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VIRUSES INFECTING CARNATIONS
AND DIANTHUS SPECIES
IN NEW ZEALAND

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of the requirements for the degree of
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ABSTRACT

Five viruses were detected in commercial carnations and these and a further four occurred in Dianthus species from gardens. Carnation mottle virus (CarMV) and carnation etched ring virus (CERV-50) were widespread in commercial carnations; arabis mosaic virus (ArMV), carnation latent virus (CLV) and carnation necrotic fleck virus (CNFV) were also detected. In Dianthus species CERV-50, CLV and CarMV were the most prevalent, whereas only a low incidence of ArMV, CNFV, carnation ringspot virus (CRSV) and carnation vein mottle virus (CVMV) was found.

Two new viruses were detected in Dianthus species: an apparently uncharacterized plant rhabdovirus, named carnation bacilliform virus, with particles ca. 260 x 55nm (in ultrathin sections), and an unidentified isometric virus (D 345) ca. 30nm in diameter.

The viruses were characterized by a variety of methods including host range, symptoms, aphid transmission and particle morphology. The three rod viruses CLV, CNFV and CVMV were differentiated by particle morphology and size. Normal lengths for CLV and CVMV were 656nm and 738nm, respectively, while CNFV had particles in the range 1,000-1,450nm. The identity of the polyhedral viruses ArMV, CarMV and CRSV, was confirmed by serology. Carnation etched ring virus was identified by its particle size, ca. 48nm in diameter, and a consistent association with refractile inclusion bodies which were readily observed by light microscopy in epidermal strips stained with phloxine/trypan blue.

Cytological observations were made on ultrathin sections of leaves from plants infected with CBV, CERV-50, CLV and CVMV. Aggregates of CBV and CLV particles were observed in the cytoplasm; CERV-50 infected plants contained typical inclusions and particles of the virus; and CVMV induced cylindrical inclusions typical of the potyvirus group.

CHAPTER 1

VIRUSES AFFECTING CARNATIONSINTRODUCTION

Carnations (Dianthus caryophyllus L.) are grown for cut flowers throughout the world and are a capital and labour intensive crop with a consequent high cost of production. Correct disease diagnosis and control are therefore of paramount importance in minimising losses in flower production. Because virus infection of carnations has been shown to cause reduction in flower quality and numbers (section 1.3) in other countries, it was considered of value to determine the identity and prevalence of viruses in this crop in New Zealand and to assess methods for their control.

1.1 VIRUSES IDENTIFIED FROM CARNATIONS

Eleven viruses have been definitively identified from carnations. They are: alfalfa mosaic virus (AMV) (Hollings & Stone, 1960); arabis mosaic virus (ArMV) (Hakkaart et al, 1972); carnation etched ring virus (CERV-50) (Hollings & Stone, 1967), which is possibly part of a complex (Hollings, Stone & Bouttell, 1968); carnation Italian ringspot virus (CIRV), a strain of tomato bushy stunt virus (Hollings, Stone & Bouttell, 1970); carnation latent virus (CLV) (Kassanis, 1955); carnation mottle virus (CarMV) (Hollings & Stone, 1964; Kassanis, 1955); carnation ringspot virus (CRSV) (Hollings & Stone, 1965b; Kassanis, 1955; Kowalska, 1972); carnation vein mottle virus (CVMV) (Hollings & Stone, 1971; Kassanis, 1955); cucumber mosaic virus (CMV) (Lovisololo et al, 1968); sowbane mosaic virus (SMV) (Hollings & Stone, 1967); and turnip crinkle virus (TCV) (Hollings & Stone, 1969b).

Recently there have been several reports of viruses with long flexuous particles, resembling beet yellows virus, in carnations. In 1968 Goethals & Verhoyen reported long filaments of about 1,000nm in Belgium and in 1971 Sutton & Taylor in Australia found particles with dimensions of about 1,200 x 12nm. Neither report provided details other than particle morphology and size. However, two more recent reports on viruses of this type in carnations provide more information. Inouye & Mitsuhashi (1973) in Japan partially characterised an aphid-borne flexuous rod virus (1,400-1,500nm) which they named carnation necrotic fleck virus (CNFV) while from Israel Smookler & Loebenstein (1974) reported the isolation of an elongated virus (ca. 1,250 x 13nm) which they called

carnation yellow flock virus (CYFV) and which had an identical host range and similar transmission characteristics to CNFV. From these two reports it would appear that CNFV and CYFV are very much alike but distinct from beet yellows virus and other viruses of similar morphology; the earlier records are probably dealing with similar viruses.

