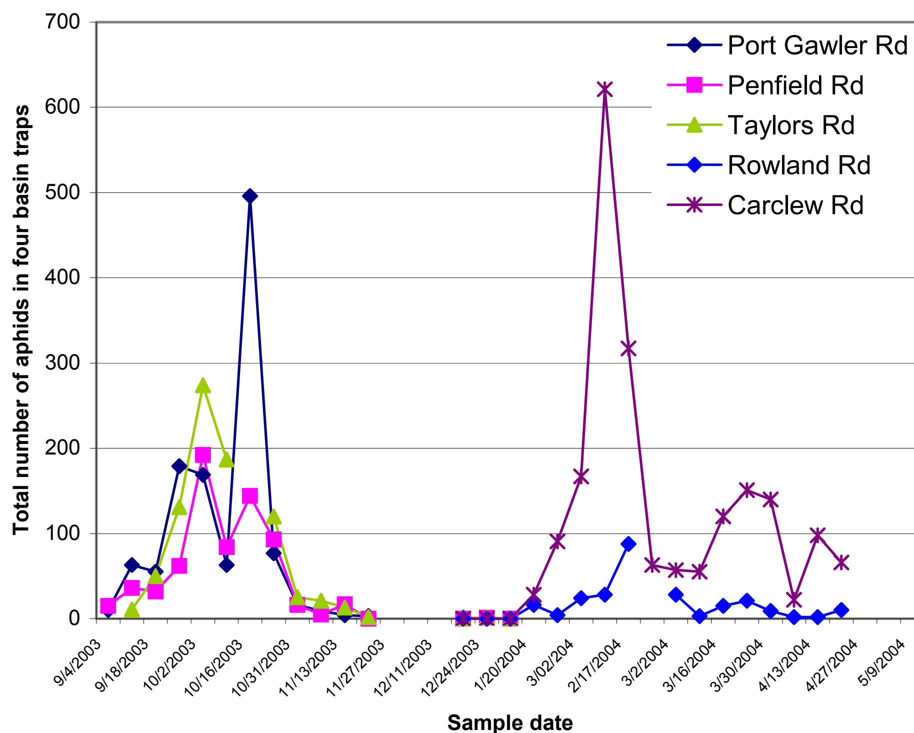


Fig. 1. Number of aphids caught in basin traps in the Northern Adelaide Plains, 2003-2004.

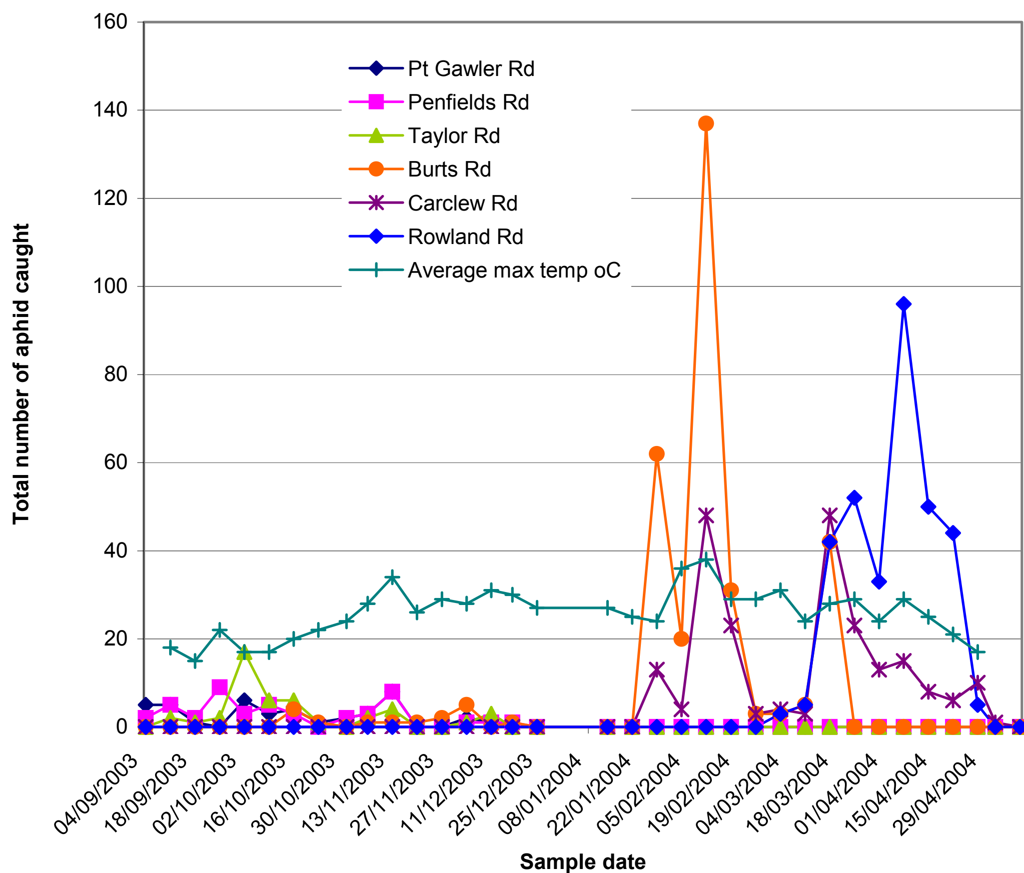


Fig. 2. Number of aphids caught on sticky traps at six locations in the Northern Adelaide Plains, 2003-2004.

Carrot Virus Y in the Northern Adelaide Plains 2003-2004

CarVY was not detected in carrot plantings of Ricardo, Stefano and Nelix until late February and March 2004. During this period CarVY was detected after the peak aphid flights in early January 2004 to mid February 2004. At this time the incidence of CarVY detected in crops ranged from 2% to 21%. Plantings that occurred between December 2003 and January 2004 had higher levels of CarVY at harvest in April 2004 and ranged from 12% to 41%.

In February 2003, 100% of Stefano carrots at the Penfield Rd sample site were infected with CarVY (L. Latham pers.comm.). In February 2004 new plantings of Stefano, 50m E of the old plantings, had 6% CarVY. A windbreak of pine trees and Allocasurina trees separated the two sites and the presence of feral carrots remaining after the 2003 harvest probably acted as the main reservoir for the virus. These findings indicate that

tree barrier zones may not be sufficient to stop aphid spread, and greater than 50m separation between carrot plantings may be required to stop virus spreading.

During the sampling period, aphid flight warnings were issued by direct mail to growers and newsletters were sent out when there was a potential for viral spread. The warnings were issued for 20/1/04, 3/2/04, 30/3/04 and 7/4/04.

Aphid incidence on the Northern Adelaide Plains

Of a total of 22 aphid species identified during the 8-month sampling period from September 2003 to May 2004, nearly 5,000 were trapped in yellow basins at five sites and over 1,000 were caught on sticky traps in six locations (Appendix Tables 2,3,5).

Of the total catch approx., 67% were *Acyrthosiphon kondi* (blue alfalfa aphid), 18% *Myzus persicae* (green peach aphid) and15% each for *Macrosipum avenae* (grain aphid = *Sitobion miscanthi*), *Rhopalosiphum maidis* (corn leaf aphid) and *Hyadaphis foeniculi* (honey suckle aphid). *M. persicae*, the most important aphid vectors for virus transmission, was trapped in the Northern Adelaide Plains during most of the sampling period, with 22% of the catch being taken in September 2003, 44% in October 2003 and 27% in February 2004. *A. kondi* was trapped in greater numbers than was any other species, and of this catch almost 97% was collected during late September to early October 2003 (Fig. 3).

