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Distribution and incidence of *Carrot virus Y* in Australia

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Abstract. In 2000–2002, *Carrot virus Y* (CarVY) was found infecting carrot crops in six Australian states. Its occurrence was greater where carrot production was continuous (New South Wales, South Australia, Victoria and Western Australia), than where production was restricted mostly to the summer (Tasmania) or winter (Queensland) months. The percentages of farms and crops infected, respectively, were New South Wales (71%, 56%), Queensland (5%, 4%), South Australia (56%, 56%), Tasmania (4%, 4%), Victoria (93%, 74%) and Western Australia (28%, 19%). Infection was detected in 30 of 36 carrot cultivars. Possible explanations for the widespread distribution and incidence of CarVY in Australian carrots are discussed.

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

Introduction

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

Carrot virus Y (CarVY) is one of three serologically related potyviruses infecting species of Apiaceae in Australia. It has been reported only in this country where it is found only in carrots (Moran *et al*. 2002; Latham and Jones 2000, 2002, 2004). *Celery mosaic virus* (CeMV) commonly occurs in celery (*Apium graveolens*) in Australia (Latham and Jones 2003) and has been found once here in a self-sown, wild carrot. Apium virus Y (ApVY) infects cultivated parsley (*Petroselinum crispum*) and several apiaceous weed species in Australia. Both CeMV and ApVY are also found overseas. These three viruses form a distinct subgenus within the *Potyviridae* (Moran *et al*. 2002).

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

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

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Methods

Glasshouse grown plants

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

Virus isolates and inoculations

Isolates used in WA were CarVY WA-1 from a symptomatic carrot collected at Guilderton, WA (Latham and Jones 2000), and CeMV WA-1 described by Latham and Jones (2003). Isolates used in VIC were CarVY WA-1 and CeMV Vic-1. Those used in NSW were freeze-dried CarVY NSW-1 and CeMV NSW-1, in TAS CarVY WA-1 and CeMV Tas-1, while in QLD CarVY WA-1 and CeMV DPI 972 (Moran *et al.* 2002) were used. CarVY was maintained in carrot by aphid transmission using *Myzus persicae.* CeMV was maintained in celery by manual inoculation. These cultures of CarVY and CeMV were used as positive controls in enzyme-linked immunosorbent assay (ELISA).

ELISA

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

Field surveys of carrot crops

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

Results

Differentiation of CarVY from CeMV

After 1 h of incubation at room temperature, extracts from CarVY-infected carrot leaves (control isolates) gave ELISA absorbance values (A_{405nm}) that were 10–40 times greater than that of the healthy carrot control with the generic potyvirus antibody, but only 5–15 times greater than that of the healthy carrot control with the CeMV antibodies. Extracts from CeMV-infected celery leaves (control isolates) gave absorbance values (A_{405nm}) that were 50–90 times greater than that of the healthy celery control with the generic potyvirus antibody and 40–120 times greater than that of the healthy celery control with the CeMV antibodies. Extracts from naturally infected carrot samples had absorbance values (A_{405nm}) which were 10–90 times greater than that of the healthy carrot control with the generic monoclonal potyvirus antibody, and 9–15 times greater than that of the healthy carrot control with the CeMV polyclonal antibodies. The weakness of the reactions observed with the CeMV antibodies relative to those observed with the generic potyvirus monoclonal antibody suggest that all ELISA positive samples collected in our surveys were CarVY.

Surveys

In WA, CarVY was found in carrot crops in northern and southern metropolitan Perth and Myalup, but not at Augusta (Fig. 1; Table 1). Incidences of infection in most affected crops were 1–2% but on two carrot export farms in the Guilderton region, they exceeded 50% in 11 crops. In VIC, CarVY was detected in crops in all five carrot-growing regions (north-western irrigation, northern irrigation, Port Phillip, south Gippsland and central Gippsland). In SA, CarVY was detected in six carrot-growing regions (Blanchetown, Kybybolite, Mount Gambier, Nuriootpa, Virginia and Waikerie) but not at Parilla. Incidences of infection in production crops were from 1 to 11%, but were as high as 98% in seed crops at Binnum. In NSW, carrot crops were surveyed only in the Murrumbidgee irrigation region where incidences in infected crops were 2–100%. In

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

State	Seasons when crops sampled	Number of farms surveyed	% farms where infection found	Number of crops sampled	% crops infected	Range of infection in infected crops (%)
New South Wales	Autumn, winter, spring	7	71	25	56	2–100
South Australia	Autumn, winter, spring, summer	25	56	25	56	1–98
Victoria	Winter, spring, summer	16	93	54	74	ND
Western Australia	Spring, summer, autumn	18	28	67	19	1–95
Queensland	Winter, early spring	20	5	27	4	1
Tasmania	Summer, early autumn	25	4	25	4	3

