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Management of celery mosaic virus

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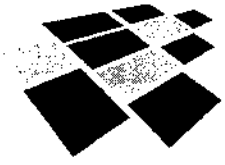

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Management of celery mosaic virus

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HRDC Project VG97103 - Management of celery mosaic virus

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VICTORIAN CELERY GROWERS ASSOCIATION

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MEDIA SUMMARY

The aim of this study was to investigate the outbreak of *Celery mosaic virus* (CeMV) in celery and other related crops in Australia. Our main purpose was to gain a better understanding of what viruses (if there were more than one) were infecting our Apiaceous crops, what their host ranges were and how widespread the virus was. Specifically we sought to determine what were the effects of virus on celery and carrots, and to assess alternative management strategies.

This study revealed that CeMV was indeed prevalent in all celery growing districts in Australia. It had severe affects on celery quality and production.

Three distinctly different but closely related viruses were found in the Apiaceous crops: *Celery mosaic virus* (CeMV), *Carrot virus Y* (CVY) and *Apium virus Y*. Our research shows that the virus (CVY) found in carrots does affect carrot production but this is dependent on variety. Preliminary investigations showed that virus did not affect postharvest performance.

As part of a total management system for CeMV in celery, petroleum oil sprays and plastic reflective mulches were trialed. Petroleum oils prays interfere with virus transmission and plastic reflective mulches modify aphid behaviour. Both showed great promise for industry. The petroleum oil sprays helped delay infection of CeMV and the plastic reflective mulches helped reduce the number of aphids (the vectors of CeMV) landing in celery crops.

Recommendations to industry to control CeMV are:

1. Plant tolerant varieties; note that no resistant varieties are known
2. Plant healthy seedlings
3. Plant seedlings as far away as possible from mature crops
4. Plant celery seed beds as far away as possible from celery crops
5. Control wild fennel and feral carrot on the farm
6. Plough in old crops and crop debris as soon as possible
7. Consider a break in production - US studies recommend at least 2-3 months.

Six of the seven recommendations can be immediately applied by industry. Industry must make some difficult decisions on whether or not they will take a break in production and this must be organised within grower groups in the same region.

TECHNICAL SUMMARY

The aim of this study was to investigate the outbreak of *Celery mosaic virus* (CeMV) in celery and other related crops in Australia. CeMV was causing major crop losses in Australia and growers had serious concerns about the quality of their crops and whether production would still remain viable.

The objective of the study investigating CeMV in Australia were to:

- determine what viruses are infecting carrots, celery and related crops
- determine the incidence of *Celery mosaic virus* (CeMV) in the major carrot (*Daucus carota*) and celery (*Apium graveolens*) growing districts in Australia
- determine the nature of spread of the virus
- determine the effect of the virus found in carrots on carrot production, harvest performance and storage
- evaluate petroleum oils sprays (DC-tron plus) and coloured reflective mulches to include into a management strategy in celery and carrots.

Two new potyviruses closely related to *Celery mosaic virus* (CeMV) have been found in the Apiaceae growing in Australia: *Apium virus Y* (APY) and *Carrot virus Y* (CVY) (Part 1). Although closely related to CeMV, they do not appear to readily move between plant species in the field. CVY and CeMV are prevalent in Australia's major carrot and celery growing areas respectively.

The spread of CeMV in celery is linked to aphid pressure. High levels of CeMV in the field correspond with high aphid numbers in Spring and Autumn.

In carrots, virus can reduce yield (measured as weight), carrot length and carrot collar width, but it is dependent on variety. However, virus had no effect on storage quality. The five varieties assessed were: *Senior*, *Leonore*, *Nantes*, *Steffano* and Red Brigade.

Two alternative control strategies to help reduce the impact of CeMV were tested: petroleum spray oils and coloured reflective mulches. Both showed great promise. The petroleum spray oil used in the trial delayed CeMV infection in the field and reduced CeMV infection overall. Plastic reflective mulches were also effective in deterring aphids for landing in celery crops. Silver mulch was more effective than white which in turn was better than bare soil (Part 5).

Recommendations to industry to control CeMV in various Apiaceous crops are as follows:

1. Plant healthy celery seedlings in the field
2. Plant tolerant varieties; note that no resistant varieties are known
3. Plant new crops as far away as possible from mature crops
4. Plant celery seed beds as far away as possible from celery crops
5. Control wild fennel and feral carrot on the farm
6. Plough in old crops and crop debris as soon as possible
7. Take a break in production - US research suggests at least 2-3 months.

Introduction: *Celery mosaic virus* - a review of the biology and management

The disease overseas

Outbreaks of *Celery mosaic virus* (CeMV), classified as a potyvirus, have occurred in most celery (*Apium graveolens* L.) growing regions around the world (Chod 1984; Pemberton & Frost 1986). A number of different strains of CeMV are known to occur in nature, however the host range of these strains can vary but are restricted to plants belonging to the Apiaceae.

The disease in Australia

CeMV was first reported in the South Australian celery growing district in 1985 (Alberts *et al.* 1989). Since then it has spread to all the celery growing districts in Australia (Quarterly newsletters Appendix I). The symptoms of CeMV in celery include distinct mosaic patterns on the leaves, exaggerated rosette growth habit with varying degrees of leaf distortion and stunting (Alberts *et al.* 1989; Traicevski *et al.* 1999). In Australia, many varieties of celery that are infected early do not produce a saleable crop. Some celery varieties have some tolerance to CeMV under Australian conditions (Traicevski *et al.* 1999).

CeMV has been epidemic in Victorian celery crops for the since 1997 and for the first time was detected in carrots (*Daucus carota*) (Traicevski *et al.* 1999). CeMV has been reported to naturally infect carrots in Europe (Brandes & Luisoni 1966; Chod 1984) and North America (Millbrath 1948; Kemp & Frowd 1975). Carrot growers in Australia expressed concern as to whether similar losses could occur in carrots as has occurred in celery. In Australia, carrot symptoms range from mild mosaic patterns on the leaves, feathery appearance of the leaves to a reddening on the leaf tips. Overseas the natural host range of CeMV is limited to Apiaceae family but in Australia it is unknown.

Management strategies used overseas

Control strategies on celery crops for CeMV in the USA are based on a two to three month celery-free period (Brunt *et al.* 1997; Shepard & Grogan 1971). In cases where the strain of CeMV is known to infect other commercial crops, the celery-free period may require expansion to include carrot, parsley and coriander crops. In the UK, the CeMV strain also infects local weed populations and in this instance a celery-free period is less effective (Pemberton & Frost 1986). In both instances the importance of virus-free seedlings is paramount.

Epidemiological studies have identified infected seedlings transplanted to the field as potential principle sites of infection from which vectors can transmit viruses of the Potyviridae to susceptible healthy plants (Shukla *et al.* 1994). The elimination of principal sites of infection is fundamental to minimising virus spread. Other primary sites of infection include infected weeds, volunteers, biennials and perennial crops.

What we need to know in Australia

In order to develop control strategies for CeMV, knowledge of the particular CeMV strain and its natural host range is required. In addition, an understanding of the epidemiology of CeMV must also be acquired. The aims of this study were to investigate the outbreak of

viruses that affect the Apiaceae, in order to develop control strategies for the affected industries. The results of the study are presented in five distinct parts:

Part 1 reports on the viruses that are found in the Australian Apiaceae, including weed, crop and native flora species. The experimental host range of these strains is also discussed.

Part 2 reports on the surveys that were undertaken to determine the incidence of *Celery mosaic virus* and related viruses in the major carrot and celery growing districts in Australia.

Part 3 reports on the effect of virus on carrot production, post harvest performance and storage.

Part 4 reports on the epidemiology of *Celery mosaic virus*.

Part 5 reports on the use of alternative control strategies for CeMV using petroleum oil sprays and reflective mulches.

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Part 1. Viruses in the Australian Apiaceae

Introduction

Background

A number of different strains of CeMV are found to occur in nature. The host range of these strains can vary but are restricted to plants belonging to the Apiaceae family. Here we set out to ascertain which strains we had in Australia (if we had more than one) and what crops, other than celery these viruses were affecting. It is important to understand what viruses (if more than one) we are dealing with, where they are located, and their host range because this determines the management strategy adopted.

Management

Control strategies in the USA for CeMV are based on a celery-free period (Brunt *et al.* 1997). In cases where the strain of CeMV is known to infect other commercial crops, the celery-free period may require expansion to include carrot, parsley and coriander crops. In the UK, the CeMV strain also infects local weed populations and in this instance a celery-free period is less effective (Pemberton & Frost 1986). Consequently, knowledge of the particular CeMV strain is required to develop control strategies as well to understand how these viruses are transmitted. Here we report on the incidence and variability of the viruses found in the Australian Apiaceae. We also report on the mechanical transmission trials done to obtain preliminary information on the experimental host ranges in order to help us understand the potential host ranges of the virus in the growing regions.

Celery mosaic virus (CeMV)

CeMV, also known as *Western celery mosaic virus* and *Crinkle leaf virus*, belongs to the family Potyviridae. The potyviridae is the largest of the 47 plant virus groups (Shukla *et al.* 1994). The group is characterised partly by the ability of aphids to transmit the virus in a non-persistent manner. Some aphid species mainly due to their fecundity, polyphagy and mobility, are often responsible for high virus incidence in crops (especially those from the genera *Aphis*, *Myzus* and *Macrosiphum*), even though they may not be efficient at transmitting the virus (Murant *et al.* 1988). The transmission efficiency of different aphid species, and of different populations or races of individuals species can differ substantially, and can be affected by environmental conditions (Goodell & Hampton 1983; Castle et al. 1992; Fereres *et al.* 1992). Although viruliferous aphids characteristically spread potyviruses over relatively short distances, the aphids can be carried under unusual meteorological conditions for many kilometres from the primary source of infection. Potyviruses also have very restricted natural and experimental host ranges, which are often confined to a few species within one genus or closely related genera (Shulka *et al.* 1994).

Outbreaks of CeMV have occurred in most celery (*Apium graveolens* L.) growing regions in the world (Götte 1957). In Australia, CeMV has been epidemic in Victorian celery crops for the past three years and recently as a result of this project, a new potyvirus related to CeMV has been found. The virus was found in carrots for the first time in Australia (Traicevski *et al.* 1999).

Materials & Methods

Virus sequencing
Samples from wild and cultivated Apiaceae with symptoms typical of a potyvirus infection were collected from around Australia as part of the nation-wide survey (the survey is reported on, in Part 2 of this report). Plant samples collected around Australia were first screened using enzyme-linked immunosorbent assay (ELISA) (Clark 1981) (using a celery specific DSMZ™ kit and protocols described by the manufacturer).

Total nucleic acid was extracted from each isolate and a specific fragment was amplified using potyvirus specific degenerate primers in a RT-PCR reaction. These fragments were then sequenced and compared against the international databases (http://biology.anu.edu.au/Groups/MES/vide/descr186.htm) using the BLAST program before a neighbour-joining tree was calculated.

Reference isolates of carrot and celery potyviruses were obtained from Brazil (*Celery yellow mosaic virus* and CeMV), The Netherlands (CeMV) and the USA (CeMV).

Mechanical transmissions
Mechanical transmission tests to determine the experimental host range for both the virus found in carrots and the virus found in celery were made by grinding leaves with symptoms in a mortar in 0.01M potassium phosphate buffer pH 7.0. The sap was applied to leaves dusted with carborundum. The experimental plants used were: celery (*Apium graveolens*), sweet fennel (*Foeniculum vulgare*), coriander (*Coriandrum sativum*), parsnip (*Pastinace sativa*), lovage (*Luvisticum officnale*), carrot (*Daucus carota*), dill (*Anethum graveolens*), parsley (*Petroselinum crispum*), *chervil (Anthriscus cerefolium*), Queen Anne's lace (*Daucus carota*), celeriac (*Apium graveolens racaceum*), caraway (*Carum carvi*), and anise (*Pimpinella anisum*)). Plants of each species were challenged with the viruses isolated from carrot and from celery and then assessed for virus infection by ELISA using DSMZ™ antisera.

Results

Virus sequencing
Sequence analysis revealed three different, but closely related, potyviruses; CeMV, and two new potyviruses tentatively named *Apium virus Y* (AVY) and *Carrot virus Y* (CVY) (Figure 1.1). Phylogenetic analysis revealed that CeMV, CVY and AVY were most closely related to each other and that plum pox virus was their closest relative (Figure 1.2). Three other potyviruses were detected, one in pennywort (*Hydrocotyle* sp.) and one in parsley (*Petroselinum crispum*), both as yet undescribed, and a strain of clover yellow vein virus in *Ammi magus* (Moran *et al.* 1999).

The natural host range of these two new viruses seems to be very limited, but this is further investigated in Part 2 of this report. AVY was only found in *Apium prostratum* and CVY was only detected in carrots.

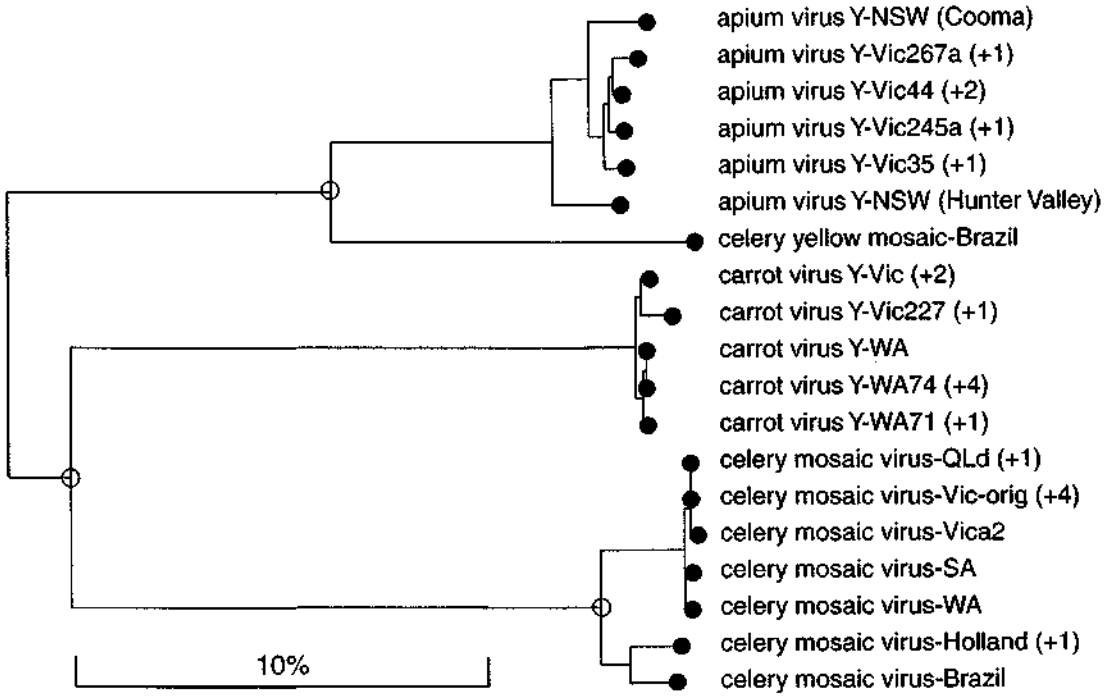

Figure 1.1. Phylogenetic neighbour-joining tree of the nucleotide sequences from the NIB-CP region (650kb) of the viruses found in the Australian Apiaceae.

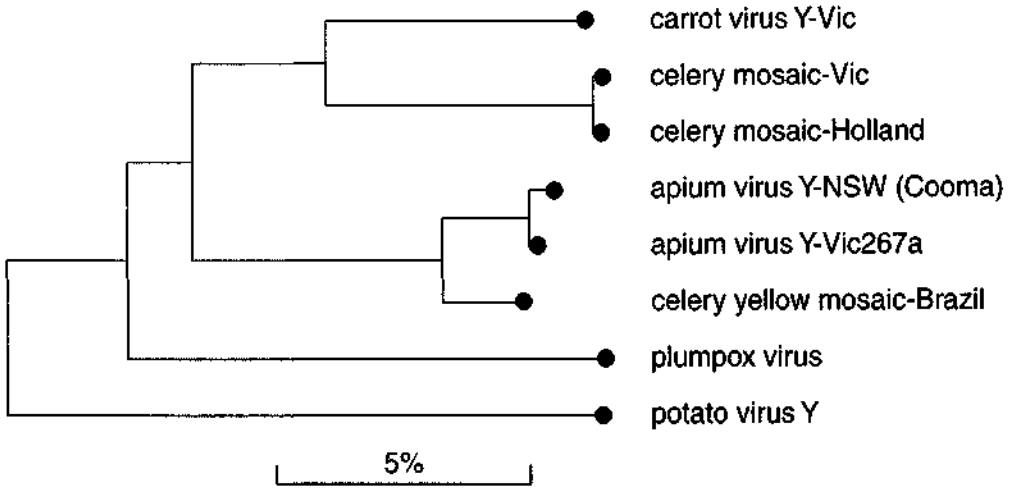

Figure 1.2 Phylogenetic neighbour-joining tree of the nucleotide sequence from the NIB-CP region of the viruses found in the Australian Apiaceae and other closely related potyviruses.

Mechanical transmissions

The results from the mechanical transmission of the viruses found in carrots and celery on other Apiaceae species are presented in Table 1.1. Although the number of plants successfully infected by mechanical inoculation are small, the virus isolate from celery was more readily transmitted to the other host plants and seem to have a greater experimental host range than the carrot isolate (Table 1.1).

Table 1.1. Experimental host ranges within the Apiaceae of viruses isolated from carrots and celery. The numbers in the brackets represent the number of plants that were successfully inoculated with CeMV. This was verified by testing with ELISA using DSMZ™ antisera.

Host plant	**Common name**	**Sample size**	**Carrot isolate**	**Celery isolate**
Apium graveolens L.	Celery	26	-	+ (1)
Foeniculum vulgare (Miller)	Fennel	26	+ (3)	+ (2)
Coriandrum sativum L.	Coriander	26	+ (4)	+ (4)
Pastinaca sativa L.	Parsnip	26	-	+ (2)
Levisticum officinale L.	Lovage	26	-	+ (1)
Daucus carota L.	Carrot	26	+ (2)	+ (1)
Anethum graveolens L.	Dill	26	-	+ (2)
Petroselinum crispum (Mill.)	Parsley	26	-	+ (1)
Anthriscus cerefolium L.	Chervil	20	-	-
Daucus carota L. var.?	Queen Anne's Lace	22	+ (2)	+ (3)
Apium graveolens L. cv. *racaceum*	Celeriac	22	-	+ (1)
Carum carvi L.	Caraway	22	-	+ (1)
Pimpinella anisum L.	Anise	22	-	+ (3)

Discussion

The results from this study reveal that two new potyviruses were found in the Australian Apiaceae: *Apium virus Y* (APY) and *Carrot virus Y* (CVY). This is the first report of CVY and APY world-wide. These are two new potyviruses that have been described.

These two new viruses are closely related to CeMV but are different. Our results suggest that in nature, the potyviruses found in Australia do not readily move between Apiaceous plant species. There is, however, some evidence that CeMV naturally infects wild carrots and poison hemlock (*Conium maculatum*) in Australia. In the early 1980's researchers from West Australia reported CeMV to be found naturally in 9% of the wild carrots and 21% of poison hemlock (Howell & Mink 1981). These findings were reported prior to the sequencing data now available and may imply that the CeMV found in carrots and poison hemlock may have indeed been CVY. However, CeMV has not been found to naturally infect high numbers of wild Apiaceae in Victoria (Part 2). Even if CeMV is present in wild Apiaceae, a celery free period may still be the best option for growers for effective control of CeMV in celery crops in Australia. The cycle of the virus between the weeds and crop could be broken with a break in production. A break in production may result in a decrease of virus in local weed virus reservoirs, which in turn would result in a reduction of virus in crops.

The results from the mechanical transmission trials suggest that the host range of the celery isolate is greater than that of the carrot isolate. CeMV from celery has been previously reported to mechanically infect a number of host plant species belonging to the Apiaceae. These include, dill, chervil, celery, celeriac, caraway, poison hemlock,

coriander, carrot, parsnip, parsley, anise and lovage (Alberts *et al.* 1989; Cadilac *et al.* 1972; Frowd & Tomlinson 1972: Fry & Proctor 1968; Gracia & Feldman 1977; Howell & Mink 1981; Kemp & Frowd 1975; Kitajima & Costa 1978; *Marchouxn et al.* 1969; Oliveira & Kitajima 1981; Pemberton & Frost 1974; Purcifull & Shepard 1967; Rubies-Autonell & Bellardi 1996; Severin & Frietag 1938; Shepard & Grogan 1971; Sutabutra & Campbell 1971; Walkey *et al.* 1970). The results presented here show that CVY has an experimental host-range that includes fennel, coriander and Queen Anne's Lace. The host range of APY was not determined.

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Part 2. Survey's of CeMV and CeMV-like viruses in celery and related Apiaceous crops in Australia.

Introduction

Distribution of CeMV in Australia and overseas

The history of CeMV in Australia extends back to 1984, when CeMV was first described in celery crops in Western Australia (McLean & Price 1984). In 1985, an outbreak of CeMV was reported in South Australia, and by 1987, had almost decimated the industry there (Alberts *et al.* 1989). CeMV has now been detected and identified as a problem in all celery growing districts of Australia.

Celery mosaic virus (CeMV) is distributed worldwide. It has been recorded in Argentina (Gracia & Feldman 1977), Canada (Kemp & Frowd 1975), the former Czechoslovakia (Chod 1984), France (Marchoux et al. 1969), Germany (Brandes & Luisoni 1966), Italy (Avgelis & Quacquarelli 1972), Japan (Iwaki & Komuro 1970), New Zealand (Fry & Proctor 1968), The Netherlands (Van-Dijk & Bos 1989), the UK (Walkey *et al.* 1970) and the USA (Purcifull & Shepard 1967).

CeMV, like many other potyviruses, has a very restricted natural and experimental host range (Pemberton & Frost 1986). The Chenopodiaceae and the Apiaceae are the two known susceptible plant families of CeMV (Severin & Freitag 1938; Sutabutra & Campbell 1971; Frowd & Tomlinson 1972; Walkey *et al.* 1970; Wolf 1969; Wolf & Schmelzer 1972).

Carrot virus Y (CVY) is a newly described potyvirus that naturally infects carrot. CVY was first identified and described through sequence analysis as a closely related potyvirus very similar to CeMV (see Part 1 of this report).

Here we present results from an Australian-wide survey of CeMV and CVY in celery, carrot and other Apiaceous crops. The major objective of this survey was to determine the incidence of CeMV and CVY Australia-wide.

Results are also presented here from a more intensive survey of wild Apiaceae taxa in Victoria. This survey was undertaken to assess the distribution of Apiaceous species in the major celery growing areas and to gauge the extent of infection by CeMV and CeMV-like viruses. Such information would aid in the identification of wild virus reservoirs and in the formulation of virus control programs.

Materials and Methods

Surveys of carrot and celery crops Australia-wide

Surveys were conducted in Queensland, New South Wales, Tasmania, Victoria, and Western Australia. Leaf samples were taken at random from carrot and celery crops and tested using a CeMV specific ELISA kit as directed by the manufacturer (DSMZ™, Germany) or a general 'Potyvirus Group' kits (Agdia Corp., USA). Representative positive samples were further analysed by sequencing as reported in Part 1.

Queensland

Three surveys of the major celery growing areas of Queensland for CeMV were conducted in 1997, 1998 and 1999. Identification of CeMV was based on electron microscopy and ELISA using the German DSMZ™ ELISA kits.

In August 1997, surveys were conducted in the Lockyer Valley where, 3000 celery plants were visually inspected for symptoms at three properties. In November in the Granite Belt area of South Queensland, 5000 celery plants were visually inspected on the largest celery growing properties.

In April 1998, a second survey was conducted in the same celery growing districts.

The third survey was done in April 1999 on three celery properties in the Darling Downs area near Toowoomba in Queensland and in five carrot growing properties in the Fassifern-Kalbar areas.

New South Wales

All samples collected were tested for CeMV in grouped samples of 20 leaves using both the DSMZ and Agdia ELISA kits to test for CeMV. Carrots were the main plant species sampled in NSW, as celery is not extensively grown in this state. The surveys were conducted in the state's two main Apiaceae production areas - the NSW Riverina and the Sydney Basin. Five targeted surveys were conducted between May 1999 and May 2000 on 22 properties. The surveys targeted plants with possible virus symptoms - a total of 124 samples were tested for virus. Random samples were also collected without visible symptoms.

Parsley and other Apiaceae herbs and vegetable seedlings were also surveyed on 12 market gardens, and nurseries in the Sydney Basin. The herb garden and selected Apiaceae specimens were also sampled form the Royal Botanic Gardens, Sydney. Samples were tested as above but were also examined under an electron microscope.

ACT

Conium maculatum (poison hemlock) samples were collected with distorted leaves and mosaic patterns on the leaves in Canberra by Anne Mackenzie (ANU). They were tested by the research group in Melbourne using ELISA and the CeMV specific DSMZ™ antisera and the electron microscope.

Tasmania

Carrots

Carrots were the main crop surveyed in Tasmania as Tasmania has no celery production areas. Surveys for CeMV were carried out between February-April 1999. Twenty-six carrot crops and one crop each of parsnip and celery in Northwest Tasmania were surveyed. 100 leaf samples were randomly selected from each crop and tested by ELISA using the DSMZ™ Germany antiserum. They were tested with ELISA in grouped samples of 10 leaves.

Victoria

Surveys of celery and other cultivated Apiaceae

Celery and herb crops in the celery growing regions of Victoria (Clyde-Cranbourne and Koo-Wee-Rup and Peninsula districts) were surveyed in April-June 1998. Celery and other Apiaceae crops were visually inspected for the presence of mosaic symptoms that

were characteristic of CeMV. The assessment was made by walking though the crop and counting the number of plants with virus-like symptoms and representative samples were taken back to the laboratory to test for CeMV by ELISA using DSMZ™ antisera. Nine celery farms and three herb farms were inspected.

Carrots
In June 1998, three carrot properties on the Mornington Peninsula and Clyde-Cranbourne areas were inspected visually for symptoms of CeMV. Samples from each property with classic mosaic, mottling and stunted growth were collected and tested individually with the antiserum from DSMZ™, for CeMV.

A small survey of the two properties in major carrot growing area in Swan Hill and surrounding districts, was also done in August 1998. Seven carrot samples with symptoms were collected and tested individually using ELISA with the antiserum from DSMZ™, Germany, for CeMV.

Surveys of non-cultivated Apiaceae
In December 1998 three areas on the western edge of the Gippsland Plain were surveyed as part of the Mornington Peninsula Apiaceae survey. The areas were of 10km radius, each centred around the celery or carrot growing districts nearby.

The surveys were undertaken by road, with drive-by checking for conspicuous species (*Conium maculatum, Daucus carota, Foeniculum vulgare, Trachymene anisocarpa*) and on-foot surveys through roadside reserves, reserved land, the coastal strip and remnant bushland for the less conspicuous species (*Apium prostratum, Berula erecta, Centella cordifolia and Hydrocotyle* sp.).

Whenever an individual or colony of a target species was found a herbarium voucher and a sample for testing was collected and the GPS location recorded. Where multiple plants were present at a collection locality a single leaf from up to six individuals was taken for virus analysis. Abundant species (ie. those present continuously or very commonly along roadsides) were collected at 1 – 1.5km intervals.

In Victoria, a voucher collection was made from one plant from each locality. A subset of vouchers have been added to the main collection at the National Herbarium of Victoria for permanent retention, and the remainder placed in storage for the duration of this project.

West Australia
Celery and related Apiaceous crops
A total of 11 celery growing properties were visited in late 1997. 100 random leaf samples were collected from each crop and plants with suspect leaf symptoms were collected and tested. A total of 3,300 samples were collected. In early November 1998, one celery farm was inspected around the Perth area. Levels of CeMV infection were estimated by eye and later confirmed with ELISA. Other Apiaceous crops including coriander (*Coriandrum sativum*) and parsley (*Petroselinum crispum*) grown on this one property were tested by ELISA for CeMV, using a general potyvirus detection kit from Agdia.

800 celery seedlings from a celery nursery were also tested for CeMV in 1997 and 1998.

Carrots
Three major carrot growing properties were surveyed for CeMV and CVY in late November 1998. 4000 random leaf tips from different carrot plants were collected and tested in batched samples of 10 leaves for the presence of CeMV using ELISA and a general potyvirus detection kit.

South Australia
Throughout 1999 and 2000 random leaf samples were taken from four celery and 14 carrot crops in the North Adelaide Plains, Riverland and South-East regions of South Australia and tested for CeMV by ELISA.

A general overview of the crops surveyed for virus, each year, in each state of Australia is presented below (Table 2.1).

Table 2.1. General overview of the crops surveyed in each state of Australia. Those marked with an × represent the crops surveyed each year. Those left blank were not surveyed.

	1997	1998	1999	2000
Queensland				
Celery	×	×	×	
Carrots			×	
New South Wales				
Celery				
Carrots			×	×
Other Apiaceae			×	
ACT				
Other Apiaceae			×	
Tasmania				
Carrots			×	
South Australia				
Celery			×	
Carrots			×	
West Australia				
Celery	×	×	×	
Carrots		×	×	
Victoria				
Celery	×	×	×	
Carrots		×	×	×
Other Apiaceae		×		

Results

Queensland

No CeMV was found in any of the major celery growing properties surveyed in the Lockyer Valley and Granite Belt in 1997, 1998 and 1999. However, CeMV was discovered in celery crops in early 1999 in the Eastern Darling Downs. Estimated levels of infection in the celery crops, calculated from the ELISA results ranged from 15-57%.

The survey in 1999 found no virus in celery crops in the Locker Valley and in the Glenore Grove area. Five properties in the Fassifern-Kalbar with carrot crops were also survey but no virus was found. However, an historic sample of a potyvirus found in the Fassifern area in the 1980's was recently identified as carrot virus Y (Part 1).

New South Wales

Carrot Survey

No CeMV or other potyviruses were found in carrot crops in the Camden district in the spring of 1999. However, the four surveys in the Riverina area of seven properties in spring and autumn of 1999/2000 revealed that potyviruses were present in NSW carrot crops. Just over one-third of the samples tested positive for Potyvirus (Appendix V).

Three samples of swamp pennywort (*Centella asiatica*) were found to contain a potyvirus using the electron microscope. However, these samples failed to react with the DSMZ™ CeMV antisera and the general potyvirus antisera from Agdia. Sequence analysis from the ANU team suggested that the virus was possibly a strain of Clover yellow vein virus (another potyvirus) and not CeMV or CVY.

Parsley, coriander and dill samples form six market gardens in the Sydney Basin all contained potyvirus. However, all these samples failed to react with both the German and Agdia antisera. More than 50% of the coriander beds were infected although parsley had a lower infection rate at less than 10%.

ACT

Potyvirus particles were observed in the *Conium maculatum* (poison hemlock) samples collected in Canberra but when tested with the CeMV antisera from Germany there was no reaction. CeMV was not present in the poison hemlock. Upon genetic sequencing, isolates from the wild poison hemlock revealed that the potyvirus observed under the electron microscope was indeed a potyvirus, and one that was closely related to CeMV known as *Apium virus Y* (Part 1).

Tasmania

No CeMV nor any other potyvirus was found in the celery or parsnip crops surveyed. There was also no CeMV or any other potyvirus found in any of the 26 carrot crops surveyed in 1999. Neither CeMV nor any CeMV-related viruses seem to be present in Tasmania (Appendix III - Quarterly Newsletter No. 5).

South Australia

No CeMV symptoms were observed in the four celery and 14 carrot crops surveyed and no CeMV was detected by the ELISA tests.

Western Australia

Celery

In 1997, one celery grower was visited and no CeMV was detected. In 1998, eleven different celery plantings were surveyed on the same property, ten of which were found to be infected with CeMV. Infection levels ranged from 33% in Cv. Yarralong, 37% in cv. Excelsior and 57% in cv. Tendercrisp all estimated by eye.

Celery seedlings were also tested for CeMV in 1997, and 1998. No CeMV was found in these seedlings. CeMV was also not found in the coriander and parsley surveyed.

In late 1999, CeMV was confirmed in celery using ELISA on three properties north of Perth on the Swan coastal Plain. Incidence was high on two properties (between 40-90%) and substantial crop losses were reported.

Carrots

In late 1998, three major carrot growing properties were surveyed for carrot virus. Of the 4000 random leaf tips from different carrot plants collected and tested only one tested positive to the general potyvirus kit (this was later confirmed to be CVY). This property had CVY with levels up to 68% in cvs. Steffano, Paris and Koya. Two more carrot growing properties in the Swan Coastal Plain in late 1999, were identified with high levels of CVY.

Victoria

Celery

In 1997, two major celery growing properties were known to contain CeMV. In 1998, a major survey of all the celery growing districts was conducted and all virus levels were estimated by walking through a representative area of the crop and counting plants showing visual symptoms. In all the plantings surveyed, CeMV was detected in all but one celery growing property in 1998. CeMV infection levels varied in celery from 10% to greater than 90%.

Other Apiaceae

In 1998, CeMV was found for the first time in coriander and parsley in Victoria. Of the three herb growing properties surveyed only one was found to have symptoms of CeMV in their coriander and parsley and was later confirmed with ELISA and DSMZ™ CeMV specific antisera.

Carrots

Of the carrot properties surveyed in 1998, all three properties had carrots with classic virus symptoms (mosaic patterns on the leaves, mottling and stunted growth). These carrots all reacted to the CeMV German antisera, but later were identified as CVY (Part 1).

In August 1999 of three properties surveyed, two were found to have potyvirus in their carrot crops. This was later identified as CVY (Part 1). A survey of the same properties in early 2000, revealed that all of the properties had CVY.

Summary of CeMV and related viruses found in Victoria in an intensive survey undertaken in November 1999

The southernmost search area covered a large portion of the lower Mornington Peninsula, centring on Boneo, and taking in Arthurs Seat State Park, Rosebud, Rye, Cape Schanck and much of the Mornington Peninsula National Park. This area incorporated a wide range of environments, including primary dune and ocean cliff vegetation, coast tea tree woodland, tall Eucalyptus forest, Allocasuarina woodland and weedy agricultural land. Nine species of Apiaceae were found in this area and the details can be found in Appendix III.

A summary of the incidence of CeMV related viruses in native plants and weeds in the three areas surveyed in Victoria are presented in Table 2.2 with the percentage of samples that tested positive to CeMV using ELISA and DSMZ ™ antisera.

Table 2.2. Incidence of CeMV related viruses in native plants and weeds in Victoria in November, 1999.

Plant species	Mornington Peninsula		Cranbourne/Clyde		Cora Lynn	
	Number of samples	Percentage positive	Number of samples	Percentage positive	Number of samples	Percentage positive
Native species						
Flannel flower	12	0%	1	100%	-	-
Native celery	38	2.5%	-	-	-	-
Centella cordifolia	5	0%	3	33%	-	-
Pennywort	16	0%	1	0%	-	-
Trachymene anisocarpa	-	-	9	0%	-	-
Xanthosia hueglii	1	0	-	-	-	-
Weed species						
Poison hemlock	6	0%	1	0%	2	0%
Feral carrot	-	-	91	19%	96	55%
Wild fennel	10	20%	8	37%	4	100%

A summary of the viruses found in the native plants and weeds in Victoria is given in Table 2.3. The term 'feral carrot' refers to carrots (*Daucas carota*) that were growing wild along the roadside and on waste-land. These carrots were not volunteers in a crop. Full locality and latitude and longitude co-ordinate information from the isolates found in Victoria are available in Appendix IV. Maps showing the areas sampled are also in Appendix IV.

Table 2.3. The types of virus found in native plants and weeds.

Region	Plant type	Strain
Flinders	Native celery	Apium virus
Rye	Native celery	Apium virus
Clyde	Feral carrot	Carrot virus
Tooradin	Feral carrot	Carrot virus
Tooradin	Feral carrot	Celery virus
Cora Lynn	Feral carrot	Carrot virus

In order to give a clearer picture of Australia-wide state of viruses present in celery, carrots, and related crops a summary of the viruses found in the Apiaceae crops are shown below (Table 2.4).

APY was detected in *A. prostratum* (native celery) samples from Victoria and in *Conium maculatum* samples from Victoria, Australian Capital Territory and New South Wales. CVY was detected in carrot samples (cultivated and feral) from Victoria, West Australia and Queensland. CeMV was detected in isolates from celery samples from Victoria, South Australia, West Australia and Queensland and in one feral carrot sample from Victoria. The hosts and locations of the viruses are shown in table 2.4.

Table 2.4. Incidence of potyviruses in cultivated, native and weed Apiaceae in Australia.

Virus found	Host and Location
Celery mosaic virus	Celery (Qld, SA, Vic, WA), feral carrot (Vic)
Apium Virus Y	*Conium maculatum* (NSW, Vic) *Apium prostratum* (Vic)
Carrot Virus Y	Carrot (WA, Vic, Qld)
Clover yellow vein virus	*Ammi magus* (ACTU)
Unknown potyvirus	Parsley (Qld), pennywort (NSW)

Discussion

The results from the survey indicate that CeMV and CVY are prevalent in Australia's major carrot and celery growing areas. CeMV has now been recorded in all major celery growing districts Australia-wide and there are records of CVY in West Australia, Victoria and Queensland.

The more intense Apiaceae survey in Victoria indicates that the native Apiaceae pose no threat to the carrot or celery growers because no CeMV or CVY was detected using ELISA in the native Apiaceae, although a new closely related virus has been identified in the native celery (*Apium prostratum*) - *Apium virus Y* (APY).

Epidemiological studies have established that infected weeds, other crop species, volunteers and propagules of vegetatively-propagated species are often the primary foci of infection from which vectors transmit viruses of the Potyviridae to susceptible healthy plants (Shukla *et al.* 1994). Often these infection sources are either within or immediately adjacent to susceptible crops. Host plant reservoirs mean that aphids flying through an area nearby can acquire the virus whether it be CeMV or CVY from the weeds and transmit it to the carrot or celery crops. The elimination of primary foci of infection

(infected weeds, volunteers) is the major strategy to minimise virus spread. In this particular case, the biggest threat to the celery and carrot industries is the host plant reservoirs of poison hemlock, feral carrot and wild fennel. In Australia, poison hemlock is a noxious weed throughout most of the celery and carrot production areas. Poison hemlock is also considered a natural reservoir for CeMV in England (Pemberton & Frost 1974), Argentina (Gracia & Feldman 1977) and California (Sutabutra & Campbell 1971). To date, no CeMV has been isolated from poison hemlock in Australia.