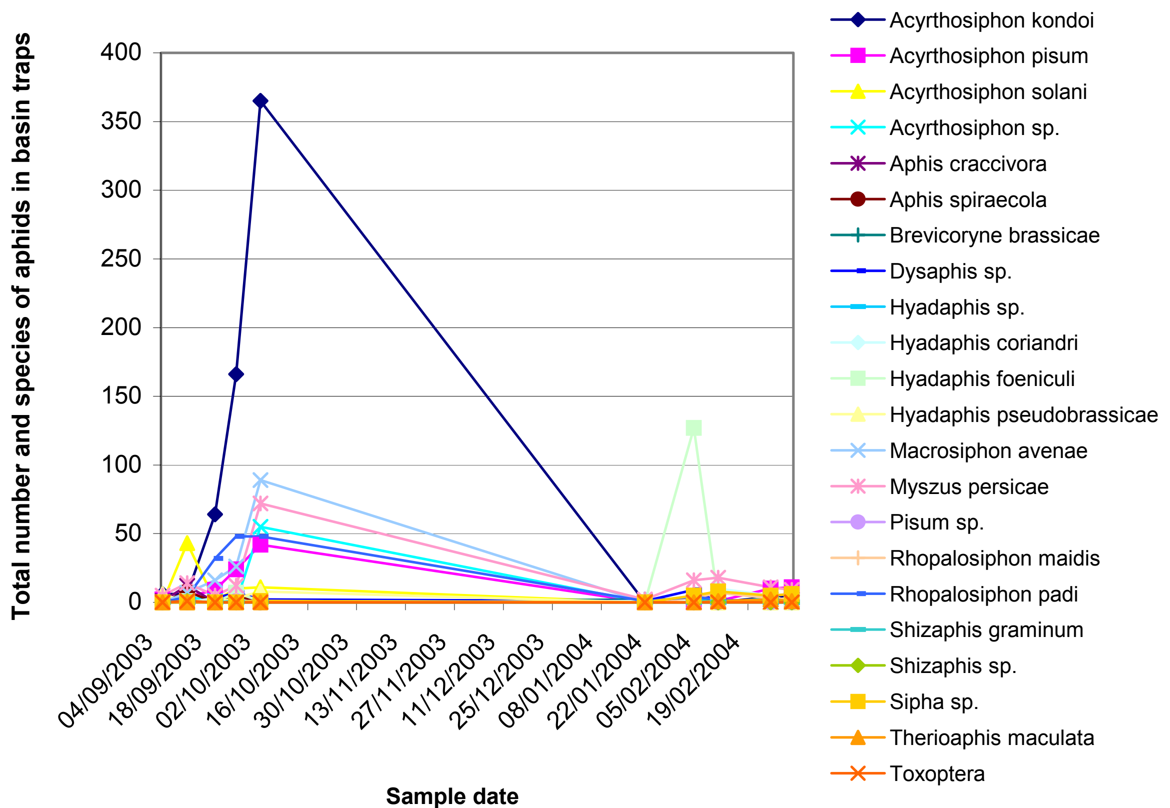


Fig. 3. Incidence of aphid species in carrot crops caught in basin traps in the Northern Adelaide plains, 2003 to 2004.

The yellow tray traps were effective in trapping most of the important virus vector species. Although the catches from the different areas varied in the total species numbers caught, the times of peak occurrence were similar in each district. Trapping results indicate that in this area, *Acyrthosiphon kondi*, which favours the plants lucerne and vetch, may be of more importance as a vector for CarVY than *Myzus persicae* as it occurred in large numbers during spring. *Macrosiphum avenae* and *Rhopalosiphum maidis*, both grain-feeding aphids probably moved from cereal and grass verges into carrot crops during October 2003, when grasses began to die off. *Hyadaphis* spp. may have moved from brassica crops into carrots during spring and late summer when plantings were harvested or incorporated as rotation crops. Temperature influenced the number of aphids caught on sticky traps. When weekly average minimum and maximum temperatures were between 13°C to 29°C aphid flight activity was recorded, out side of this temperature range little flight activity occurred (Appendix, Table 3).

CarVY Host plant survey

During the SA study eight additional plant species found adjacent to carrot crops were tested for CarVY using Elisa tests (Latham et al, 2004). Two other Umbelliferous species were collected from watercourses near the River Murray and tested for susceptibility to CarVY infection. Between two and ten plant samples for each species were tested. They included: Sheoak, Lesser Water Parsnip*, Rape, Lincoln Weed, Fumitory, Shield Penny Wort, Soursob, Ponderosa Pine and Stinging Nettle. In a shade house study aphids feeding on CarVY infected carrots were shown to cross infect the introduced species "Lesser Water Parsnip" (*Berula erecta*), Table 3. Also wild specimen collections of *B. erecta* revealed a possible positive CarVY result from one location near Paiwilla Swamp on the River Murray. A borderline positive CarVY reading was also detected in negative control plants grown for six months in the open at the Lenswood research centre. These plants also showed a slightly more positive value than negative control plants grown in growth chambers at the SARDI, Plant Research Centre.

Table 3. Additional plants tested for CarVY in the Northern Adelaide Plains

Botanical name	Common name
Allocasurina sp.	Sheoak
Berula erecta	Lesser Water Parsnip*
Brassica napus	Rape
Diplotaxis tenuifolia	Lincoln Weed
Fumaria parviflora	Fumitory
Hydrocotyl verticillata	Shield Penny Wort
Oxalis pes-caprae	Soursob
Pinus radiata	Ponderosa Pine
Urtica urens	Stinging Nettle

* Positive after cross infection with CarVY in shade house trials

WA studies demonstrated that two native plants within the Umbelliferous group: *Trachmene pilosa* and *Daucus glochidiatus* could be artificially infected with CarVY (L. Smith, pers. comm.). These species can occur in the Northern Adelaide Plains in

sandy soils, but surveys in the carrot growing areas have so far failed to locate them. However if they do occur in the study region their presence may act as further host reservoirs of CarVY.

Evaluation of systemic insecticides at planting

Materials and Methods

Seed dressings with the insecticide Gaucho were compared with Confidor sprays to determine their effect of reducing virus transmission by aphids in the Northern Adelaide Plains. Ricardo and Stefano carrot seed was treated with Gaucho 600 FS at 20ml/kg and 40ml/kg of seed and Confidor sprays 5g/5L applied 2,4,6 and 8 weeks after emergence. Only one of six insecticide trials (Insecticide Trial 1) established at different times in the Northern Adelaide Plains developed symptoms of CarVY (see SA component for Milestone 11).

Insecticide Trial 1.

Stefano seed was planted on the 11th December 2003 in beds 1.2m wide with three rows of carrots. Treatments applied are outlined below (Table 6). Each plot was 10m long with a 2m buffer gap.

Treatments were arranged in a randomised block with 5 replications (Fig. 4).

At harvest, carrots were sampled mid row for a length of 1m on the east and west side of the beds. The total numbers of carrots for the five replicates for each treatment were pooled and assessed for any CarVY symptoms on the roots. A further sample was collected and pooled 6 days later. Percentages of CarVY were averaged from approx. 250 carrots for each treatment.

Results

All trials planted up until 30th November 2003 tested negative for the virus (see Milestone 11), therefore only results from Trial 1 are reported.

CarVY levels after seed and foliar applications of imidacloprid

In the control plots, 41% of carrots developed CarVY symptoms on storage roots by April 2004 (Fig. 5). All treatments reduced the incidence of CarVY, however the lowest level of CarVY was found in the 40ml Gaucho rate at harvest, where only 13% of carrots were infested.

Table 6. Imidacloprid treatments applied, Trial 1. Northern Adelaide Plains

Treatment No.	Seed Treatment a.i.	rate	Spray Treatment a.i.	rate	Spray timing Weeks after emergence
1	–	–	–	–	–
2	Gaucho 600 FS (Imidacloprid)	20ml/kg seed	–	–	–
3	Gaucho 600 FS (Imidacloprid)	40ml/kg seed	–	–	–
4	–	–	Confidor (Imidacloprid)	170ml/ha	2 4 6 8
5	Gaucho 600 FS (Imidacloprid)	40ml/kg seed	Confidor (Imidacloprid)	170ml/ha	2 4 6 8
6	Gaucho 600 FS (Imidacloprid)	20ml/kg seed	Confidor (Imidacloprid)	170ml/ha	2 4 6 8

5 Confidor sprays	3 Gaucho 20ml/kg	1 Gaucho 40ml/kg	3 Gaucho 20ml/kg	1 Gaucho 40ml/kg
2 Gaucho 40ml/kg and Confidor sprays	4 Gaucho 20ml/kg and Confidor sprays	3 Gaucho 20ml/kg	5 Confidor sprays	4 Gaucho 20ml/kg and Confidor sprays
3 Gaucho 20ml/kg	1 Gaucho 40ml/kg	2 Gaucho 40ml/kg and Confidor sprays	Control	4 Gaucho 20ml/kg and Confidor sprays
1 Gaucho 40ml/kg	2 Gaucho 40ml/kg and Confidor sprays	Control	1 Gaucho 40ml/kg	5 Confidor sprays
4 Gaucho 20ml/kg and Confidor sprays	Control	5 Confidor sprays	2 Gaucho 40ml/kg and Confidor sprays	Control
Control	5 Confidor sprays	4 aucho 20ml/kg and Confidor sprays	4 Gaucho 20ml/kg and Confidor sprays	3 Gaucho 20ml/kg

Fig. 4. Randomised Gaucho seed treatments and Confidor sprays, 11th December 2003, cultivar Stefano, plot length 10m, width 1.2m and 2 m gaps between plots.