There are several records of particles only partially characterised from carnations. Carnation etched ring disease was first described in 1960 by Hollings & Stone (1961) and since then attempts have been made to isolate and characterize the virus or viruses involved. Initially a 28nm diameter polyhedral particle was isolated (Hollings, Stone & Norrish, 1963) but was subsequently reported not to be involved with symptoms of etched ring (Hollings, Stone & Norrish, 1964). Later, two other isometric viruses (25nm and 29nm in diameter) were found in a carnation displaying etched ring symptoms and it was suggested that together with CERV-50 they comprised the full etched ring complex (Hollings & Stone, 1965a; Hollings, Stone & Bouttell, 1968). However, these findings have not been verified.

Symptoms of a streak disease of carnations reported by Hollings (Hollings, Stone & Norrish, 1962) in the United States are reputed to correspond with symptoms found in carnations in Belgium (Verhoyen, 1974). Extracts from plants with streak symptoms contained long filaments about 1,000nm (Goethals & Verhoyen, 1968) but subsequent work by Verhoyen (1974) showed that the disease could be separated into two components by meristem-tip culture. Carnation mottle virus and a 'yellow and necrotic blotch' reaction were implicated, the latter component apparently containing CERV-50 (Horvat & Verhoyen, 1974; Verhoyen, 1974). Symptoms of streak were reproduced in the carnations showing 'yellow and necrotic blotch' by inoculating them with CarMV. Although the component viruses have not been fully detailed, it appears that carnation streak is a complex involving CarMV and the 50nm particle of the etched ring complex at least.

Hakkaart & Olpen (1971) transmitted a 29nm diameter particle from the carnation cultivar Orange Triumph to Saponaria vaccaria L. 'Pink Beauty' on which it caused local white rings; the virus was later named carnation white ring virus (CWRV) (Hakkaart, 1974) but no further details were given. A number of other virus-like diseases and viruses have been partially characterized from carnations. A tobacco ringspot-type virus has been reported (Hollings & Stone, 1960) and there are various communications on carnation mosaic (Ames & Thornberry, 1952; Brierley & Smith, 1955, 1957; Creager, 1943; Jones, 1945; Rumley & Thomas, 1951;

Wright, 1951), carnation streak (Brierley & Smith, 1957; Goethals, 1969; also see above) and carnation yellows (Jones, 1945; Rumley & Thomas, 1948). The information presented was not usually sufficient to be able to ascribe these diseases to the previously mentioned viruses and in many cases mixtures of the known viruses were most likely present. Based on the thermal inactivation data, transmission characteristics and host ranges the mosaic virus of Brierley & Smith (1957) was probably CVMV while those of Noordam et al (1951) and probably Wright (1951) correspond to CRSV. The mosaic virus of Ames & Thornberry (1952) had physical properties similar to those of CVMV but purified preparations contained polyhedral particles (ca. 31nm diameter) when examined by electron microscopy and plants of Dianthus barbatus L. inoculated with these preparations developed mosaic symptoms.

Particles about 19nm in diameter have been observed in ultrathin sections from carnations and in a purified preparation which also contained CarMV (Castro et al, 1971). The size of this virus is similar to that reported for curly top virus (Duffus & Gold, 1973; Esau & Hoefert, 1973; Mumford, 1974).

1.2 WORLD OCCURRENCE

Five of the carnation viruses (CERV-50, CLV, CarMV, CRSV and CVMV) are known to have a wide distribution.

CERV: Since carnation etched ring disease was first recorded in Britain (Hollings & Stone, 1961) it has been found in a number of other European countries as well as in Israel and the United States (Appendix 1). The prevalence of this disease or of the component viruses of the complex has not been studied in carnation crops due to the lack of reliable and efficient methods for detection. However, it appears to be fairly prevalent.

CLV: Carnation latent virus has been infrequently found in cultivated carnations in Britain and Europe (Wetter, 1971) (Appendix 1). The virus has also been reported from Japan (Yora & Yuki, 1965), U.S.S.R. (Procenko & Procenko, 1964) and Australia (Sutton & Taylor, 1971). In Australia CLV was present in most field carnations but not in glasshouse crops.