As well as reducing weed reservoirs to reduce the incidence of CeMV and CeMV related viruses in carrots and celery, another option to growers is to take a break in production. This break in production may indeed reduce the cyclic effect of virus transferring itself from weeds to the crop and vice-versa. This break in production has been successful in South Australia and the USA.

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Part 3. The effect of virus on carrot production, postharvest performance and storage.

Introduction

Since the identification of the CeMV-like virus in carrots (see Part 1 and 2), most likely to be *Carrot virus Y* (CVY) growers had expressed concern that crop losses similar to those that have occurred in celery could occur in carrots. As a result, a preliminary trial was undertaken to determine the effect of the CeMV-like virus on carrot yield and post-harvest performance.

Materials and Methods

Carrot production

Five different carrot cultivars were monitored for virus in Victoria on the Mornington Peninsula. Using visual virus symptoms as an indicator for virus presence, 100 carrots with symptoms and 100 without symptoms were collected from each variety. These were then taken back to the laboratory and were then weighed and measured to assess the effect of the virus on carrot yield (measured as weight) (g), length (cm) and the circumference of the top of the collar (mm). The five cultivars used were: Senior, Leonore, Nantes, Steffano, and Red Brigade.

Carrot postharvest performance

Two carrot varieties Senior and Leonore were tagged in the field and each individual carrot was tested using ELISA as per manufacturers directions for CeMV to identify those with and without CeMV.

The carrots were hand harvested on the 17/05/99 and brought back to the research institute where the carrots were hand-washed before being transferred into a 0°C cool room. The carrots were stored in large plastic crates wrapped in a perforated plastic bag to maintain a high relative humidity. Carrot *var.* Senior with and without virus was stored for 6 weeks at 0°C and quality was assessed on 30/06/99. Carrot *var.* Leonore with and without virus was stored for 14 weeks at 0°C and quality assessed on the 24/08/99.

Visible signs of post-harvest disorders such as botrytis, sclerotinia, rhizoctonia rot, fusarium rot, rhizopus rot and bacterial soft rot were also monitored in the stored carrots.

Quality assessment after storage

Quality assessment parameters used to evaluate the carrot quality after storage are shown below (Table 3.1).

Table 3.1. Quality assessment parameters used to evaluate the carrot quality after storage.

Score	White blush	Root turgor
1	None	Fully turgid
2	Trace	Trace limpness
3	Slight	Slight limpness
4	Moderate	Moderate limpness
5	Severe	Severe limpness

The colour of the internal root (root cortex) 2cm from the shoulder of the carrot was measured using a Minolta CR200 Chroma meter using the white calibration tile (L=97.3, a = 0.49, b = 1.91). The a-value = green/red hue component and the b-value = yellow/blue hue component. The values were used to calculate the hue angle. Hue angle (h°) = arc tangent b/a where 0° = red, 90° = yellow, 180° = green, 270° = blue. Forty-six carrots from each treatment were used for colour determination after storage.

Results

Carrot production

Virus does have effect the yield of carrots but this is dependent on variety. The results for five different cultivars are summarised below (Table 3.2).

Table 3.2. The effect of the CeMV-like virus on carrot yield (g), length of the root (cm) and the circumference of the top of the collar (cm) for five carrot cultivars (N=100) with the results of a two-sample t-test. The effect of virus on forking with the results of a Chi-square test are also included. Bolded P-values are significantly different.

Cultivar	Yield (g) ± SEM	Length (cm) ± SEM	Collar width (cm) ± SEM	Forking (χ^2)
Senior	P>0.05	P>0.05	P>0.05	
Positive	140.9 ± 6.2	18.86 ± 0.39	42.41 ± 0.70	P>0.05
Negative	139.5 ± 7.7	18.67 ± 0.46	41.59 ± 0.76	
Leonore	**P=0.049**	P>0.05	**P=0.026**	
Positive	134.6 ± 8.4	20.65 ± 0.53	40.00 ± 0.95	P>0.05
Negative	153.8 ± 4.6	19.55 ± 0.25	42.00 ± 0.46	
Nantes	P>0.05	P>0.05	P>0.05	
Positive	134.6 ± 8.2	17.63 ± 0.54	43.24 ± 1.1	P>0.05
Negative	130.0 ± 8.0	16.77 ± 0.50	41.56 ± 0.91	
Steffano	**P<0.0001**	**P<0.001**	**P<0.0001**	
Positive	46.6 ± 2.8	12.60 ± 0.36	27.03 ± 0.80	P>0.05
Negative	69.9 ± 2.7	14.26 ± 0.32	32.53 ± 0.50	
Red Brigade	**P<0.001**	**P<0.001**	**P<0.0001**	
Positive	60.5 ± 3.9	16.13 ± 0.48	29.02 ± 0.81	P>0.05
Negative	77.2 ± 3.4	18.27 ± 0.35	32.90 ± 0.55	

Carrot postharvest performance

The results of virus on storage quality for two varieties of carrot: *Senior* and *Leonore* are summarised in Table 3.3 ± SEM (standard error of the mean). The carrot var. *Leonore* stored better than the var. Senior. After 6 weeks at 0°C the var. *Leonore* with or without virus was still in a saleable condition.

The results from the storage trial suggest that although the storage quality of *Var. Senior* and *Leonore* had significantly declined overall there was no difference in storage quality between carrots that were infected with virus and those that were not. Root cortex colour remained a healthy orange colour.

Table 3.3. The effect of storage on carrots varieties *Senior* after 6 weeks at O°C and *Leonore* after 14 weeks at O°C on limpness, white blush and root cortex colour ± SEM with and without virus.

Variety	Virus status	Limpness[1] ± SEM	White blush[2] ± SEM	Root cortex colour (Hue angle h°)[3] ± SEM
Senior	Positive	2.3 ± 0.1	3.0 ± 0.1	68.0 ±0.1
	Negative	2.8 ± 0.2	3.0 ± 0.1	71.7 ± 1.4
Leonore	Positive	2.8 ± 0.2	2.2 ± 0.2	65.5 ± 0.4
	Negative	2.3 ± 0.1	2.4 ± 0.1	65.0 ± 0.4

[1]. Root turgor: 1 = fully turgid, 2 = trace limpness, 3 = slight limpness, 4 = moderate limpness and 5 = severe limpness.
[2]. White blush: 1 = none, 2 = trace, 3 = slight, 4 = moderate and 5 = severe.
[3]. Root cortex colour: hue angle 0° = red and 90° = yellow. Mid-range values represent orange hues.

No visible signs of post-harvest disorders such as botrytis, sclerotinia, rhizoctonia rot, fusarium rot, rhizopus rot and bacterial soft rot were found in the stored carrots.

Discussion

Carrot production

These studies show that virus has an effect of yield, quality and storage but depends on cultivar. The virus has a detrimental affect on carrot yield, carrot length and carrot collar width. Some carrot cultivars infected with virus were lighter, shorter and smaller in the collar than those that had no virus.

Carrot postharvest performance

The results from this preliminary study did not show any adverse affects of the virus on storage quality. The results from our trial suggest that virus (most likely to be CVY) has no effect on storage capacity for the two varieties tested. Others varieties not examined here may be different.

Mature carrots that have been topped generally have a reasonably long postharvest life. Carrots can normally be stored for 4-5 months under optimum storage conditions of 0°C with 98% to 100% relative humidity when they have been promptly precooled (Thompson 1996; Anon. 1986). Carrots of the var. Senior and Leonore were stored for a maximum of 3 months at 0°C which is to be expected for mature carrots that have not been pre-cooled prior to storage. The carrot var. Leonore stored better than var. Senior: after 6 weeks at 0°C the var. Leonore with and without virus was still in a saleable condition.

The storage periods of 1.5 months and 3 months for *var.* Senior and Leonore under optimal storage conditions was to be expected for carrots that were not hydro-cooled. It is likely that without proper post-harvest handling ie.hydro-cooling, the carrots may have suffered respiratory heating and moisture loss which all would have a detrimental effect on the shelf life of the carrots. Hydro-cooling provides some benefit to the carrots in the

form of rehydration of slightly wilted roots, as well as reducing decay problems and sprouting (Rubatzky *et al.* 1999).

The most likely disorders at postharvest are wilting, bitterness, and other diseases such as grey mould rot (Botrytis), water soft rot (*Sclerotinia*), Rhizoctonia rot, Fusarium rot, Rhizopus rot and bacterial soft rot however, these were not found in this trial.

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Part 4. Epidemiology of *Celery mosaic virus*.

Introduction

The spread of an insect-transmitted plant virus like CeMV from one plant to another requires three basic components: the host plant, the insect vector and the virus itself. In trying to understand the way a virus spreads one must try to ascertain where the virus is, (what plant it is on) what the vectors are doing, and where the vectors are moving the virus. All this is imperative in determining control approaches.

Here we present results on the incidence of CeMV in celery seedlings and celery crops over time together with aphid numbers in the field. The results from this study are aimed to help determine the pattern of spread of the virus and the aphid species that are present in celery crops to develop management strategies.

Materials and Methods

Incidence of virus in celery seedlings

One of our main grower collaborators who produced his own seedlings provided us with 500 random celery leaf samples every week just prior to him planting the same batch out in the field. The samples collected were tested for the presence of CeMV over the first year of the project. Seedlings were tested in batched samples (N=15) for CeMV using enzyme-linked immunosorbent assay (ELISA) and the German, DSMZ™ ELISA kits. Estimated levels of infection were calculated using the formula given by Burrows (1987).

Incidence of virus in the field

Each week 250 leaves were collected from each crop (this crop was derived from the already surveyed seedlings) and tested for CeMV using ELISA in batches of either 10 or 5 depending on the virus levels observed in the previous week. Estimated levels of infection were calculated using the formula given by Burrows (1987). This was done in conjunction with the regular testing of virus incidence in the seedlings to determine if indeed there was a correlation between virus levels in the field with virus levels in the seedlings.

Aphid numbers

Yellow water pan traps were established in the celery crop (where CeMV infection levels in the seedlings were known) to monitor aphid pressure through the growing season of that crop. Yellow water pan traps are a standard method to monitor aphids (Upton 1991). The yellow water pan traps had an overflow hole drilled near to the rim of the container and covered with wire gauze so that no insects could escape. Each trap was 38 (cm) in length and 30 (cm) in width and 15 (cm) deep. All traps were filled to their overflow with water containing sprinkles of detergent (Pyroneg Powder™) and copper sulphate ($CuSo_4$). Detergent was added to reduce the surface tension of the water so that the arriving insects would sink. Copper sulphate was used to prevent any algae build up in the traps. The water in these traps was changed weekly.

The number of winged aphids trapped were collected weekly and taken back to the laboratory for identification to species level. Only winged aphids were counted as these are the migratory aphids and have the potential to spread the virus over long distances (Dixon 1985).

An estimate of aphid pressure at any particular time on a crop was described here as an aphid index. The aphid index is an estimate of aphid numbers at a particular time in the crop based on the mean number of aphids four weeks after the celery seedlings were transplanted into the field.

Results

Seedling and field infection

The estimated level of infection (using the formula by Burrows 1987) of CeMV in the seedlings over the 52 week testing period varied between 0-6.5% (Figure 3.1). Only two batches of seedlings had infection levels higher than 3.1% and only one batch had an infection level higher that 6%.

Estimated levels of CeMV infected celery in the field were much higher than the estimated levels of CeMV in celery seedlings in the nursery over time. Estimated levels of infection using ELISA varied in the field from 0-100% (Figure 4.1) Week number in Figure 4.1 and batch number in Figure 4.2 correspond to when the seedlings were planted out in the field - week one and batch one corresponds to the first week of the new financial year. The correlation between infection levels in the seedlings and out in the field is unknown, however there seems to be a trend indicating that disease incidence in the nursery may be correlated with disease incidence in the field. Estimated levels of CeMV infection in celery crops in the field are expected to be higher than in the nursery as the crops in the field have greater exposure to aphids and are thus more vulnerable to virus infection.

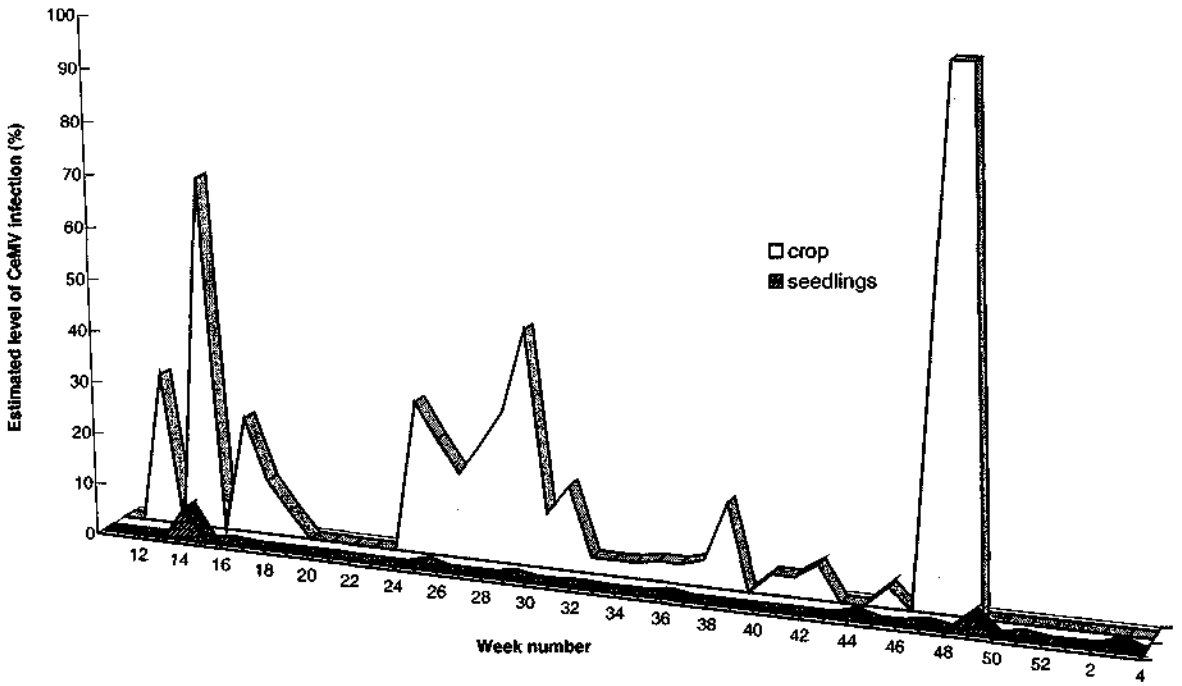

Figure 4.1. Estimated level of infection in the crop and in the nursery seedlings versus week number. Week 1= first week of the new financial year.

Aphid numbers and infection in seedlings and the field
The data of the estimated level of CeMV infection in the nursery seedlings together with aphid numbers are presented in Figure 4.2. The figure shows that when aphid numbers increased so did the virus level with a 3-6 week lag. CeMV has a latent period in which symptoms take 3-6 weeks to become evident.

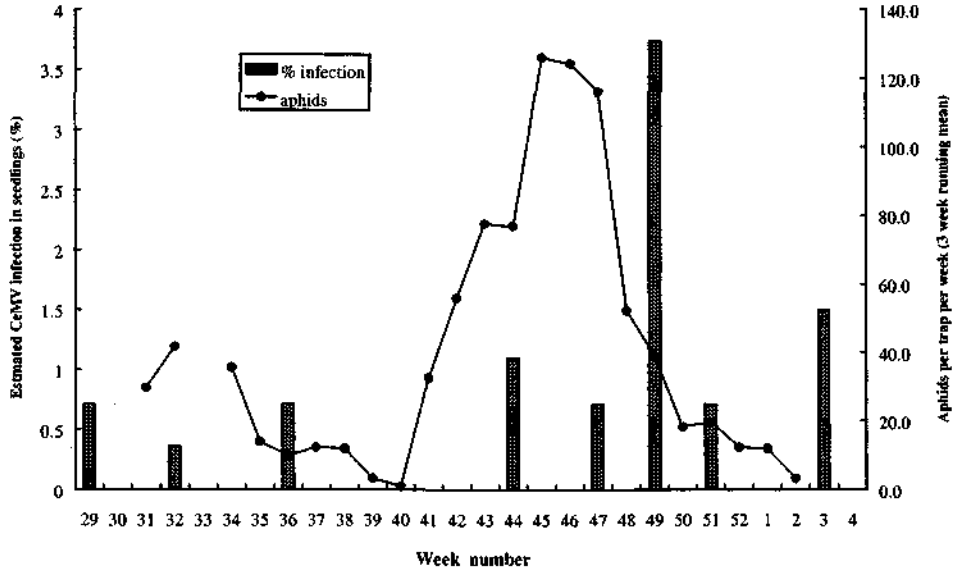

Figure 4.2. Estimated levels of CeMV in celery seedlings versus mean aphid catch per trap (3 week running mean prior to sampling) over time. Week 1 corresponds to the first week of the new financial year.

Infection levels of CeMV in celery in the field was usually much higher than the celery seedlings in the nursery. Figure 4.3 shows the estimated level of CeMV in the crop at harvest with aphid numbers. Aphid number here has been calculated as an aphid index. Aphid index is equal to the mean number of aphids captured in the crop 4 weeks prior to the time the estimated level of infection in the crop was calculated. High aphid numbers are shown in autumn and spring which is consistent with aphid behaviour - aphids are more active at these times. Increased aphid activity also results in higher level of CeMV infection (Figure 4.3).

Key aphid species found in celery crops in Cranbourne and Clyde (Victoria).
Aphid trapping was done in the first year of the project (1998). The key species found in the celery crops are presented below with their common hosts. All aphids are potential vectors of CeMV but some are much more efficient than others. Those aphids that were captured and are known vectors of CeMV are identified in Table 4.1. No experiments were undertaken to determine the transmission efficiency of each aphid species for CeMV.

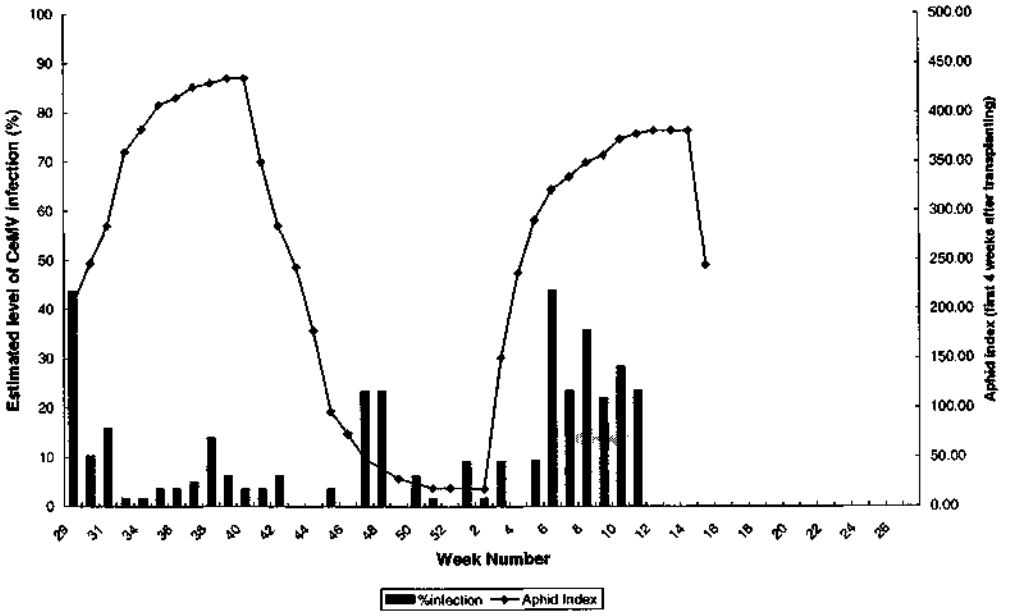

Figure 4.3. Estimated levels of CeMV infection of celery at harvest versus mean aphid index four weeks after transplanting. Batch number corresponds to the week the celery were planted out in the field. Week 1 = first week of the new financial year.

Table 4.1. Key aphid species with their common hosts found in the celery crops of the Cranbourne - Clyde area of Victoria, and their previously known ability to transmit CeMV naturally in the field.

Aphid species	**Ability to Transmit CeMV**	**Common hosts found in Victorian crops**
Brachycaudus rumexicolens		Dock
Dysaphis aucupariae		Plantain
Myzus persicae	×	Mallow, Celery, Brassica
Lipaphis erysimi		Brassica
Aphis sp.	×	Clover
Dysaphis apiifolia		Celery
Uroleucon sonchi		Sowthistle
Hyperomyzus lactucae		Sowthistle
Rhopalosiphum maidis		Sweet corn
Tetraneura nigriabdominalis		Grass roots
Rhopalosiphum rufiabdominalis		Grass roots
Brevicoryne brassicae		Brassica
Aploneura lentisci		Grass roots
Acyrthosiphon pisum		Clover
Rhopalosiphum padi	×	Grass
Therioaphis trifolii f. maculata		Lucerne

Discussion

The infection levels in the nursery seedlings were very low (Figure 4.1) which suggests that most of the infection is occurring out in the celery fields. This implies that seedling infection plays a minor role in CeMV spread when infections in seedlings are low and the field infection pressure is high, however the reverse is true in new production areas where field infection pressure is low - infected seedlings do play a major role in field infections. Thus, starting with virus-free nursery seedlings is imperative to minimising the spread of CeMV into new districts.

The results from this study indicate that aphid pressure is linked to virus spread, however, the exact effect of the high aphid numbers on the incidence of CeMV is not known. There is a definite pattern of high aphid numbers in spring and autumn and this seems to correlate with high levels of CeMV in the field (Figure 4.3). Hence, we can predict that the extent of CeMV spread is related to aphid pressure.

Three of the 16 key aphid species found are known vectors of CeMV. Of the main aphid species present in the celery crop the exact effectiveness of their ability to transmit CeMV is unknown. Further experimental work is needed to answer this question.

There are several indirect virus control approaches as described by Harpaz (1982) which include cultural and technical measures. The cultural measures include:

- *genetic manipulation* which aim to produce plant varieties which are resistant to infection
- *culturing plant tissue fragments* for obtaining virus-free propagative material;
- *elimination of inoculum sources* whether it be by legislation or actual eradication of infected material
- *breaking the cultivation practices* by introducing wide gaps in the availability of susceptible host plants to the virus eg. bare fallowing and rotation of crops.

Technical measures include:

- *reduce the number of vectors* that are active in the field or interfere with virus transmission process.

In California, CeMV epidemics have been controlled in celery crops by the implementation of a celery-free period, which aids in the elimination of the source of virus inoculum (Shepard & Grogan 1971). This is feasible in Victoria, however, the growers must be responsible for this to be implemented. It is recommended that nurseries producing celery seedlings are located outside of the celery growing districts. This will minimise the chance of seedlings being infected and minimise the chances of new plantings of Apiaceous crops becoming infected with CeMV.

Cultural measures such as a break in production may eliminate sources of inoculum. This should be considered by the growers to help manage CeMV, as this is something that can be implemented immediately.

Spraying insecticides to minimise virus spread does not work, because present day insecticides rarely act fast enough to prevent aphids making the brief probes (5-30 seconds) needed to acquire and transmit non-persistent viruses. In fact, the use of insecticides may potentially increase the amount of virus transmission because aphids that have been exposed to insecticides tend to visit more plants than those that have not been exposed to sublethal doses of insecticides (Broadbent *et al.* 1963, Münster & Murbach

1952). Thus, other technical measures to reduce aphid numbers other than spraying with insecticides should be further investigated.

Other technical control strategies for CeMV have been investigated and are reported in Part 5 of this report.

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Part 5. Manging aphids to control CeMV - mineral oils and reflective mulches.

Introduction

There are two basic methods to controlling the incidence and spread of non-persistent aphid borne viruses. Aphids need to be prevented either from reaching plants, or from transmitting the virus. Many different methods with varying degrees of success have been tested worldwide in an attempt to achieve one or both of these outcomes (Loebenstein *et al.* 1980: Raccah *et al.* 1980; Gibson & Rice 1989; Harrewijn *et al.* 1991; Jones 1994). These include traps, coloured mulches and mineral oils. As mentioned earlier insecticides DO NOT prevent the spread of non-persistent viruses.

Mineral oils

Mineral oils have been shown to be effective in reducing the incidence of non-persistent viruses in the field (Bradley et al. 1962, 1966, Vanderveken 1977, Lobenstein & Raccah 1980, Simons & Zitter 1980). The oil appears to interfere with the virus transmission by insects - brief contact between the labium (lip-shaped structure forming the lower lip) and oil reduces both acquisition and inoculation (Powell 1992). Protection depends on obtaining an even covering of oil over the plant, thereby increasing the chance of aphid mouthparts' contacting the oil before probing (Powell et al. 1994).

Reflective mulches

Reflective mulches have also been used to reduce virus spread in crops by the way of altering vector behaviour. Mulches work through visual stimuli on the insect or aphid. Visual stimuli can be either attractive, promoting the use of traps or disruptive (ie. unattractive) in the form of coloured mulches. It is nevertheless possible to use such stimuli to control aphids (Kring 1972; Cohen & Marco 1973; Lobenstein & Raccah 1980; Budnik et al. 1996).

To date there have been many successful studies incorporating a large number of mulch types resulting in either the delay of virus onset, a reduction of viral incidence, or both (Jones 1991; Cartwright *et al.* 1990; Brown *et al.* 1993; Orozco-Santos *et al.* 1994, Csizinszky *et al.* 1995; Summers *et al.* 1995). Budnik *et al.* (1996) provided evidence that white plastic mulch reduced viral infection by 50%. Similarly, Stapleton *et al.* (1995) showed that various polyethylene, nylon, net and sprayable mulches, coloured either silver or white, resulted in a three-to five-fold increase in marketable yields of squash (*Cucurbita pepo*) compared with non-mulched controls. They claimed that this was a direct result of a reduction in *Cucumber mosaic virus*, *Watermelon mosaic virus* and *Zucchini yellow mosaic virus*.

Kring (1972) points out that it is unlikely that all aphid species will be equally attracted to any one colour. There will always be those individuals who for one reason or another, be it wind or something else, land and settle on a crop. Here we have trialed coloured reflective mulches with the aim of using them to deter aphids form landing in celery crops and hence, minimising CeMV transmission.

Presented here are results from two trials to help reduce aphid pressure with the aim to reduce the impact of CeMV in the field: mineral spray oils and coloured reflective mulches.

Materials and Methods

1. Mineral spray oils

Two spray oil trials were undertaken in 1999 in a commercial celery crop in Victoria. The mean levels of CeMV in sprayed and unsprayed plots were monitored in the celery crop over 12 weeks. Regular applications of mineral spray oil (C24 - Caltex DC-tron Plus®) at a concentration of 0.075% was applied to the crop at regular intervals. For trial I (autumn 1999) the spray oil was applied every 7-11 days. For trial II (spring 1999), the spray oil was applied every 5-7 days. The crops were irrigated regularly using fixed overhead sprinklers. The spray oil was first applied 11 days after planting for both trials. The sprayed and unsprayed plots were arranged as four, 2x2 Latin squares, giving 8 replicates of the sprayed and unsprayed treatments.

Estimating virus levels in the field, and assessing weight and quality of celery

Each fortnight 50 leaves were collected from each plot and tested for CeMV using ELISA in batches of 5, 2 or 1 depending on the virus levels observed in the previous fortnight. Estimates of infection percentage were calculated using the formula given by Burrows (1987). To assess fresh weight, 50 celery plants were collected at harvest using a random systematic sample from each plot. After trimming each individual celery plant was weighed and length and circumference of the base measured. The quality of these plants was visually assessed at the packing shed and graded into four different grades: 20, 16, 12, and 9. These numbers correspond to the number of celery bunches that can be packed per box, with nine being the highest and 20 being the lowest grade.

The results were analysed using analysis of variance (ANOVA). The statistical program used was GENSTAT™.

2. Reflective mulches

Coloured reflective mulches were trialed at IHD, Agriculture Victoria, Knoxfield, Victoria. There were two trials at IHD and both trials employed two treatments: white plastic mulch and silver plastic mulch.

- White plastic mulch: this was a black/white co-extruded plastic film (manufactured by Australian Challenge P/L) which was white on top and black on the underside. It was 1200mm wide and 25µm thick. It was laid with the white side up.
- Silver plastic mulch: This was a black plastic film with silver coating on the upper side manufactured by IAMA Yarra Valley, Victoria. As with the white mulch, it also appeared black on the underside. It was 1420mm wide and 25µm thick. It was laid silver side up.

Watering system

Owing to the impermeable nature of the mulches, a dripper system, placed under the mulch was used to water the crops as opposed to the normal commercial use of overhead sprinklers.

The watering system was modified in trial two because of the very poor growth of plants in trial one. Not one, but two lines of t-tape were placed down the centre of each land, so the crop received twice as much water as trial one. Water was drawn from an on-site irrigation dam, which was not subject to any treatment in any way.

Design Trial 1
The total site for trial one was 21 lands (approx. 30m) and 40m long giving a total area of approximately 1200m^2. The site was divided into twelve plots, giving four replicates for each treatment as well as control plots. Each plot was 7 lands wide (approx. 10m) and 10 m in length, covering approximately 100m^2.

The treatment and control plots were arranged as randomised 3x3 Latin square with an extra randomised added row, giving a final 3x4 rectangular field. All three plot types (i.e. control, white mulch and silver mulch) were represented in each row. However, owing to the presence of the fourth row, a plot type appeared twice in each column.

Design Trial 2
The design for trial two was modified. Poor celery growth had been experienced in trial one necessitating alteration to the watering regime. This alteration coupled with a change in seasons meant that it would be impossible to directly compare both trials. As a result, the opportunity was taken to change the location and the original field design so as to increase the number of replicates of each treatment.

The trial two site was located directly east of the trial one site; approximately 60m form the hedge. The field design facilitated the same two treatments (silver and white mulch) and control (bare soil) being used at an increased incidence. Plot size was sacrificed due to a limitation of celery seedlings necessary for maintaining equivalent density levels. The reduction in plot size permitted a total of eighteen plots giving an increase in replications to six plots per treatment plus control, the new design comprised two 3x3 Latin square arrangements next to each other, giving a three column x six row rectangular field. Each plot was 5 lands wide (approx. 8m) by 7m long, giving a plot area of approximately 64m^2. The total site for trial two, was 15 lands wide (approx 21m) and 42m long, giving a total area of 1152m^2).

General maintenance, such as weeding and repairs to the watering system, was conducted as necessary. Both mulches were laid using an agricultural plastic-laying machine. Holes for the plants were made using a gas fuelled circular cutter.

In both trials, green plastic water pan traps, similar to the colour of the celery foliage were used. The pan traps comprised one green plastic container. An overflow hole was drilled near to the rim of the container and covered with wire gauze so that no insects could escape, each trap was 14cm in diameter and 4 cm deep and was mounted via a clamp to a single 1cm x 1cm square steel stake, the clamp allowed the trap height to be progressively adjusted in accordance with the crop height. All traps were filled with water containing detergent (Pyroneg Powder™) and copper sulphate ($CuSO_4$). Detergent was added to reduce the surface tension of the water so that the arriving insects would sink.

Aphid trapping
Aphids were monitored in the crop through the placement of water pan traps. Aphids caught in these traps were collected and counted twice weekly in trial 1 and only once a week in trial 2. There were 48 traps used in trial 1, (ie. 4 traps per plot). For trials 2, a total of 54 traps were used (ie. 3 traps per plot). The water pan traps were set every day for the duration of each of the trials.

Ten plants were randomly selected in each plot once a week and checked for any resident (wingless) aphids to determine whether aphids were settling on the celery plants.

Statistical analysis was applied using the computer package SPSS version 8.0. Before analysis, all data were log transformed or transformed by regressing logV (variance) against logm (mean) as per Southwood (1978) to homogenise the variances. The data was then analysed by nested General Linear Models (GLM), and post-hoc tests as appropriate. Those data sets for which the variances could not be homogenised were analysed using non-parametric tests e.g. Kruskal-Wallis mean rank tests and Mann-Whitney U tests.

Results

1. Spray oils

The mean level of estimated infection of CeMV in the field 21 days before harvest in the sprayed and unsprayed plots for both trials are presented in Table 5.1. No ELISA tests were done in the last 21 days leading to harvest for Trial 1 and the last 28 days leading to harvest for Trial II because any infection that was incurred in this time was unlikely to have an effect on weight, quality or number of celery as the infection would be to late.

Table 5.1. Estimated levels of CeMV (formula given by Burrows 1997) in celery that was sprayed with the petroleum oil and crops left unsprayed for both field trials 21 days before harvest.

	Sprayed	Unsprayed
Trial 1. (Autumn 1999)	74.3%	81.4%
Trial 2. (Spring 1999)	50.2%	69.6%

The relationship between the percentage infection of CeMV and time in Trial I was sigmoidal in shape and logistic curves were fitted. ANOVAs were performed on each of the four parameters of the fitted curves. This revealed that there was a significant ($P<0.001$) divergence of the curves; celery sprayed with the oil had a lower incidence of CeMV. This implies that the spray oil delayed the onset of CeMV (Figure 5.1).

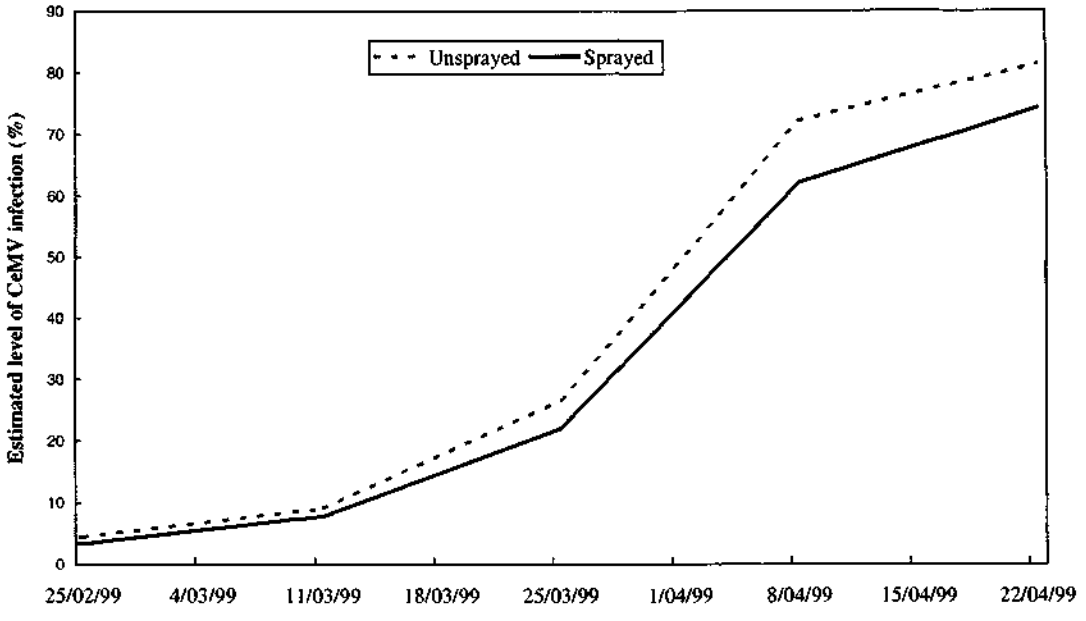

Figure 5.1. Results from ELISA analysis with estimates of virus infection levels in celery in the field over time for Trial I.

The relationship between the percentage infection of CeMV and time in Trial II is shown in Figure 5.2. Celery that was unsprayed had a higher incidence of CeMV that celery that was sprayed.

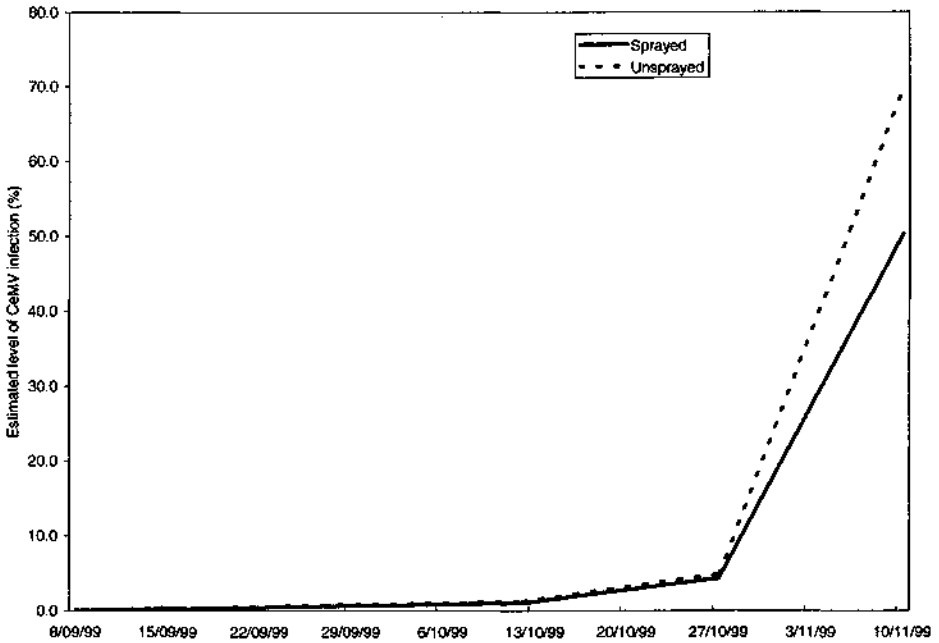

Figure 5.2. Results from ELISA analysis with estimates of virus infection levels in celery in the field over time for Trial II.

The effect of spray oil treatments on celery fresh weight, length, circumference, quality and percent culled

The results from both trials are presented below.

For Trial I (Table 5.2), celery left unsprayed with oil had a marginally higher fresh weight (5%) than celery that was sprayed with the oil ($P<0.05$). However, spray oil had no significant effect on length, circumference at the base, quality or the percentage of celery culled.

Table 5.2. Fresh weight (g), length (cm), circumference (cm), quality and percentage of celery culled for Trial 1(autumn 1999). Bolded numbers are significantly different.