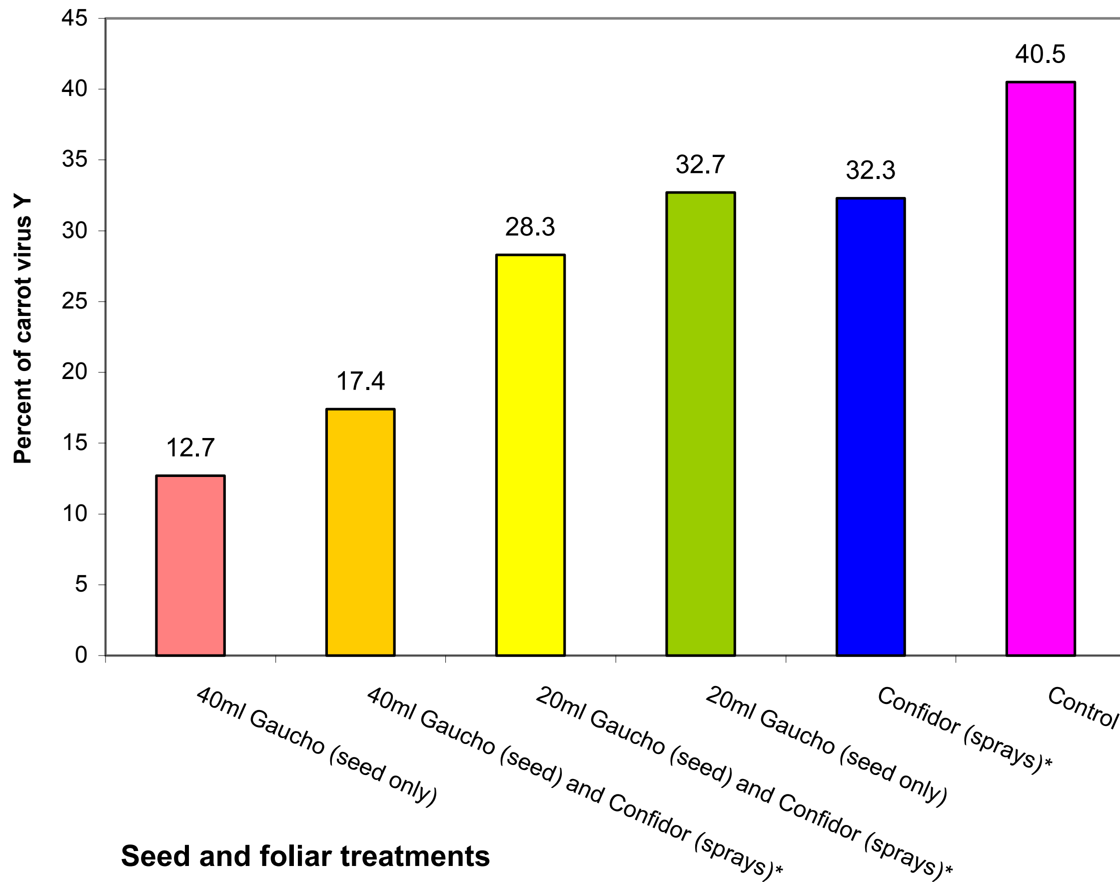


Fig. 5. Effect of Gaucho seed treatments and Confidor sprays on the incidence of Carrot Virus Y in the Northern Adelaide Plains 2003 to 2004.

*Confidor (50gm/kg imidacloprid) was sprayed at 5g/5L of water at 2,4,6,8 weeks after emergence.

Carrot yields after seed and foliar applications of imidacloprid

The seed treatments of Gaucho 600 FS at 40ml/kg and 20ml/kg of seed produced the highest yield of 6.9kg when compared to 4.9kg in the controls (Fig. 6). Confidor sprays alone or when combined with Gaucho seed treatments of 40ml/kg and 20ml/kg produced a yield increase of 6.2kg, 6.9kg and 6.2kg. The lowest yields of 3.6kg were in Gaucho seed treatment at 20ml/kg of seed with Confidor sprays and the controls with 4.9kg.

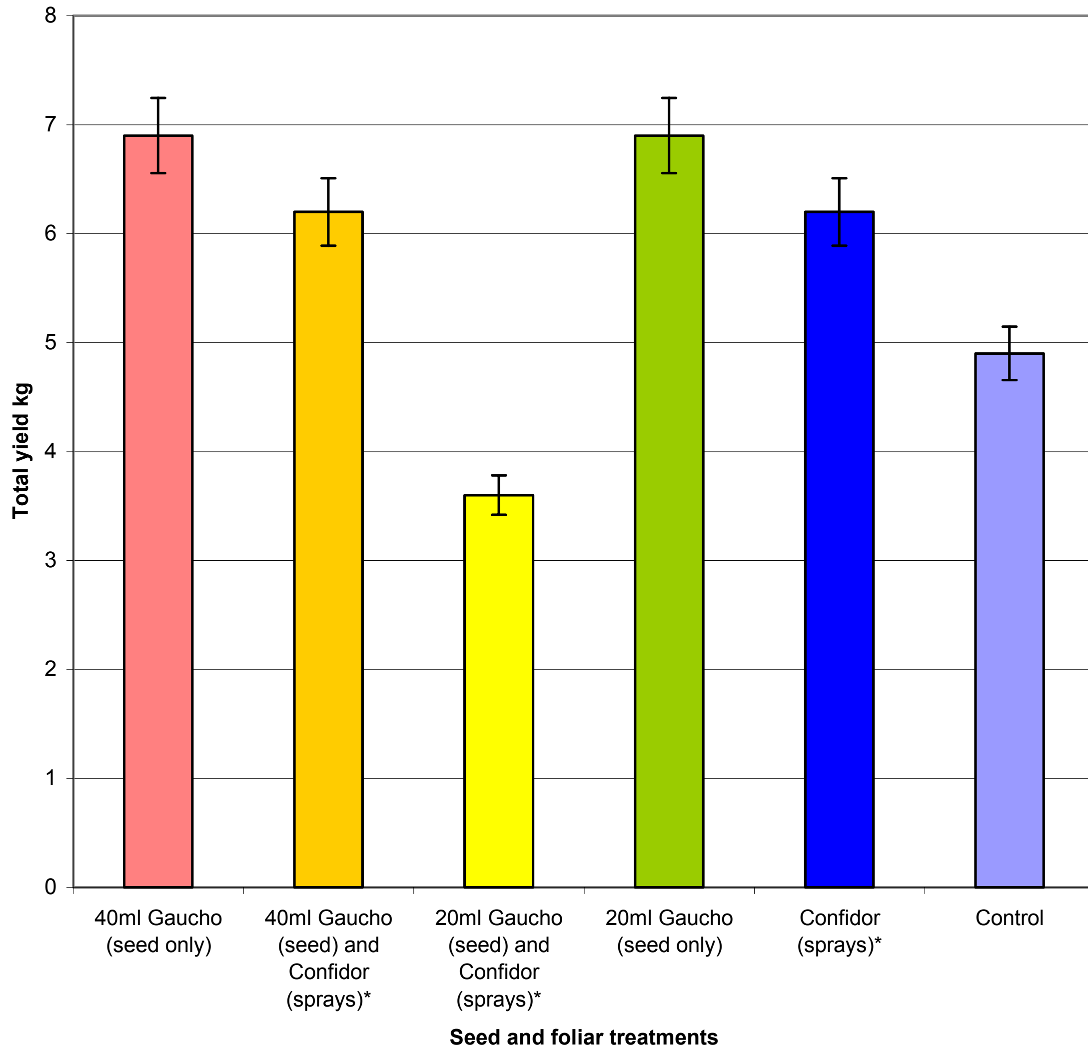


Fig. 6. Carrot yields after Gaucho seed treatments and Confidor sprays in the Northern Adelaide Plains 2003 to 2004.