CarMV: Carnation mottle virus has been recorded in Britain and Europe (Appendix 1) and is also known to be present in the United States (Brierley & Smith, 1957), Canada (Kemp, 1964) and Australia (Sutton & Taylor, 1971). The virus is reported to be prevalent in carnations (Brierley & Smith, 1957; Hollings, 1961; Kowalska, 1973).

CRSV: Like CarMV, CRSV has also been found throughout Europe (Appendix 1) and in the United States (Brierley & Smith, 1955, 1957) and Canada (Kemp, 1964), but though widespread the virus is apparently not prevalent in crops. Kowalska (1973) found 177 out of 1440 plants (12%) were infected with CRSV while Hakkaart (Wasscher, 1965) reported 17.7% of presumably healthy carnations contained CRSV, but the distribution was irregular. Although Kristensen (1957) found that CRSV was very common in Denmark, Paludan (1965) reported only 13% infection. It is probable that this virus is even less common now due to roguing based on the obvious symptoms it produces and the use of 'high-health' planting stock.

CVMV: The vein mottle virus has been recorded in Britain (Kassanis, 1955), Europe (Appendix 1), U.S.S.R. (Korneeva, 1964), the United States (Brierley & Smith, 1957) and Australia (Sutton & Taylor, 1971). However, it is apparently uncommon in N.W. Europe but more prevalent in S. Europe and the United States (Hollings & Stone, 1971). Although common in sweet william (D. barbatus) in gardens in Britain, the incidence of CVMV in glasshouse crops is very low (Hollings & Stone, 1971).

Other viruses characterised from carnations are apparently less widespread.

CNFV/CYFV: Carnation necrotic fleck virus particles were detected in 13 out of 18 varieties of carnation in Japan (Inouye & Mitsuata, 1973), but the incidence of CYFV in Israel (Smookler & Loebenstein, 1974) was not indicated. The long rod from carnations in Australia was present in both glasshouse and field varieties, but was more prevalent in the latter varieties (Sutton & Taylor, 1971).

Reports for AMV (Hollings & Stone, 1960), ArMV (Hakkaart et al, 1972), CMV (Lovichio et al, 1968), SMV (Hollings & Stone, 1967) and TCV (Hollings & Stone, 1969b) indicate only isolated cases of the viruses infecting carnations. The widespread occurrence and wide host range of AMV, ArMV and CMV, however, means that they must always be considered potential pathogens of carnation and therefore likely to be present in carnations wherever they are grown. Carnation Italian ringspot virus was isolated from carnations obtained from Italy and the United States but was reported to be extremely rare in Britain and also difficult to mechanically transmit to carnations (Hollings, Stone & Bouttell, 1970).

1.3 THE EFFECT OF CARNATION VIRUSES ON FLOWER QUALITY AND YIELD

Several experiments have been performed to determine the effect of some carnation viruses on flower quality and yield. Both Hakkaart (1964a) and Brierley (1964) found that CarMV, CRSV and CVMV (= Brierley's mosaic virus) depressed yields but the most important consequence was the economic loss resulting from the reduction in flower quality. Carnation ringspot virus was reported to be the most serious virus in the cultivar 'William Sim' (Hakkaart, 1964a) and the reduction in flower quality was even greater when CarMV was also present. However, the loss due to CVMV infection in 'William Sim' was not fully obvious in Hakkaart's results as flowers were graded regardless of colour breaking. Carnation vein mottle virus caused the largest loss in quality and number of saleable flowers of single virus infections in the cultivar 'King Cardinal', but plants infected with all the above viruses plus streak "virus" gave even fewer flowers although the number of rejects was about the same (Brierley, 1964). Streak "virus" alone caused only a slight reduction in quality and yield.

Carnation vein mottle virus is known to cause colour breaking in flowers (Hakkaart, 1964a; Poupet, 1971) which accounts for the severe reduction in flower quality of CVMV-infected carnations. Plants infected with CRSV produced a large proportion of flowers with split calices, but 'King Cardinal' was reported to produce no splits as it has a small calyx. Virus infection also caused a decrease in the average flower weight (Hakkaart, 1964a) which reflects the lower quality.

In contrast to the above findings, Paludan & Rehnström (1968) reported that although CarMV reduced the yield in the first six months, over longer periods it had no effect on number or quality of flowers although the weight per flower was reduced. Trials with carnation cultivars free of known viruses indicate that better yields and quality can be obtained when viruses are absent (Hollings, Stone & Smith, 1972).