	Fresh weight (g)	Length (cm)	Circumference (cm)	Quality	Culled (%)
Unsprayed	1439	49	33.0	15	7.6
Sprayed	1371	49	32.4	15	5.5
l.s.d (P=0.05)	**36**	1.2	0.8	0.6	2.2

For Trial II, celery sprayed was shorter than celery left unsprayed and had lower quality celery than the celery left unsprayed. The spray oil had a marginal effect on length (1%): celery sprayed was shorter than celery that was left unsprayed. Celery sprayed with oil were also 12% poorer in quality than celery that was left unsprayed. This reduction of quality was a result of phytotoxicity caused by an interaction of the oil spray and a herbicide after transplanting. The spray oil can act as an adjuvant for the sprays that a grower may use, however, some chemical combinations can cause phytotoxic problems as was the case here. However, the results showed that the spray oil had no significant effect on fresh weight or on the circumference of the percentage of celery culled.

Table 5.3. Fresh weight (g), length (cm), circumference (cm), quality and percentage of celery culled for Trial II (spring 1999). Bolded numbers are significantly different.

	Fresh weight (g)	Length (cm)	Circumference (cm)	Quality	Culled (%)
Unsprayed	1354	48	35.2	14.3	7.2
Sprayed	1265	47	34.1	16.2	9.2
l.s.d (P=0.05)	120.3	**0.91**	1.4	**0.88**	11.1

2. Reflective mulches and effect on aphid landing rates

The effects of treatment on total aphid catch were similar in the two trials. A greater number of aphids landed in the control plots (no mulch); white plots received fewer, and silver plots the least (Figures 5.3 and 5.4). A nested GLM for trial 1 and a Kruskal-Wallis mean rank test for trial 2 showed that the effect of colour on winged aphids landing rates was highly significant for both trials (Tables 5.4 and 5.5). In trial 1, significantly more winged aphids landed in control plots (bare soil) than white plots, and significantly more landed in white than in silver plots (Table 5.4). Similarly in trial 2, significantly more aphids landed in the control plots than white, while significantly more landed in white than silver. Thus, the number of winged aphids on control plots differed significantly from that on silver plots (Table 5.5).

No resident aphids were found on any of the plots in any of the trials.

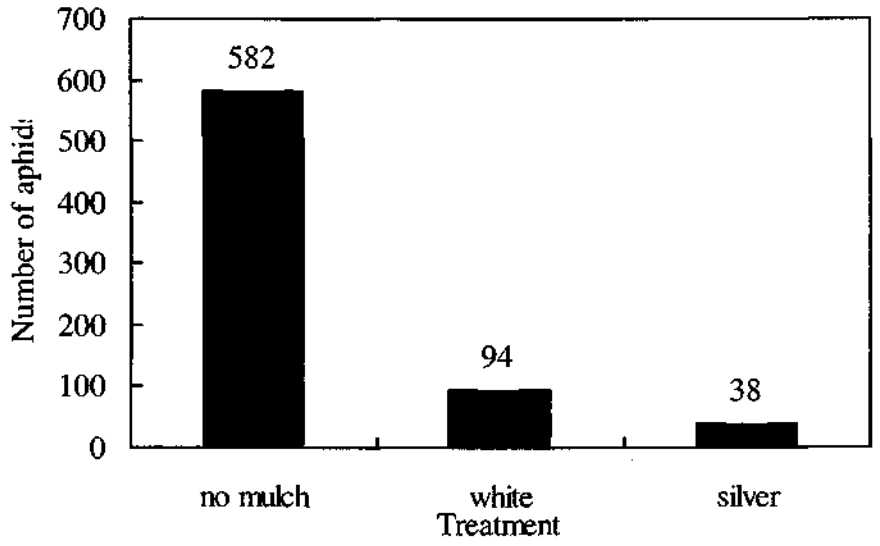

Figure 5.3. Aphid landing on the white, silver plots and no mulch plots (Trial 1).

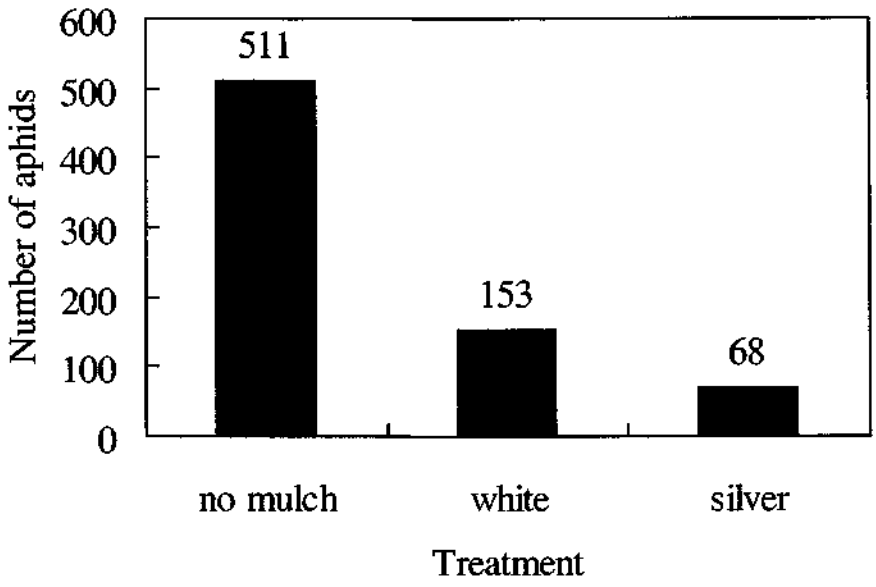

Figure 5.4. Number of aphids landing on the white, silver plots and no mulch plots (Trial 2).

Table 5.4. Nested GLM and LSD analysis of the effects of treatment and plots within treatments on aphid landing rates for trial 1. Bold type indicates significance $p<0.05$. n(number of traps) =48; C=control; W=white; S=silver.

Trial 1	df	F	P(GLM)	Treatment	Mean difference	SE	P (LSD)
Treatment	2,9	43.124	**≤0.0005**	C and W	0.703	0.054	**≤0.0005**
Plot (treatment)	9,36	4.500	**=0.001**	W and S	1.042	0.054	**≤0.0005**
residual	1,9	402.987	**≤0.0005**	C and W	0.340	0.054	**≤0.0005**

Table 5.5. Non parametric Kruskal-Wallis and Mann-Whitney U test analyses of the effects of treatment on aphid landing rates for Trial 2. Bold type indicates significance $p<0.05$; df=2; n(number of plots)=18.

Trial 2	χ^2	P (K-W)	Mean rank		Treatment	U	P (M-W)
Total	14.392	**=0.001**	C	15.50	**C and W**	0.00	**=0.004**
			W	9.17	**W and S**	2.0	**=0.010**
			S	3.83	**C and S**	0.0	**=0.004**

Discussion

1. Spay oils

The use of the spray oil as a tool to manage CeMV is promising. The estimated incidence of CeMV infection in celery in the field was delayed (in the first trial) and reduced (in both trials) when sprayed with oil. A delay in the infection of CeMV can affect quality of the celery - later infected celery has a greater chance of reaching its full growth potential than celery infected early (Severin & Freitag 1938).

Although the incidence of CeMV was lower in the sprayed crop and the quality of the celery was not significantly different in Trial I, the mean fresh weight was 5% lower. This reduction in weight may be explained by the phytotoxicity caused by the interaction of the spray oil and herbicide used early after transplanting which burnt the tips of the celery compromising the normal growth habit. This effect of phytotxicity recurred in the second trial, so exact estimates of the benefits associated with using the spray oils are not present. However, they do show great promise given that they are used with compatible chemicals that will not cause a phytotoxic effect and that the spray oil is implemented with a comparable spraying regime by the grower.

A limitation of using the spray oil to decrease the level of CeMV in trial one was the incidence of maximum temperatures exceeding 30°C. High temperatures meant that the crop needed to be watered more often, making the application for the oil difficult, as it could not be applied to a wet crop, nor above temperatures of 30°C.

Over time, the application of the spray oil on the celery crop may potentially reduce the source of inoculum and thus reduce overall infection of CeMV in the area. Although there was some phytotoxicity experienced in the crop, the application of oil did reduce the estimated incidence of CeMV in the sprayed plots and did not affect the number of celery culled. This implies that the oil has the potential to be used as part of a management strategy to control CeMV.

2. Reflective mulches

Reflective aluminium mulches have been proven effective as aphid deterrents (Johnson *et al.* 1967: Loebenstein *et al.* 1975: Brown et al. 1993: Sapleton *et al.* 1995; Loebenstein & Raccah 1980; Lamont *et al.* 1990; Jones 1991; Pinese 1994; Summers *et al.* 1995), however coloured mulches such as blue and black have proved less effective (Pinese 1994; Lamont *et al.* 1990). White mulch as was tested here, has been less extensively tested and with mixed results (Cartwright *et al.* 1990; Brown *et al.* 1993). The results for both reflective mulch trials agree with the literature - reflective mulches are effective aphid deterrents. No resident aphid populations were found on the crop, which suggests that the mulches were having an effect on aphid landing rates. Not only did the mulches significantly reduce aphid landing rates in the celery crop, but silver mulch resulted in a lower aphid count than either white mulch or bare soil (Tables 5.4 & 5.5).

There are some concerns associated with the use of plastic mulch, which is likely to compromise its commercial attractiveness. The pre- and post-laying practices are also labour intensive. Because plastic is impermeable, it does not allow the use of overhead sprinklers for watering nor the application of fertiliser after planting. Hence, a watering system has to be located under the plastic, and the plot must be fertilised enough prior to planting to last the entire growing period.

One of the reasons for the change of design of trial 1 and 2 was the very poor growth of plants in trial 1. This was directly attributed to the lack of water. Only one dripper pipe was used in trial 1. Hence for trial 2, two dripper pipes were placed under the plastic mulch and celery growth was observed to be much better.

Other problems associated with the use of plastic mulches is that after holes have been made in the plastic to allow the planting of seedlings, planting must be done manually. As well as planting, all weeding has to be done manually with simple farm implements, since most machines are likely to damage mulch. Therefore, high costs are incurred at each step in the process. Furthermore, the plastic cannot be reused for the same purpose owing to deterioration in its physical properties, which in turn necessitates specific disposal policies. It means that for plastic mulch to be an economically viable alternative, their cost/benefit must be attractive. Although the results here indicate that white mulch significantly reduces aphid landing rates and silver mulch is a significantly better aphid repellent, the use of silver mulch may not currently be commercially viable on a large scale, it could still be used to provide some benefit against CeMV.

Past research on other crops has shown that the use of reflective mulch is most beneficial in an integrated management strategy, which incorporates, eg. insecticides and mineral oils (Lowery 1980; Brown *et al.* 1993; Pinese 1994). The use of silver mulch in an integrated management strategy for controlling CeMV is therefore likely to contribute a benefit.

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Technology Transfer

The list below indicates the activities undertaken throughout the life of the project to ensure the research has been made public as well as adopted by growers.

Conference Papers and posters

Moran J., Ridland P., Rodoni B.C., Eagling D., Jones R., Latham L., Persley D., Thomas D., Hepworth G., and Constable F.E. (1997). Research strategies for the Management of Celery Mosaic Virus. Proceedings of the Australian Plant Pathology Society Biennial Conference, Perth.

Moran J., Gibbs A., van Rijswijk B., Mackenzie A., Gibbs M. and Traicevski V. (1999). Potyviruses in the cultivated and wild Apiaceae in Australia and the implications for disease control. Proceedings of the Australian Plant Pathology Society Biennial Conference, Canberra.

Traicevski V., Schreurs T., Rodoni B., Ridland P. and Moran J. (1999). Celery mosaic virus occurring naturally in cultivated Apiaceae in Victoria, Australia. *Australasian Plant Pathology* 28, 92.

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Traicevski V., Ridland P., van Rijswijk B., Rundle B. and Moran J. (2000). Celery Mosaic Potyvirus - epidemiology and implications for control in celery (Apium graveolens L.). Proceedings of the Australian Entomological Society 31st Conference, Darwin, 2000.

Reports

Quarterly newsletters 1-6. (See Appendix I). These newsletter were distributed to all celery growers and carrot growers regularly throughout the project.

Honours thesis - The effects of two reflective mulches on aphid landing rates in a celery crop, Apium graveolens (Linnaeus). Author; Brad Rundle, LaTrobe University.

Technical reports and extension material

Traicevski V. (2000). Agnote AG0939: Celery mosaic virus. Resource and external Web sites.

Quarterly newsletters 1-6 (See Appendix I).

Hand out to growers (Poster with CeMV symptoms)

Meetings

- Carrot growers R & D meeting (1998 & 1999)
- Victorian celery growers meetings (1998, 1999 & 2000)
- Meetings with consultants (Carl Reidel - E.E. Muirs & Sons, and Tony Kourmouzis - Private consultant)

Recommendations

It is possible to manage the spread of CeMV. The recommendations to industry to manage CeMV are as follows:

1. **Plant healthy celery seedlings in the field.** Seedlings sourced from outside the celery growing areas are less likely to be infected with CeMV. In addition, future options for growers would be to test seedlings before they are transplanted out into the field, but this may be cost prohibitive.
2. **Plant tolerant varieties.** At present, no resistant varieties are known, however further research is currently being undertaken by staff at IHD, Knoxfield to address this. In the near future it is hoped that growers will be able to plant virus-resistant crops to combat both CeMV and CVY.
3. **Plant new crops as far away as physically possible from mature crops.** This is a relatively simple and effective control method that can be implemented immediately by growers. Growers need to be encouraged to allocate some time to reorganising their planting regimes to cater for this.
4. **Plant celery seed beds as far away as possible from celery crops.** The longer the plants are in the ground the more likely the plants are to acquire virus. Because aphids are more likely to feed on older more challenged plants they are more likely to acquire the virus from the celery seed beds and pass on the virus to other plants.
5. **Control wild fennel and feral carrot on the farm.** The importance of controlling weeds which act as virus reservoirs as well as alternative food sources for the aphids is paramount in helping control the transmission of virus from weeds to crops. This is a cultural control method that can be immediately implemented by the growers.
6. **Plough in old crops and crop debris as soon as possible.** This too is another recommendation that can be immediately implemented by growers. The sooner the plants are ploughed the less likely aphids will be to acquire the virus from the old crop and pass it on to the new crop.
7. **Take a break in production** - studies from the US recommend at least 2-3 months. The break in production will help break the cyclic effect of virus from one crop to another. This type of cultural control has proved to be very successful in South Australia and the US.

Although there seems to be no evidence available world-wide with regard to the seed transmissibility of CeMV this question has not been thoroughly addressed. Further research to investigate whether the virus is seed borne is worthwhile. Other potyviruses are known to be seed transmitted, eg. *Lettuce mosaic potyvirus* and growers in the US now use certified lettuce seed when planting crops. If CeMV and CVY are seed transmitted, implementing a certified seed program, together with all the cultural control recommendations above will help control the viruses in the Apiaceae.

DNRE can help facilitate communication between growers and other relevant people in the industry to help implement a break in production. This could be done through our extension specialists.

Acknowledgments

The assistance of the steering committee members, who provided constructive comments and information, is gratefully acknowledged. Members of the group were the Victorian Celery Growers Association, in particular Tom Schreurs, and Silvio Favero.

A large number of individuals provided support throughout the duration of the project allowing us to use field sites and gathering of important information. We thank the following people:

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- Persley Thomas
- Rowles Alexie
- Rundle Brad
- Teserierioro Len
- van Rijswijk Bonny
- Victorian Celery Growers Association
- Wilson Calum
- Zeihrl Angeleika

Appendix I. Quarterly Newsletters. The control of *Celery mosaic virus*. Editions 1-6.

The control of *Celery mosaic virus* - Quarterly Newsletter No 1.

THE CONTROL OF CELERY MOSAIC VIRUS

Quarterly Newsletter

Edited by Violeta Traicevski

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1. NEWS FROM VICTORIA

Jane Moran, Violeta Traicevski and Peter Ridland, Institute for Horticultural Development, Agriculture Victoria.

a) Staff

Violeta Traicevski was appointed on the 27th January 1998 as the project scientist in Victoria. She has recently finished a PhD on aphid behaviour and aspects of their biology at La Trobe University. Violeta is an expert on aspects of aphid behaviour that are critical to virus spread.

Dennis Persley (QDPI) and Roger Jones (WADA) recently visited Victoria and spent time observing infected celery crops in the Clyde district.

b) Laboratory work

Three viruses have been found in Victorian celery, tomato spotted wilt (**TSWV**), celery mosaic (**CeMV**) and cucumber mosaic virus (**CMV**). To date **TSWV** has only been found rarely and **CMV** appears to be more prevalent than **CeMV.**

Evita Alberts (PISA) has kindly provided antiserum to **CeMV** for all of the states working on this project. We have used this antiserum to establish an ELISA that will enable rapid assessment of disease samples. ELISA kits have already been purchased for **CMV** and **TSWV**.

c) Testing of seedlings

One of our main grower collaborators in this project has organised a weekly collection of 500 random leaf samples from seedlings just prior to planting. These samples have been collected since October 1997. The same grower has also organised 300 random samples to be taken from the crops grown from these seedlings at harvest. The samples collected will be tested for the presence of all three viruses using ELISA. This study will help us to understand if infected seedlings are contributing to the virus epidemic in the field. The results of these tests will also enable us to determine virus levels at different planting dates and at different locations. The results will be available in the next few months.

d) Field sites

Two sites have been chosen to enable us to study how the disease is spreading. At each site, disease levels are monitored and aphids are trapped weekly. The study sites were established in December 1997. **Aphid numbers have been low**. This is to be expected in the summer months but as the weather cools down we expect aphid numbers to increase.

Site 1: This site is within a large area in which only celery is grown. The crop was planted in early November 1997 and to date infection levels have remained below 20%.

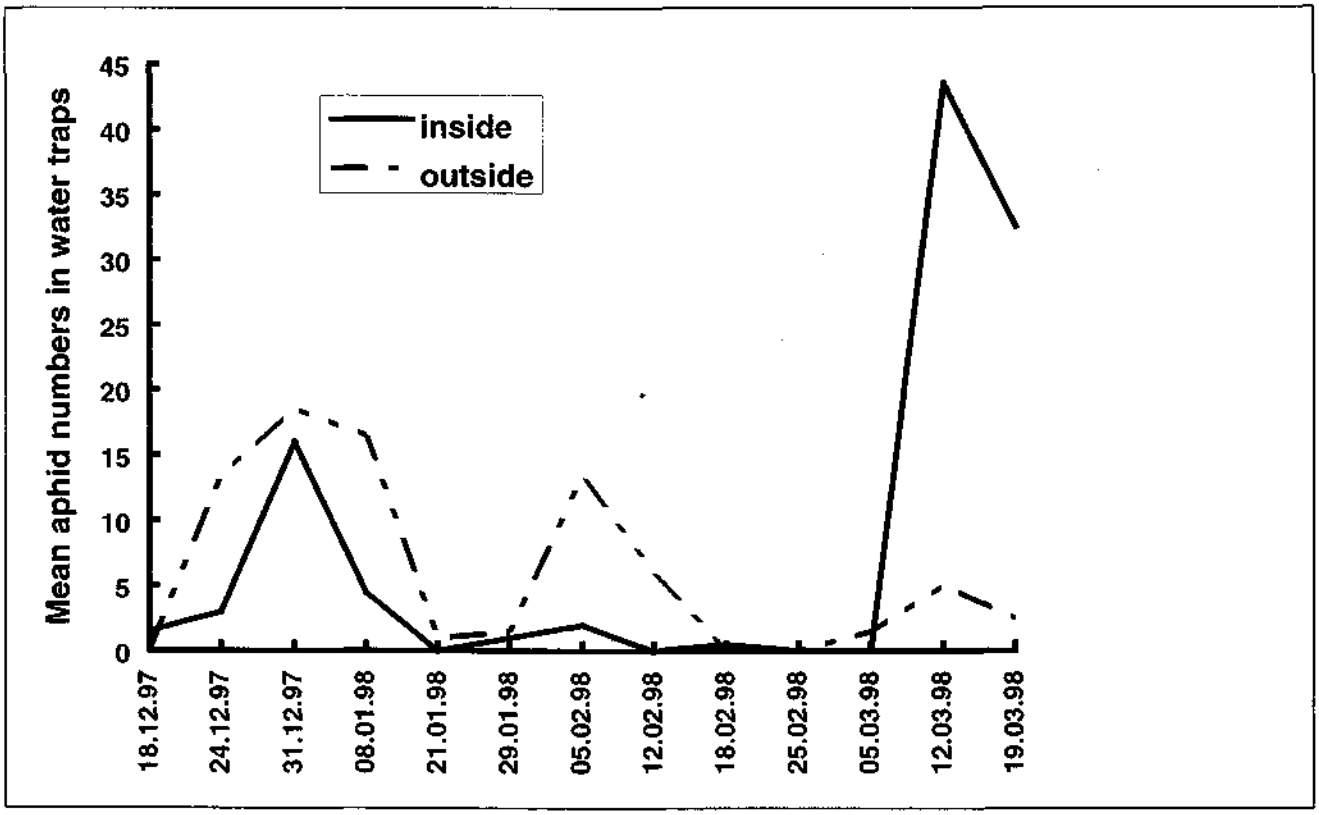

Figure 1. Numbers of aphids caught in yellow pan traps located within and just outside celery crops at Site 1.

Site 2: This site is within a mixed cropping area with other vegetables, including some celery seed crops. The crop was planted in early November 1997. Plants showing symptoms of virus first appeared three weeks after planting. The crop now appears to be 100% infected with virus.

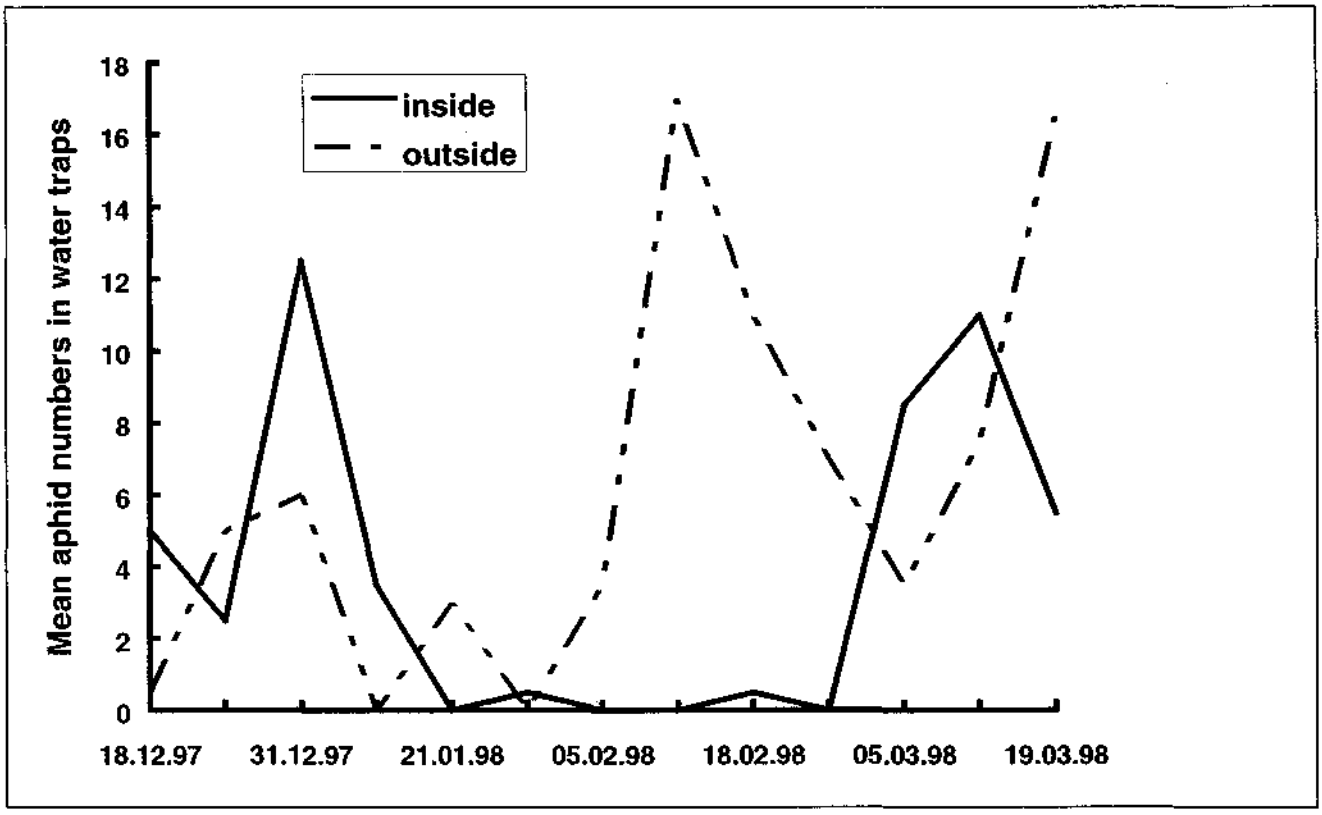

Figure 2. Number of aphids caught in yellow pan traps located within and just outside the celery crop at Site 2.

e) Parsley

Parsley plants with virus symptoms were found in the Clyde district in February 1998. Preliminary tests indicate that **CeMV** is the most likely cause of the symptoms. This situation will be closely monitored.

Future surveys of celery crops in the district will be expanded to include parsley and other related crops.

2. NEWS FROM WESTERN AUSTRALIA

Lindrea Latham and Roger Jones, Western Australian Department of Agriculture.

A survey of celery crops in the south west of Western Australia was done over the past few months looking for three viruses - celery mosaic virus (**CeMV**), tomato spotted wilt virus (**TSWV**) and cucumber mosaic virus (**CMV**).

Thirteen properties growing celery were visited and on four of these plants infected with **CeMV** were found. This is the first confirmed report of **CeMV** in Western Australia. Symptoms of a leaf mottle and stunting were strongest in cv. Tendercrisp. The cvv. Excelsior and Yarralong were also found to be infected.

One property had very high levels of infection up to 60% in most plantings and it is thought that the virus must have been present for a number of years to reach such high levels. That grower is seriously considering abandoning celery production. The other three properties had less that 0.01% infection. All the three properties purchased their seedlings from the same nursery. It is not known where the virus originated from; whether it was already present on their own property in the weeds or it was imported from the seedling nursery. It is a concern that **CeMV** was detected at all and raises a few important questions:

> *Is this a recent virus introduction, and are we likely to see more devastating levels in coming years?*
>
> *Has the virus been here a long time but never reached the levels which are currently found in Victoria?*
>
> *Do we have a different strain of **CeMV** in Western Australia?*

At the time of the survey no aphids were observed. This was presumably due to a drop in aphid populations as a result of the hot weather.

It is also important to note that only one celery sample was found to be infected with **CMV**.

3. NEWS FROM QUEENSLAND

Dennis Persley and John Thomas, Queensland Horticulture Institute, Plant Pathology group.

The two major celery growing areas of Queensland were surveyed for viruses in 1997.

Celery is grown during winter in the Lockyer Valley and crops of the three major growers were surveyed in August 1997. The cultivars were Toowoomba Early and Tendercrisp. About 3000 plants were visually inspected at each property, and plants with virus-like symptoms were collected. Celery mosaic virus (**CeMV**) was not found by electron microscope examination of sap extracts. Tomato spotted wilt virus was detected by ELISA in two samples.

Celery is grown during the spring to autumn period in the Granite Belt area of south Queensland. Crops on Haslettes farms, the largest and one of the few celery growers in the district, were surveyed in November 1997. About 5000 plants were inspected and virus-like symptoms were seen on a small number of plants. **CeMV was not detected** in these samples. One sample was positive for tomato spotted wilt virus. This area will be surveyed again in February.

CeMV had not previously been found on celery in Queensland and has not been detected in the current work. The virus, however, has previously been isolated from parsley in south Queensland. An important reason for the apparent freedom of Queensland crops from CeMV is likely to be the break in production which occurs in each district - summer in the Lockyer Valley and mid-winter in the Granite Belt.

Positive samples of CeMV and antiserum have been obtained from Victoria for future work. Celery crops will again be surveyed during the 1998 season.

The control of *Celery mosaic virus* - Quarterly Newsletter No 2.

The Control of Celery Mosaic Virus

No. 2 JULY 1998

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Edited by Violeta Traicevski

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1. EDITORIAL

By Jane Moran

It is now six months since we started full swing on trying to understand the celery mosaic virus (CeMV) epidemic. We have spent this time gathering data on the incidence of the disease in the field and in seedlings, as well as the aphid populations in the growing areas. Our collaborators interstate have also been looking at their own celery crops. The report from WA was in the first Newsletter and a report from Queensland is in this edition (p. 14).

Our results to date show that the situation is a very serious one which will take some time to resolve. The results of our recent survey (pp. 6 & 7) showed that CeMV was present in celery in all growing districts; we also found some infected coriander and parsley crops. What was heartening was that we found two crops that appeared to be free of the disease, showing us for the first time that it is possible to grow a celery crop without CeMV. Unfortunately just recently we found a number of carrot crops to be infected with CeMV (p. 5). This certainly complicates the situation and indicates to us that we may be dealing with a disease with a wider host range then we expected.

We have included a new section in the Newsletter, a "Who's Who" of researchers around Australia involved with CeMV and their contact details (page 16 & 17). We would also love to include a section on comments from industry. So if you have anything to say please let us know.

WHAT IS SPECIAL ABOUT BATCH 41?

Recently we were out in the field and we visited a number of celery crops. Two crops stick in my mind. Both were planted on the same day (March 11). Both were planted with the same seedling batch grown in the same glasshouse. Both were the same variety. One crop had 70% virus, the other had less than 5% virus. Why, with all things being equal, should one crop escape a high infection level? The crops were planted on different farms, but both farms have a history of bad virus infection.

The major difference between these two plantings was how close to older infected crops the seedlings were planted. The crop with 70% virus was planted in an area containing infected crops that were close to harvest. The crop with less than 5% virus was planted in an area that had not grown celery for the previous 8 weeks. The lesson here is that, if it is possible to schedule plantings, a break in production knocks the virus levels right down.

I would like to take this opportunity to introduce Bonnie van Rijswijk and Brad Rundle. Bonnie is a work placement student from Dookie who has been helping Violeta with various things in the laboratory and glasshouse. Bonnie has been responsible for sowing and rearing various species of the Apiaceae family. She has contributed to this newsletter and her report is presented on page 11. Brad has just started with the CeMV team and is working on an Honours project that will investigate the landing rates of aphids in celery crops that have been sprayed with different mulches that are aimed at repelling aphids from landing. His trials should start in September and we should have some results early in the new year.

2. Aphids and Viruses

By Violeta Traicevski

a) Aphid behaviour

Most aphid species found in Australia are parthenogenetic (female populations that reproduce without males). They often produce two morphs: winged and wingless. The migratory form is the winged morph, but wingless aphids are capable of movement as well - they are able to travel across bare ground (although not very far) and colonise new plants. As a general rule winged aphids are not very strong fliers and do not cover very long distances. Winged aphids rely on the wind to disperse and are not really conscious migrants.

b) How do aphids pass on virus?

Probing by the aphids is the way in which viruses are spread. Probing by the aphid involves the aphid ingesting a small amount of cell sap (in the leaf or stem), and the infection occurs when the aphid probes on another plant and the virus is on its mouthparts. Virus retention is usually low due to the inactivation of the virus by the aphid's saliva.

c) Control of non-persistent viruses

Celery mosaic virus is a non-persistent virus, a group of viruses which are characterised by:

- virus acquisition and infection during brief probes of the aphid (aphid does not have to colonise plant),
- no inactive period after acquisition,
- loss of virus by aphids after short feeding periods,
- loss of virus by aphids after moulting
- low virus-vector specificity (many vector species).

d) Insecticides do not work

Controlling the vectors that pass on non-persistent viruses by using insecticides has generally been unsuccessful; present day insecticides rarely act fast enough to prevent aphids making the brief probes needed to acquire and transmit non-persistent virus. Spraying may control colonisation by aphids but not the transmission of the virus. The use of insecticides may potentially increase the amount of virus transmission because aphids that have been exposed to insecticides tend to visit more plants than those that have not been exposed to sublethal doses of insecticides.

e) Breeding

Breeding for resistance is one of the most effective ways to control viruses provided that stable sources of resistance can be obtained. Breeding for resistance is a slow process and, even after years of extensive research,

resistant lines may not be found. There is no known resistance to CeMV in celery. Therefore, alternative control measures must be addressed. To date there are a series of methods being tried to control for non-persistent viruses, these are outlined below.

Plants can also be made tolerant or resistant to plant viruses using genetic engineering. Genetically engineered virus resistant potatoes for example have been trialed in Australia.

f) Alternative control measures for viruses

1. Oil spraying This is where the plant is coated with mineral oil. It is still not fully understood how it exactly works, but the oil is thought to interfere with the attachment or removal of virus particles from the aphid's mouthparts. Phytotoxicity can be a problem.

2. Reflective surfaces Aphids are attracted by yellow and green spectral wave-lengths (500-580 nm) and so alight on bare ground or plants. Short wave lengths repel them and so covering the ground between plants or covering the plants themselves with reflective surface sprays will repel the aphids. White wash and aluminium (highly reflective mulches) have been investigated and have had some success, mainly with cucurbits and other high-value crops.

3. Border plants The idea behind plant border plants is that these plants will be visited by the aphids first and the aphids will lose the virus on these. Consequently when the aphids move into the crop they will be free of virus, but present cultivation practices may not make this feasible.

4. Alarm pheromones These chemicals are released by aphids when attacked by predators. Spraying alarm pheromones onto the crop has the potential to discourage winged aphids from settling on the plants which means that the aphid will not probe and will not pass on viruses from plant to plant. Although alarm pheromones can be prepared readily for commercial use they have their drawbacks: they are very volatile and persist only briefly in a crop. But, this disadvantage may be overcome by using slow release formulations.

5. Sticky yellow sheets Placing very large sticky sheets around the crop with the aim of attracting the winged aphids and thereby reducing their movement into the field. This has not been used extensively and its effectiveness is still unknown, it is also likely to be expensive on a large scale.

6. White nets or floating row covers Placing white nets over the crop can significantly reduce the incidence of virus in some plots. The nets seem to obscure the plants from the

aphids. But this method is very expensive and can have potential problems associated with climatic changes such as high winds.

3. News from Victoria

By Violeta Traicevski

a) Important new development:

CeMV has now been detected in carrots. The carrot plants showed classic CeMV symptoms; light and dark mottling between the leaf veins, the top of the plants have a flattened appearance and their is a definite narrowing of the leaf tips. The electron microscope preparation showed potyvirus-like particles, consistent with those of CeMV, and the positive results from serology tests using German antisera confirmed the presence of CeMV in the carrot. A small survey of carrot crops near the celery growers on the Peninsula and in the Clyde-Cranbourne district revealed that of the 5 crops visited, 4 of them tested positive to CeMV. This may have important consequences not only for the future sustainability of celery but also for carrots in the regions.

b) Celery and Herb Survey

Celery and herb crops in the celery growing regions of Victoria were surveyed in April-June 1998. On most properties virus-like symptoms were observed and the level of infection was estimated by eye. The results from the survey of celery and herb crops in Victoria (April-June 1998) in the various districts are presented in the tables following.

Table 1. Results from survey of celery growers in the Clyde-Cranbourne district

Grower	Crop	Estimated level of infection	Age of crop (weeks)	Seedling source (in or out of district)
1.	Summit	49 %	13	in
	Summit	32 %	10	in
	Summit	25 %	8	in
2.	Summit	31 %	14	in
	own *var.1a.*	10 %	13	in
	own *var.1b.*	30 %	8	in
	own *var.1c.*	66 %	8	in
3.	Green Giant	20 %	7	in
	Green Giant	9 %	11	in
4.	Green Giant	22%	5	in
5.	Green Giant	42%	6	in

Table 2. Results from survey of celery and herb growers in the Koo-Wee-Rup district

Grower	Crop	Estimated level of infection	Age of crop (weeks)	Seedling source (in or out of district)
1.	Green Giant	>90 %	15	in
2.	Parsley			
	a) Italian	0 %	>10	grown from seed
	b) curly leaf	0 %	8-9	grown from seed
	c) continental	0 %	16	grown from seed
	Dill	0 %	7-8	grown from seed
	Coriander	0 %	7-8	grown from seed
3.	Tendercrisp	0 %	>20	out
	Parsley	0 %	>10	grown from seed

Table 3. Results from survey of celery and herb growers in the Peninsula district.