The results of this work were presented at a talk to growers at the Virginia Horticulture Centre on the 22nd April 2004:-

Control of CarVY in SA relies on implementing management strategies:

- to avoid plantings during peak aphid flights,
- maintain at least 50m buffer zones between adjacent carrot crops
- vigorous control of feral and volunteer carrots.

Future work in the Northern Adelaide Plains

- monitoring aphid flights and relating these to levels of crop infection
- continuing to monitor alternative hosts and feral carrots as reservoirs of the virus
- fine tune the use of systemic insecticides to deter aphid feeding after planting

- evaluate the development levels of systemic insecticide resistance
- evaluate the effectiveness of barrier crops and barrier zones between crops

Acknowledgments

We thank Jo Kent for aphid identifications and compilation of the aphid species list with numbers caught in basin traps in the Northern Adelaide Plains and also Sue Pederick for elisa tests carried out in SA.

Reference:

Latham LJ and Jones RAC (2004) Carrot virus Y: symptoms, losses, incidence, epidemiology and control. *Virus Research* 100, 89-99.

SECTION 9.0

TECHNOLOGY TRANSFER

1. Communication and extension activities

(i) Scientific Refereed Publications

Latham, L.J. and Jones, R.A.C (2004). *Carrot virus Y*: incidence, symptomology, yield losses, epidemiology and control. *Virus Research* **100**, 89-99.

Latham, L.J., Traicevski, V., Persley, D., Wilson, C. R. and Jones, R.A.C. (2004). Distribution and incidence of *Carrot virus Y* in Australia. *Australasian Plant Pathology* **33:** 83-86.

Jones, R.A.C., Smith, L. J., Smith, T. N. and Latham, L. J. (2005). Relative abilities of different aphid species to act as vectors of Carrot virus Y. (submitted).

Jones, R.A.C., Smith, L. J., Gajda B. E. and Latham, L. J. (2005). Patterns of spread of *Carrot virus Y* in carrot plantings and validation of control measures. (submitted).

Jones, R.A.C., Smith, L. J., B. E. Gadja, Smith, T. N. and Latham, L. J. (2005). Further studies on *Carrot virus Y*: hosts, symptomatology, search for resistance and tests for seed transmissibility. (submitted)

(ii) Book Contribution

Latham LJ, Jones RAC (2001) Carrot Virus Y. In*: Compendium of Umbelliferous Diseases (Ed. M Davis, R Raid)* American Phytopathological Society Press *p. 53.*

(iii) Conference Abstracts

Jones, R. A. C, Coutts, B. A., and Gajda, E. (2004). Collecting spatial data from different epidemic scenarios to help validate recommendations over cultural control measures against plant viruses. In: *Proceedings of 6th Australasian Plant Virology Workshop*, Sea World Nara Resort, Gold Coast, Queensland, p.62 (Abstr.)

Latham L, Smith L, Jones R (2003). Current understanding of the epidemiology and management of *Carrot virus Y. Australasian Plant Pathology* **32**, p. 436. (Abstr.)

Latham L, Smith L, Jones R (2003). Current understanding of the epidemiology and management of *Carrot virus Y* In: *Plant Virus Epidemiology Workshop 8th International Congress of Plant Pathology*, Christchurch New Zealand. (Abstr.)

Latham, L. J and Jones, R. A. C. (2003). Epidemiology and control of carrot virus Y. *Journal of Plant Diseases and Protection* **110**, p. 79 (Abstr.).

Latham L, Jones R (2002). Epidemiology and control of carrot virus Y. In *VIII International Plant Virus Epidemiology Symposium*. Aschersleben, Germany, May 12-17, 2002. p. 64. (Abstr.)

Latham L, Jones R (2002). Epidemiology and control of carrot virus Y In: *The 10th Meeting of the International Working Group on Vegetable Viruses*. Bonn, Germany, August 4-9, 2002. p.22. (Abstr.)

Latham L, Jones R (2002). Carrot virus Y a new and devastating disease of carrots. In *Proceedings of the 29th International Carrot Conference.* Bakersfield, California, February 10-12th 2002 vric.ucdavis.edu/carrot. (Abstr.)

Latham LJ, Jones RAC (2000) Yield and quality losses in carrots infected with carrot virus Y. In *Proceedings and abstracts of carrot conference Australia* (Ed. E Davison, A McKay) Perth, Western Australia 24-28, October 200 p.48. (Abstr.)

(iv) Newsletter/Magazine/Newspaper articles

Jones R. (2004) Carrot virus Y controlled by good habits. AUSVEG Review – Vegetable industry report, p.10.

Latham L, Jones RAC, Smith L (2003) Learning to Control Carrot virus Y. *Good Fruit and Vegetables,* Vol. 14 (No. 5), p. 61.

Latham L, Jones RAC, Smith L (2003). Carrot virus Y. In *Horticulture Workshop 2003 – Good Partnerships, Great Results*. Department of Agriculture Biennial Conference, Mandurah, WA, 18-19 September 2003. p.35.

Latham L, Smith L (2002) Growers on top of carrot virus disease. *Countryman*, Horticulture Section. August p. 3.

Latham LJ, Jones RAC (2001). Causal agent of devastating carrot disease is identified. *Good Fruit and Vegetables*, Vol. 11 (No. 10) p.21.

Latham LJ, Jones RAC (2001) Carrot virus destroys quality, yield. *Countryman* March 8th p.20.

Latham L, Jones R (2001). Carrot virus springs up down under. *Carrot Country, USA* Vol. 9 (No. 2) p. 8.

Latham LJ, Jones RAC (2001) Yield and quality losses in carrots infected with carrot virus Y. In *Horticulture Workshop 2001 - Leading Today, Shaping Tomorrow.* Department of Agriculture Biennial Conference*,* Mandurah, WA, 18-19 September 2001, p. 20-21

Latham LJ, Jones RAC (2000). Carrot virus Y. In *D'Carota. Newsletter of the Carrot Association for Research and Development WA*, Vol. 3. (No. 2) p.3.

Latham L (2000) News from Western Australia: Celery mosaic virus and carrot virus Y: In '*The control of celery mosaic virus – Newsletter,* February 2000'. (Ed. V. Traicevski) p. 5.

(v) Farmnote

Latham LJ, Jones RAC, Smith LJ (2003) Carrot Virus Y, Department of Agriculture Farmnote No. 29/2003.

(vi) Other

Wide dissemination of Farmnote on CarVY by the WA Vegetable Industry Development Officer to the National industry, other Newspaper and Newsletter articles, field day presentations, talks to carrot growers in SA, VIC and WA, updates on project progress at 2 monthly meetings of the Carrot Association for R & D (CARD), telephone hook ups with the HAL CarVY Project Steering Committee.

SECTION 10.0

RECOMMENDATIONS

(i) Scientific

- The means by which CarVY rapidly gets introduced to new areas for growing carrots that are geographically isolated from older, CarVY- infected carrot growing areas needs to be determined.
- Further artificial host range studies with native Apiaceous hosts are needed along with surveys of native bush to determine whether they might occsaionally act as CarVY reservoirs for spread to carrot crops.
- The potential role of alternative Apiaceous weed and crop plants hosts as CarVY reservoirs also requires further investigation.
- The CarVY vector efficiencies of additional aphid species often found flying over carrot crops, such as *A. gossypii* and *H. lactucae,* still need to be determined.
- More trapping and transmission tests with aphids caught live downwind of CarVY-infected carrot plantings is required to determine which species are actually transmitting the virus in the field.
- Field trials are needed to validate the recommendation for deployment of non-host barrier crops in between old and new carrot plantings to help control CarVY spread.

(ii) Industry

- The project devised, extended to growers and validated a robust integrated disease management strategy for CarVY in carrots (page 27). Further promotion of the strategy to the carrot industry is warranted to increase its uptake and adoption in CarVY-affected carrot growing areas, especially in Victoria, South Australia and New South Wales.

