Know-how for Horticulture™

Management of celery mosaic virus

Violeta Traicevski, et al
Institute for Horticultural Development Victoria

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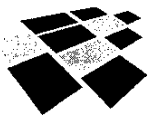
Level 1
50 Carrington Street
Sydney NSW 2000
Telephone: (02) 8295 2300
Fax: (02) 8295 2399
E-Mail: horticulture@horticulture.com.au



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HRDC Project VG97103 - Management of celery mosaic virus

Project leader: Jane Moran
Author: Violeta Traicevski, Bonny van Rijswijk, Alexei Rowles, Angelika Ziehrl, Brad Rundle and Jane Moran.

Institute for Horticultural Development
Agriculture Victoria
Department of Natural Resources & Environment
Knoxfield, 3176
Phone: 03 9210 9222
Fax: 03 9800 3521
Email: violeta.traicevski@nre.vic.gov.au

Contributors:
Peter Ridland
Len Tesoriero
Adrian Gibb
Calum Wilson
Lindrea Latham
Evita Alberts
Dennis Persley
John Thomas
Roger Jones

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HRDC

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).

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.

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

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).

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.

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.

Traicevski V., van Rijswijk, Hepworth G., Ridland P. and Moran J (1999). Influence of petroleum spray oil on the incidence of celery mosaic potyvirus in celery (Apium graveolens L.) (Cornales: Apiaceae). Proceedings of Spray Oils Beyond 2000, Sustainable Pest & Disease Management, International Conference, Sydney, 1999.

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.

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