Grower	Crop	Estimated level of infection	Age of crop (weeks)	Seedling source (in or out of district)
1.	Green Giant	0 %	4-5 & 10	out
	Summit	0 %	4-5 & 10	out
2.	Summit (a)	15 %	6	in
	Summit (b)	19 %	8-9	in
	Tendercrisp	17 %	8-9	in
3.	Tall Utah	<5 %	10	?
4.	Parsley	<1%	12	grown from seed
	Coriander	<1%	12	grown from seed

c) General observations and information:

- celery at 6-8 weeks looks far worse than at later stages. The celery crops appear to grow out of the severe effects of the virus. How and why this happens is still unclear.
- a comparison of Tendercrisp to Summit on one grower's property suggested that although the symptoms of CeMV are more dramatic and seem more severe on Tendercrisp, the estimated infection level of CeMV was similar for both varieties.

d) Infection levels of CeMV in celery seedlings in the nursery

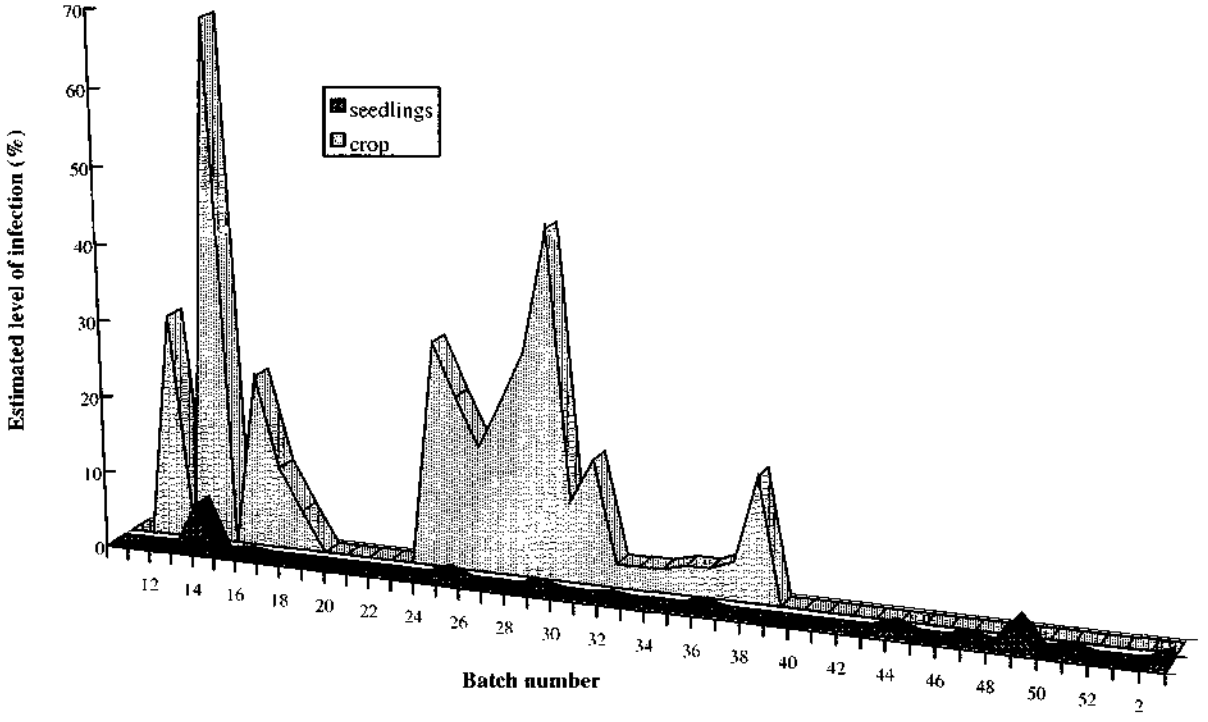
Results from serological tests of seedlings collected from a nursery show that the estimated level of infection of CeMV varies between 0 and 6.5% (Figure 1). There was usually no CeMV detected in nursery seedlings but if there was CeMV detected the estimated level of infection was very low. Only two batches of seedlings had infection levels higher than 3.1% and only one batch had an infection level higher than 6%.

The data of the estimated level of CeMV infection in the nursery seedlings together with aphid numbers are presented in Figure 2 and shows that when the aphid numbers increased so did the virus level after a 3-6 week lag.

e) Infection levels of CeMV in celery at harvest

Infection levels of CeMV in celery in the field was much higher than the celery seedlings in the nursery. Estimated levels of infection using serology tests (ELISA), varied in the field from 0 - 45% (Figure 1). Estimated levels of CeMV infection in celery crops were expected to be higher than in the nursery as the crops in the field have greater exposure to aphids and thus are more susceptible to virus infection. Figure 3 shows the estimated level of CeMV in the crop at harvest with aphid numbers: our data set is still incomplete but if the seedling infection levels do play a significant role in the incidence of CeMV in the field we expect higher levels of CeMV to be detected in the crop. Personal communications with several celery growers have suggested that levels of CeMV infection in their crops is expected to be high for those seedlings planted out in Autumn. This higher incidence of CeMV found in the field seedlings may be correlated with the Autumn aphid peaks.

Figure 1. Estimated level of infection in the crops and in the nursery seedlings versus batch number



NB: Levels of CeMV in the crop after batch 40 have not yet been determined using ELISA

Figure 2. CeMV infection of celery seedlings versus mean aphid catch per trap (3 week running mean prior to sampling)

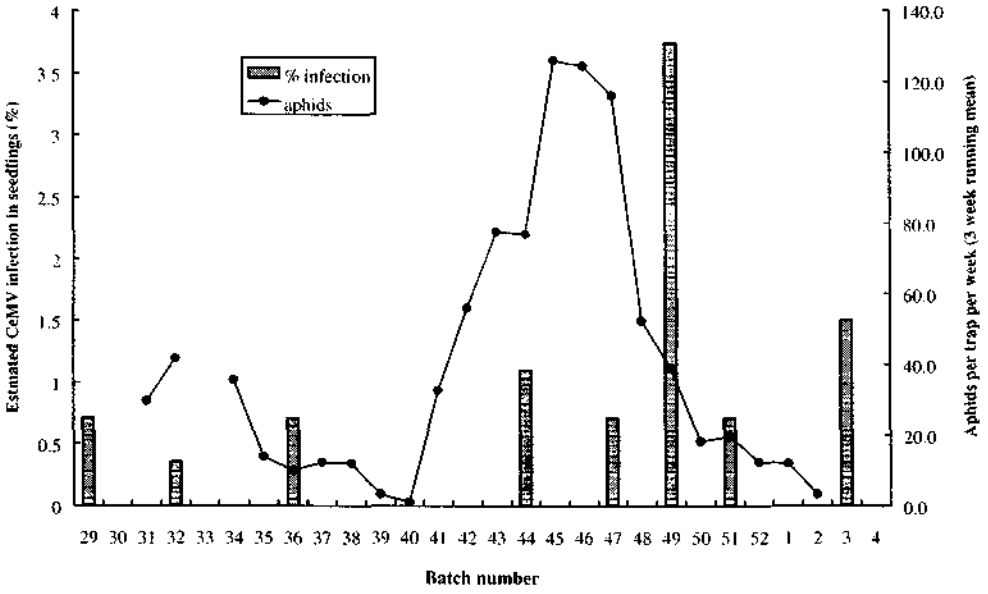
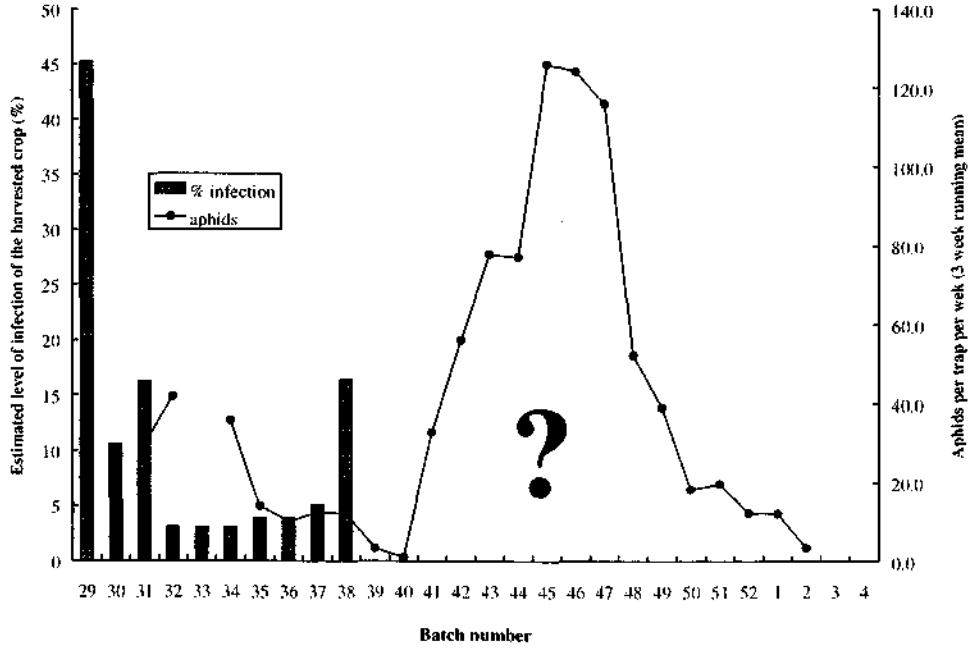


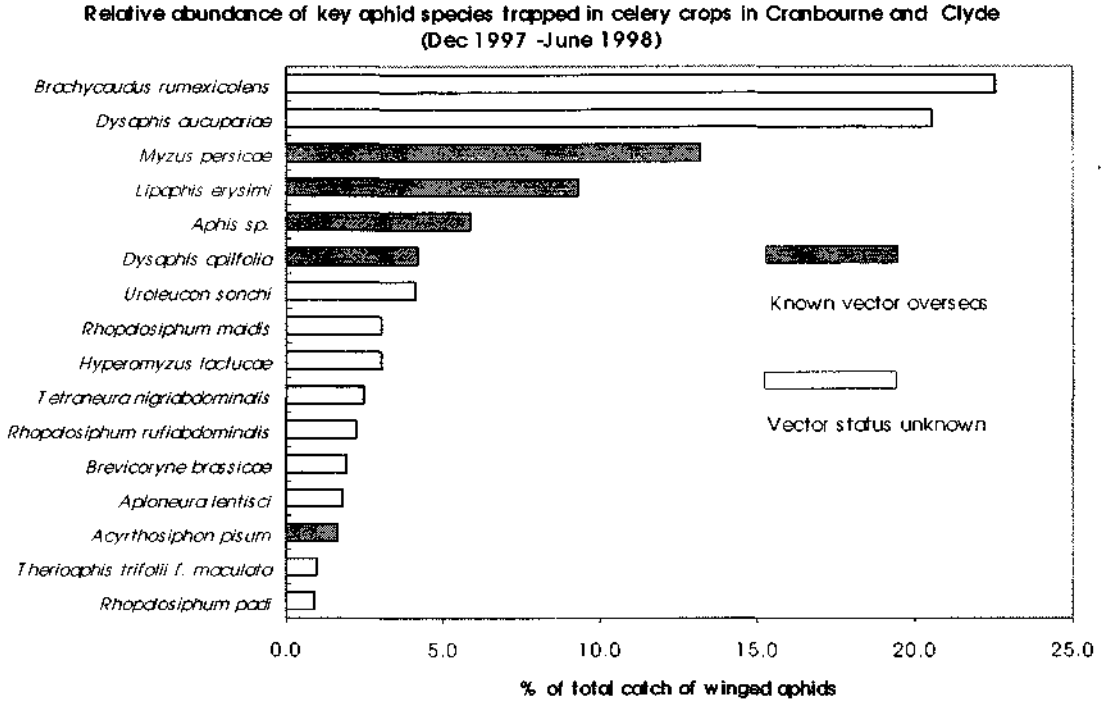
Figure 3. CeMV infection of celery at harvest versus mean aphid catch per trap (3 weeks running prior to transplanting) - virus infection yet to be determined for batches 40 onward



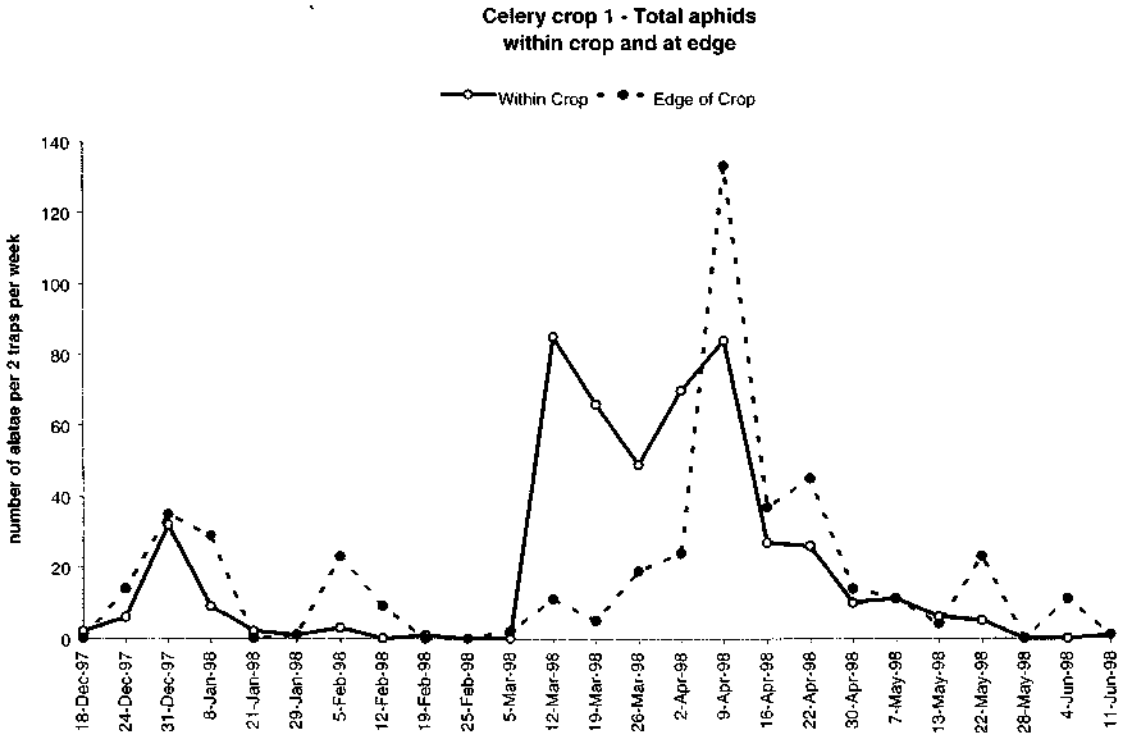
f) Aphid abundance and key species found in celery crops in Cranbourne & Clyde

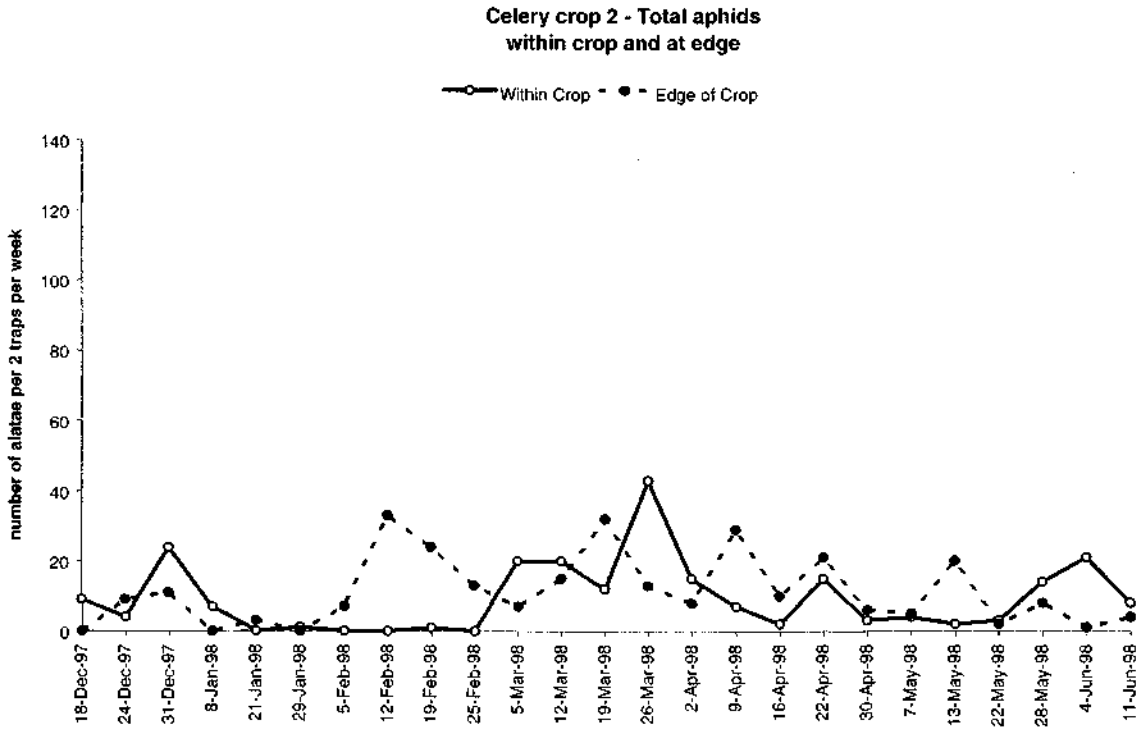
by Peter Ridland

Aphid trapping commenced in December and the data on aphid numbers and the key species found and their common hosts are presented below.



Aphid Species	Common Hosts
Brachycaudus rumexicolens	Dock
Dysaphis aucupariae	Plantain
Myzus persicae	Mallow, Celery, *Brassica*
Lipaphis erysimi	Brassica
Aphis sp.	Clover
Dysaphis apiifolia	Celery
Uroleucon sonchi	Sowthistle
Hyperomyzus lactucae	Sowthistle
Rhopalosiphum maidis	Sweet corn
Tetraneura nigriabdominalis	Grass roots
Rhopalosiphum rufiabdominalis	Grass roots
Brevicoryne brassicae	*Brassica*
Aploneura lentisci	Grass roots
Acyrthosiphon pisum	Clover
Therioaphis trifolii f. *maculata*	Lucerne
Rhopalosiphum padi	Grass





Aphid numbers were higher in Crop 1 with *Myzus persicae* being the predominant aphid trapped during the peak in March and April. The peak catch from the edge of Crop 1 on 9 April consisted largely of *Dysaphis aucupariae*, an aphid breeding on plantain (lamb's tongue). In Crop 2, the main aphid trapped was *Brachycaudus rumexicolens,* an aphid breeding on dock. We do not yet know whether these two abundant aphids are important vectors of CeMV.

g) Investigating the host range of Celery Mosaic Virus

By Bonny van Rijswijk

In Victoria, at present CeMV has been causing production losses for farmers growing celery. Because celery is a member of the Apiaceae family, it is thought that other cultivated plants, weeds and native plants belonging to the same family could also act as hosts for CeMV. In this situation, the use of a celery-free period is unlikely to prevent the occurrence of CeMV.

Cultivated Apiaceae

A glasshouse trial is currently being conducted at IHD, Knoxfield to determine which cultivated plant members of the Apiaceae family can indeed act as hosts for the Victorian isolate of CeMV. Plants being trialed include: anise, anise hyssop, caraway, carrot, celery, chervil, celeriac, coriander, Queen Anne's lace, dill, lovage, parsnip, parsley (Italian flat & curled) and sweet fennel. Plants have been mechanically inoculated with CeMV.

Weeds

There are six Apiaceae weed species found in Victoria: blue devil, fennel, hemlock, pennywort, wild parsnip and wild carrot. To date only seeds of fennel and wild carrot have been collected and these are included in the glasshouse trials.

Natives

The Australian native Apiaceae occur in cooler climates and in Victoria are found on the coast of the Mornington Peninsula. The native species of the Apium group includes sea celery (*Apium prostratum*), Australian celery (*Apium australe*), wild parsley (*Apium leptophyllum* and *Apium annum*). These native species are very difficult to identify as they vary quite considerably within the same species.

4. News from Queensland

By Denis Persley and John Thomas

Celery crops in the Granite Belt and Lockyer Valley were again surveyed in April 1998. Virus or virus-like symptoms were not seen on any plants during a thorough inspection of crops of varying age in the Granite Belt. Symptoms of celery mosaic virus were not found on crops in the Lockyer Valley. A few plants with symptoms suggesting alfalfa mosaic virus infection were found but virus could not be detected in the laboratory tests.

At this stage we have no evidence that CeMV occurs on celery In Queensland.

An extension article on the virus and means of preventing its introduction to Queensland crops was published in the Queensland Fruit and Vegetable News. A copy of this article is attached.

Celery Mosaic Virus

Queensland Fruit and Vegetable News

By Denis Persley and John Thomas, Queensland Horticultural Institute, Plant Pathology Group, Indooroopilly

Celery mosaic virus is one of the major diseases of celery worldwide and is currently causing problems for growers in southern States, especially Victoria.

Symptoms: The virus causes light-green/dark-green mosaic and mottling patterns on leaves. Prominent vein chlorosis may occur and leaves can be curled and crinkled. leaf size may be reduced and plants stunted with a flattened appearance. The severity of symptoms varies between varieties with Tendercrisp being severely affected. Cucumber mosaic virus can also cause similar symptoms and this virus has been frequently isolated from plants with mosaic symptoms in Victoria. Plants can be infected by both celery mosaic and cucumber mosaic viruses.

Spread: The virus is spread from plant to plant by aphids. A large number of species can transmit the virus with only very brief feeding periods required for transmission. Aphids can acquire and then transmit the virus to another plant during feeding periods of less than one minute. Celery mosaic virus is not known to be carried in celery seed. The

virus can infect crop and weed species in the celery family (Umbelliferaceae), for example, carrot, parsley, coriander and the common weed, slender celery.

Australian situation: A major outbreak of celery mosaic virus in South Australia in the late 1980s was a major factor in the demise of the industry in that State. The virus was first found in Victorian celery crops in 1996 and is now causing serious losses in production.

As a result of this outbreak, a national project was begun in 1998 with HRDC support to investigate the distribution and management of celery mosaic virus in Australia. The project is based at the Institute for Horticultural Development, Knoxfield, Victoria. The virus has recently been found for the first time in Western Australian celery crops as a result of surveys in this project. In Queensland, celery crops in the Granite Belt and Lockyer Valley have been surveyed for virus in 1997 and 1998. Celery mosaic virus has not been found. The only virus found has been a very low level of tomato spotted wilt virus.

Maintaining freedom from celery mosaic in Queensland: Queensland celery crops are apparently free of celery mosaic virus. To maintain this competitive advantage, it is important that:

- seedling plants of celery, carrot, parsley and other related species are not imported onto Queensland celery farms from southern States
- boxed celery from interstate production areas are not transhipped or stored on Queensland farms as infected plants and aphids may be present in these consignments

The year round production of celery in southern states favours spread of celery mosaic virus. The break in the production cycle in Queensland districts during winter or summer would be a major asset in limiting the spread of the virus should it be found in Queensland.

Celery crops will continue to be surveyed during the 1998/99 season for celery mosaic and other virus diseases.

5. WHO'S WHO IN CeMV

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The control of *Celery mosaic virus* - Quarterly Newsletter No 3.

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Edited by Violeta Traicevski

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1. Note from Jane Moran

Hi everyone and welcome to our Christmas/New Year edition of the newsletter. Some of you will be aware that the Celery Mosaic Virus project has undergone a major expansion to encompass the virus situation in carrots, and also to include three more states, NSW, SA and TAS. The contact details of the new people involved in the project can be found in the Who's Who at the back. We would like to welcome **Len Tesoriero** (NSW Ag), **Calum Wilson** (TIAR) and **Evita Alberts** (PISA) who will all be surveying carrot and celery crops in their states to determine if virus levels are significant. We would also like to welcome **Professor Adrian Gibbs** from the Australian National University who is working on the DNA fingerprinting of the viruses in carrots, celery and related crops.

In Victoria, **Bonnie van Rijswijk**, has joined the team and will be working very closely with Violeta. Recently Bonnie has been conducting a survey of the disease outbreak area to look for viruses in the weeds and native plants that may be harbouring the viruses we have in carrots and celery. She was working with **Sandy Cochrane** from the Royal Botanic Gardens in Melbourne and some of you may have met them as they traipsed around Cranbourne and the Peninsula.

The news from WA is interesting and Lindrea Latham has found carrot crops with very high virus levels. At present the team in Queensland is just about to begin a survey of the celery growing district. We still don't yet know how the virus is affecting carrots, and in the new year we will be working very closely with the Post Harvest Team at IHD to try and resolve some of these questions.

Although a little late, we hope that everyone has had a lovely Christmas and we wish everybody a happy and safe New Year.

Jane Moran

2. News from Victoria

a) Results from the preliminary Apiaceae survey in and around the major celery and carrot growing areas in Southern Victoria

Bonny van Rijswijk and Sandy Cochrane

The areas surrounding Apiaceae (carrot, celery, parsley and coriander) cropping land on the western edge of Gippsland Plain were surveyed in early December 1998, to determine if CeMV was present in the native Apiaceae. Two regions were surveyed, each based on areas within a 10 km radius of the main carrot and celery cropping areas in South Eastern Victoria. The first region was around Boneo, on the south-western end of the Mornington Peninsula, and the second was a continuous area with Pearcedale, Clyde and Cora Lynn as its centres.

Nine species of Apiaceae were located and identified within the two search regions.

- ***Apium prostratum***

Within our search area *A. prostratum* subsp. *prostratum* var. *filiform* was found on primary sand dunes and cliff tops along the ocean-facing beaches of the Peninsula, and on the clay sides of creek ravines immediately upstream of their entrance to the beach. It was common along the southern and south-western coasts of the Mornington Peninsula.

- ***Berula erecta***

This plant was found once during the survey. It was found growing in sandy soils on a stream floor and in clayish soils up the stream bank.

- ***Centella cordiflia***

Centella cordifolia was found in damp depressions. Three collections were made, two from still inundated roadside depressions in grazing land, and one on a track through a disturbed bushland environment.

- ***Conium maculatum*** (Poison hemlock)

Hemlock was found in two localities: in a sandy foreshore at Flinders, protected form ocean spray by a thicket of *Leucopogon parviflorus* and amongst roadside weeds in grazing land near Pearcedale. Both infestations were relatively small (less than 30 plants), perhaps indicating they were recent introductions.

- ***Daucus carota*** (carrot weed)

Carrot weed was very common along weedy roadsides in the Cranbourne, Devon Meadows, Pearcedale, Dalmore, Koo Wee Rup and Cora Lynn areas. *D. carota* was found along roadsides in grazing and cropping country almost always growing amongst the abundant weedy grass *Phalaris aquatica*.

In the area around Cora Lynn, plants were present for up to 50% of the roadside length. Plants were commonly associated with roadside drains, with many growing on the upper halves of drainage ditches, and on the outside of drainage levee banks. However, plants were also often seen growing away from drains and, in some cases, on small rises.

D. carota was very rarely observed growing in shade. A handful of plants were seen growing in partial shade. The plants may be excluded by low light or by competition for soil moisture from trees and shrubs.

- ***Foeniculum vulgare***

Individual or small groups of Fennel (*Foeniculum vulgare*) plants were found throughout the survey region, growing along roadsides amongst weedy grasses. Fennel was not observed dominating the environment at any sites, as it is often observed to do in other regions.

- ***Hydrocotyle***

Hydrocotyle hirta/laxiflora (id?) was found growing in a variety of relatively undisturbed woodland types. It was most commonly found under Coast tea tree (*Leptospermum laevigatum*), where there was no competing ground flora and heavy shade. Several collections of *Hydrocotyle* were also made in *Eucalyptus obliqua* woodland. Hydrocotyle was not sighted in areas with weedy grasses.

- ***Trachymene anisocarpa***

Trachymene anisocarpa was found to be common on disturbed sandy soils, commonly under a canopy of Eucalyptus species. It was located at Five Way, Warneet, Cannons Creek, near Langwarrin and slightly north west of Cranbourne.

- ***Xanothosia huegelii***

Xanthosia huegelii was found only once during the survey, occurring in a largely undistributed bushland reserve within the Mornington Peninsula National Park. *X. huegelii* was common in the open understorey of an Allocasyarina community growing on shallow, rocky soils.

At present all the samples collected are being tested for CeMV using ELISA.

b) Preliminary results from a trial investigating the effects of coloured plastics mulches on aphid landing rates in celery crops

Brad Rundle and Violeta Traicevski

In October 1998, Brad Rundle set up a small field trial at IHD, to test the effects of coloured plastic mulches and their effect on aphid landing rates in celery crops. The trial was run for 10 weeks, with water traps being sampled twice weekly to monitor aphid numbers. Preliminary results have revealed that insects were generally more attracted to the bare ground as opposed to the coloured plastics and it seems that silver plastic was slightly better at discouraging insects to land than the white plastic (Figure 1). The results were similar for the total of aphids landing. Aphids tended to be found in higher numbers where there were no plastics laid and again silver was slightly better than the white plastic at discouraging aphids to land (Figure 2).

At present Brad is doing another replicate to take place early in the new year to confirm his preliminary findings.

Figure 1. Total insect numbers vs treatment.

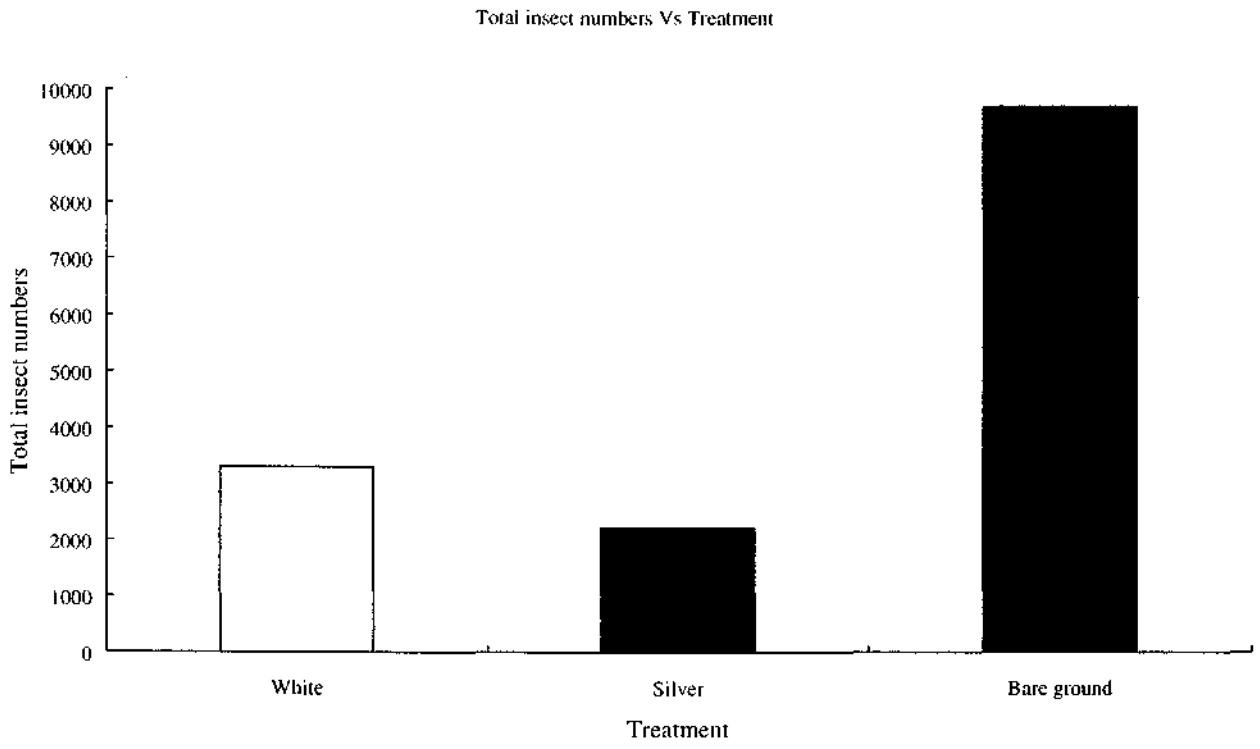
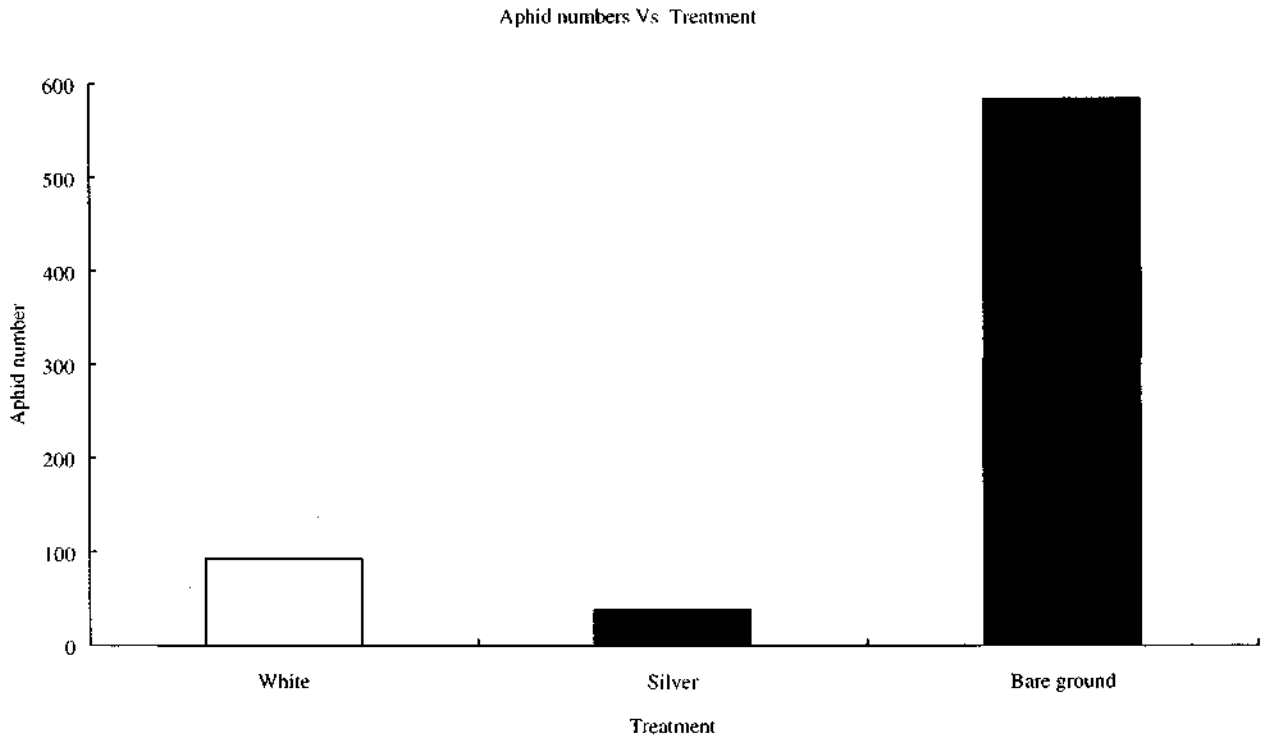


Figure 2. Total aphid numbers vs treatment.

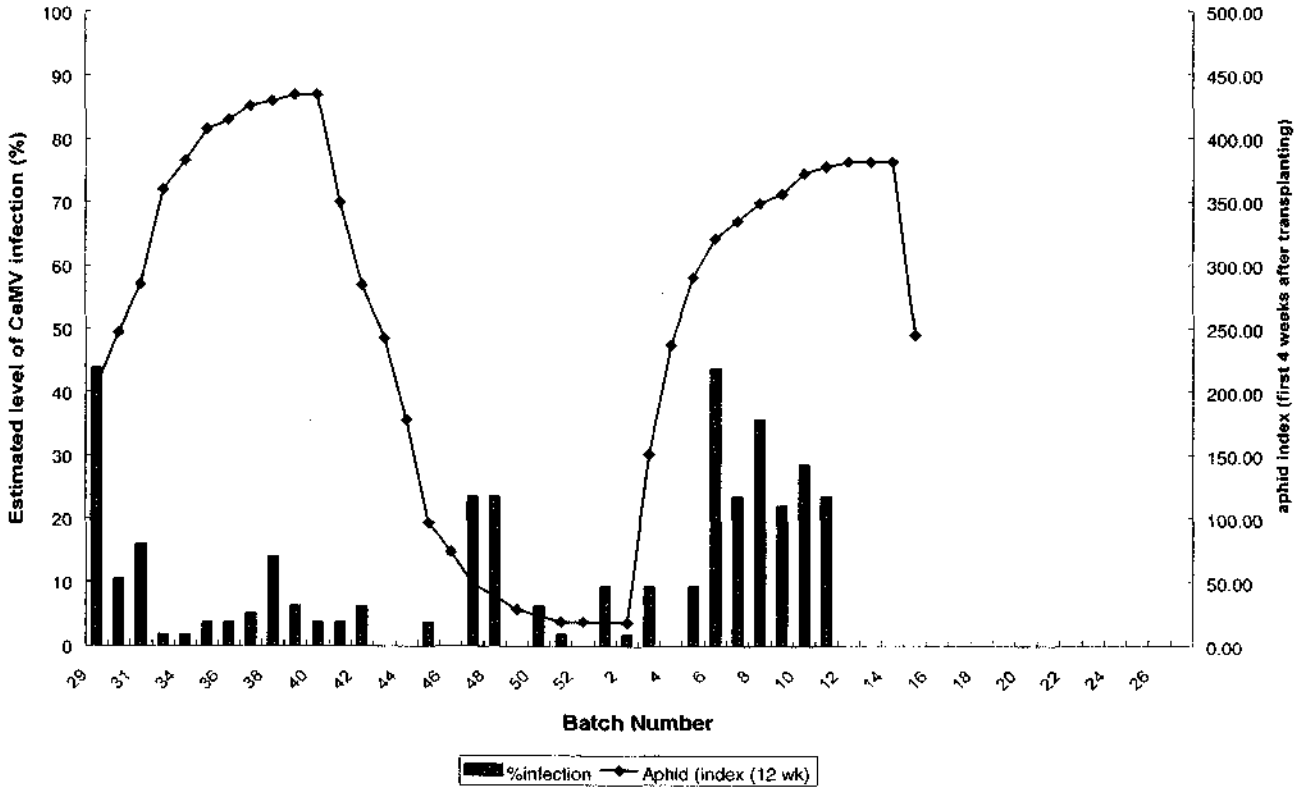


c) The incidence of CeMV in celery at harvest and aphid abundance in Clyde

Peter Ridland and Violeta Traicevski

Estimated levels of infection of CeMV in celery in the field using serology tests (ELISA) have shown that CeMV infection varies from 0-43.7%. Figure 3 shows that aphid numbers increased in both the Autumn (1997) and Spring (1997) but the exact effect of the high aphid numbers on the incidence of CeMV is not known.

Figure 3. CeMV infection of celery at harvest versus mean aphid index 4 weeks after transplanting.



d) What is being planned at IHD

*i) **Oil spray trial.*** Oil sprays are the next thing being trialed at Knoxfield (as a means to control the spread of CeMV). Control of aphid-borne non-persistent viruses with the use of oil sprays is aimed at interfering with the transmission process. The spread of non-persistent viruses (eg. CeMV) in the majority of field situations occurs between crop plants (secondary spread) and only a relatively small part of the diseased population is comprised of plants infected by virus brought in from the outside (primary spread). Observations reported by other researches trialing oil sprays in the USA indicate that oil sprays can lose their effectiveness as the inoculum potential increases. Other factors that affect the effectiveness of oil sprays are: the numbers of winged aphids present, the titre of transmissible virus present in infected plants and plant density. Hopefully we will have some interesting results to report in the following newsletter.

*ii) **Mechanical transmission trials.*** Bonny will be further investigating alternate hosts for CeMV in glass-house trials. Preliminary results from the work done by both Bonny and Violeta last year show some interesting results but still need further rigorous testing before the results can be confidently reported. The new glass-house trial will evaluate whether some of native Apiaceae species collected by Bonny and Sandy in December 1998 are able to be infected with CeMV. The other Apiaceae included in the glass-house trial are: anise, anise hysssop, caraway, carrot, celery, chervil, celeriac, coriander, Queen Anne's lace, dill, lovage, parsnip, parsley and sweet fennel.