SECTION 11.0

APPENDIX A – Details of South Australian trap aphid data

Table 1. Aphid numbers caught in yellow basin traps at five locations in the Northern Adelaide Plains, 2003-2004

	Port Gawler Rd	Penfield Rd	Taylors Rd	Rowland Rd	Carclew Rd
04/09/2003	10	15			
11/09/2003	63	36	11		
18/09/2003	55	32	50		
25/09/2003	179	62	131		
02/10/2003	169	192	274		
09/10/2003	63	84	187		
16/10/2003	496	144			
23/10/2003	77	93	120		
31/10/2003	16	16	25		
08/11/2003	9	5	21		
13/11/2003	4	17	13		
20/11/2003	3	0	2		
27/11/2003	24	6	0		
04/12/2003					
11/12/2003					
17/12/2003	1	0	0	0	0
24/12/2003	0	1	1	0	0
13/01/2004	0	0	0	0	0
20/01/2004				16	28
27/01/2004				4	91
3/02/2004				24	167
10/02/2004				28	621
17/02/2004				88	317
24/02/2004					63
02/03/2004				28	57
09/03/2004				3	55
16/03/2004				15	120
23/03/2004				21	151
30/03/2004				9	140
06/04/2004				2	22
13/04/2004				2	98
18/04/2004				10	66
27/04/2004					
02/05/2004					
09/05/2004					

Table 2. Number of aphids caught on sticky traps in six locations in the Northern Adelaide Plains

04/09/2003	5	2	0	0	0	0
11/09/2003	5	5	2	0	0	0
18/09/2003	1	2	1	0	0	0
25/09/2003	0	9	2	0	0	0
02/10/2003	6	3	17	0	0	0
09/10/2003	3	5	6	0	0	0
16/10/2003	4	3	6	4	0	0
23/10/2003	1	0	1	1	0	0
31/10/2003	2	2	0	0	0	0
06/11/2003	3	3	2	1	0	0
13/11/2003	8	8	4	1	0	0
20/11/2003	0	0	0	1	0	0
27/11/2003	0	0	0	2	0	0
04/12/2003	2	1	1	5	0	0
11/12/2003	1	1	3	0	0	0
17/12/2003	0	1	0	1	0	0
24/12/2003	0	0	0	0	0	0
13/01/2004	0	0	0	0	0	0
20/01/2004	0	0	0	0	0	0
27/01/2004	0	0	0	62	13	0
03/02/2004	0	0	0	20	4	0
10/02/2004	0	0	0	137	48	0
17/02/2004	0	0	0	31	23	0
24/02/2004	0	0	0	3	3	0
02/03/2004	0	0	0	3	4	3
09/03/2004	0	0	0	5	3	5
16/03/2004	0	0	0	42	48	42
23/03/2004	0	0	0	0	23	52
30/03/2004	0	0	0	0	13	33
06/04/2004	0	0	0	0	15	96
13/04/2004	0	0	0	0	8	50
20/04/2004	0	0	0	0	6	44
27/04/2004	0	0	0	0	10	5
02/05/2004	0	0	0	0	1	0
09/05/2004	0	0	0	0	0	0

Weather data for Northern Adelaide Plains*

Table 4. Aphid flight peaks on sticky traps and weekly averages of daily weather observations for the Northern Adelaide Plains, September 2003 to May 2004.

Date period 2004	Max Aphid No	Min ^{o}C	Max ^{o}C	Wind km/hr	Rainfall mm
5/9-11/9	5	8	18	17	3.2
12/9-18/9	5	9	15	29	21.4
19/9-25/9	9	9	22	29	4.4
26/9-2/10	17	7	17	26	14.8
3/10-9/10	6	6	17	9	0.8
10/10-16/10	6	8	20	20	2.2
17/10-23/10	1	10	22	28	5.2
24/11-6/11	3	9	24	16	1.6
7/11-13/11	8	11	28	8	0
14/11-20/11	1	18	34	22	1.2
21/11-27/11	2	14	26	22	0
28/11-4/12	5	17	29	18	0.4
5/12-11/12	3	14	28	22	0.6
12/12-17/12	1	17	31	25	0
18/12-24/12	0	17	30	19	15
25/12-13/1	0	15	27	17	11.8
14/1-20/1	0	15	27	19	0
21/1-27/1	62	13	25	8	0
28/1-3/2	20	14	24	14	0
4/2-10/2	137	17	36	14	0
11/2-17/2	31	22	38	12	0
18/2-24/2	3	17	29	9	8
25/2-2/3	4	13	29	12	0
3/3-9/3	5	18	31	21	7.2
10/3-16/3	48	11	24	6	0
17/3-23/3	52	12	28	8	0
24/3-30/3	33	13	29	18	13.2
31/3-6/4	96	14	24	15	0
7/4-13/4	50	14	29	10	0.6
14/4-20/4	44	14	25	13	1.6
21/4-27/4	10	12	21	14	7
28/4-2/5	1	8	17	16	5.2

* Recorded from the Edinburgh Air Base

Table 5. Aphid species and numbers caught in basin traps in the Northern Adelaide Plains, 2003-2004.

(Identifications with "?" after name are not confirmed, as specimens were either not complete or not in good condition.)
Locations: Musolino = Port Gawler, Zerella = Penfields Rd, Nicol = Taylors Rd, Burt Rd and Rowland Rd, Carclew = Carclew Rd.

Trap	Location	Date	Genus and Species	Total No.s
M1	Musolino	04/09/2003	*Acyrthosiphon pisum*	1
			Sipha/Dysaphis	1
			Total	**2**
M2	Musolino	04/09/2003	*Acyrthosiphon kondoi*	3
			Acyrthosiphon pisum	1
			Aphis craccivora?	1
			Total	**5**
M4 (no M3)	Musolino	04/09/2003	*Acyrthosiphon kondoi*	2
			Hyadaphis pseudobrassicae?	1
			Myzus persicae	1
			Total	**4**
Z5	Zerella	04/09/2003	*Hyadaphis pseudobrassicae?*	1
			Myzus persicae	1
			Total	**2**
Z6	Zerella	04/09/2003	*Acyrthosiphon kondoi*	1
			Acyrthosiphon solani	1
			Hyadaphis pseudobrassicae?	3
			Myzus persicae	4
			Total	**9**
Z8 (no Z7)	Zerella	04/09/2003	*Hyadaphis pseudobrassicae?*	*1*
			Unassigned?	1
			Total	**2**
M1	Musolino	11/09/2003	*Acyrthosiphon kondoi*	11
			Acyrthosiphon pisum	2
			Acyrthosiphon solani	1
			Macrosiphum avenae	1
			Myzus persicae	1
			Toxoptera sp.	1
			Total	**17**
M2	Musolino	11/09/2003	*Acyrthosiphon kondoi*	5
			Acyrthosiphon pisum	2
			Hyadaphis foeniculi	1
			Myzus persicae	2
			Miscellaneous-broken?	1
			Total	**11**
M3	Musolino	11/09/2003	*Acyrthosiphon kondoi*	6
			Aphis craccivora	6
			Aphis spiraecola	3
			Macrosiphum avenae	1
			Myzus persicae	3
			Miscellaneous-broken?	1