1.4 VIRUSES OF CARNATIONS IN NEW ZEALAND

A mosaic disease of carnation was first observed in New Zealand in 1949 and was subsequently found in Auckland, Christchurch and Timaru, although the incidence was comparatively low (Chamberlain, 1954).

Carnation mottle virus is the only virus characterized from carnations in this country (Thomson, 1962; Thomson & Reynolds, 1963) and was detected in all plants from 27 out of the 29 varieties tested (Thomson, 1962).

1.5 SURVEY OF VIRUSES INFECTING CARNATIONS AND DIANTHUS SPECIES IN NEW ZEALAND

An extensive survey involving 410 samples of glasshouse (commercial) and home garden carnations, as well as Dianthus species or hybrids from gardens, was carried out. As a result eight viruses were distinguished using a variety of techniques. The viruses identified were: ArMV, CERV-50, CLV, CarMV, CRSV, CVMV, a very long rod in the same size range as CNFV/CYFV, and a new bacilliform virus (CBV).

1.5.1 Materials and methods

Indicator plants were propagated in a sand-peat-fertiliser medium in a glasshouse in which temperatures ranged between 16-23 C. Pest and disease control was implemented using the insecticide methomyl (Lannate*), the acaricides cyhexatin (Plictran*) and dicofol (Kelthane*), and the fungicide benomyl (Benlate*), while maldison (Malathion*) drenches were used to control fungus gnat (Sciara spp.).

Inoculation: Inoculum was prepared by grinding tissue (usually leaf tissue) in a small volume of buffer plus celite using a pestle and mortar. Indicator plants were inoculated by gently rubbing the leaves with the pestle and then washing the leaves with water. Graft inoculation was carried out by inserting small pieces of leaf tissue, from which the epidermis was removed, into the stem of the test plant.

Electron microscopy: Plant material was indexed rapidly with an electron microscope using negatively stained crude sap. The crushed homogenate method (Walkey & Webb, 1968) was preferred to the epidermal strip (Hitchborn & Hills, 1965) or leaf dip (Brandes, 1957) techniques because of ease and speed of preparation. A small amount of leaf tissue was macerated on a spotting tile in two drops of negative stain and a 300 mesh formvar-carbon-coated copper grid held face down on the solution for a few seconds, after which excess stain was removed by touching the grid to filter paper. The grids were scanned within two days of preparation at a magnification of about 20,000 using a Philips EM-200 electron microscope.

Negative stains used were ammonium molybdate (AM), phosphotungstic acid (PTA) and uranyl acetate (UA). These were prepared as 2% solutions and the pH then adjusted with either 2M potassium hydroxide (for PTA and UA) or 1M ammonium hydroxide (for AM).

* registered trade names

The size of virus particles was measured from electron micrographs and the magnification calculated using tobacco mosaic virus (TMV) as a standard, assuming a normal length of 300nm for TMV. The normal length (NL) of rod viruses was determined by the method of Brandes & Wetter (1959). Particle measurements were made from electron micrographs of crude homogenates only and after calculating the actual sizes the particles were grouped into 10nm divisions and the NL obtained as the mean of the major peak of the distribution, assuming a 'normal distribution'. The size range of polyhedral viruses was determined and the mean diameter calculated in a manner similar to that used to determine the NL of rod viruses.

Light microscopy: Epidermal strips were removed from leaves and stained for 1-5 minutes in a mixture of trypan blue (0.5% in 0.85% NaCl) and phloxine (0.5% in 0.85% NaCl) in the ratio of 15:1. Strips were then rinsed and mounted in 0.85% NaCl and examined for inclusion bodies of CERV-50 with a light microscope at a magnification of 200 .

Serology: Ouchterlony double diffusion was carried out in 0.75% agar medium with 0.01% sodium azide as preservative in 9cm plastic petri dishes. Usually a pattern with six peripheral wells around a central well was employed, the wells being 5mm in diameter with 6mm between wells. Leaf tissue of virus-infected and healthy plants was respectively ground in 0.1M K/K₂ phosphate buffer, pH 7.5. Serology plates were incubated at 20-24 °C in large petri dishes lined with moist filter paper, to prevent drying of the agar, and occurrence of precipitin lines recorded over several days.