3. News from Western Australia

By Lindrea Latham

a) News about celery

The common celery production practice in and around the Perth area is to plant the celery varieties Tendercrisp' and 'American Stringless' in rotation all year round. In early November 1998 we were contacted by a grower from the northern metropolitan horticultural growing area of Perth that we had visited in November 1997. In 1997, no CeMV was detected on his property but this time CeMV was found in all crop planted between late August to mid-October. The estimated levels of infection in these planting's (estimated by eye) ranged between 4% to 15%. As well as growing celery, the grower also cultivates other Apiaceaeous crops including coriander (*Coriandrum sativum*) and parsley (*Petroselinum* crispum) which are known alternative hosts of CeMV. The coriander and parsley on this property were tested for CeMV, but none was detected.

The celery inspected on this property showed typical CeMV virus symptoms: light and dark mottling between the leaf veins, the top of the plants had a flattened appearance and the leaf tips were narrow. Celery leaves from the farm were collected for CeMV testing in the laboratory. There were eleven different plantings from August through to October. The percentage of CeMV infection for each transplanting date was plotted over time (Figure 1). The results from the estimated levels of infections revealed that infection levels peaked in early October and rapidly declined towards the end of October. This can be attributed to the spring flush of aphid activity in the Perth area. Fewer plants with virus symptoms observed in counts in the later celery transplants can perhaps be due to symptoms not having enough time to fully develop.

Levels of CeMV infection levels estimated by eye were confirmed with ELISA on one random sample taken form the property. The visual estimate of virus levels in the crop was 12% but the ELISA testing revealed that 24% of the celery crop was infected. This suggests that the visual virus counts actually underestimate the true virus infection levels.

Batches of celery seedlings from a celery seedling nursery were also tested for CeMV in both 1997 and 1998. No CeMV was found which suggests that the nursery is not the virus source.

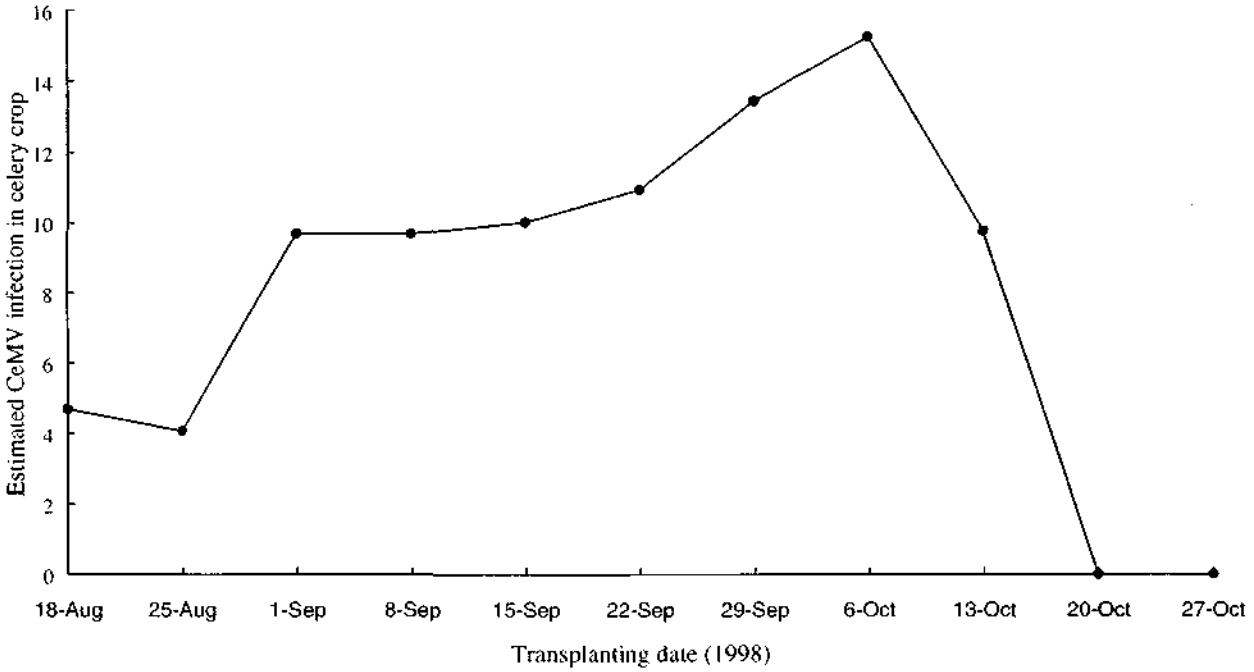


Figure 1. Estimated levels of CeMV infection in celery crop Vs. transplanting date

CeMV may become a limiting factor for celery production in the Perth area in the future. To date already one of the celery growers has stopped his celery production due to the losses he faced last year (60% CeMV infection levels in his celery crop (*var.* Tendercrisp)). The appearance of CeMV this year on other properties is a cause for concern in WA celery growers given the South Australian experience in the late 1980's which wiped out the celery industry and what has happened Victoria in recent years.

b) News about carrots

Western Australia accounts for more than 90% of Australia's carrot exports. The three major carrot growing properties that supply 75% of WA's domestic and export carrots were surveyed for carrot viruses and in particular CeMV in mid to late November. 4000 random leaf tips from different carrot plants were collected and tested for the presence of CeMV using ELISA. Only one sample tested positive for CeMV.

Until recently the only known virus infecting carrots in the Perth metropolitan area was the carrot motley dwarf virus complex (CMDV). However, our survey of this area has revealed that at one property, there are symptoms of an as yet unidentified virus which was found to be present in up to 65% of carrot plants causing great concern to the grower. The unidentified virus failed to react against antiserum specific to alfalfa mosaic virus (AMV), bean yellow mosaic virus (BYMV)

and a potyvirus monoclonal kit (*Agdia Inc.*™). Samples of carrots with the unidentified virus were forwarded to Adrian Gibbs (ANU) for identification using DNA fingerprinting techniques. Adrian found the virus to be a potyvirus which may or may not be related to CeMV. Work is continuing to identify the virus. A research station where carrot research is undertaken south of Perth was also visited. No CeMV was detected there, but a sample with virus-like symptoms was found but the virus remains unidentified. Further surveys of carrot growing properties south of Perth on the Swan Coastal Plain will be visited next month.

4. News from ACT

By Adrian Gibb and Anne Mackenzie

Work has started on the "DNA fingerprinting of viruses of celery and carrots" part of the project. Lots of potyviruses have been isolated by RT-PCR from celery and carrots collected by many collaborators around Australia. Even at this stage it is clear that those potyviruses from celery are different from those from carrots, as they yield different secondary fragments in addition to the specific ~1.6kb fragment selected by the potyvirid primers we are using. It will become clear when RFLP analysis and sequencing of the fragments are done, whether or not all that give one type of RT-PCR pattern are the same. Sequences have already been obtained form a Victorian and WA isolate of the celery virus, and they have been found to be nearly identical. Whereas that of a Victorian carrot isolate shows it to be closely related but distinct species, and that from *Conium maculatum* growing near Canberra is a third member of the cluster. We are making a major effort to obtain other potyviruses of Apiaceae from around the world, so that the relationships and possible sources of the Australian viruses are known. We are keen to obtain isolates of all Australian viruses, especially potyviruses of Apiaceae, both cultivated and wild, so please check your memories, fridges, gardens, etc. and contact us as soon as possible.

5. WHO'S WHO IN CeMV

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The control of *Celery mosaic virus* - Quarterly Newsletter No 4.

The Control of Celery Mosaic Virus

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Edited by Violeta Traicevski

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1. Introduction

By Jane Moran

We are now well over 12 months into the research program. Virus continues to be a problem in celery crops and control is going to be difficult. We are now learning much more about the viruses that are in the celery production area. Three viruses have been found: Celery Mosaic Virus (CeMV), a carrot potyvirus and a conium virus. The carrot and conium virus will soon have a name once Adrian and Anne have finished the DNA sequencing (p3).

The news from the Apiaceae survey is good and bad. The good news is that our Australian native plants were not found to harbour the carrot virus or CeMV. Unfortunately carrot weed and fennel (which are widespread) were found to harbour high levels of both carrot virus and CeMV (p 7). This means that weed control on roadside verges may become necessary. A very difficult proposition. We as yet have no evidence that the carrot and celery viruses are moving between the two crops. Work is continuing in this area. Bonny is preparing maps showing the location of the weeds and viruses for the next edition.

The oil spray trial is promising (p3). Virus infection appears to have been delayed despite some initial hitches with the spraying. This trial will be repeated in Spring when aphid pressure is again high.

Brad's work on the reflective mulches shows that aphid landing rates can be drastically reduced (p5). The challenge is to get the use of the reflective mulches cost effective for the industry.

Violeta has organised the carrot yield/storage trial (p9) and results should be available for the next newsletter. Hopefully, we will know by then whether the carrot virus is having a detrimental effect on carrot production.

News from interstate is mixed. Calum Wilson has found no virus in carrots grown in Tasmania (p10). Unfortunately CeMV has been found in Queensland celery crops (p11) with infection levels exceeding 50%. Cucumber mosaic virus is also a problem in these crops. This is very like the situation we first encountered in Victorian celery. At the beginning of the research program cucumber mosaic virus was found at high levels. For some reason it is no longer a problem in Victoria. We do not understand why, and hope that the same happens for the Queensland growers. One virus in a crop is enough to deal with.

Violeta will be attending the International Oil spray conference in Sydney in October where she will present the oil spray work. This is a great opportunity for her to make links with researchers overseas. Jane will be attending the Australian Plant Pathology Congress in Canberra in October and will

meet with the interstate project team members.

2. News from Victoria

i) Oil spray trial

By Violeta Traicevski and Bonnie van Rijswijk

A trial to investigate the use of oil sprays for virus control is well underway. The oil being trialed is D-C-Tron Plus®, a highly refined, emulsifiable, agricultural spray oil. The oil was initially developed to improve the targeting, spreading and wetting action of selected plant protection chemicals. Research done overseas has shown that the use of similar oil sprays has an effect on the transmission of viruses by aphids. The oil has been known to inhibit virus transmission thus reducing virus spread. In this trial plants were sprayed weekly. It must be noted that the oil can only be sprayed under certain conditions. It is important that the crop is dry and that the temperature does not exceed 30°C.

The results indicate that the oil has delayed the initial infection celery seedling transplants with CeMV. The incidence of CeMV early after transplanting is lower in the sprayed plots when compared to the unsprayed ones (Figure 1). We were concerned at one stage in the trial that cucumber mosaic virus may be present and confusing some of our results. Consequently we tested plants for cucumber mosaic virus and found that infection levels to be below 1%. This indicates that cucumber mosaic virus is not posing a problem for celery growers at present.

Although the estimated levels of CeMV in the field are still high and the sprayed and unsprayed plots do not differ significantly in levels of infection, it is important to note that the level of CeMV already present in the field is very high. Over time the use of the oil may potentially reduce the source of inoculum and thus reduce overall infection of CeMV in the field.

The long-term effects of using such an oil spray and its effects on yield and whether the spray oil can be used as a part of a management strategy for the control of CeMV is still unclear. The current trial will soon be harvested and the effect of the oil spray on yield and quality will be measured.

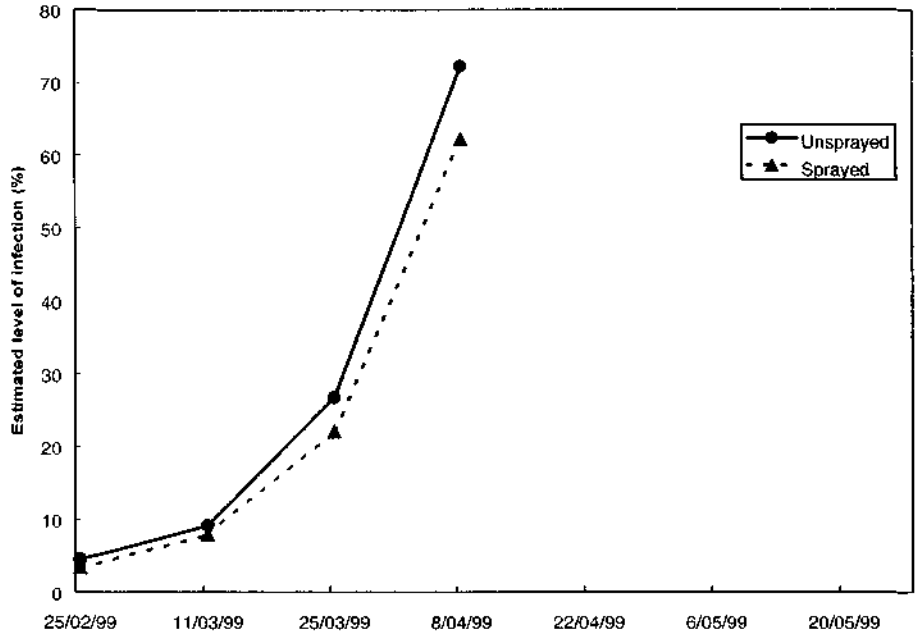


Figure 1. Estimated level (%) of CeMV infection at the time of transplant (25/02/99) and the level of infection over time in the field.

ii) Reflective mulches and aphid landing rates

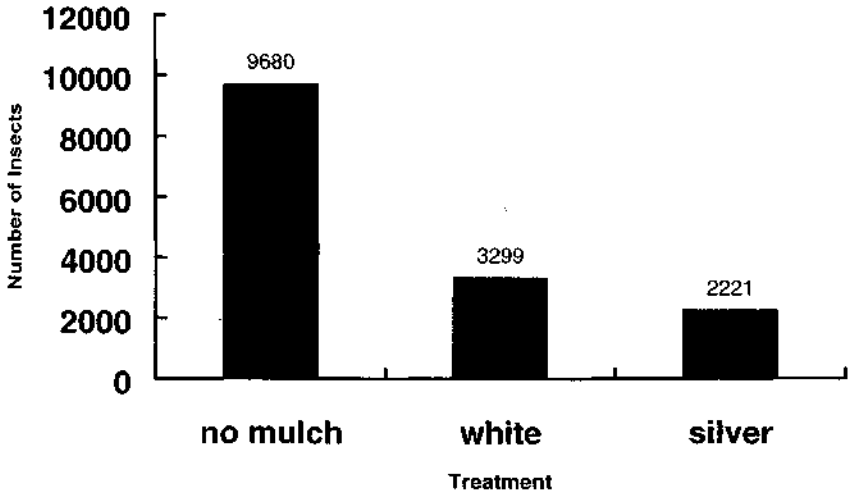
By Brad Rundle and Violeta Traicevski

As explained in the previous newsletter, Brad had undertaken two trials to investigate the effects of coloured reflective plastic mulches on aphid landing rates in celery crops.

The coloured plastics have been successful in deterring aphids as well as other insects from landing on celery in both trial 1 and trial 2 (Figures 2, 3, 4 & 5).

The effect of coloured reflective plastic mulches and their effect on CeMV levels in celery need to be tested and assessed in a field trial.

Figure 2. Trial One
Number of insects per treatment
(n=15200)



Trial Two
Number of insects per treatment
(n=6018)

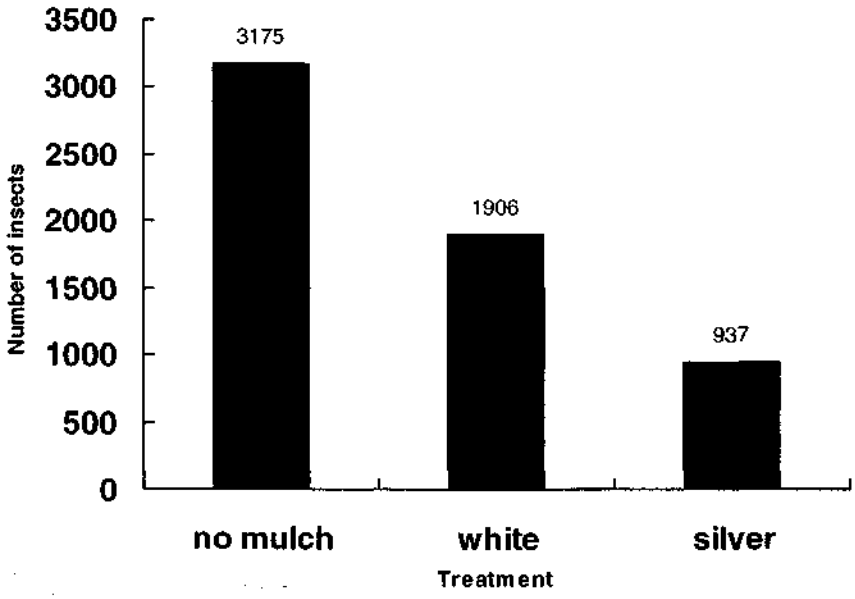


Figure 4. Trial One
Number of aphids per treatment
(n=714)

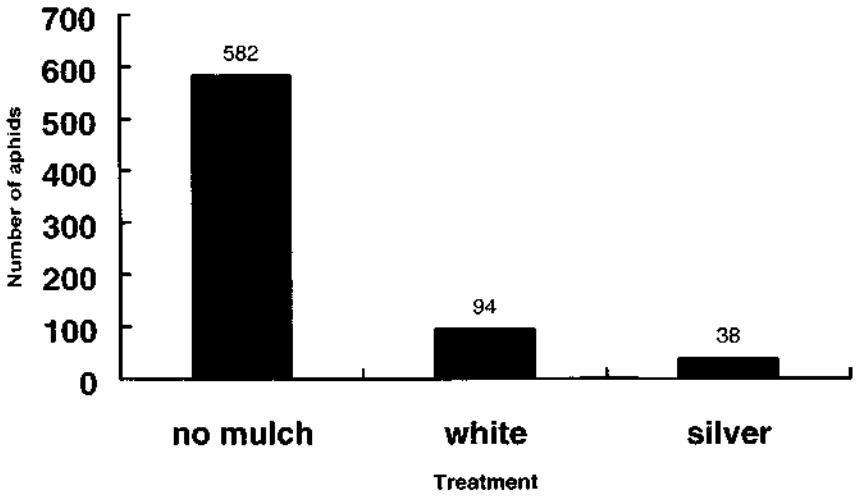
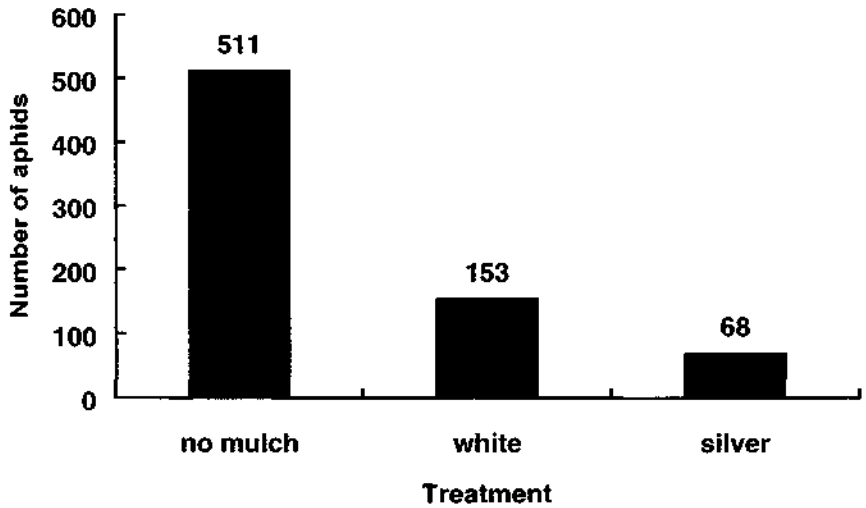


Figure 5. Trial Two
Number of aphids per treatment
(n=732)



The use of reflective mulches to discourage aphids landing on the celery crop has shown great potential to be used as a part of a management strategy to control CeMV. However, at present the cost of the reflective mulches seems to be cost prohibitive.

iii) Apiaceae survey report

By Bonny van Rijswijk, and Sandy Cochrane

The Apiaceae survey aimed to identify alternative hosts for celery mosaic virus within the native and weed plant populations in the celery growing district. The information collected from this survey will be used to help formulate a management plan to control CeMV. One of the proposed strategies is to have a celery-free period to reduce virus levels. However, if the virus is found in high levels in surrounding vegetation then this strategy may not be completely effective.

The results from the survey indicate that the native Apiaceae **do not** pose a threat to growers. No CeMV was found in the native samples collected, but other closely related species were found to be infected with CeMV. Nonetheless, even if CeMV was being harboured by the native Apiaceae, they have such a limited habitat and distribution that they would not be a threat to celery production. Virus was found in the weeds; and carrot weed looks to be a major source of virus.

Survey method and results:

Three major production areas of celery and carrots were surveyed for weed plant species belonging to the Apiaceae family (Table 3). Within each area, only sites that met the specific growing conditions of the Apiaceae were surveyed. All sites surveyed were on public land, such as roadsides, reserves and State parks, with the exception of flannel flower being grown commercially.

Ten plant species were identified: nine Apiaceae and one Chenopodiaceae. Each sample (consisting of leaves collected from one to six plants) was tested for CeMV, using ELISA. The results for the ELISA are presented in Table 4. All samples testing positive to CeMV were forwarded to Prof. Adrian Gibbs and Anne Mackenzie at the Australian National University (ANU) for genetic analysis to identify the strain of the virus.

Prof. A. Gibbs and Anne Mackenzie have identified three closely related viruses: CeMV, a carrot potyvirus and a conium (poison hemlock) potyvirus (Table 5).

Table 3. Three production areas surveyed.

Mornington Peninsula	Cranbourne/Clyde	Cora Lynn
Arthur's Seat	Baxter	Bayles
Boneo	Cannon's Creek	Cora Lynn
Cape Shanck	Cardinia	Iona
Flinders	Clyde	Tynong
Main Ridge	Cranbourne	Vervale
Rosebud	Devon Meadows	
Rye	Officer	
	Pearcedale	
	Somerville	
	Tooradin	

Table 4. Incidence of CeMV related viruses in native plants and weeds.

Plant species	Mornington Peninsula		Cranbourne/Clyde		Cora Lynn	
	Number of samples	Percentage positive	Number of samples	Percentage positive	Number of samples	Percentage positive
Native species						
Flannel flower	12	0%	1	100%	-	-
Native celery	38	2.5%	-	-	-	-
Centella cordifolia	5	0%	3	33%	-	-
Pennywort	16	0%	1	0%	-	-
Trachymene anisocarpa	-	-	9	0%	-	-
Xanthosia hueglii	1	0	-	-	-	-
Weed species						
Poison hemlock	6	0%	1	0%	2	0%
Feral carrot	-	-	91	19%	96	55%
Wild fennel	10	20%	8	37%	4	100%

Table 5. The types of virus found in native plants and weeds.

Region	Plant type	Strain
Flinders	Native celery	Conium virus
Rye	Native celery	Conium virus
Boneo	Cultivated carrot	Carrot virus
Clyde	Cultivated celery	Celery virus
Clyde	Wild carrot	Carrot virus
Tooradin	Wild carrot	Carrot virus
Tooradin	Wild carrot	Celery virus
Cora Lynn	Wild carrot	Carrot virus

iv) Update on CeMV in carrots

By Violeta Traicevski and Bonny van Rijswijk

Our assessment of the carrot potyvirus-like effect on carrots is in its preliminary stages. Two carrot crops were surveyed and ELISA diagnosis suggested that the level of virus in the two crop varieties varied between 4 and 11%. Symptoms were very difficult to identify at the 20-week stage. Recently however, distinct mosaic symptoms have been identified on carrot tops and ELISA diagnosis confirmed the presence of CeMV – although this has yet to be confirmed with DNA fingerprinting. The estimated level of infection in the two varieties were 34.3% and 41%.

Carrots from these crops will be used to assess the effect of virus on yield, harvest performance and storage.

3. News from ACT

By Adrian Gibbs and Anne Mackenzie

- ***Update on the DNA fingerprinting of viruses affecting celery and carrots***

Genetic fingerprints obtained from different potyvirus isolates has confirmed the differences noted in our last report. These tests have shown that:

a) all 8 tested isolates from celery are of celery mosaic virus and this virus was also obtained from one feral carrot plant

b) all 9 tested isolates from carrot crops in Victoria and W.A are of a different, but closely related virus (possibly carrot thin leaf potyvirus) and this was also isolated from 5 feral carrot samples from Victoria

c) both tested isolates from wild poison hemlock plants in N.S.W are of a third closely related virus (possibly wrongly called the poison hemlock strain of CeMV in the U.S.A) and this was also isolated from two feral carrot plants in Victoria.

More isolates are now being tested and various type specimens obtained from overseas to extend the survey. We are sequencing part of the genome of many of these isolates – watch this space.

4. News from New South Wales

By Len Tesoriero

In February and March a survey for viruses in the Apiaceae in nurseries around the Sydney basin were completed. To date only one potyvirus in a pennywort (*Hydrocotyle* spp.) sample has been found. Fresh material was sent to the CeMV team at Knoxfield for confirmation of virus – which was identified as borderline positive. Samples were also sent to Adrian Gibbs in the ACT for DNA fingerprinting. This month I will be surveying the carrot crops in the Riverina.

5. News from Tasmania

By Calum Wilson

Nine carrot crops in Tasmania have been surveyed for CeMV. Samples of poor looking carrots were taken and a 100 leaf random sample was taken per crop. The "sick" looking carrots were tested individually and the 100 leaves were tested in groups of 10. All ELISA diagnosis tests were negative for CeMV and universal potyvirus.

6. News from Queensland

By John Thomas and Dennis Persley

CeMV has recently been detected on three properties in the Darling Downs area near Toowoomba in Queensland. The identification was based on electron microscopy and ELISA diagnosis using the German antiserum. This is the first record of CeMV found in celery in Queensland.

The incidence of CeMV in the crop of cv. American Stringless at harvest assessed by ELISA was 57%. The incidence of cucumber mosaic virus (CMV) in the same crop was 33% and 17% of plants had both viruses. CeMV was at three properties in close proximity to each other. At estimated levels of 10-50% in various planting's. Cultivars affected were Tendercrisp, American Stringless and Green Giant. On a fourth neighbouring property, CMV, but not CeMV was detected.

Plants of American Stringless and Green Giant infected with CeMV showed symptoms of mild stunting and chlorosis. Leaves were narrower with down-curled tips and a mild mosaic was evident. Severe vein chlorosis and stunting was evident in young crops in other districts in Queensland. Plants infected with CMV alone displayed a very mild chlorosis only.

7. News from South Australia

By Evita Alberts

i) The terrible 80's

South Australian celery growers experienced devastating losses due to CeMV during 1986-87 although the virus had not been observed in South Australian celery crops before this. The yield losses alone were very severe, but of even greater concern was the loss of quality resulting in severely reduced shelf life. Often exported produce would arrive in semi-liquid form at its destination.

The South Australian celery industry was quick to adopt both a celery free period and off farm seedling production strategies to break the cycle of virus transmission between successive crops, but many growers had already suffered enormous losses and the celery industry wound down not long after.

ii) The current situation in celery and carrot

Celery

South Australian celery production is now limited to a few growers mainly located in the Virginia area in the northern Adelaide plains. No research has been conducted on celery in South Australia since the late 80's to determine if CeMV has been controlled or to survey current practices in relation to celery free periods or off farm seedling production. The northern Adelaide plains

supports intensive mixed vegetable where celery and carrot crops may be grown in close proximity and the diverse weed populations are also more difficult to manage given the diversity of vegetable production.

Carrots

Carrot production at Virginia is practically for 12 months of the year with some farms producing both celery and carrots. Other carrot production areas in the state, apart from Virginia, include large farms grown under centre pivot irrigation located in Riverland and South East regions. These production areas are rigidly maintained as weed free as possible and are grown in isolation from related crops.

iii) Proposed research

Although the celery production areas in South Australia may be small, the risk of CeMV infection to our extensive carrot industry is of concern, particularly where celery crops may serve as an important source of CeMV infection to adjacent carrot crops.

Carrot and celery crops grown at Virginia will be the initial focus of the virus survey, as it seems there are more opportunities for the virus to exist in this environment than in the large isolated production areas. Discussion with growers also suggests that not all celery growers adhere to the celery free period or off farm seedling production control strategies.

Once survey results from Virginia have been obtained, to determine whether the virus is actually present, the survey will be extended to include the larger carrot production areas.

South Australia joined the CeMV project late in 1998 and has only recently obtained antiserum from overseas for virus testing. Sampling strategies have been discussed with project collaborators to ensure some standardisation of sampling protocols, and sampling will commence this month. It is proposed to collect random samples from celery and carrot crops in the Virginia area on a fortnightly basis. All samples with virus symptoms and weeds adjacent to the crops collected will be tested for virus.

The CeMV isolate from 1986 has been sent to Adrian Gibbs to determine its relatedness to other celery and carrot viruses.

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The control of *Celery mosaic virus* - Quarterly Newsletter No 5.

The Control of Celery Mosaic Virus

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Edited by Violeta Traicevski

VICTORIAN CELERY GROWERS ASSOCIATION

HRDC

Agriculture Victoria

A business of the Department of Natural Resources and Environment

Natural Resources and Environment

Victoria on the move

Table of Contents **Page**

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1. Introduction

By Jane Moran

Once again we have good news and bad news. We have at last named the viruses that we have found in carrots, celery and weeds (see Page 5). The carrot virus we have named **carrot virus Y (CVY),** and the virus found in hemlock and the native Apium we have named **Apium virus Y (AVY).** The good news for the celery and carrot growers is that the viruses do not seem to be moving from carrots to celery and vice versa. This means that a celery-free period has a good chance of effectively reducing the levels of **CeMV**. We do not have to contemplate the almost impossible Apiaceae free period that we originally thought may be necessary.

The bad news is for the carrot growers, Violeta has found that some cultivars suffer very badly when infected with **CVY** with yield losses of over 30% in the cultivar Steffano (see page 5). Other varieties seem to be hardly affected at all. We are beginning to wonder if some of the past unexplained poor crops have indeed been due to virus. The big danger for the carrot crops is the feral carrot that is common in the Cranbourne/Clyde area. These are cultivated carrots that have gone wild and they line many of the roadways in the district and we have found **CVY** in many of them. Unfortunately we have also found **CVY** in some of the new plantings up on the Murray.

The third virus **APY**, does not seem to be playing a role in the carrot or celery disease outbreaks. Adrian believes it is an Australian native virus, whereas **CeMV** and **CVY** appear to be fairly recent introductions into Australia.

The second spray oil trial is about to start and we hope that we have ironed out all the problems we identified in the first trial. From the first trial we know that the spray oil is suitable for celery, lets hope we can see some good effects on virus levels in this second trial. Finally good news from Tasmania, Calum has found no virus in carrot crops.

Staff update

Alexei Rowles has joined the Celery Mosaic team in Victoria replacing Bonny van Rijswijk. Alexei will be working closely with Violeta. Currently Alexei is setting up aphid colonies to do some virus transmission work in the laboratory and is working with Violeta on the second spray oil trial on celery. He is also helping with various things in the laboratory and the glasshouse. Bonny has moved on to other projects at IHD, and is now working on tobacco yellow mosaic virus and powdery mildew in tomatoes.

2. News from Victoria

i) Oil spray trial

By Violeta Traicevski and Bonnie van Rijswijk

The first trial to investigate the use of oil sprays for CeMV control is now complete. The oil spray trial was carried out for a period of 16 weeks on celery crops in the Clyde/Cranbourne district. As reported in the last newsletter, the oil trialed was D-C-Tron Plus™, a highly refined, emulsifiable agricultural spray oil. The oil was sprayed every 7-11 days in an attempt to control the spread of CeMV.

As this oil had never before been sprayed on celery the results showed that:

• **The oil spray delayed the onset of CeMV** (Figure 1). Although the oil spray did not prevent CeMV infection in the field it did delay the onset of CeMV. This is an encouraging result in a crop that was exposed to a high virus inoculation pressure. Over time, the application of the oil on the celery crop may potentially reduce the source of inoculum and thus reduce overall infection of CeMV in the field.

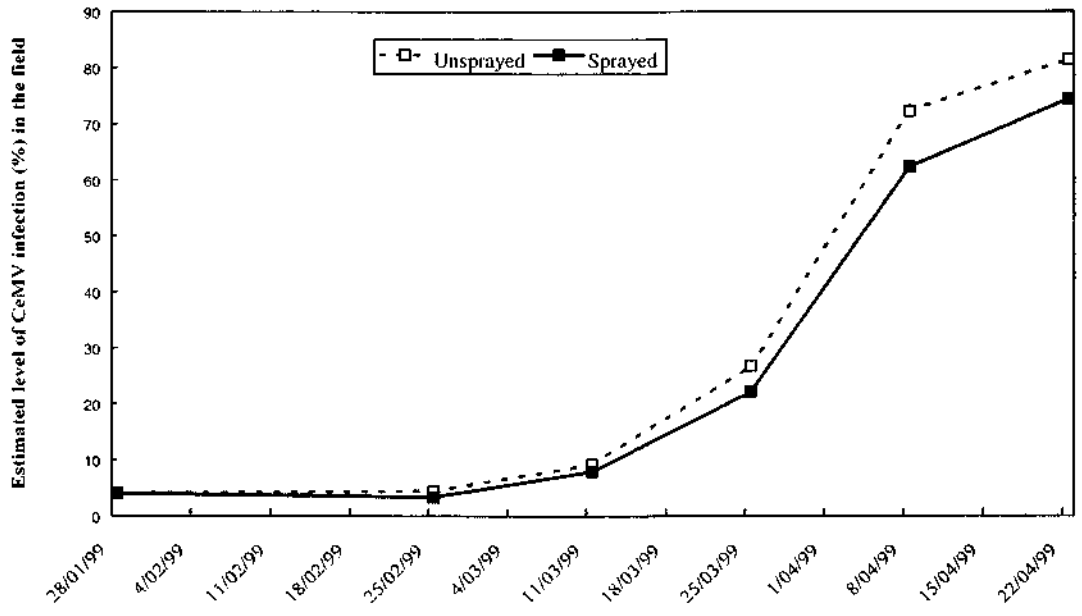

Figure 1. Estimated level (%) of CeMV infection at the time of transplant (28/01/99) and the level of infection over time in the field.

The celery trial was harvested and the effect of the oil spray on weight, length, base diameter as well as quality was measured. The summary of the results are presented in Figure 2. The results revealed that the oil spray had a significant effect on weight.

• **Celery sprayed with oil weighed less than the celery that was not sprayed** (ANOVA, P=0.003) (Figure 2). In fact those celery that were unsprayed weighed 5% more that those that were sprayed. Although weight was reduced, the weight difference can be attributed to:

- problems associated with phytotoxicity caused by an interaction of the oil spray and herbicide. The oil reacted badly with one of the herbicides and burnt the tips of the celery and compromised its normal growth habit thus the reduction in yield.

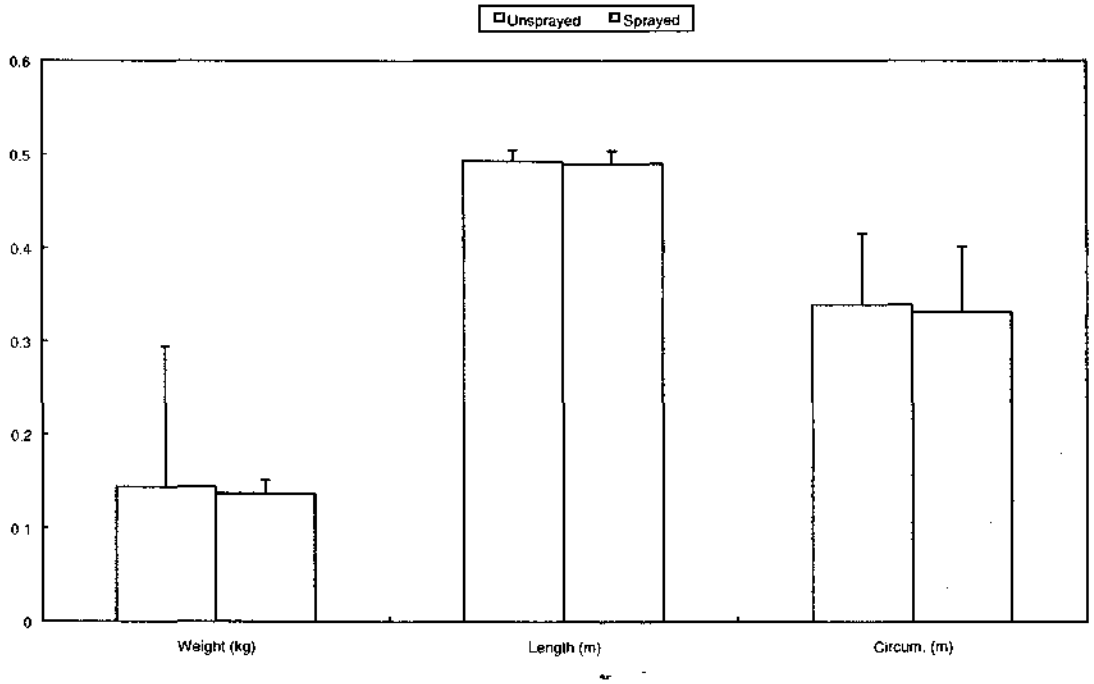

Figure 2. The effect of oil spray on celery weight, length and circumference of the root mean ± SEM.

• **Celery sprayed with oil were no taller than celery that was not sprayed** (ANOVA, P>0.05) (Figure 2). Thus spraying oil has no effect on celery height.

• **Celery sprayed with oil were no wider at the base than celery that was not sprayed** (ANOVA, P>0.05) (Figure 2). Spray has no effect of the circumference of the base of the celery.

• The percentage of celery culled in sprayed versus unsprayed plots were comparable – there was no effect of oil spray on the percent of celery culled.

The long-term effects of using such an oil spray and its effects on yield and whether the spray oil can be used as a part of a management strategy for the control of CeMV is still unclear and does need further assessment. Another oil spray trial is being planned for the Spring 1999.

ii) Virus names

Sequence analysis by Prof. Gibbs has revealed three different, but closely related, potyviruses: celery mosaic virus (CeMV), and two new potyviruses tentatively named Apium virus Y (APY) and carrot virus Y (CVY). Three other potyviruses were detected, one in pennywort (*Hydrocotyle* sp.) and one in parsley (*Petroselinum crispum*), both as yet undescribed, and a strain of clover yellow vein virus in *Ammi magus*.