			Exuviae?	2
			Total	**22**
Trap	**Location**	**Date**	**Genus and Species**	**Total No.s**
M4	Musolino	11/09/2003	*Acyrthosiphon kondoi*	2
			Dysaphis sp.	1
			Myzus persicae	3
			Rhopalosiphon padi	4
			Unassigned?	1
			Total	**11**
N1	Nicols	11/09/2003	*Acyrthosiphon kondoi*	1
			Acyrthosiphon solani	1
			Total	**2**
N2	Nicols	11/09/2003	*Acyrthosiphon pisum*	1
			Total	**1**
N3	Nicols	11/09/2003	*Acyrthosiphon solani*	1
			Myzus persicae	1
			Total	**2**
N4	Nicols	11/09/2003	*Acyrthosiphon kondoi*	2
			Acyrthosiphon pisum	1
			Myzus persicae	1
			Total	**4**
Z1	Zerella	11/09/2003	*Acyrthosiphon kondoi*	3
			Aphis sp.	1
			Macrosiphum avenae	3
			Myzus persicae	1
			Total	**8**
Z2	Zerella	11/09/2003	*Acyrthosiphon kondoi*	4
			Aphis craccivora	3
			Aphis spiraecola	1
			Macrosiphum avenae	2
			Myzus persicae	1
			Total	**11**
Z3	Zerella	11/09/2003	*Acyrthosiphon kondoi*	7
			Acyrthosiphon solani	1
			Aphis craccivora	3
			Aphis spiraecola	4
			Macrosiphum avenae	1
			Total	**16**
Z4	Zerella	11/09/2003	*Acyrthosiphon kondoi*	2
			Myzus persicae	2
			Schizaphis?	1
			Total	**5**
M1	Musolino	18/09/2003	*Acyrthosiphon kondoi*	5
			Acyrthosiphon pisum	2
			Macrosiphum avenae	5
			Rhopalosiphon padi	7
			Total	**19**
M2	Musolino	18/09/2003	*Acyrthosiphon kondoi*	23
			Acyrthosiphon pisum	3
			Aphis craccivora	1

			Dysaphis sp.	
			Total	**28**

Trap	Location	Date	Genus and Species	Total No.s
			Hyadaphis foeniculi	1
			Macrosiphum avenae	2
			Myzus persicae	1
			Rhopalosiphon padi	5
			Total	**38**
M4	Musolino	18/09/2003	*Acyrthosiphon kondoi*	8
			Dysaphis sp.	1
			Myzus persicae	1
			Rhopalosiphon padi	1
			Total	**11**
N1	Nicols	18/09/2003	*Acyrthosiphon kondoi*	3
			Acyrthosiphon pisum	2
			Myzus persicae	1
			Rhopalosiphon padi	4
			Total	**10**
N2	Nicols	18/09/2003	*Acyrthosiphon kondoi*	2
			Hyadaphis foeniculi	1
			Macrosiphum avenae	1
			Rhopalosiphon padi	2
			Miscellaneous & exuvia?	1
			Total	**7**
N3	Nicols	18/09/2003	*Acyrthosiphon kondoi*	9
			Hyadaphis foeniculi	1
			Macrosiphum avenae	1
			Rhopalosiphon padi	4
			Total	**15**
N4	Nicols	18/09/2003	*Acyrthosiphon kondoi*	9
			Acyrthosiphon pisum	2
			Acyrthosiphon solani	1
			Macrosiphum avenae	2
			Myzus persicae	1
			Rhopalosiphon padi	3
			Total	**18**
Z1	Zerella	18/09/2003	*Acyrthosiphon kondoi*	3
			Myzus persicae	2
			Rhopalosiphon padi	4
			Macrosiphum avenae	1
			Total	**10**
Z2	Zerella	18/09/2003	*Macrosiphum avenae*	3
			Rhopalosiphon padi	3
			Total	**6**
Z4 (no Z3)	Zerella	18/09/2003	*Acyrthosiphon kondoi*	2
			Macrosiphum avenae	1
			Myzus persicae	1
			Rhopalosiphon padi	2
			Miscellaneous exuvia?	1

			Total	8
M1	Musolino	25/09/2003	*Acyrthosiphon kondoi*	39
			Acyrthosiphon pisum	4

Trap	Location	Date	Genus and species	Total No.s
			Dysaphis sp.	1
			Hyadaphis pseudobrassicae?	1
			Macrosiphum avenae	11
			Myzus persicae	2
			Rhopalosiphon padi	1
			Sipha/Dysaphis	1
			Miscellaneous & exuviae?	6
			Total	**67**
M2	Musolino	25/09/2003	*Acyrthosiphon kondoi*	25
			Acyrthosiphon pisum	6
			Acyrthosiphon solani	1
			Macrosiphum avenae	1
			Myzus persicae	1
			Rhopalosiphon padi	2
			Miscellaneous?	1
			Total	**37**
M3	Musolino	25/09/2003	*Acyrthosiphon kondoi*	18
			Acyrthosiphon pisum	3
			Aphis craccivora	2
			Aphis spiraecola	1
			Hyadaphis foeniculi	2
			Hyadaphis pseudobrassicae?	1
			Macrosiphum avenae	3
			Myzus persicae	2
			Rhopalosiphon padi	4
			Miscellaneous?	1
			Total	**36**
M4	Musolino	25/09/2003	*Acyrthosiphon kondoi*	14
			Acyrthosiphon pisum	2
			Acyrthosiphon solani	1
			Aphis spiraecola	4
			Dysaphis sp.	1
			Macrosiphum avenae	2
			Rhopalosiphon padi	6
			Miscellaneous & exuviae?	9
			Total	**39**
N1	Nicols	25/09/2003	*Acyrthosiphon kondoi*	10
			Aphis craccivora	1
			Aphis spiraecola	1
			Hyadaphis foeniculi	2
			Myzus persicae	1
			Rhopalosiphum padi	2
			Schizaphis graminum?	1

			Miscellaneous-broken?	9
			Total	**28**
N2	Nicols	25/09/2003	*Acyrthosiphon kondoi*	11
			Acyrthosiphon pisum	1
			Acyrthosiphon sp. solani?	3
			Total	**15**

Trap	Location	Date	Genus and Species	Total No.s
			Hyadaphis foeniculi	1
			Macrosiphum avenae	1
			Myzus persicae	3
			Rhopalosiphum padi	3
			Miscellaneous-broken?	4
			Total	**28**
N3	Nicols	25/09/2003	*Acyrthosiphon kondoi*	27
			Acyrthosiphon pisum	4
			Aphis spiraecola	1
			Hyadaphis foeniculi	1
			Macrosiphum avenae	6
			Rhopalosiphum padi	7
			Rhopalosiphum padi	5
			Rhopalosiphon maidis?	1
			Pisum or Schizaphis sp?	1
			Miscellaneous-broken?	2
			Total	**55**
N4	Nicols	25/09/2003	*Acyrthosiphon kondoi*	5
			Acyrthosiphon pisum	1
			Acyrthosiphon solani	2
			Aphis craccivora	1
			Myzus persicae	2
			Rhopalosiphon padi	3
			Miscellaneous-broken	6
			Total	**20**
Z1	Zerella	25/09/2003	*Rhopalosiphon padi*	3
			Total	**3**
Z2	Zerella	25/09/2003	*Acyrthosiphon kondoi*	9
			Acyrthosiphon solani	1
			Aphis craccivora	1
			Aphis spiraecola	3
			Dysaphis sp.	3
			Hyadaphis pseudobrassicae?	*1*
			Rhopalosiphon padi	7
			Miscellaneous & exuviae	1
			Total	**26**
Z3	Zerella	25/09/2003	*Acyrthosiphon kondoi*	5
			Acyrthosiphon pisum	1
			Acyrthosiphon solani	1
			Aphis craccivora	1
			Aphis spiraecola	1
			Macrosiphum avenae	1

			Rhopalosiphon padi	6
			Total	**16**
Z4	Zerella	25/09/2003	*Acyrthosiphon kondoi*	3
			Acyrthosiphon pisum	2
			Acyrthosiphon solani?	1
			Dysaphis sp.	2
			Hyadaphis foeniculi	1

Trap	**Location**	**Date**	Genus and species	**Total No.s**
			Total	**9**
			Macrosiphum avenae	1
			Myzus persicae	1
			Rhopalosiphon padi	4
			Miscellaneous & exuviae?	2
			Total	**17**
M1	Musolino	02/10/2003	*Acyrthosiphon kondoi*	32
			Acyrthosiphon pisum	8
			Acyrthosiphon solani	1
			Aphis sp.	1
			Hyadaphis foeniculi	1
			Macrosiphum avenae	7
			Myzus persicae	3
			Rhopalosiphon padi	8
			Miscellaneous & exuviae?	5
			Total	**66**
M2	Musolino	02/10/2003	*Acyrthosiphon kondoi*	13
			Acyrthosiphon pisum	3
			Hyadaphis pseudobrassicae?	1
			Myzus persicae	1
			Rhopalosiphon padi	5
			Miscellaneous & exuviae?	4
			Total	**27**
M3	Musolino	02/10/2003	*Acyrthosiphon kondoi*	27
			Acyrthosiphon pisum	3
			Macrosiphum avenae	4
			Myzus persicae	2
			Rhopalosiphon padi	5
			Sipha/Dysaphis	1
			Total	**42**
M4	Musolino	02/10/2003	*Acyrthosiphon kondoi*	21
			Acyrthosiphon solani	1
			Macrosiphum avenae	5
			Rhopalosiphon padi	7
			Total	**34**
N1	Nicols	02/10/2003	*Acyrthosiphon kondoi*	20
			Acyrthosiphon pisum	6
			Acyrthosiphon solani	3
			Dysaphis sp.	1
			Macrosiphum avenae	18