ND = not determined

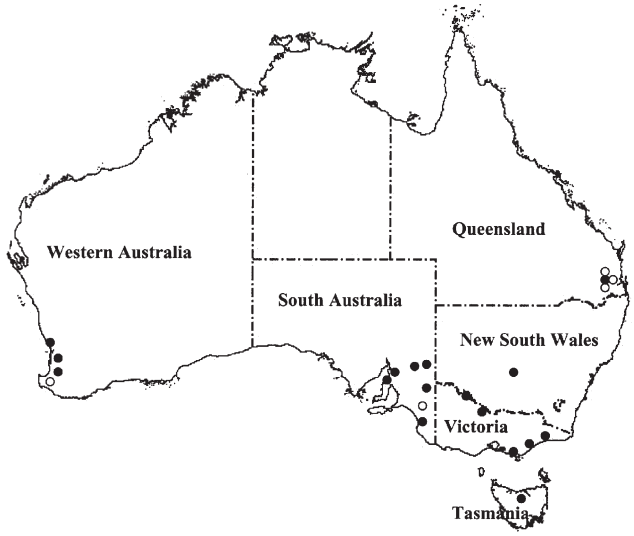

Fig. 1. Locations where carrots were surveyed (● = regions where CarVY found; ○ = no CarVY found).

QLD, carrot crops were surveyed in the Fassifern, Granite Belt, Lockyer Valley and southern Darling Downs regions. CarVY was found in only one crop in the southern Darling Downs, with an incidence of 1%. In TAS, CarVY was found in only one crop at Forth, with an incidence of 3%.

CarVY was detected in 30/36 cultivars (Table 2). In WA, cv. Stefano was the most frequently surveyed and 20/61 crops of this cultivar were found infected. In VIC, cvv. Stefano and Mokum were the most frequently surveyed, with 13/15 and 3/9 crops infected, respectively. In SA, cvv. Carisma, Ricardo and Stefano were most frequently surveyed, with 1/2, 1/5 and 2/3 crops of each infected, respectively. In NSW, cvv. Western Red, All Seasons and Kamaran were the most frequently surveyed, with 5/5, 2/3 and 1/3 crops infected, respectively. All but one of the carrot crops surveyed in QLD were of cv. Stefano, including the one infected crop. Senior, Coral II and Stefano were the predominant cultivars surveyed in TAS but only cv. Senior was infected.

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

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

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

Discussion

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

(Kusterer *et al*. 2002), does not cross-react with CeMV antibodies in ELISA (Latham and Jones 2003), thus excluding confusion between ApVY and CarVY in our surveys.

CarVY was detected in carrot crops in all six Australian states surveyed and in 17 different carrot-growing regions. Infection was found in 30/36 cultivars. Incidences of CarVY infection in NSW and SA, where carrots are grown continuously throughout the year, were often high, sometimes exceeding 90%. Such high incidences are also found in VIC (Traicevski *et al*. 2001). The highest incidences of infection were on farms where carrot production was intensive with carrot plantings sown close to one another throughout the year. Incidences of infection in WA were generally low, except on two large export carrot farms where they were greater than 50%. These two farms practice continuous production under irrigation whereas other farms in the state usually rotate carrots with other crops under irrigation and have sufficient space to sow new crops at large distances from old ones. In QLD and TAS, where carrots are grown for only 6 months of the year, infection did not exceed 3%. Crops for seed production are usually grown for 2 years, which provides a greatly extended period for additional virus spread. The two carrot seed crops tested in our surveys had very high incidences of CarVY infection.

These findings suggest that a break in carrot production can greatly diminish the extent of virus carryover between carrot crops. Short of such a drastic approach at sites where carrots are produced all year round, the best control strategy is through phytosanitary and cultural control measures using integrated disease management tactics (Latham and Jones 2004).

The reason for the occurrence of CarVY infection in carrot crops in isolated and climatically diverse production regions throughout Australia is unknown. Seed transmission and contamination of commercial seed stocks of carrots provides one possible explanation. Preliminary studies suggest that CarVY may be seed-borne in carrot but at very low levels (Latham and Jones 2004). Such seed transmission might also sometimes occur in alternative apiaceous hosts. Another possible explanation is presence of infection reservoirs within alternative hosts belonging to certain introduced apiaceous weeds, native Australian apiaceous plants or other apiaceous crop plants These could provide sources for spread when carrot crops are first introduced to new areas. Further investigations into the seed transmissibility of CarVY and studies to determine if there are alternative infection reservoirs are underway.

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