The hosts and locations of the viruses are shown in Table 1. APY was detected in seven *A. prostratum* samples from VIC and in four *Conium maculatum* samples from VIC, ACT and NSW. CVY was detected in 10 carrot samples (cultivated and feral) from VIC, WA and QLD. CeMV was detected in eight isolates, seven from celery samples from VIC, SA, WA and QLD and in one feral carrot sample from VIC. These preliminary results suggest that in nature the potyviruses do not readily move between species. Further sampling and testing will be done to clarify the natural host range of these viruses and aphid transmission studies are planned to ascertain if the viruses can be experimentally transferred between hosts.

Table 1. Incidence of potyviruses in cultivated, native and weed Apiaceae in Australia.

Virus	**Host and location**
Celery mosaic virus	celery (WA, SA, Vic, Qld), feral carrot (Vic)
Apium virus Y	*Conium maculatum*, (NSW, Vic), *Apium prostratum* (Vic)
Carrot virus Y	carrot (WA, Vic, Qld)
Clover yellow vein virus	*Ammi magus* (ACT)
Unknown potyvirus	parsley (Qld), pennywort (NSW)

iii) The effect of virus on carrots

Yes, the virus does affect carrots, but it depends on the cultivar. The effect of virus on carrot yield (measured as weight (mg)), length of the root (cm) and the circumference of the top of the collar (mm) were investigated in five carrot cultivars: Senior, Leonore, Nantes, Steffano and Red Brigade. The results are summarised below (Table 2, Figures 3, 4 , 5, 6 & 7).

Table 2. The effect of virus on carrot yield (g), length of the root (cm) and the circumference of the top of the collar (mm) for five carrot cultivars.

Cultivar	Effect on yield	Effect on length	Effect on collar width	Effect on forking
Senior	NONE	NONE	NONE	NONE
Leonore	YES, 12.5% lighter	NONE	Yes, 4.8% smaller	NONE
Nantes	NONE	NONE	NONE	NONE
Steffano	YES, 33.3% lighter	YES, 11.6% shorter	YES, 16.9% smaller	NONE
Red Brigade	YES, 21.63% lighter	YES, 11.7% shorter	YES, 11.8% smaller	NONE

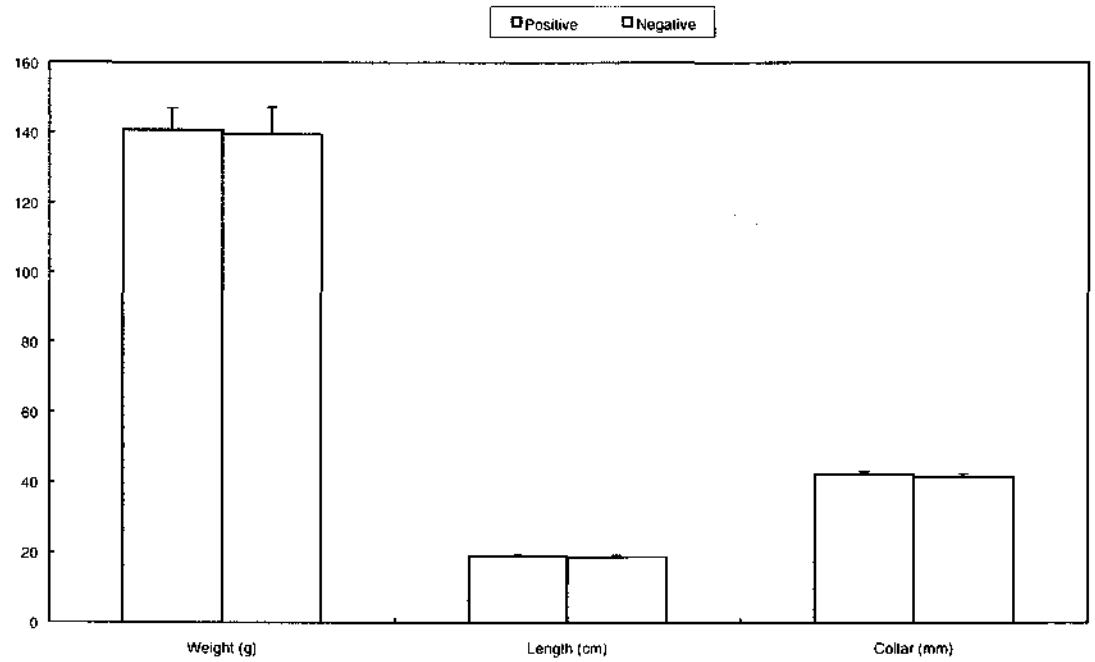

Figure 3. Carrot cv. Senior: the effect of virus on weight (g), length (cm) and collar circumference (cm).

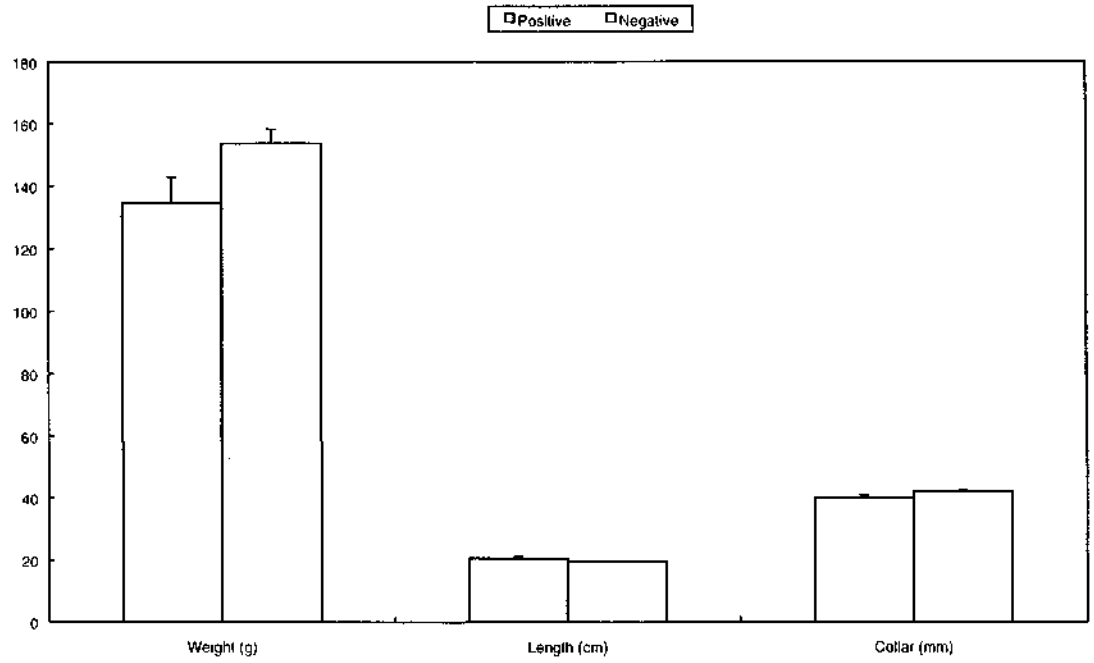

Figure 4. Carrot cv. Leonore: the effect of virus on weight (g), length (cm) and collar circumference (cm).

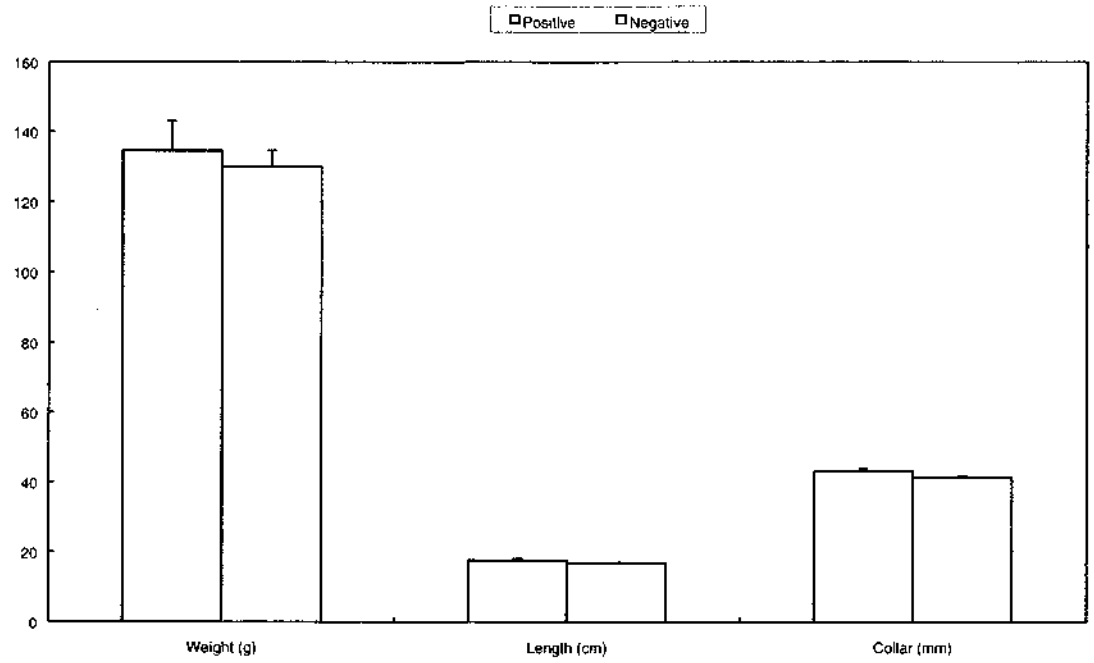

Figure 5. Carrot cv. Nantes: and the effect of virus on weight (g), length (cm) and collar circumference (cm).

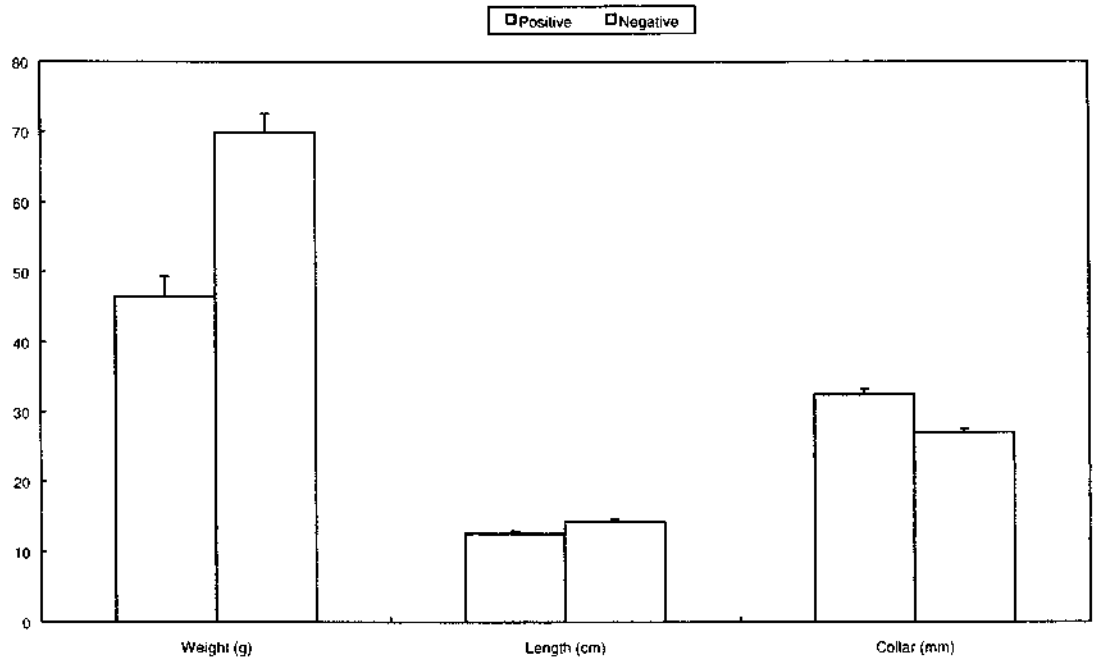

Figure 6. Carrot cv. Steffano: the effect of virus on weight (g), length (cm) and collar circumference (cm).

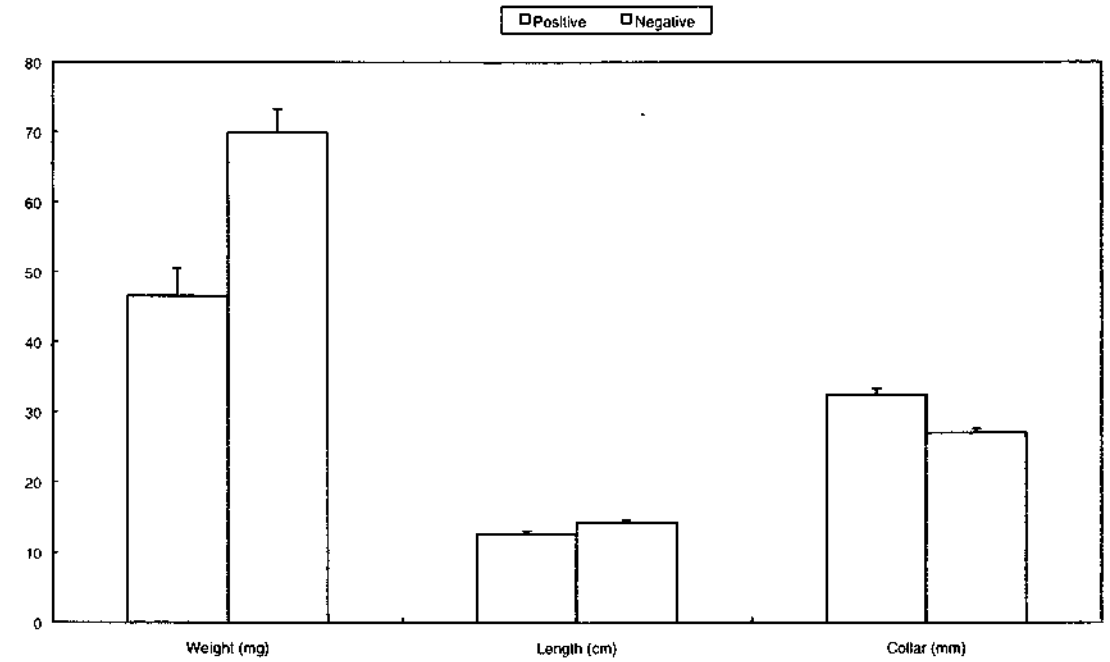

Figure 7. Var 5 – Red Brigade and the effect of virus on weight (g), length (cm) and collar circumference (cm).

Post-harvest performance of two cultivars: Leonore is still being assessed and results should be available for the next newsletter.

3. News from Tasmania

By Calum Wilson

Surveys for CeMV and general potyvirus infections were carried out during February and April of 1999 on 26 carrot crops and one crop each of parsnip and celery in North West Tasmania. 100 leaf samples were randomly selected from each crop and tested by ELISA in grouped samples of 10 leaves. Any plants with suspicious symptoms were also sampled and tested individually.

No CeMV was found in any crop, nor any potyvirus as determined from Agdia Universal Poty test kit. This reflects a similar survey done many years ago by D. Munro who found that the presence of potyviruses amongst the Apiaceae in Tasmania to be very infrequent. Seed source may also play a role in Tasmanian freedom of viruses in carrots. The majority of carrot crops tested were of Japanese origin seed for the export market. The results of the surveys are summarised in the table below (Table 3).

Table 3. CeMV in Carrot, Celery and Parsnip crops in North West Tasmania

CROP	CULTIVAR	DATE PLANTED	REGION	VISUAL ASS.	ELISA	DATE OF SAMPLING
Carrots	Kuroda	12/10/98	Kindred	-	-	11/02/99
Carrots	Kuroda	16/11/98	Forth	-	-	11/02/99
Carrots	Kuroda	18/11/98	Melrose	-	-	11/02/99
Carrots	Kuroda	17/11/98	Melrose	-	-	11/02/99
Carrots	Kuroda	25/11/98	Lake Barrington	-	-	11/02/99
Carrots	Kuroda	4/12/98	Sassafras	-	-	12/02/99
Carrots	Kuroda	8/12/98	Sassafras	-	-	12/02/99
Carrots	Kuroda	14/12/98	Sassafras	-	-	12/02/99
Carrots	Kuroda	17/12/98	Sassafras	-	-	12/02/99
Carrots	Kuroda	22/12/98	Sassafras	-	-	13/04/99
Carrots	Kuroda	18/12/98	Thirlstane	-	-	13/04/99
Carrots	Kuroda	30/12/98	Thirlstane	-	-	13/04/99
Carrots	Kuroda	4/01/99	New Ground	-	-	13/04/99
Carrots	Kuroda	12/01/99	Northdown	-	-	13/04/99
Carrots	Kuroda	18/01/99	Sassafras	-	-	13/04/99
Carrots	Hi Pak	5/11/98	Forthside	-	-	15/04/99
Carrots	Hi Pak	5/11/98	Forthside	-	-	15/04/99
Carrots	Hi Pak	4/11/98	Forth	-	-	15/04/99
Carrots	Hi Pak	26/11/98	Braddon's	-	-	15/04/99
Celery	Excelsior	10/12/98	Forth	-	-	14/04/99
Carrots	Hi Pak	30/01/99	Westleigh	-	-	14/04/99
Parsnip	Lamatina	12/11/98	Westleigh	-	-	14/04/99
Carrots	Hi Pak	10/12/98	Kindred	-	-	14/04/99
Carrots	Hi Pak	18/12/98	Kindred	-	-	14/04/99
Carrots	Hi Pak	30/11/98	Kindred	-	-	14/04/99
Carrots	Hi Pak	23/12/98	Abbotsham	-	-	15/04/99
Carrots	Hi Pak	18/12/98	Abbotsham	-	-	15/04/99
Carrots	Hi Pak	23/12/98	Abbotsham	-	-	15/04/99

Two dried historical Tasmanian samples of potyviruses from carrot and from hemlock collected by D. Munro have been sent to Prof. Adrian Gibbs for characterisation alongside current isolates from this project.

4. News from Queensland

By Dennis Persley and John Thomas

No further records of CeMV have been made since the first detection in Queensland on celery in April 1999. The four growers involved in this outbreak have agreed to have a celery free period in late winter.

Host range studies with Queensland CeMV and other potyviruses isolated from Apiaceae species in Queensland over several years are in progress.

Virus has not been detected in carrot crops surveyed in south east Queensland this season. This work is continuing. CeMV and other Apiaceae virus isolates were sent to Adrian Gibbs in the ACT as part of the genetic fingerprinting work.

5. WHO'S WHO IN CeMV

Victoria

- **Jane Moran** is a plant virologist with extensive experience in the control of virus diseases in horticultural crops

  Jane.Moran@nre.vic.gov.au

- **Peter Ridland** is an entomologist with experience in IPM programs and aphid ecology

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- **Brendan Rodoni** is a diagnostic virologist

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- **Violeta Traicevski** is an entomologist

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- **Alexi Rowles** is the new technical officer appointed to the CeMV project

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The control of *Celery mosaic virus* - Quarterly Newsletter No 6.

The Control of Celery Mosaic Virus

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Edited by Violeta Traicevski

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Victoria 3176

1. Hard decisions for industry

Jane Moran

We are coming to the close of this research project and are busily analysing results and writing reports. The project has been very interesting scientifically and has challenged us greatly to come up with practical solutions for the industry.

Our findings indicate that industry has some very hard decisions to make. We have shown that coloured plastic mulches have a lot of promise, but the cost is too high and would require a change to trickle irrigation. Oil sprays show some promise but phytotoxicity is a problem.

We also believe we have evidence that aphids **not** leafhoppers are spreading the virus (see next column).

The bottom line is that currently industry has two choices:

- grow tolerant varieties and live with the virus or
- instigate a break in production.

Our work has shown that a break in growing is likely to be successful, as we now understand the viruses tend to stay put in their own crops, ie. the carrot virus does not move into celery and vice versa. However, a break in production has far reaching ramifications for industry extending from the nursery right through to the marketing chain.

2. The leafhopper versus aphid debate: who is the culprit?

By Adrian Gibbs

Recently three viruses have been identified in Apiaceous weeds and crops, especially celery and carrots, in Australia. So far the main natural insect vectors of these viruses have not been definitively identified, but our work on the relationships of the viruses indicates the most likely vectors. There have been some concerns that leafhoppers have been spreading the viruses. We believe this not to be so and here is the evidence.

The potyviruses

The three viruses we have found are celery mosaic virus in celery crops, and two, new to science, that we have named carrot virus Y in carrot crops and wild carrots, and Apium virus Y in other weed Apiaceae, especially wild celery (*Apium* spp.) and hemlock (*Conium maculatum*). All are members of the potyvirus genus of the family Potyviridae; the biggest genus of the biggest known family of plant viruses.

How do the potyviruses spread?

All potyviruses that have been tested by scientists are transmitted in nature, and in experiments, **by aphids**, and some are also transmitted through seed. In the 1996 edition of "Viruses of Plants", which recorded the known properties of all plant viruses, there were records for **106 potyvirus species and 105 were known to be transmitted by aphids, and none by other vector organisms** (i.e. insects such as leafhoppers, nematodes, fungi, etc). All 99 tested had been shown to be transmitted by the aphids in the "non-persistent manner", meaning that they were acquired and transmitted by aphids when they were briefly probing plants, which often occurs when migrating aphids are seeking their preferred host plant species. Only 16 species of potyvirus out of 68 were transmitted by seed, 52 were not, and 3 species out of 20 were transmitted by pollen.

Why do only some insects transmit these viruses?

Vector transmission is usually a very specific property of each virus; some, such as 'myxoviruses' are transmitted by mosquitoes or fleas, which act as 'flying pins', but for the majority there is a very special relationship between the virus and its vector. Even a highly contagious virus like tobacco mosaic virus that is readily transmitted when tobacco plants touch, or when they are touched by contaminated tools, has never been transmitted by aphids probing infected and then healthy plants, although the virus is sometimes transmitted on the feet of the aphid. Likewise potyviruses have **not been** transmitted in tests with other 'plant bugs', such as **leafhoppers** or beetles.

How many aphids do you need to have an epidemic?

Each potyvirus may be transmitted in a crop by several different aphid species, but some will probably be more efficient than others. There are subtle specificities in the efficiency of different aphid species as vectors of aphid-borne viruses (n.b. potyviruses are not the only viruses transmitted by aphids, other viral genera use aphids too). Some aphid species are much better vectors of particular viruses than others; for example, in the U.K. in the 1950s and 60s, sugar beet crops were seriously damaged by beet yellowing viruses, and also by the direct feeding of

large populations of the black aphid, *Aphis fabae*. In glasshouse experiments the black aphids transmitted the yellowing viruses, but careful experiments showed that they were almost totally ineffective in the crops. In the field, almost all transmission was by the green peach-potato aphid, *Myzus persicae*, even though they were present in such small numbers that they were often difficult to find; the ratio of black to green aphids was 1000 to 1 or more.

An aphid species that doesn't like the crop can often do the most damage.

The main aphid vector of a potyvirus is often a species that does not normally settle and reproduce on that crop; potyviruses are transmitted by probing aphids flitting between plants, and so anything that keeps them on the move will enhance transmission. Thus, given that the vector aphid may come from elsewhere, and best transmits during very brief probes (2-10 seconds), it is not surprising that treating a crop with insecticide often has no effect on the spread of potyviruses. Indeed in some trials insecticides increased virus spread, presumably because they disturbed the aphids before it killed them.

How can we tell which aphid is doing the damage in celery and carrot crops?

Only field experiments over several seasons will firmly establish which aphid, or aphids, are the main vectors of the viruses of Apiaceae in Australia, and hence which plants are the source of the main vector species. Aphid specificities may be responsible for the fact that we have found only celery mosaic virus in celery crops, and only carrot virus Y in carrot crops in the same districts, even though in glasshouse tests both viruses happily infect both crop species.

So when you see leafhoppers in potyvirus-infected celery or carrot crops don't blame them. Very probably the flight of aphids that passed by over a fortnight ago is to blame.

3. News from Western Australia

By Lindrea Latham

Celery Mosaic Virus (CeMV)

Over the last couple of months, CeMV has been confirmed in celery using ELISA on three properties north of Perth on the Swan Coastal Plain. Virus incidence was high on two properties (between 40-90%) and substantial crop losses were and are still being experienced. Several other properties have celery that are showing typical CeMV symptoms in cv. Tendercrisp but are yet to be confirmed using ELISA.

The spread of the virus seems to be perpetuated by:

1. Continuous cropping all year around (usually cv. Tendercrisp) without a fallow break, the older crops providing a potent source of virus infection for newly planted celery seedlings and
2. The close proximity of many of the celery growing properties to one another, again providing the potential for spread from one property to the other.

Carrot Virus Y (CVY)

Since the last carrot survey (12 months ago) we have identified two more carrot growing properties in the Swan Coastal Plain with high levels of CVY.

Samples collected in early December 1999 from one of the properties showed carrots (cv. Murdoch) with severe root distortion and knobbiness. The vascular tissue was also severely distorted. Carrots with root distortion and knobbiness were linked with typical CVY symptoms on the shoots. Leaf symptoms included a chloritic mottle, general chlorosis and increased subdivisions of the leaflets giving a feathery appearance. Infected plants were stunted. Large areas of carrot plantings have been severely affected and in some instances the total crop has been unsaleable. This is causing considerable concern to the carrot industry.

The carrot situation appears very similar to celery production in that carrot growers plant carrots all year round providing a continuous virus source.

4. News from Victoria

i) Oil spray trial II

By Violeta Traicevski and Alexei Rowles

As reported in the previous newsletter (September 1999) the first spray oil trial showed great promise. The results of the second oil spray trial will be reported in the next issue.

ii) Update on the study of the effect of virus on the storage quality of carrot varieties Senior and Leonore

Two carrot varieties 'Senior' and Leonore', with and without virus, were tested to ascertain if the virus was detrimental to carrot storage life. Carrot var 'Senior' was stored for six weeks at 0°C and var. 'Leonore' was stored for 14 weeks at 0°C prior to quality assessment. The following parameters were measured:

- the presence of white blush at varying levels (from low to high by assigning values from 1-5),
- root turgor (from turgid to limp by assigning values from 1-5) and
- the colour of the internal root using a chroma meter.

The results revealed that the virus had no significant affect on the outturn quality. The variety Leonore however did store better than var. Senior.

iii) Publications

a) Paper presented at the 12th Biennial Australasian Plant Pathology Society Conference, 27-30 September 1999.

Potyvirus in the cultivated and wild apiaceae in Australian and the implementations for disease control.

Jane Moran[A], *Adrian Gibbs*[B], *Bonny van Rijswijk*[A], *Anne Mackenzie*[B], *Mark Gibbs*[B] *and Violeta Traicevski*[A].

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INTRODUCTION

Outbreaks of celery mosaic potyvirus (CeMV) have occurred in most celery (*Apium graveolens*) growing regions around the world. CeMV has been epidemic in Victorian celery crops for the past two years and we recently detected, for the first time in Australia, a potyvirus in carrots. A number of different strains of CeMV are known to occur in nature and carrots naturally infected with CeMV have been reported previously in the United States and Europe. The host range of these strains can vary but are restricted to plants belonging to the Apiaceae family.

Control strategies in the USA for CeMV are based on a celery-free period. In cases where the strain of CeMV is known to infect other commercial crops, the celery-free period may require expansion to include carrot, parsley and coriander crops. In the UK, the CeMV strain also infects local weed populations and in this instance a celery-free period is less effective. Consequently, knowledge of the particular CeMV strain is required to develop control strategies. This paper reports on a survey to determine the incidence and variability of viruses in the Apiaceae in Australia.

MATERIALS AND METHODS

Samples from cultivated Apiaceae with symptoms typical of a potyvirus infection were collected from around Australia. Isolates were also obtained from national and international virus collections reference isolates of carrot and celery potyviruses were obtained from Brazil (celery yellow mosaic virus and CeMV), The Netherlands (CeMV) and the USA (CeMV).

Total nucleic acid was extracted from each isolate and a specific fragment was amplified using potyvirus specific degenerate primers in a RT-PCR reaction. These fragments were then sequenced and a neighbour-joining tree calculated.

RESULTS AND DISCUSSION

Sequence analysis revealed three different, but closely related, potyviruses; CeMV, and two new potyviruses tentatively named Apium virus Y (AVY) and carrot virus Y (CVY) (Figure 1.). Phylogenetic analysis revealed that CeMV, CVY and AVY were most closely related to each other and that plum pox virus was their closest relative (Figure 2.). Three other potyviruses were detected, one in pennywort (*Hydrocotyle* sp.) and one in parsley (*Petroselinum crispum*), both as yet undescribed, and a strain of clover yellow vein virus in *Ammi magus*.

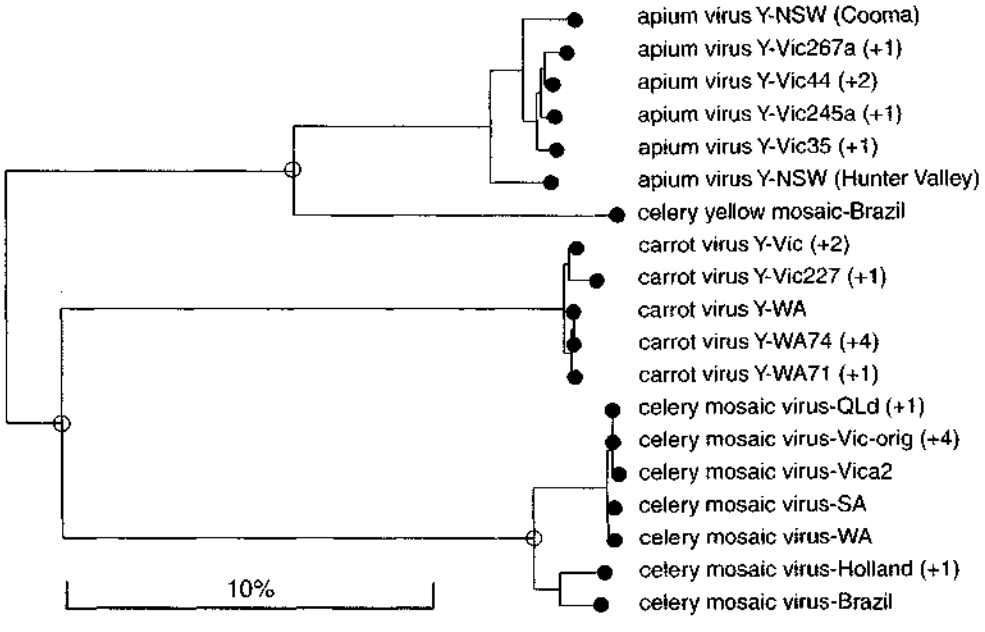

Figure 1. Phylogenetic neighbour-joining tree of 650 nucleotides from the NIB-VP region of the viruses found in the Australian Apiaceae.

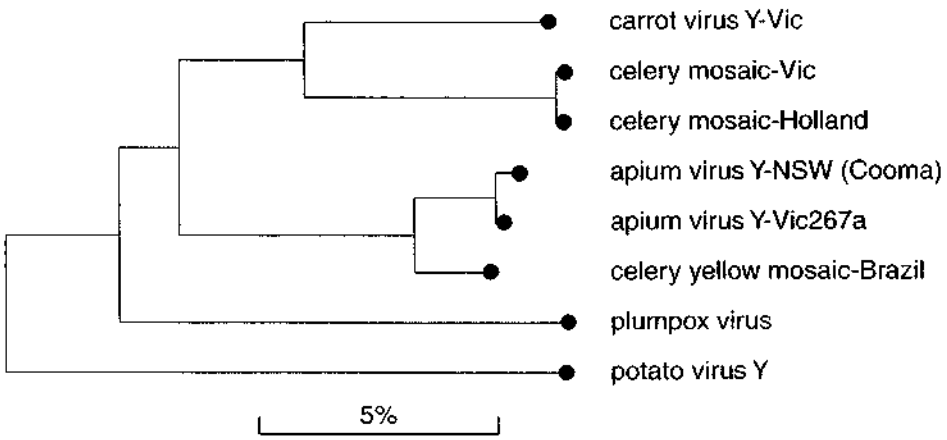

Figure 2. Phylogenetic neighbour-joining tree of 650 nucleotides from the NIB-VP region of the viruses found in the Australian Apiaceae and their nearest relatives.

The hosts and locations of the viruses are shown in Table 1. APY was detected in seven *A. prostratum* samples from VIC and in four *Conium maculatum* samples from VIC, ACTU and NSW. CVY was detected in 10 carrot samples (cultivated and feral) from VIC, WA and QLD. CeMV was detected in eight isolates, seven from celery samples from VIC, SA, WA and QLD and in one feral carrot sample from VIC. These preliminary results suggest that in nature the potyviruses do not readily move between species. Consequently a celery free period may be effective for CeMV control in Australia.

Table 1. Incidence of potyviruses in cultivated, native and weed Apiaceae in Australia.

Virus	**Host and location**
Celery mosaic virus	celery (WA, SA, Vic, Qld), feral carrot (Vic)
Apium virus Y	*Conium maculatum*, (NSW, Vic), *Apium prostratum* (Vic)
Carrot virus Y	carrot (WA, Vic, Qld)
Clover yellow vein virus	*Ammi magus* (ACTU)
Unknown potyvirus	parsley (Qld), pennywort (NSW)

b) Paper presented at the "Spray oils beyond 2000 - Sustainable Pest & Disease Management", Sydney (October 25-29, 1999).

Influence of petroleum spray oil on the incidence of celery mosaic potyvirus in celery (*Apium graveolens* L.)

Violeta Traicevski, Bonny van Rijswijk, Graham Hepworth, Peter Ridland and Jane Moran.

Institute for Horticultural Development, Agriculture Victoria, Knoxfield, Private Bag 15, South Eastern Mail Centre Vic 3176

ABSTRACT

Regular applications of a petroleum spray oil C24, Caltex DC-tron Plus®, applied every 7-11 days at 750 L/ha to a celery crop (*Apium graveolens*) were effective in reducing overall incidence of celery mosaic potyvirus (CeMV) and delaying the onset of CeMV. Enzyme-linked immunosorbent assay was used to estimate the level of virus infection in the field each fortnight. The sprays had no effect on celery quality; the number of celery culled or on the circumference of the celery bunches, although weights were marginally reduced in sprayed plots. This was caused by phytotoxicity with a herbicide. The results suggest that the spray oil could be used as part of a management strategy to control CeMV.

INTRODUCTION

Celery mosaic potyvirus (CeMV) was first diagnosed in cultivated celery (*Apium graveolens*) in Victoria in mid-June 1996 (Traicevski *et al.* 1999). Since then, the problem has spread throughout the Victorian celery-growing region with infection levels ranging from 5%-100% in celery with early-infected plants rendered unsaleable.

Spray oils have been shown to be effective in reducing the incidence of non-persistent viruses in the field (Bradley *et al.* 1962, 1966, Vanderveken 1977, Lobenstein & Raccah 1980, Simons & Zitter 1980). The oil appears to interfere with virus transmission by insects – brief contact between the labium and oil reduces both acquisition and inoculation (Powell 1992).

The purpose of our study was to investigate the effectiveness of petroleum spray oil for the control of CeMV in celery and to determine the effect of the spray oil on celery quality, the number of celery culled, the circumference of the celery bases and weight.

MATERIALS AND METHODS

Crop used and experimental design

Celery seedlings were transplanted into the field on 28/01/1999 (in the Clyde area, 50 km south-east of Melbourne, Victoria) and petroleum spray oil (C24 – Caltex DC-tron

Plus®) at a concentration of 0.75% was applied to the crop every 7-11 days using a boom spray (750 L/ha) in conjunction with the normal spraying regime implemented by the grower. The crop was irrigated regularly using fixed overhead sprinklers. The spray oil was first applied 11 days after planting, then on days 20, 28, 34, 41, 48, 55, 63, 70, and 78. The sprayed and unsprayed plots were arranged as four, 2×2 Latin squares, giving 8 replicates of the sprayed and unsprayed treatments. There were approximately 1200 plants per plot which were planted in 8 raised beds. Between each plot there were buffer zones of 8 raised beds.

Estimating virus levels in the field, and assessing weight and quality of celery

Every fortnight, fifty leaves were collected from each plot and tested for CeMV using enzyme-linked immunosorbent assay (ELISA) in batches of either 5, 2 or 1 depending on the virus levels observed in the previous fortnight. Estimates of infection percentage were calculated using the formula given by Burrows (1987). To assess fresh weight, fifty celery plants were collected using a random systematic sample from each plot. After trimming, each individual celery plant was weighed and length and circumference of the base measured. Quality of these plants was visually assessed at the packing shed and graded into four different grades: 20, 16, 12 and 9. These numbers correspond to the number of celery bunches that can be packed per box. The numbers of celery bunches left behind in the field in each of the sixteen plots were also counted to compare the number of celery culled between the sprayed and unsprayed plot.

The results were analysed using analysis of variance (ANOVA). For estimated infection levels over time, a separate curve was fitted for each plot, and the parameters of the fitted curve compared using ANOVA. The statistical program used was GENSTAT™.

RESULTS

Virus levels in the field at harvest and the effect of the petroleum spray oil

The mean levels of CeMV in sprayed and unsprayed plots was monitored in the celery crop over 12 weeks. The mean level of estimated infection of CeMV in the field 21days before harvest in the sprayed and unsprayed plots were 74.3% and 81.4% respectively. No ELISA tests were done in the last 21 days leading to harvest because any infection that was incurred in this time was unlikely to have an effect on weight, quality or number of celery as the infection would be too late. The relationship between the percentage infection and time was sigmoidal in shape and logistic curves were fitted. ANOVAs were performed on each of the four parameters of the fitted curves. This revealed that there was a significant ($P<0.001$) divergence of the

curves; celery sprayed with the oil had a lower incidence of CeMV. This implies that the spray oil is delaying the onset of CeMV.