			Myzus persicae	19
			Rhopalosiphon padi	3
			Miscellaneous – broken?	1
			Total	**71**
N2	Nicols	02/10/2003	*Acyrthosiphon kondoi*	12
			Acyrthosiphon pisum	2
			Hyadaphis pseudobrassicae?	*3*
			Macrosiphum avenae	7
			Myzus persicae	4
			Total	**25**

Trap	Location	Date	Genus and Species	Total No.s
			Acyrthosiphon sp.	9
			Total	**38**
N3	Nicols	02/10/2003	*Acyrthosiphon kondoi*	23
			Acyrthosiphon pisum	12
			Acyrthosiphon solani	3
			Acyrthosiphon sp.	16
			Hyadaphis pseudobrassicae?	1
			Macrosiphum avenae	11
			Myzus persicae	17
			Rhopalosiphon padi	5
			Sipha/Dysaphis	1
			Total	**89**
N4	Nicols	02/10/2003	*Acyrthosiphon kondoi*	29
10/tube			*Acyrthosiphon pisum*	4
			Acurthosiphon solani	2
			Acyrthosiphon sp.	12
			Dysaphis sp.	1
			Macrosiphum avenae	17
			Myzus persicae	5
			Rhopalosiphon padi	6
			Total	**76**
Z1	Zerella	02/10/2003	*Acyrthosiphon kondoi*	13
			Acyrthosiphon pisum	1
			Acyrthosiphon sp.	10
			Macrosiphum avenae	1
			Myzus persicae	4
			Rhopalosiphon padi	2
			Total	**31**
Z2	Zerella	02/10/2003	*Acyrthosiphon kondoi*	16
			Acyrthosiphon pisum	2
			Acyrthosiphon sp.	10
			Dysaphis sp.	1
			Hyadaphis pseudobrassicae?	3
			Macrosiphum avenae	3
			Myzus persicae	8
			Rhopalosiphon padi	5

			Miscellaneous – broken?	1
			Total	**49**
Z3	Zerella	02/10/2003	*Acyrthosiphon kondoi*	17
			Acyrthosiphon pisum	1
			Acyrthosiphon solani	1
			Acyrthosiphon sp.	13

Trap	Location	Date	Genus and Species	Total No.s
			Dysaphis sp.	2
			Macrosiphum avenae	13
			Myzus persicae	6
			Rhopalosiphon padi	3
			Total	**56**
Z4	Zerella	02/10/2003	*Acyrthosiphon kondoi*	24
			Acyrthosiphon sp.	12
			Macrosiphum avenae	10
			Myzus persicae	3
			Rhopalosiphum maidis?	1
			Rhopalosiphum padi	4
			Rhopalosiphum sp.	1
			Miscellaneous – broken?	1
			Total	**56**
C2	Carclew	20/01/2004	*Brevicoryne brassicae?*	3
			Myzus persicae	1
			Total	**4**
Carclew	Carclew	20/01/2004	*Aphis craccivora*	1
			Brevicoryne brassicae?	2
			Myzus persicae	1
			Rhopalosiphon sp.	1
			Total	**5**
N1	Nicols	20/01/2004	*N/A*	**N/A**
N2	Nicols	20/01/2004	*Brevicoryne brassicae*	1
			Hyadaphis coriandri	1
			Hyadaphis/Dysaphis spp?	1
			Sihpa/Dysaphis spp?	1
			Total	**4**
C1	Carclew	27/01/2004	*Aphis craccivora*	1
			Aphis spiraecola	1
			Brevicoryne brassicae	1
			Dysaphis sp.	1
			Hyadaphis coriandri	7
			Myzus persicae	1
			Sihpa/Dysaphis	3
			Total	**15**
C2	Carclew	27/01/2004	*Dysaphis sp.*	1
			Hyadaphis coriandri	11
			Macrosiphum avenae	1
			Myzus persicae	4
			Sihpa/Dysaphis spp.?	10
			Toxoptera sp.	1

			Total	**28**
C3	Carclew	27/01/2004	*Dysaphis sp.*	1
			Hyadaphis coriandri	5

Trap	**Location**	**Date**	**Genus and Species**	**Total No.s**
			Sihpa/Dysaphis spp?	8
			Miscellaneous-broken?	1
			Total	**15**
C4	Carclew	27/01/2004	*Aphis spiraecola*	1
			Dysaphis sp.	1
			Hyadaphis coriandri	24
			Myzus persicae	3
			Sihpa/Dysaphis	4
			Total	**33**
N2	Nicols	27/01/2004	*Sihpa/Dysaphis spp.?*	1
			Total	**1**
C1	Carclew	03/02/2004	*Dysaphis sp.*	4
			Dysaphis sp?	1
			Hyadaphis foeniculi	9
			Myzus persicae	1
			Rhopalosiphum maidis?	2
			Rhopalosiphum padi	1
			Total	**18**
C2	Carclew	03/02/2004	*Dysaphis sp.*	1
			Hyadaphis foeniculi	35
			Myzus persicae	7
			Rhopalosiphum padi	2
			Sipha/Dysaphis	1
			Total	**46**
C3	Carclew	03/02/2004	*Dysaphis sp.*	1
			Dysaphis sp?	2
			Hyadaphis foeniculi	19
			Myzus persicae	2
			Total	**24**
C4	Carclew	03/02/2004	*Brevicoryne sp.*	2
			Dysaphis sp.	2
			Dysaphis sp?	3
			Hyadaphis coriandri	1
			Hyadaphis foeniculi	56
			Myzus persicae	6
			Rhopalosiphum maidis?	1
			Rhopalosiphum padi	1
			Sipha/Dysaphis spp.?	3
			Miscellaneous-dehydrated?	1
			Total	**76**
N1	Nicols	03/02/2004	*Dysaphis sp.*	1
			Hyadaphis foeniculi	6
			Sipha/Dysaphis spp.?	1
			Total	**8**
N2	Nicols	03/02/2004	*Hyadaphis foeniculi*	2

			Hyadaphis sp.	2
			Total	**4**
C1	Carclew	10/02/2004	*Aphis craccivora*	1
			Total	**1**

Trap	**Location**	**Date**	**Genus and Species**	**Total No.s**
C2	Carlew	10/02/2004	*Brevicoryne brassicae*	1
unfinished			*Hyadaphis coriandri*	3
			Myzus persicae	18
			Sipha/Dysaphis spp.?	4
			Therioaphis maculata	1
			Total	**27**
N1	Nicols	10/02/2004	*Acyrthosiphon pisum*	1
			Hyadaphis coriandri	9
			Hyadaphis foeniculi	1
			Hyadaphis sp.	1
			Rhopalosiphum maidis	4
			Rhopalosiphum padi	2
			Sipha/Dysaphis spp.?	1
			Total	**19**
N2	Nicols	10/02/2004	*Hyadaphis coriandri*	1
			Hyadaphis sp.	2
			Rhopalosiphum maidis	3
			Sipha/Dysaphis spp.?	3
			Total	**9**
C1	Carclew	25/02/2004	*Acyrthosiphon pisum*	1
			Aphis sp.	1
			Dysaphis sp.	1
			Dysaphis sp?	3
			Hyadaphis coriandri	2
			Sipha/Dysaphis	2
			Therioaphis maculata	1
			Miscellaneous-broken?	1
			Total	**12**
C2	Carclew	25/02/2004	*Acyrthosiphon kondoi*	1
			Acyrthosiphon pisum	5
			Hyadaphis coriandri	3
			Hyadaphis sp.	3
			Myzus persicae	3
			Rhopalosiphum maidis?	2
			Sipha/Dysaphis	2
			Total	**19**
C3	Carclew	25/02/2004	*Aphis craccivora*	1
			Acyrthosiphon kondoi	1
			Acyrthosiphon pisum	3
			Myzus persicae	3
			Rhopalosiphum maidis?	1
			Sipha/Dysaphis	3
			Total	**12**
C4	Carclew	25/02/2004	*Acyrthosiphon kondoi*	2

			Acyrthosiphon pisum	1
			Aphis craccivora	1
			Aphis spiraecola	1
			Brevicoryne brassicae	1
			Dysaphis sp.	1
			Dysaphis sp?	1
Trap	**Location**	**Date**	**Genus and Species**	**Total No.s**
			Myzus persicae	5
			Therioaphis maculata	1
			Miscellaneous-shrivelled?	1
			Total	**15**
C1	Carclew	02/03/2004	*Acyrthosiphon kondoi*	1
			Dysaphis sp?	3
			Hyadaphis coriandri	4
			Hyadaphis foeniculi	1
			Rhopalosiphum maidis?	1
			Sipha/Dysaphis spp.?	1
			Total	**11**
C2	Carclew	02/03/2004	*Acyrthosiphon kondoi*	2
			Acyrthosiphon pisum	4
			Aphis craccivora	1
			Myzus persicae	2
			Sipha/Dysaphis	1
			Miscellaneous & exuviae	1
			Total	**11**
C3	Carclew	02/03/2004	*Acyrthosiphon pisum*	3
			Aphis gossypii	1
			Aphis spiraecola	1
			Brevicoryne brassicae	1
			Myzus persicae	1
			Miscellaneous	1
			Total	**8**
C4	Carclew	02/03/2004	*Acyrthosiphon pisum*	3
			Brevicoryne brassicae	2
			Dysaphis sp?	2
			Macrosiphum avenae	1
			Myzus persicae	4
			Total	**12**
N3	Nicols	02/03/2004	*Acyrthosiphon pisum*	1
			Aphis craccivora	1
			Myzus persicae	2
			Rhopalosiphum maidis?	1
			Sipha/Dysaphis	4
			Therioaphis maculata	1
			Total	**10**

Summary of *Carrot Virus Y* advice for growers in Virginia 2003-2004

Aphid flights in Virginia were monitored weekly from the 4/9/03 to 2/5/04. In three different regions using a combination of (A) basin and (B) sticky traps.

- Basin traps gave estimates of aphid numbers landing in crops.
- Sticky traps gave an indication of vertical movement and flight direction.

Basin trap catches indicated high numbers of aphids landing in carrot crops during two periods:

- early spring 2003 (September to October)
- late summer to early autumn 2004 (January to March 2004)

These periods corresponded with times when aphids flew from one feeding site to another.

Sticky trap catches indicated that major flights occurred in early to mid January 2004 and later in February to April 2004 with flight directions mainly from the North, West and East.

CarVY was not detected in the three regions of Virginia until late March 2004 with an incidence of between 12% to 40% occurring in crops near harvest.

Carrot plantings times between December 2003 and January 2004 allowed aphid to transmit CarVY to newly emerged seedlings during periods when peak aphid flights were recorded.

The presence of feral carrots in these areas showed significant CarVY levels that were still persistent from previous crops planted in 2003.

The feral carrots probably acted as the main reservoir for the virus to spread. During the sampling period, aphid flight warnings were issued by direct mail to growers and newsletters provided when potential viral spread could occur. The warnings were issued for 20/1/04, 3/2/04, 30/3/04 and 7/4/04.

Control of CarVY in Virginia still relies on implemented management strategies:

- avoid plantings during peak aphid flights
- maintain 50m or more buffer zones between adjacent crops
- vigorous control of feral and volunteer carrots

Future work still needs to be done on:

- Monitoring aphid flights and relating them to levels of crop infection
- Monitoring alternate hosts and feral carrots as reservoirs of the virus
- Fine tuning the use of systemic insecticides to deter aphid feeding
- Evaluate the effectiveness of barrier crops and barrier zone between crops

SECTION 12.0

Appendix B

Farmnote No. 29/2003. - Carrot Virus Y

[Figures removed]

By Lindrea Latham, Lisa Smith and Roger Jones, Department of Agriculture for WA.

Carrot Virus Y (CarVY) is spread by aphids and causes mild leaf and severe root symptoms in carrots. It seriously diminishes quality of carrots if plants are infected at an early growth stage. CarVY infects some other plants belonging to the same plant family as carrots (Apiaciae), such as anise, chervil, coriander, cumin, dill, and parsnip. It does not infect celery, fennel, parsley, and several other related herbs.

Distribution

CarVY has only been detected in Australia. It is found in carrot crops in New South Wales, Queensland, South Australia, Tasmania, Victoria and Western Australia. It is detected at higher incidences when carrot crops are grown throughout the year without a break in production.

Symptoms and Losses

In carrot plants, leaf symptoms of CarVY include chlorotic mottle (see Fig. 1), marginal necrosis, increased subdivision of leaflets giving a feathery appearance, and affected plants show mild stunting. In the roots, the most severe symptoms are seen when infection occurs early (carrot seedlings infected up to six weeks after germination) and include stubby or shortened roots, knobbliness and severe distortion rendering them unmarketable (see Fig. 2). Later infection (carrot plants infected more than six weeks after germination), produces milder symptoms of thinner carrots with only slight distortion but still with a substantial overall yield loss (around 30%). In some cases crops have been abandoned due to large-scale early infection and the severe root symptoms that result. All commonly grown carrot cultivars are susceptible to CarVY.

Spread

CarVY is spread by aphids in a non-persistent manner, ie. they rapidly acquire the virus when feeding on infected carrot plants and then just as rapidly lose the virus from their mouthparts after feeding on a healthy or non host plant. A range of aphid species can transmit it, including species that do not normally colonise carrots. The green peach aphid *Myzus persicae* is a very efficient vector. Volunteer carrots and nearby carrot crops infected with CarVY are the main sources of virus for spread to newly planted crops. Other Apiaceous crops such as anise, chervil, dill, coriander, cumin and parsnip are hosts and are potential alternative virus sources. Some native Apiaceaous plants may possibly be other sources, but this has yet to be demonstrated. As CarVY has a very narrow host range, it is unlikely that weeds and crop plants that do not belong to

the Apiaciae family are ever a source of infection. Whether CarVY is seed-borne at very low levels is yet to be confirmed but other similar viruses belonging to the same virus family are seed-borne at low levels. If it is seed-borne, this would provide a means of introduction of the virus to new sites.

Control

Once a carrot plant becomes infected with CarVY, there is no cure. The best means of control is adopting management practices that minimise the reservoir of infection. If the following integrated control strategy is followed, infection will be greatly reduced:

- **Avoid side-by-side plantings of carrots, grow an intervening non-host crop or have the bordering area under fallow –** Having side by side plantings of carrots of different ages leads to the younger crops being infected from nearby, older virus infected ones. Planting a barrier non-host crop or having a fallow area around the crop will reduce virus spread. The non-host crop helps because aphids carrying virus lose it if they probe a non-host before they reach a carrot crop.

- **Destroy all volunteer carrots and any finished crops -** Carrots that remain after the crop has been harvested may be infected with CarVY and act as a source for its spread to newly sown carrot crops. Crops that are finished or abandoned should immediately be ploughed in well below the soil surface.

- **Monitor aphid numbers, manipulate the sowing date so that young carrot seedlings are not present during peak aphid periods and protect the crop with insecticides while the crop is young –** It is important to monitor the area for aphid numbers by trapping. Try to avoid planting during peak aphid population times because if young seedlings (up to 6 weeks old) are infected with CarVY they develop severe root symptoms (Fig. 3). Peak aphid population times occur for short periods in spring and autumn. Chemical control of aphids in carrot field trials is being researched and preliminary results have shown a reduction in virus spread with fortnightly applications of one 'new chemistry' insecticide.

- **Introduce a carrot-free period -** Sequential plantings of carrots all year round are often associated with CarVY outbreaks, so this type of planting should be avoided. Having a fallow period after harvest will greatly reduce the likelihood of an epidemic. If a non-host crop is grown in rotation, the area can still be productive while removing the source of CarVY infection.