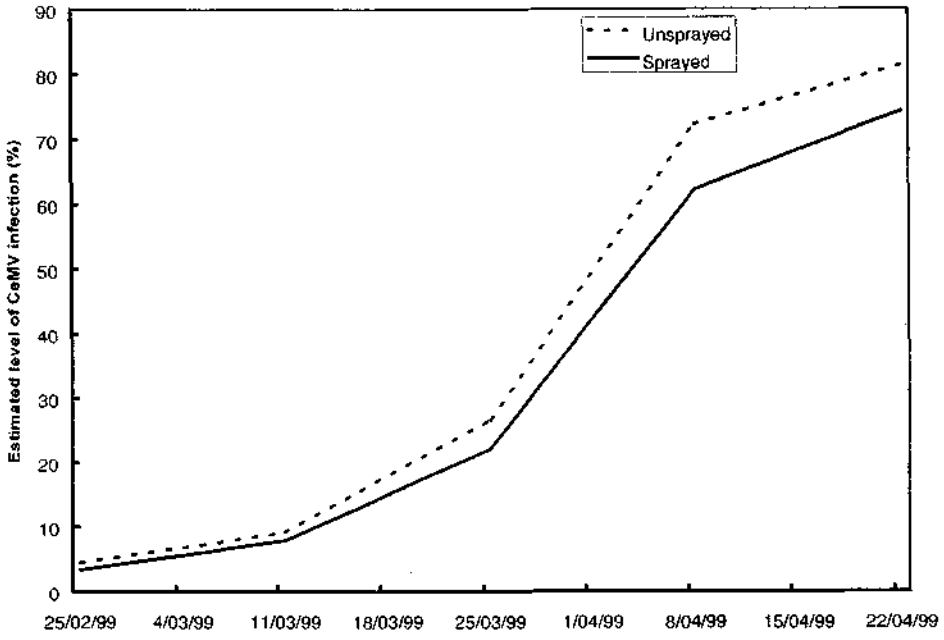

Figure 1. Results from ELISA analysis with estimates of virus infection levels in celery in the field over time.

Spray oil and its effect on celery fresh weight, length, circumference, quality and percent culled

Celery left unsprayed with oil had a marginally higher fresh weight (5%) than celery that was sprayed with the oil (Table 1). However, spray oil had no significant effect on the length, circumference at the base, quality, or percentage of celery culled (Table 1).

Table 1. Mean fresh weight, length, circumference, quality and number culled and the least significant difference (l.s.d) of celery in sprayed and unsprayed plots.

	Fresh weight (g)	Length (cm)	Circumference (cm)	Quality	% culled
UNSPRAYED	1439	49	33.0	15	7.6
SPRAYED	1371	49	32.4	15	5.5
l.s.d (P=0.05)	36	1.2	0.8	0.6	2.2

DISCUSSION

The estimated incidence of CeMV infection in celery in the field was delayed and reduced when sprayed with oil. A delay in the infection of CeMV can affect quality of the celery – later infected celery has a greater chance of reaching its full growth potential than celery infected early. Although the incidence of CeMV was lower in the sprayed crop and the quality of the celery was not significantly different, the mean fresh weight was 5% lower. This reduction in weight may be explained by the phytotoxicity caused by an interaction of the spray oil and a herbicide early after transplanting which burnt the tips of the celery compromising the normal growth habit.

A limitation of using the spray oil to decrease the level of CeMV in this trial was the high incidence of maximum temperatures exceeding 30°C. High temperatures meant that the crop needed to be watered more often, making the application of the oil difficult, as it could not be applied to a wet crop. The spray oil may have been more effective against CeMV if it had been applied more often in the early period after transplanting as celery infected early is more vulnerable to growth retardation.

Over time, the application of the spray oil on the celery crop may potentially reduce the source of inoculum and thus reduce overall infection of CeMV in the area. The fact that oil sprays did not affect the number of celery culled, although there was some phytotoxicity experienced in the crop, as well as the significant difference in estimated incidence of CeMV in the sprayed plots suggests that it has the potential to be used as a part of a management strategy to control CeMV.

In future trials to assess the role of spray oils for control of CeMV care will be needed to ensure good coverage of the plants, particularly in the immediate post-transplanting period.

ACKNOWLEDGMENTS

The authors would like to gratefully acknowledge all the staff at J. and J.M. Schreurs Pty. Ltd. for their time, help, and use of their celery paddocks in the spray oil trial. This work was supported by AusVeg and the Horticultural Research and Development Corporation.

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Appendix II. Conference presentations and publications.

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Research Strategies for the Management of Celery Mosaic Virus

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INTRODUCTION

CELERY PRODUCTION IN AUSTRALIA Celery production in Australia, in 1995, was 38 630 tonnes and valued at $22M. Exports of 4 400 tonnes valued at $3.4M, in 1996, were mostly to Malaysia (1060 tonnes), Taiwan (1330 tonnes), Singapore and Hong Kong. Celery is produced in all states with Victoria (17 200 tonnes) and Queensland (11 200 tonnes) being responsible for more than 60% of production.

CELERY MOSAIC VIRUS IN AUSTRALIA Celery mosaic potyvirus (CeMV) was first reported from a single grower in the Victorian celery growing district in 1996. The virus has since been reported through out the major Victorian celery growing district, infection levels ranging from 5% to 100% (based on visual symptoms), and infected plants are unsaleable. Overseas CeMV causes major crop losses and in South Australia, an outbreak in the celery growing district in the 1980's caused widespread economic damage (1). The incidence of CeMV in other major celery growing areas is unknown. Symptoms include a green to light green mottling, and malformation of the leaflets. Early infection can cause stunting and the petioles do not show a normal upright growth.

CONTROL Control of CeMV is difficult. Overseas celery mosaic virus is controlled by a celery free period for at least three months of every year. This also includes related crops from the Apiaceae (formerly Umbilliferae) family such as carrots. However, in England this has been shown to be less effective as CeMV inhabits the local weed populations. In Victoria, the industry has year round celery production as well as significant local weed populations and a celery free period may be difficult to implement if it is required. Research overseas has shown that at least 11 different aphid species can transmit CeMV of which at least two, *Myzus persicae* and *Aphis gossypii* are commonly found in Australia. There is no evidence for spread by seed or touch. Controlling spread of CeMV through chemical control of aphids has failed overseas (2).

CeMV is a member of the potyvirus group and several strains occur overseas (3). The strains vary in their host range yet CeMV is restricted to the family Apiaceae. Control for such a virus requires knowledge of which strains occur.

THE PROJECT "Management of Celery Mosaic Virus" is a project evolved through grower initiative following significant outbreaks of CeMV in the Victorian and South Australian industries. In response to the disease outbreaks nine key questions have been posed. Growers and researchers will work together in partnership to answer these question and manage CeMV in Australia.

THE NINE KEY QUESTIONS

1. Where is the virus?
Response Celery production areas in Victoria, Queensland and Western Australia will be surveyed for CeMV using GIS mapping techniques and ELISA for virus testing.

2. Are other viruses present in celery?
Response TSWV and CMV produce similar symptoms to CeMV and their presence will be assessed by ELISA in symptomatic celery plants.

3. What is the host range of CeMV?
Response Likely hosts will be challenged with CeMV in the glasshouse.

4. What strains of CeMV are in Australia?
Response This will be assessed by challenging alternate hosts in the glasshouse and DNA fingerprinting.

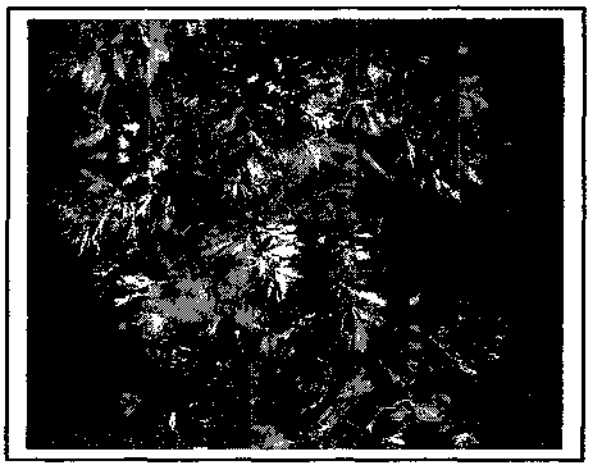
5. Is CeMV being spread on seedlings and is it seed borne?
Response Seedling nurseries will be sampled and tested for CeMV using ELISA.
Celery seed will be grown in the glasshouse and observed and tested for CeMV.

6. What aphids are spreading CeMV and where are they breeding?
Response Aphids will be trapped weekly in outbreak areas and identified. Likely vectors will be used in transmission experiments.

7. How far away from an outbreak is it safe to plant a celery crop?
Response GIS mapping techniques will be used to identify disease hot spots and this will be combined with aphid trapping data to determine zones of lowest risk.

8. Are certain times of year safer than others with respect to spread of CeMV?
Response This will be determined by identifying peak times of aphid activity.

9. Can any cultural practices, such as oil sprays, aphid repellents or reflective mulches, reduce virus spread?
Response Contact will be made with researchers and grower groups overseas to gather information.
With assistance from researchers in designing trials, growers will "do their own research" by carrying out trials on their own properties.



Celery infected with CeMV. Symptoms include: Green to light green mottling, and malformation of the leaflets. Early infection can cause stunting and the petioles do not show a normal upright growth.

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POTYVIRUSES IN THE CULTIVATED AND WILD APIACEAE IN AUSTRALIA AND THE IMPLICATIONS FOR DISEASE CONTROL

Jane Moran[A], Adrian Gibbs[B], Bonny van Rijswijk[A], Anne Mackenzie[B], Mark Gibbs[B] and Violeta Traicevski[A].
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INTRODUCTION

Outbreaks of celery mosaic potyvirus (CeMV) have occurred in most celery (*Apium graveolens*) growing regions around the world. CeMV has been epidemic in Victorian celery crops for the past two years and we recently detected, for the first time in Australia, a potyvirus in carrots. A number of different strains of CeMV are known to occur in nature and carrots naturally infected with CeMV have been reported previously in the United States and Europe . The host range of these strains can vary but are restricted to plants belonging to the Apiaceae family.

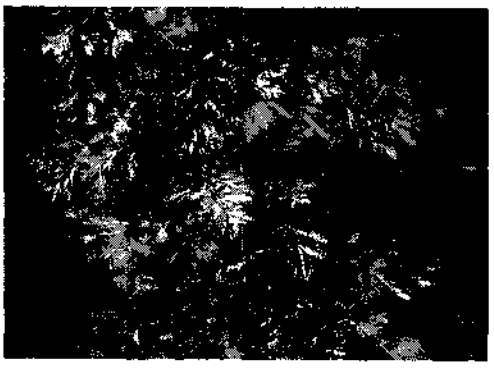

Figure 1. Celery mosaic virus in celery.

Control strategies in the USA for CeMV are based on a celery-free period. In cases where the strain of CeMV is known to infect other commercial crops, the celery-free period may require expansion to include carrot, parsley and coriander crops . In the UK, the CeMV strain also infects local weed populations and in this instance a celery-free period is less effective. Consequently, knowledge of the particular CeMV strain is required to develop control strategies. This paper reports on a survey to determine the incidence and variability of viruses in the Apiaceae in Australia.

MATERIALS AND METHODS

Samples from cultivated Apiaceae with symptoms typical of a potyvirus infection were collected from around Australia. Isolates were also obtained from national and international virus collections reference isolates of carrot and celery potyviruses were obtained from Brazil (celery yellow mosaic virus and CeMV), The Netherlands (CeMV) and the USA (CeMV).

Total nucleic acid was extracted from each isolate and a specific fragment was amplified using potyvirus specific degenerate primers in an RT-PCR reaction. These fragments were then sequenced and a neighbour-joining tree calculated.

RESULTS AND DISCUSSION

Sequence analysis revealed three different, but closely related, potyviruses; CeMV, and two new potyviruses tentatively named Apium virus Y (AVY) and carrot virus Y (CVY) (Figure 2.). Phylogenetic analysis revealed that CeMV, CVY and AVY were most closely related to each other and that plum pox virus was their closest relative (Figure 3.) Three other potyviruses were detected, one in pennywort (*Hydrocotyle* sp.) and one in parsley (*Petroselinum crispum*), both as yet undescribed, and a strain of clover yellow vein virus in *Ammi magus*.

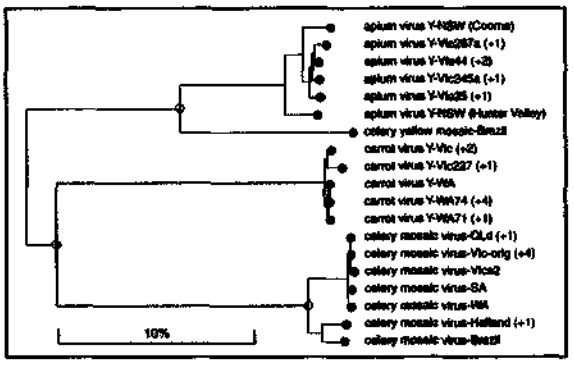

Figure 2. Phylogenetic neighbour-joining tree of 650 nucleotides from the NIB-VP region of the viruses found in the Australian Apiaceae.

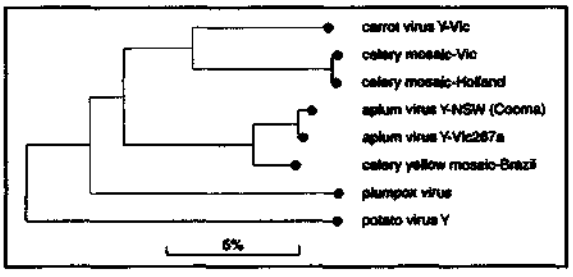

Figure 3. Phylogenetic neighbour-joining tree of 650 nucleotides from the NIB-VP region of the viruses found in the Australian Apiaceae and their nearest relatives.

The hosts and locations of the viruses are shown in Table 1. APY was detected in seven *A. prostratum* samples from VIC and in four *Conium maculatum* samples from VIC, ACTU and NSW. CVY was detected in 10 carrot samples (cultivated and feral) from VIC, WA and QLD. CeMV was detected in eight isolates, seven from celery samples from VIC, SA, WA and QLD and in one feral carrot sample from VIC. These preliminary results suggest that in nature the potyviruses do not readily move between species. Consequently a celery free period may be effective for CeMV control in Australia.

Table 1. Incidence of potyviruses in cultivated, native and weed Apiaceae in Australia.

Virus	Host and location
Celery mosaic virus	celery (WA, SA, Vic, Qld), feral carrot (Vic)
Apium virus Y	*Conium maculatum*, (NSW, Vic), *Apium prostratum* (Vic)
Carrot virus Y	carrot (WA, Vic, Qld)
Clover yellow vein virus	*Ammi magus* (ACTU)
Unknown potyvirus	parsley (Qld), pennywort (NSW)

POTYVIRUSES IN THE CULTIVATED AND WILD APIACEAE IN AUSTRALIA AND THE IMPLICATIONS FOR DISEASE CONTROL

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INTRODUCTION

Outbreaks of celery mosaic potyvirus (CeMV) have occurred in most celery (*Apium graveolens*) growing regions around the world and crop losses have been substantial. CeMV has been epidemic in Victorian celery crops for the past two years and we recently detected, for the first time in Australia, a potyvirus in carrots (*Daucus carota*) which reacted in enzyme-linked immunosorbent assay (ELISA) with antiserum made to CeMV (9). A number of different strains of CeMV are known to occur in nature (1) and carrots naturally infected with CeMV have been reported previously in the United States (6) and Europe (9). The host range of these strains can vary but are restricted to plants belonging to the Apiaceae family (2).

Control strategies in the USA for CeMV are based on a celery-free period (1). In cases where the strain of CeMV is known to infect other commercial crops, the celery-free period may require expansion to include carrot, parsley and coriander crops (3). In the UK, the CeMV strain also infects local weed populations and in this instance a celery-free period is less effective (5). Consequently, knowledge of the particular CeMV strain is required to develop control strategies. This paper reports on a survey to determine the incidence and variability of viruses in the Apiaceae in Australia. Serological methods for differentiating between potyviruses are not particularly successful (7) and therefore sequence analysis was used.

MATERIALS AND METHODS

Samples from cultivated Apiaceae with symptoms typical of a potyvirus infection were collected from around Australia. In Victoria an intensive survey of the outbreak area was conducted, and suspect weed, and native species were collected. Samples were screened by ELISA, using a DSMZ™ kit and protocols described by the manufacturer. Isolates were also obtained from virus collections around Australia. Reference isolates of carrot and celery potyviruses were obtained from Brazil (celery yellow mosaic virus and CeMV), The Netherlands (CeMV) and the USA (CeMV).

Total nucleic acid was extracted from each isolate and a specific fragment was amplified using potyvirus specific degenerate primers in an RT-PCR reaction (4). These fragments were then sequenced. All sequences were checked against the international databases using the BLAST programs. Sequences were aligned using CLUSTAL V and a neighbour-joining tree calculated.

RESULTS AND DISCUSSION

Sequence analysis revealed three different, but closely related, potyviruses; CeMV, and two new potyviruses tentatively named Apium virus Y (APY) and carrot virus Y (CVY). Three other potyviruses were detected, one in pennywort (*Hydrocotyle* sp.) and one in parsley (*Petroselinum crispum*), both as yet undescribed, and a strain of clover yellow vein virus in *Ammi magus*.

The hosts and locations of the viruses are shown in Table 1. APY was detected in seven *A. prostratum* samples from VIC and in four Conium maculatum samples from VIC, ACTU and NSW. CVY was detected in 10 carrot samples (cultivated and feral) from VIC, WA and QLD. CeMV was detected in eight isolates, seven from celery samples from VIC, SA, WA and QLD and in one feral carrot sample from VIC. These preliminary results suggest that in nature the potyviruses do not readily move between species. Consequently a celery free period may be effective for CeMV control in Australia. Further sampling and testing will be done to clarify the natural host range of these viruses and aphid transmission studies are planned to ascertain if the viruses can be experimentally transferred between hosts.

Table 1. Incidence of potyviruses in cultivated, native and weed Apiaceae in Australia.

Virus	Host and location
Celery mosaic virus	celery (WA, SA, Vic, Qld), feral carrot (Vic)
Apium virus Y	*Conium maculatum*, (NSW, Vic), *Apium prostratum* (Vic)
Carrot virus Y	carrot (WA, Vic, Qld)
Clover yellow vein virus	*Ammi magus* (ACTU)
Unknown potyvirus	parsley (Qld), pennywort (NSW)

AKNOWLEDGEMENTS

Thanks to John Thomas and Dennis Persley, QDPI, Len Tesoriero, NSW Agriculture, and Evita Alberts, PISA for virus isolates. This project was funded by the Horticultural Research and Development Corporation and the Department of Natural Resources and Environment.

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Influence of petroleum spray oil on the incidence of celery mosaic potyvirus in celery (*Apium graveolens* L. (Cornales: Apiaceae))

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Regular 1% (v/v) applications of a petroleum spray oil, C24 Caltex D-C-Tron Plus, applied every 7-11 days at 750 L/ha to a celery crop, were effective in reducing overall incidence of celery mosaic potyvirus (CeMV) and delaying the onset of CeMV. Enzyme-linked immunosorbent assay was used to estimate the level of virus infection in the field each fortnight. The sprays had no effect on celery quality, the number of celery culled or on the circumference of the celery bunches, although weights were significantly reduced in sprayed plots. This reduction in fresh weight in sprayed plots may have been caused by an interaction between the oil and a herbicide applied soon after the plants were transplanted into the field. The results suggest that the spray oil could be used as part of a management strategy to control CeMV.

CELERY MOSAIC POTYVIRUS - EPIDEMIOLOGY AND IMPLICATIONS FOR CONTROL IN CELERY (*APIUM GRAVEOLENS* L.)

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ABSTRACT

Surveys of celery (*Apium graveolens* L.) crops Australia-wide in 1998 and 1999 have shown an epidemic of celery mosaic virus (CeMV). Growers face huge financial losses. A major contributing factor to the CeMV epidemic in Australia is that celery is continually cropped which enables the virus to cycle through the crops. Control strategies overseas are based on a celery-free period, but Australian growers have been reluctant to implement this strategy.

Levels of virus infection in celery crops were correlated with aphid peaks in spring and autumn using yellow water pan traps both in the nursery (<1%) and the field (50-100%). Surveys of alternative Apiaceous hosts (native, weed and crop) have revealed two new potyviruses, apium virus Y (infecting native celery and poison hemlock) and carrot virus Y (infecting feral carrot). Research conducted overseas has shown that different coloured reflective mulches and/or the use of petroleum spray oils can reduce the spread of similar viruses. In trials, conducted at Knoxfield, silver and white reflective plastic mulches proved successful at deterring aphids from landing on the celery. Their use could be a viable control method for CeMV in celery, but this is currently not a cost effective option for growers. Petroleum spray oils were trialed on commercial celery crops and were found to be effective in delaying the onset of the virus. However, problems associated with phytotoxicity were encountered and need to be overcome.

REDUCING APHID LANDING RATES IN CELERY (*APIUM GRAVEOLENS* L.) USING REFLECTIVE MULCHES

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Reflective mulches were tested as a method of deterring aphids landing on celery. The aim was to use them as part of a control strategy for *Celery mosaic virus* (CeMV) in celery crops. CeMV is an aphid borne, non-persistent virus, mainly spread by winged immigrants. Flying aphids are known to be repelled by or attracted to different colours, and they are thought to be repelled by blue sky and attracted to plants and/or bare earth. This colour preference can be exploited for the control of aphids and the viruses they transmit. Reflective mulches have been used successfully in other crops to delay the onset of virus epidemics and or reduce virus incidence. Reflective mulches may also have some other benefits including yield increases and decreased fertiliser leaching.

Two randomised block field trials were conducted at Knoxfield to evaluate white mulch and silver mulch as a means of reducing aphid landing rates in celery. The white mulch was a low-density polyethylene, manufactured by Australia Challenge P/L, and IAMA Yarra Valley, Victoria, supplied the silver mulch. Aphid landing rates were monitored in the plots by using green-water pan traps. Aphids caught in these traps were collected, counted and identified weekly.

Significantly fewer alate aphids were trapped in celery grown with silver mulch than white mulch ($P<0.05$), and significantly fewer aphids were trapped in celery grown with white mulch than with no mulch ($P<0.05$). No insecticides were used in either of the trials and no colonising aphids were found. The results indicate that the use of silver mulches in an integrated management strategy may provide a benefit to farmers by deterring aphids from landing and thus potentially reducing virus spread and perhaps aphid infestations.

Appendix III. Technical reports and extension material

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Hand out to growers.

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Natural Resources and Environment
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Notes

Celery mosaic virus

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Celery mosaic virus (CeMV) is a virus disease of celery. CeMV was first identified in South Australia in the 1980's but has now spread throughout all Australian celery growing districts.

Symptoms

Infected plants have a mosaic pattern on the leaves (Figures 1, 2 & 3) and are stunted. Infected plants show an exaggerated rosette growth habit with varying degrees of leaf distortion. Symptoms can be confused with similar symptoms caused by *Cucumber mosaic virus*.

Suspect samples should be submitted to an accredited diagnostic laboratory for an accurate diagnosis.

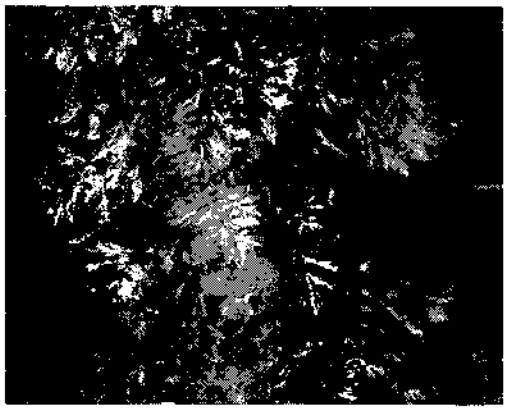


Figure 1. CeMV in celery

Figure 2. CeMV in parsley

Figure 3. CeMV in coriander

Spread and source of infection

The virus is transmitted from plant to plant by aphids. The most likely vectors are usually the winged form which migrates into the crop from surrounding crops and local vegetation. The virus is spread to healthy plants when an aphid probes on an infected plant, ingests a small amount of cell sap, and then probes on a healthy plant while the virus is on its mouthparts. Virus retention is usually low, due to the inactivation of the virus by the aphid's saliva. However, the aphid needs only to probe for a few seconds to acquire and pass on the virus.

Host range

CeMV is limited to the Apiaceae family. The virus has been recorded in Australia from celery, parsley, coriander and feral carrot.

Environmental conditions

The disease is usually most serious during late autumn and spring, following flushes of winged aphid activity.

Control

Virus diseases cannot be cured. An integrated program to manage the virus is the best approach:

- make sure seedlings planted in the field show no symptoms of virus infection
- make sure fresh seedlings planted out into the field are not planted next to "older" plants
- do not calender spray with insecticides to control aphids, sprays should only be applied as needed
- do not plant seed crops near the celery crop, and
- monitor crops to identify hot spots and avoid planting seedlings in this hot spot

Control in the crop

In the crop, the incidence of CeMV can be reduced using the following methods:

- re-plough in old crops as soon as possible
- do not crop continuously
- petroleum spray oils show great promise to inhibit the aphid from transmitting the virus, however when used in conjunction with other chemical sprays phytotoxicity may be a problem.

Control in the nursery

The key to control CeMV in the nursery is to prevent aphids carrying the virus from probing on seedlings. For example:

- the nursery should ideally be located outside of the celery growing district
- the glasshouse should be screened to be as "insect-free" as possible
- monitor the insects in the glass-house with sticky traps to minimise risk of virus spread

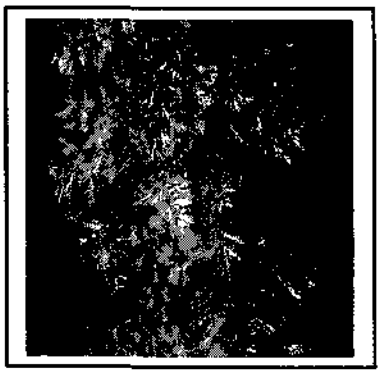
Calender chemical control of aphids, the vectors of CeMV, is of little use to growers. Aphids only need a few seconds to acquire the virus from an infected plant and can take only a few seconds to transmit the virus to another plant, hence continuous spraying is of little use. In fact, continuous calender spraying can increase virus spread. However, controlling colonising aphids in the crop may reduce secondary spread when aphids are in high numbers.

Further information

Registered chemicals:
Chemical Information Service
Ph. (03) 9210 9379

For effective pest and disease control, correct diagnosis is essential. A commercial diagnostic service is available at the Institute for Horticultural Development. For further information, contact the Diagnostic Service. ph: (03) 9210-9222 or fax (03) 9800 3521.

CELERY MOSAIC VIRUS (CeMV)



a) CeMV in celery

b) CeMV in parsley

What is CeMV & what crops are infected?

CeMV is a virus that infects celery crops but is also known to affect a variety of other related plants including parsley, coriander, chervil and dill.

The main symptoms are: mottling (light and dark green mottling on the stems and between leaf veins) and pithiness (distorted and cracked stems).

How is CeMV spread?

Celery mosaic virus is spread by aphids.

For further information please contact: Violeta Traicevski, Jane Moran or Peter Ridland at The Institute for Horticultural Development, Knoxfield. tel: 9210 9222

Appendix IV. Weed and Native survey in Victoria

A more comprehensive account of the Victorian survey is presented below.

Victorian Apiaceae Survey Report

Rationale

The survey of wild Apiaceae taxa was undertaken to assess the distribution of apiacous species, for guaging the extent of infection by CeMV, and for determining which strains of the virus were affecting which host species. It was anticipated that such information would aid in the identification of wild virus reservoirs and in the formulation of virus control programs.

Search area & personnel

In December 1998 three areas on the western edge of the Gippsland Plain were surveyed as part of the Mornington Peninsula Apiaceae survey. The areas were of 10km radius, each centred around a celery / carrot growing district.

Search method

The surveys were undertaken by road, with drive-by checking for conspicuous species (**Conium maculatum, *Daucus carota, *Foeniculum vulgare, Trachymene anisocarpa*) and on-foot surveys through roadside reserves, reserved land, the coastal strip and remnant bushland for the less conspicuous species (*Apium prostratum, *Berula erecta, Centella cordifolia, Hydrocotyle* sp. etc.).

Whenever an individual or colony of a target species was found a herbarium voucher and a sample for testing was collected. Where multiple plants were present at a collection locality a single leaf from up to six individuals was taken for viral analysis. Abundant species (ie. those present continuously or very commonly along roadsides) were collected at 1 – 1.5km intervals.

A voucher was made from one plant at each collection locality. A subset of vouchers have been added to the main collection at MEL (National Herbarium of Victoria) for permanent retention, and the remainder placed in storage for the duration of this project.

Results

<u>The far Peninsula – Boneo</u>

The southernmost search area covered a large portion of the lower Mornington Peninsula, centring on Boneo, and taking in Arthurs Seat State Park, Rosebud, Rye, Cape Schanck and much of the Mornington Peninsula National Park. This area incorporated a wide range of environments, including primary dune and ocean cliff vegetation, coast tea tree woodland, tall Eucalyptus forest, Allocasuarina woodland and weedy agricultural land.

* denotes naturalised species.

9 species of Apiaceae were found in this area:

* *Actinotus helianthi* was found once during the survey, at Baxter. The colony was on a residential building site, and may be destroyed soon.

Apium prostratum subsp. *prostratum* var. *filiforme* and second variety *A. prostratum* subsp. *prostratum* var. ? were both found growing along the primary sand dunes and coastal clay cliffs of the Mornington Peninsula National Park. *A. prostratum* was collected on fourteen occasions in this search area.

* *Berula erecta* was found once during the survey, growing in sandy soils on the stream floor, and in clayish soils up the stream bank of Main Creek.

Centella cordifolia was found once in a degraded bushland reserve at Rosebud South.

* *Conium maculatum* was found in a sandy foreshore situation at Flinders, protected from ocean spray by a thicket of Coast Tea Tree, *Leucopogon parviflorus*. The Mornington Council has been notified of the presence of this colony and I am informed they plan to destroy it.

* *Foeniculum vulgare*, was found in several places close to Boneo and Rosebud, with one massive infestation on the outskirts of Rye. *F. vulgare* was collected ten times in this search area.

Several *Hydrocotyle* sp. were found in high quality bushland reserves throughout the survey area, growing in watercourses, Coast Tea Tree woodland and Eucalyptus forest. Fifteen *Hydrocotyle* samples were collected in this search area.

Xanthosia huegelii was found only once during the survey, occurring in a largely undisturbed bushland reserve within the Arthurs Seat State Park. *X. huegelii* was common in the open understorey of an *Allocasuarina* community growing on shallow, rocky soils.

Several specimens of *Rhagodia candolleana* (Chenopodiaceae) were collected, as chenopods are known to be occasional hosts of CeMV.

Clyde – Somerville

The second area searched was a contiguous area with Sommerville, Pearcedale and Clyde as its centres. This large area included Tooradin, Warneet, Cannon Creek, Dalmore, Cardinia, Cranbourne, Baxter, Moorooduc and Tyabb. With the exception of some high quality bushland remnants close to Westernport bay and the Cranbourne Botanic Gardens, this area was very heavily weed invaded, and native species abundance and diversity was low. Agricultural weeds were very common, and *Daucus carota* was extensively distributed in the north east of the region.

* denotes naturalised species.

5 species of Apiaceae were found in this area:

Centella cordifolia was found three times during this survey in wet roadside depressions north of Cannon Creek.

* *Conium maculatum* was found at one site near Pearcedale, in a roadside ditch in agricultural land.

* *Daucus carota* was very common to the east of Cranbourne and Devon Meadows / Five-Ways, particularly near Clyde, Cardinia and Dalmore, and south of Officer. Seventy five *D. carota* collections were made in this search area.

* *Foeniculum vulgare* was collected nine times in this search area, growing singly or in small colonies.

Trachymene anisocarpa was found in reasonably high quality bushland remnants with a degree of disturbance. Nine collections were made near Warneet, Cannons Creek, and Cranbourne.

Cora Lynn

The final search area centred on the Apiaceae cropping area of Cora Lynn. This area was originally an enormous swamp, and the draining of the swamp last century has resulted in an extensively modified environment with almost no native vegetation. The pasture grass *Phalaris aquatica* is by far the dominant species, and *Daucus carota* is very common.

3 species of Apiaceae were found in this area:

* *Conium maculatum* was collected at two sites close to the towns of Vervale and Iona.

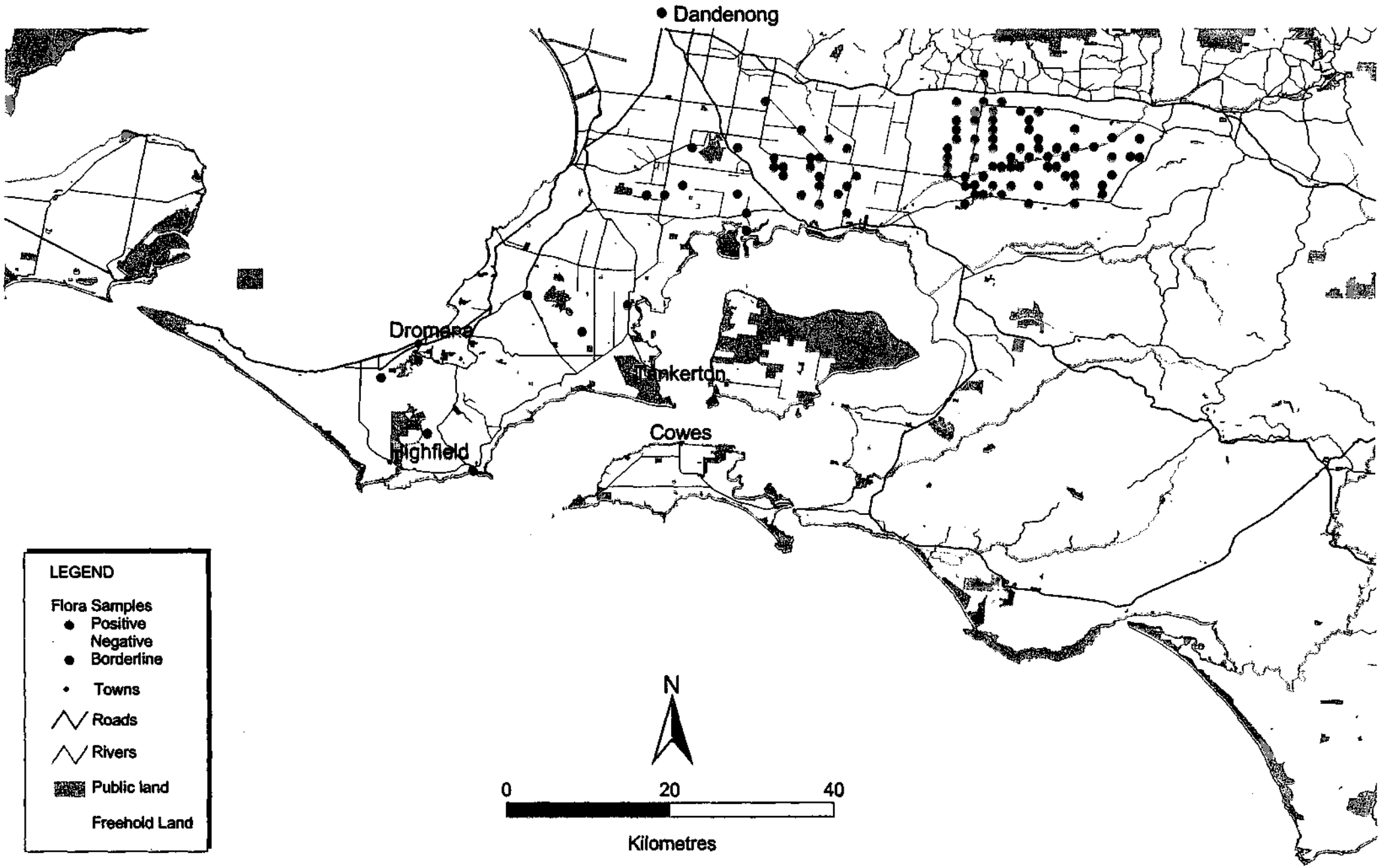
* *Daucus carota* was abundant throughout the search area, being present on some roadsides almost continuously. Sixty seven collections were made in this search area.

* *Foeniculum vulgare* was collected at four localities, growing singly or in small colonies.

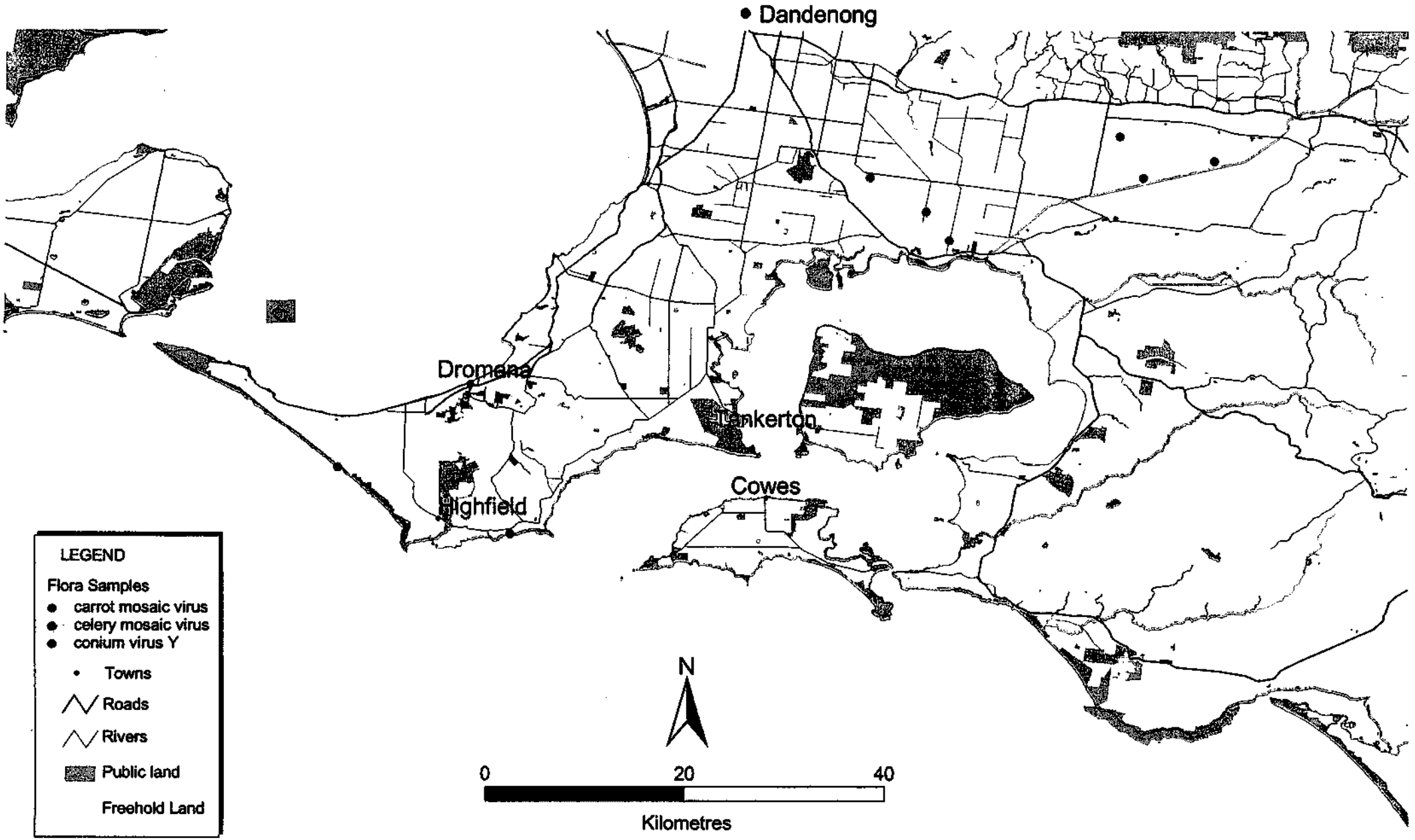
Maps of the areas surveyed with the diagnostic results using ELISA and PCR are attached.

* denotes naturalised species.

Mornington Peninsula flora samples infected with celery mosaic virus diagnosed using ELISA



Celery mosaic virus and it's related viruses isolated by PCR



Appendix V. Report from NSW

Report on NSW Apiaceae Virus Surveys, 1999-2000

Len Tesoriero, Plant Protection Officer, NSW Agriculture EMAI, Menangle

Acknowledgments for surveying NSW Riverina crops to Andrew Watson, Plant pathologist, Herman Kuipers & Mark Hickey, District Horticulturists, from NSW Agriculture's Vegetable Research Centre, Yanco. Assistance from colleagues at EMAI is also acknowledged: Lowan Turton for photography; Mukesh Srivastava for Electron Microscopy and Fiona Bertus for ELISA.

Summary

Surveys were conducted in the NSW Riverina and the Sydney Basin (the state's two main production districts for Apiaceae crops). Four samples were intercepted in late 1998 from the NSW Agriculture Plant Health Diagnostic Service (PHDS). Five targeted surveys commenced in May 1999 and terminated in May 2000 on twenty-two properties. A total of 124 samples were tested for viruses by transmission electron microscopy (negative stains of plant sap) and ELISA (using 'Potyvirus Group' kits [Agdia Corp., USA], and CeMV kits [DSMZ, Germany]). Selected samples were forwarded to Professor Adrian Gibbs' laboratory at ANU, Canberra for RT-PCR and cDNA sequence analysis. All crop surveys targeted plants with possible virus symptoms (leaf mosaics, mottles and yellowing, red leaf, distorted growth, stunting, leaf blistering, vein clearing and feathering). Random samples were taken where no symptoms were visible. In such cases, bulked samples of 20 symptomless leaves were collected and tested by ELISA for CeMV and/or 'Potyvirus Group'.

Carrots are the most important crop from the Apiaceae in NSW and grown predominantly between Griffith and Darlington Point in the Riverina. Lesser quantities are grown near Camden in the Sydney Basin. NSW has a negligible celery industry. Coriander has been planted for broad-acre seed production in the Riverina over the last few years, but production is relatively unstable and therefore excluded from surveys (except for two samples received from crops grown near Wagga Wagga in late 1998 through the PHDS). Parsley, coriander, and dill are grown as bunching vegetables in several market gardens around the Sydney Basin. Several seedling nurseries in the Sydney Basin supply herbal and medicinal plants from the Apiaceae (swamp pennywort, fennel, chervil, anise, dill, caraway, angelica, parsley, and coriander) for home gardens and amenity plantings.

Viruses were detected in each survey, although no CeMV was confirmed in the two celery samples tested. Significant Potyvirus infections were present in parsley and coriander on market gardens in the Sydney Basin. Crop losses were noted in 'Continental' cultivars of parsley and in coriander since they had stronger virus symptoms than 'frilly' parsley cultivars. Carrots from the Riverina were also infected with Potyvirus but the symptoms were more variable and the impact on yield appeared to be negligible. Results are discussed further below.

Carrot Surveys

To reflect their importance, carrots were the main plant species sampled. Survey sites were at one property near Camden (winter and spring 1999) and at seven properties in the Riverina (autumn, winter, spring 1999; and autumn 2000). Carrot crops that were approaching maturity were selected for sampling. Results from surveys are presented in Appendix Table 1. No viruses were found in 14 bulked samples from the two surveys on the Camden property.

Just over one-third (22/60) of samples tested positive for Potyvirus (likely Carrot Virus Y [CVY]) from the four surveys of the Riverina crops. Potyvirus was detected on three of the seven properties surveyed. Potyvirus was detected on one property (Benerembah 2) in all 4 surveys. The property 'Griffith 2' was surveyed on three occasions and Potyvirus was detected on each occasion. The property 'Darlington Point' was surveyed in winter 1999 and autumn 2000 and Potyvirus was only detected on the latter occasion. These results may suggest local reservoirs for the virus at least on the properties 'Benerembah 2' and 'Griffith 2'. Both properties are large production enterprises and often have successions of plantings that span most of the year. Results also suggest that virus-like symptoms are not a reliable indicator of true infection in carrots since positive test results were obtained from symptomless plant as well as those showing strong mosaics, feathering and stunting. Several samples in the autumn 1999 survey had 'mild mottle' leaf symptoms but were apparently free of virus infection. Clearly other factors (such as nutrition and chemical phytotoxicities) can mimic certain virus symptoms and carrot cultivars may express symptoms differently (which may also vary with climatic and seasonal variables). Unfortunately cultivar information was not always available to clearly correlate symptoms x cultivar x infection data. Cultivars *Western Red* and *Red Hat* were infected with Potyvirus despite being symptomless in the winter 1999 survey. No significant symptoms were evident on tap roots despite Potyvirus infection as has been recorded in WA carrots infected with CVY.

Three samples (one from Camden and two from the Riverina) were found to have Rhabdovirus-like particles in their plant sap. Particles showed distinct sub-unit and central axis structure and measured 220-260 nm x 35nm from negatively stained preparations (see Figure 1). A bacilliform virus has been described in carrots from Japan (Carrot latent nucleorhabdovirus, CLV) which has similar length (220-260nm) (Ohki *et al.*, 1978). The diameter CLV from Japan was measured from thin sections to be 70nm, whereas particles from NSW were measured to be 35nm diameter. The discrepancy could be due to the labile nature of CLV in dip preparations, which lose their envelopes (Ohki *et al.*, 1978). One further carrot sample from the Riverina with 'red leaf' symptoms was shown to contain virus-like isometric particles consistent with a Luteovirus (likely to be Carrot red leaf luteovirus).

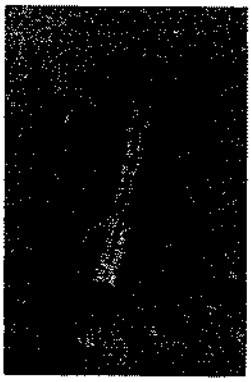
(Ohki, S., Doi, Y. & Yora, K., 1978. Carrot latent virus: A new Rhabdovirus of carrot. *Annals of the Phytopathological Society of Japan* 44:202-204).

Apiaceae Herb Surveys

Parsley, other Apiaceae herbs and vegetable seedlings were surveyed on twelve market gardens and nurseries in the Sydney Basin. The herb garden and selected Apiaceae specimens were also sampled from the Royal Botanic Gardens (RBG), Sydney. The only virus detected from the nurseries and RBG was an unassigned Potyvirus in 3 independent samples of swamp pennywort (*Centella asiatica*, known in Asia as the common name Gotu Kola) with chlorotic and necrotic leaf markings and stunted growth. Virus particles consistent with a Potyvirus were detected by EM but failed to react to the two antisera in ELISA. Sequence analysis from the ANU team has suggested that this virus is possibly a strain of Clover yellow vein virus.

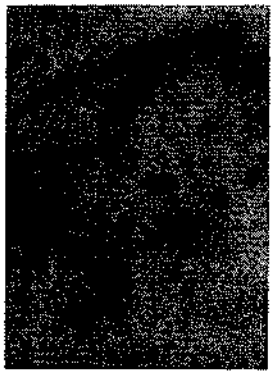
Parsley, coriander and dill samples with distinct virus symptoms from a six market gardens in the Sydney Basin were shown to contain Potyvirus. Both the Agdia 'Potyvirus Group' and the CeMV kits failed to detect these viruses by ELISA. It is likely that this virus is similar to those found in these species in other states. Mosaics, vein-clearing and stunting on coriander and dill were often so pronounced that significant grower losses would be encountered. More than 50% of some coriander beds were infected. Parsley symptoms were less pronounced, especially in the 'frilly' cultivars. Symptom expression in crops was noted as low (<10%) on all farms surveyed. Losses would be expected to be minimal (only where yellow leaf markings are obvious). Continental cultivars of parsley showed stronger mosaic and vein-clearing symptoms but again <10% of plant were symptomatic.

FIGURE 1. Electron Micrographs of Virus-like particles from NSW Apiaceae Survey



(a) Rhabdo-like from carrot (99/492)

(b) Potyvirus from Parsley (00/320)



(c) Potyvirus from Coriander (00/322)

APPENDIX TABLE 1 NSW Apiaceae Virus Survey 1999-2000

Sample No	Specimen	Virus Symptoms	Location	EM (Negative stain
98/721	Swamp pennywort (*Centella asiatica*)	chlorotic & necrotic spots	Bomaderry (Sth. Coast)	potyvirus
98/751	pennywort	chlorotic & necrotic spots	Seedling Nursery/Warnervale	potyvirus
98/1125#1	Coriander (*Coriandrum sativum*)	Nil (Bacterial)	Wagga Wagga	-ve
98/1125#2	coriander	Nil (nutrition)	Berrigan	-ve
99/281	Anise (*Pimpinella anisum*)	Nil	Royal Botanic Gardens/Sydney	-ve
99/282	Chervil (*Anthriscus cerifolium*)	Nil	Royal Botanic Gardens/Sydney	-ve
99/283	chervil	Nil	Royal Botanic Gardens/Sydney	-ve
99/284	Celeriac (*Apium graveolens*)	Nil	Royal Botanic Gardens/Sydney	-ve
99/285	*Eryngium campestre*	Nil	Royal Botanic Gardens/Sydney	-ve
99/286	*Angelica archangelica*	Nil	Royal Botanic Gardens/Sydney	-ve
99/287	celeriac	Nil	Royal Botanic Gardens/Sydney	-ve
99/288	Parsley (*Petroselinum crispum*)	Nil	Royal Botanic Gardens/Sydney	-ve
99/296	Fennel (*Foeniculum vulgare*)	Nil	Royal Botanic Gardens/Sydney	-ve
99/297	fennel	Nil	Royal Botanic Gardens/Sydney	-ve
99/298	Dill (*Anethum graveolens*)	Nil	Royal Botanic Gardens/Sydney	-ve
99/323#1	Swamp pennywort	chlorotic & necrotic spots	Seedling Nursery/Warnervale	potyvirus
99/323#2	Swamp pennywort	Nil	Seedling Nursery/Warnervale	-ve
99/323#3	Swamp pennywort	Nil	Seedling Nursery/Warnervale	-ve
99/323#4	Swamp pennywort	Nil	Seedling Nursery/Warnervale	-ve
99/327#1	parsley - curly	Nil	Seedling Nursery/Warnervale	-ve
99/327#2	parsley - Italian	Nil	Seedling Nursery/Warnervale	-ve
99/328	coriander	Nil	Seedling Nursery/Warnervale	-ve
99/329	fennel	Nil	Seedling Nursery/Warnervale	-ve
99/330	Caraway (*Carum carvi*)	Nil	Seedling Nursery/Warnervale	-ve
99/331	dill	Nil	Seedling Nursery/Warnervale	-ve
99/336#1	fennel	Nil, aphids	Camden	-ve
99/336#2	fennel	Nil, aphids	Camden	-ve
99/337#1	carrot	Nil	Camden	-ve
99/337#2	carrot	Nil	Camden	-ve
99/337#3	carrot	Nil	Camden	-ve
99/337#4	carrot	Nil	Camden	-ve
99/491#1	carrot	Red leaf	Warrawidgee	Luteovirus?
99/491#2	carrot	mottle	Warrawidgee	-ve
99/492#1	carrot	mottle	Griffith 1	-ve

Sample No	Specimen	Virus Symptoms	Location	EM (Negative stain
99/492#2	carrot	mottle	Griffith 1	-ve
99/492#3	carrot	mottle	Griffith 1	Rhabdo-like
99/492#4	carrot	mottle	Griffith 1	-ve
99/492#5	carrot	mottle	Griffith 1	-ve
99/493#1	carrot	Red leaf	Griffith 2	Rhabdo-like
99/493#2	carrot	mottle	Griffith 2	-ve
99/493#3	carrot	mottle	Griffith 2	potyvirus
99/493#4	carrot	Yellowing (herbicide?)	Griffith 2	-ve
99/494#1	carrot	mottle, aphids	Benerembah 2	potyvirus
99/494#2	carrot	mottle, aphids	Benerembah 2	-ve
99/494#3	carrot	mottle, aphids	Benerembah 2	-ve
99/707#1a	carrot - Red Brigade	Nil	Griffith 3	-ve
99/707#1b	carrot - Red Brigade	Nil	Griffith 3	-ve
99/707#1c	carrot - Red Brigade	Nil	Griffith 3	-ve
99/707#2a	carrot - Western Red	Nil	Griffith 2	-ve
99/707#2b	carrot - Western Red	Nil	Griffith 2	-ve
99/707#2c	carrot - Western Red	Nil	Griffith 2	potyvirus
99/707#3a	carrot - Red Hat	Nil	Benerembah 2	potyvirus
99/707#3b	carrot - Red Hat	Nil	Benerembah 2	potyvirus
99/707#3c	carrot - Red Hat	Nil	Benerembah 2	potyvirus
99/707#4a	carrot - Red Brigade	Nil	Griffith 1	-ve
99/707#4b	carrot - Red Brigade	Nil	Griffith 1	-ve
99/707#4c	carrot - Red Brigade	Nil	Griffith 1	-ve
99/707#5a	carrot - Red Hat	Nil	Darlington Point	-ve
99/707#5b	carrot - Red Hat	Nil	Darlington Point	-ve
99/707#5c	carrot - Red Hat	Nil	Darlington Point	-ve
99/707#6a	carrot - Condor	Nil	Darlington Point	-ve
99/707#6b	carrot - Condor	Nil	Darlington Point	-ve
99/707#6c	carrot - Condor	Nil	Darlington Point	-ve
99/707#7a	carrot - Ringo	Nil	Darlington Point	-ve
99/707#7b	carrot - Ringo	Nil	Darlington Point	-ve
99/707#7c	carrot - Ringo	Nil	Darlington Point	-ve
99/726#1	flannel flower (*Actinotus helianthi*)	Nil	Narara	-ve
99/726#2	flannel flower	Nil	Narara	-ve
99/726#3	flannel flower	Nil	Narara	-ve
99/803	celery	Nil	Mulgoa	-ve
99/897	Swamp pennywort	chlorotic & necrotic spots, mottled leaves, stunting	Glenhaven	potyvirus
00/182	parsley	Nil	Currans Hill	potyvirus
00/320#1	Parsley - Flamenco	Yellow leaflets	North Richmond	potyvirus
00/320#2	Parsley - Flamenco	Yellow edges, small leaflets	North Richmond	potyvirus
00/320#3	Parsley - Flamenco	Yellow leaflets, stunted plant	North Richmond	potyvirus

Sample No	Specimen	Virus Symptoms	Location	EM (Negative stain
00/320#4	Parsley - Flamenco	Vein-clearing, yellow tips	North Richmond	potyvirus
00/321#1	Parsley	Mosaic, yellowing	Kellyville	potyvirus
00/321#2	Parsley - continental	Mosaic	Kellyville	-ve
00/321#3	Parsley - continental	Mosaic	Kellyville	potyvirus
00/321#4	Parsley - continental	Mosaic	Kellyville	potyvirus
00/321#5	Parsley	Mosaic	Kellyville	potyvirus
00/322#1	Coriander	Mosaic	Austral	potyvirus
00/322#3	Dill	Purpling, yellowing, mosaic, stunted	Austral	potyvirus
00/322#4	Parsley - Continental	Strong mosaic	Austral	potyvirus
00/322#5	Parsley - Continental	Slight mosaic	Austral	potyvirus
00/325#A	Coriander	Yellowing, stunting, mosaic, puckering	Rossmore	potyvirus
00/325#B	Parsley - continental	Slight mosaic	Rossmore	potyvirus
00/325#C	Parsley - continental	Strong mosaic, stunted	Rossmore	potyvirus
00/326	Celery	Nil	Badgery's Creek	-ve
00/327#A	Parsley – continental	Mosaic, vein-clearing	West Hoxton	potyvirus
00/327#B	Parsley – continental	Strong mosaic, vein - clearing	West Hoxton	potyvirus
00/327#C	Coriander	Yellowing, stunting, mosaic, puckering	West Hoxton	potyvirus
00/346#1	Carrot	Distortion, flecking, purpling	Darlington Point	potyvirus
00/346#2	Carrot	Strong mosaic, lesions on leaf stalk	Darlington Point	potyvirus
00/346#3	Carrot	Strong mosaic	Darlington Point	potyvirus
00/347#1	Carrot	Slight leaf distortion	Benerembah 1	-ve
00/347#2	Carrot	Slight leaf distortion	Benerembah 1	-ve
00/347#3	Carrot	Mild flecking	Benerembah 2	-ve
00/348#1	Carrot	Distortion, purpling	Benerembah 2	-ve
00/348#2	Carrot	Feathering, mottle, distortion, slight mosaic, faint purple tips	Benerembah 2	potyvirus
00/348#3	Carrot	Purpling, yellowing, mild mosaic	Benerembah 2	potyvirus
00/349#1	Carrot	Flecking, slight purpling, twisting	Benerembah 2	-ve
00/349#2	Carrot	Feathery mosaic, forked roots, purpling	Benerembah 2	potyvirus
00/349#3	Carrot	Very mild mosaic, some flecking, purpling	Benerembah 2	-ve
99/934#1	Carrot	Nil	Camden	-ve
99/934#2	Carrot	Nil	Camden	-ve
99/934#3	Carrot	Nil	Camden	-ve
99/934#4	Carrot	Nil	Camden	-ve
99/934#5	Carrot	Nil	Camden	-ve
99/934#6	Carrot	Nil	Camden	Rhabdo-like

Sample No	Specimen	Virus Symptoms	Location	EM (Negative stai
99/934#7	Carrot	Nil	Camden	-ve
99/934#8	Carrot	Nil	Camden	-ve
99/996#1	Carrot	mild mosiac	Benerembah 2	potyvirus
99/996#2	Carrot	Nil or mild mosiac	Benerembah 2	potyvirus
99/996#3	Carrot	mild mosiac	Benerembah 2	potyvirus
99/996#4	Carrot	Nil or mild mosiac	Benerembah 2	potyvirus
99/996#5	Carrot	mild mosiac	Benerembah 2	potyvirus
99/996#6	Carrot	mild mosiac	Benerembah 2	potyvirus
99/996#7	Carrot	Nil	Benerembah 2	-ve
99/996#8	Carrot	mild mosiac	Benerembah 2	potyvirus
99/997#1	Carrot	Nil	Griffith 2	-ve
99/997#2	Carrot	Nil or mild mosiac	Griffith 2	potyvirus
99/997#3	Carrot	Nil or mild mosiac	Griffith 2	potyvirus
99/997#4	Carrot	Nil	Griffith 2	-ve
99/997#5	Carrot	Nil or mild mosiac	Griffith 2	potyvirus

* A Gibbs/A McKenzie, ANU

Table 3.3. The effect of storage on carrots varieties *Senior* after 6 weeks at O°C and *Leonore* after 14 weeks at O°C on limpness, white blush and root cortex colour ± SEM with and without virus.

Variety	Virus status	Limpness[1] ± SEM	White blush[2] ± SEM	Root cortex colour (Hue angle h°)[3] ± SEM
Senior	Positive	2.3 ± 0.1	3.0 ± 0.1	68.0 ±0.1
	Negative	2.8 ± 0.2	3.0 ± 0.1	71.7 ± 1.4
Leonore	Positive	2.8 ± 0.2	2.2 ± 0.2	65.5 ± 0.4
	Negative	2.3 ± 0.1	2.4 ± 0.1	65.0 ± 0.4

[1]. Root turgor: 1 = fully turgid, 2 = trace limpness, 3 = slight limpness, 4 = moderate limpness and 5 = severe limpness.
[2]. White blush: 1 = none, 2 = trace, 3 = slight, 4 = moderate and 5 = severe.
[3]. Root cortex colour: hue angle 0° = red and 90° = yellow. Mid-range values represent orange hues.

No visible signs of post-harvest disorders such as botrytis, sclerotinia, rhizoctonia rot, fusarium rot, rhizopus rot and bacterial soft rot were found in the stored carrots.

Discussion

Carrot production

These studies show that virus has an effect of yield, quality and storage but depends on cultivar. The virus has a detrimental affect on carrot yield, carrot length and carrot collar width. Some carrot cultivars infected with virus were lighter, shorter and smaller in the collar than those that had no virus.

Carrot postharvest performance

The results from this preliminary study did not show any adverse affects of the virus on storage quality. The results from our trial suggest that virus (most likely to be CVY) has no effect on storage capacity for the two varieties tested. Others varieties not examined here may be different.

Mature carrots that have been topped generally have a reasonably long postharvest life. Carrots can normally be stored for 4-5 months under optimum storage conditions of 0°C with 98% to 100% relative humidity when they have been promptly precooled (Thompson 1996; Anon. 1986). Carrots of the var. Senior and Leonore were stored for a maximum of 3 months at 0°C which is to be expected for mature carrots that have not been pre-cooled prior to storage. The carrot var. Leonore stored better than var. Senior: after 6 weeks at 0°C the var. Leonore with and without virus was still in a saleable condition.

The storage periods of 1.5 months and 3 months for *var.* Senior and Leonore under optimal storage conditions was to be expected for carrots that were not hydro-cooled. It is likely that without proper post-harvest handling ie.hydro-cooling, the carrots may have suffered respiratory heating and moisture loss which all would have a detrimental effect on the shelf life of the carrots. Hydro-cooling provides some benefit to the carrots in the

form of rehydration of slightly wilted roots, as well as reducing decay problems and sprouting (Rubatzky *et al.* 1999).

The most likely disorders at postharvest are wilting, bitterness, and other diseases such as grey mould rot (Botrytis), water soft rot (*Sclerotinia*), Rhizoctonia rot, Fusarium rot, Rhizopus rot and bacterial soft rot however, these were not found in this trial.

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Part 4. Epidemiology of *Celery mosaic virus*.

Introduction

The spread of an insect-transmitted plant virus like CeMV from one plant to another requires three basic components: the host plant, the insect vector and the virus itself. In trying to understand the way a virus spreads one must try to ascertain where the virus is, (what plant it is on) what the vectors are doing, and where the vectors are moving the virus. All this is imperative in determining control approaches.

Here we present results on the incidence of CeMV in celery seedlings and celery crops over time together with aphid numbers in the field. The results from this study are aimed to help determine the pattern of spread of the virus and the aphid species that are present in celery crops to develop management strategies.

Materials and Methods

Incidence of virus in celery seedlings

One of our main grower collaborators who produced his own seedlings provided us with 500 random celery leaf samples every week just prior to him planting the same batch out in the field. The samples collected were tested for the presence of CeMV over the first year of the project. Seedlings were tested in batched samples (N=15) for CeMV using enzyme-linked immunosorbent assay (ELISA) and the German, DSMZ™ ELISA kits. Estimated levels of infection were calculated using the formula given by Burrows (1987).

Incidence of virus in the field

Each week 250 leaves were collected from each crop (this crop was derived from the already surveyed seedlings) and tested for CeMV using ELISA in batches of either 10 or 5 depending on the virus levels observed in the previous week. Estimated levels of infection were calculated using the formula given by Burrows (1987). This was done in conjunction with the regular testing of virus incidence in the seedlings to determine if indeed there was a correlation between virus levels in the field with virus levels in the seedlings.

Aphid numbers

Yellow water pan traps were established in the celery crop (where CeMV infection levels in the seedlings were known) to monitor aphid pressure through the growing season of that crop. Yellow water pan traps are a standard method to monitor aphids (Upton 1991). The yellow water pan traps had an overflow hole drilled near to the rim of the container and covered with wire gauze so that no insects could escape. Each trap was 38 (cm) in length and 30 (cm) in width and 15 (cm) deep. All traps were filled to their overflow with water containing sprinkles of detergent (Pyroneg Powder™) and copper sulphate ($CuSo_4$). Detergent was added to reduce the surface tension of the water so that the arriving insects would sink. Copper sulphate was used to prevent any algae build up in the traps. The water in these traps was changed weekly.

The number of winged aphids trapped were collected weekly and taken back to the laboratory for identification to species level. Only winged aphids were counted as these are the migratory aphids and have the potential to spread the virus over long distances (Dixon 1985).

An estimate of aphid pressure at any particular time on a crop was described here as an aphid index. The aphid index is an estimate of aphid numbers at a particular time in the crop based on the mean number of aphids four weeks after the celery seedlings were transplanted into the field.

Results

Seedling and field infection

The estimated level of infection (using the formula by Burrows 1987) of CeMV in the seedlings over the 52 week testing period varied between 0-6.5% (Figure 3.1). Only two batches of seedlings had infection levels higher than 3.1% and only one batch had an infection level higher that 6%.

Estimated levels of CeMV infected celery in the field were much higher than the estimated levels of CeMV in celery seedlings in the nursery over time. Estimated levels of infection using ELISA varied in the field from 0-100% (Figure 4.1) Week number in Figure 4.1 and batch number in Figure 4.2 correspond to when the seedlings were planted out in the field - week one and batch one corresponds to the first week of the new financial year. The correlation between infection levels in the seedlings and out in the field is unknown, however there seems to be a trend indicating that disease incidence in the nursery may be correlated with disease incidence in the field. Estimated levels of CeMV infection in celery crops in the field are expected to be higher than in the nursery as the crops in the field have greater exposure to aphids and are thus more vulnerable to virus infection.

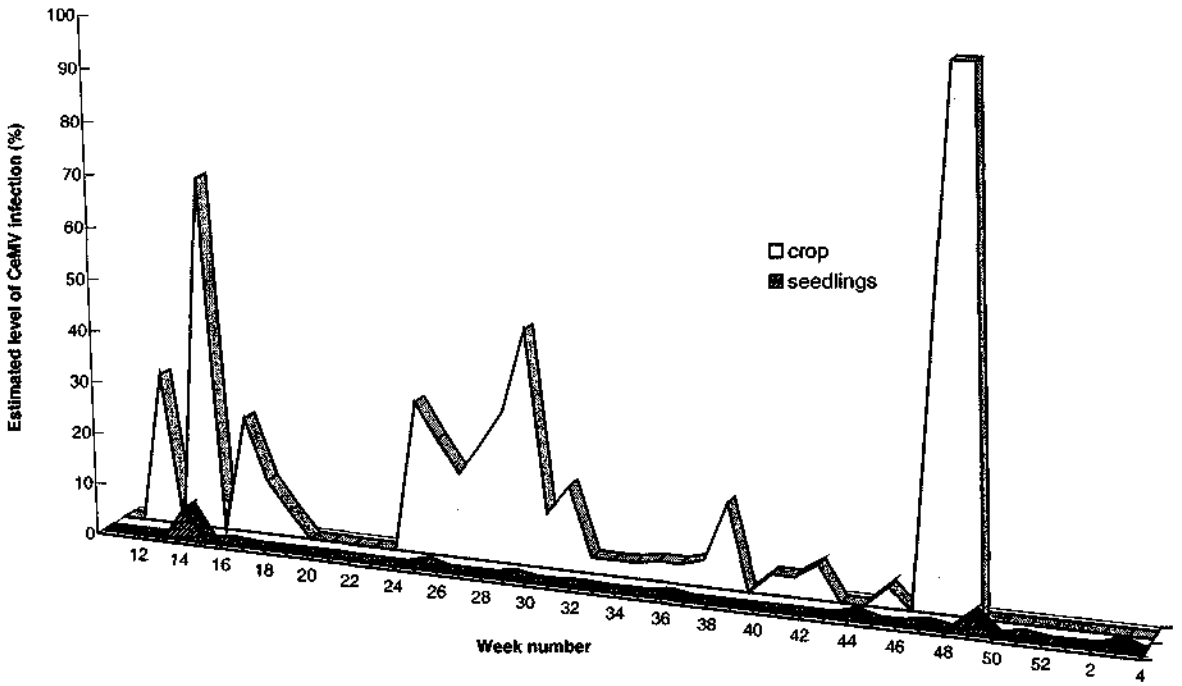

Figure 4.1. Estimated level of infection in the crop and in the nursery seedlings versus week number. Week 1= first week of the new financial year.

Aphid numbers and infection in seedlings and the field
The data of the estimated level of CeMV infection in the nursery seedlings together with aphid numbers are presented in Figure 4.2. The figure shows that when aphid numbers increased so did the virus level with a 3-6 week lag. CeMV has a latent period in which symptoms take 3-6 weeks to become evident.

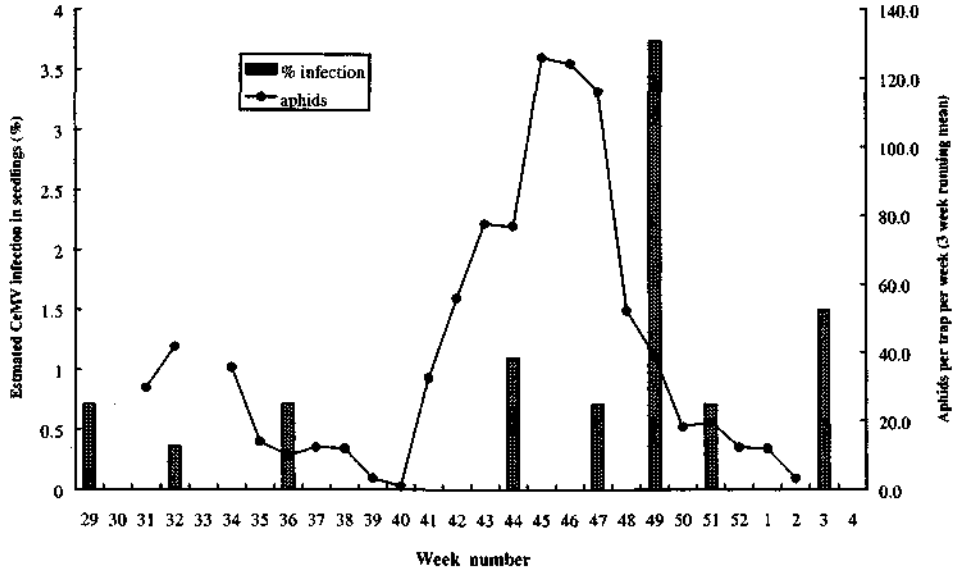

Figure 4.2. Estimated levels of CeMV in celery seedlings versus mean aphid catch per trap (3 week running mean prior to sampling) over time. Week 1 corresponds to the first week of the new financial year.

Infection levels of CeMV in celery in the field was usually much higher than the celery seedlings in the nursery. Figure 4.3 shows the estimated level of CeMV in the crop at harvest with aphid numbers. Aphid number here has been calculated as an aphid index. Aphid index is equal to the mean number of aphids captured in the crop 4 weeks prior to the time the estimated level of infection in the crop was calculated. High aphid numbers are shown in autumn and spring which is consistent with aphid behaviour - aphids are more active at these times. Increased aphid activity also results in higher level of CeMV infection (Figure 4.3).

Key aphid species found in celery crops in Cranbourne and Clyde (Victoria).
Aphid trapping was done in the first year of the project (1998). The key species found in the celery crops are presented below with their common hosts. All aphids are potential vectors of CeMV but some are much more efficient than others. Those aphids that were captured and are known vectors of CeMV are identified in Table 4.1. No experiments were undertaken to determine the transmission efficiency of each aphid species for CeMV.

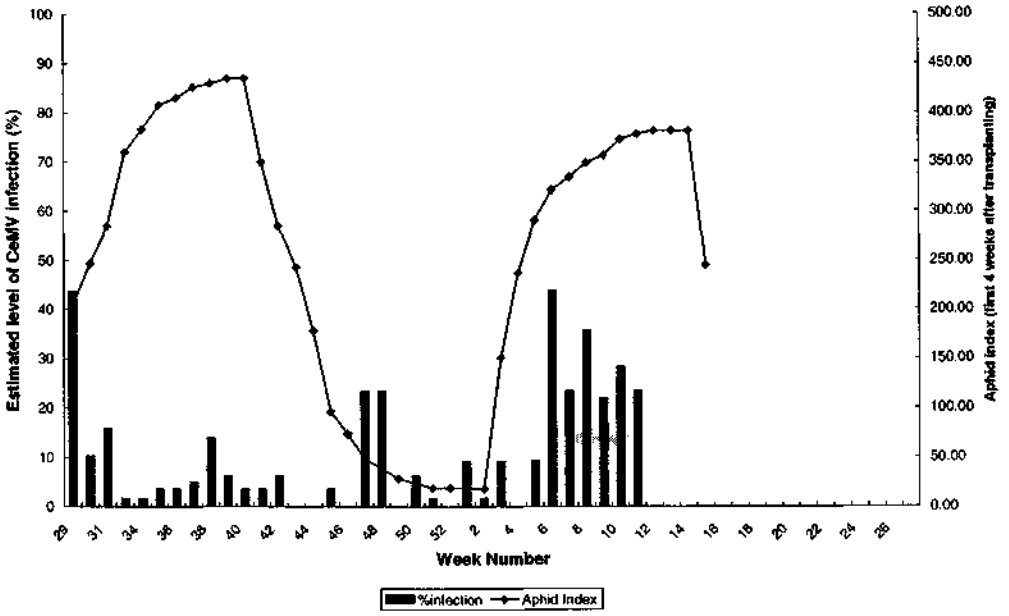

Figure 4.3. Estimated levels of CeMV infection of celery at harvest versus mean aphid index four weeks after transplanting. Batch number corresponds to the week the celery were planted out in the field. Week 1 = first week of the new financial year.

Table 4.1. Key aphid species with their common hosts found in the celery crops of the Cranbourne - Clyde area of Victoria, and their previously known ability to transmit CeMV naturally in the field.

Aphid species	**Ability to Transmit CeMV**	**Common hosts found in Victorian crops**
Brachycaudus rumexicolens		Dock
Dysaphis aucupariae		Plantain
Myzus persicae	×	Mallow, Celery, Brassica
Lipaphis erysimi		Brassica
Aphis sp.	×	Clover
Dysaphis apiifolia		Celery
Uroleucon sonchi		Sowthistle
Hyperomyzus lactucae		Sowthistle
Rhopalosiphum maidis		Sweet corn
Tetraneura nigriabdominalis		Grass roots
Rhopalosiphum rufiabdominalis		Grass roots
Brevicoryne brassicae		Brassica
Aploneura lentisci		Grass roots
Acyrthosiphon pisum		Clover
Rhopalosiphum padi	×	Grass
Therioaphis trifolii f. maculata		Lucerne

Discussion

The infection levels in the nursery seedlings were very low (Figure 4.1) which suggests that most of the infection is occurring out in the celery fields. This implies that seedling infection plays a minor role in CeMV spread when infections in seedlings are low and the field infection pressure is high, however the reverse is true in new production areas where field infection pressure is low - infected seedlings do play a major role in field infections. Thus, starting with virus-free nursery seedlings is imperative to minimising the spread of CeMV into new districts.

The results from this study indicate that aphid pressure is linked to virus spread, however, the exact effect of the high aphid numbers on the incidence of CeMV is not known. There is a definite pattern of high aphid numbers in spring and autumn and this seems to correlate with high levels of CeMV in the field (Figure 4.3). Hence, we can predict that the extent of CeMV spread is related to aphid pressure.

Three of the 16 key aphid species found are known vectors of CeMV. Of the main aphid species present in the celery crop the exact effectiveness of their ability to transmit CeMV is unknown. Further experimental work is needed to answer this question.

There are several indirect virus control approaches as described by Harpaz (1982) which include cultural and technical measures. The cultural measures include:

- *genetic manipulation* which aim to produce plant varieties which are resistant to infection
- *culturing plant tissue fragments* for obtaining virus-free propagative material;
- *elimination of inoculum sources* whether it be by legislation or actual eradication of infected material
- *breaking the cultivation practices* by introducing wide gaps in the availability of susceptible host plants to the virus eg. bare fallowing and rotation of crops.

Technical measures include:

- *reduce the number of vectors* that are active in the field or interfere with virus transmission process.

In California, CeMV epidemics have been controlled in celery crops by the implementation of a celery-free period, which aids in the elimination of the source of virus inoculum (Shepard & Grogan 1971). This is feasible in Victoria, however, the growers must be responsible for this to be implemented. It is recommended that nurseries producing celery seedlings are located outside of the celery growing districts. This will minimise the chance of seedlings being infected and minimise the chances of new plantings of Apiaceous crops becoming infected with CeMV.

Cultural measures such as a break in production may eliminate sources of inoculum. This should be considered by the growers to help manage CeMV, as this is something that can be implemented immediately.

Spraying insecticides to minimise virus spread does not work, because present day insecticides rarely act fast enough to prevent aphids making the brief probes (5-30 seconds) needed to acquire and transmit non-persistent viruses. In fact, the use of insecticides may potentially increase the amount of virus transmission because aphids that have been exposed to insecticides tend to visit more plants than those that have not been exposed to sublethal doses of insecticides (Broadbent *et al.* 1963, Münster & Murbach

1952). Thus, other technical measures to reduce aphid numbers other than spraying with insecticides should be further investigated.

Other technical control strategies for CeMV have been investigated and are reported in Part 5 of this report.

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