1.5.2 Mechanical Transmission

Although CLV, CarMV and CVMV were readily transmissible from carnations the other viruses posed some problems. Arabis mosaic virus was reported not to be transmissible from crude carnation sap to Chenopodium quinoa Willd. or Chenopodium amaranticolor Coste & Reyn. without the addition of bentonite (Hakkaart, Hoof & Maat, 1972). Consequently bentonite was added at the rate of 0.5% in the inoculation buffer (0.1M K/K₂PO₄, pH 7.5). Yarwood's (1966) solution (0.5% bentonite in 0.5% K₂HPO₄) was also found suitable for the transmission of ArMV to C. quinoa (section 2.1).

Carnation etched ring virus was sap transmitted fairly readily, but only to Cypsophila elegans Bieb. and Saponaria vaccaria L. 'Pink Beauty'. Concentric red rings were not induced on the inoculated leaves of S. vaccaria, which contrasts with the results of Hakkaart (1974) (who

supplied the seed line). Although the virus did not cause obvious symptoms in G. elegans and S. vaccaria the presence of CERV-50 in these hosts was demonstrable in inoculated and systemic tissues by light and electron microscopy. Graft transmission of CERV-50 to the carnation cultivar 'Joker' occurred readily in the limited number of tests carried out. Symptoms of CERV-50 in 'Joker' are detailed in section 2.3.

The very long flexuous rod was mechanically transmitted by sap inoculation in one case only, a result which is in accord with the reports on CNFV (Inouye & Mitsuhashi, 1973) and CYFV (Smookler & Loebenstein, 1974). Carnation bacilliform virus was not transmitted by sap inoculation.

1.5.3 Electron microscopy - indexing

Electron microscopy of negatively stained crude homogenates was found to be a useful method for the detection of both rod and polyhedral plant viruses, despite reports (Brandes, 1966; Schmidt, 1967) to the contrary for polyhedral viruses. However, the results varied depending on the stain, pH, age of infection, the plant host and the particular tissue. Therefore a preliminary trial was carried out with carnation leaf tissue and three common EM stains at varying pH: AM at pH 5.6 and 7, PTA at pH 4 and 7, and UA at pH 4.

Ammonium molybdate gave preparations of poor contrast, but had good spreading properties. Phosphotungstic acid at both pHs provided good contrast generally, although poor spreading occurred at times. With uranyl acetate very poor spreading occurred and this stain was not subsequently used for survey work. Because of the spreading properties of AM, mixtures with PTA at three ratios (AM:PTA at 2:1, 1:1, 1:2) were tried. The best spreading and contrast was obtained with PTA at pH 7 in the ratio of AM:PTA = 1:1 or 1:2 (pH 5.5 and 5.6 respectively). These stain mixtures were subsequently used for most routine indexing of carnations. No obvious damage to virus particles occurred using AM, PTA or the mixtures although this has been observed with some stain and virus combinations (Atkey et al, 1973; Tomlinson et al, 1973).

The carnation rod viruses, CLV, CVMV and CNFV/CYFV and an isometric virus CERV-50, could all be identified by particle size and morphology using electron microscopy. However, the spherical viruses with diameters of about 30nm could not be differentiated on this basis and further definitive tests were required to establish their identity. In the few cases where only ARMV occurred in carnations, particles were seldom observed in the EM.

1.5.4 Light microscopy indexing for CERV-50

The most suitable epidermal strips for light microscopy were obtained from young fully expanded leaves. Inclusion bodies were detected in leaves of various ages, except in very young leaves; in older leaves the inclusion bodies tended to be much less prevalent.

Inclusion bodies have been reported for CVMV (Castro et al, 1971; Norrish, 1962, 1963; Rubio-Huertos, 1959), CLV (Castro et al, 1971) and CNFV (Inouye & Mitsuhashi, 1973) but with the methods employed in this study inclusion bodies were correlated only with CERV-50 infection (section 2.3); inclusion bodies were neither found in carnations infected with CLV, CVMV and CNFV/CYFV, nor in plants experimentally infected with these viruses, unless CERV-50 was also present.

There are two other reports of inclusions in carnations, and in both instances only summaries were available. Reiter (1961) found crystalline inclusions, both hexagonal and tetrahedral, amorphous X-bodies and striated inclusions; the identity of the viruses involved is not clear. Thaler et al (1970) used electron microscopy to examine striated virus inclusions which were very similar to those found by Reiter by light microscopy. The inclusions consisted of fibrillose virus particles in regular array. The particles were reported to be about 8-10nm in diameter and 300nm long.

In the present study crystalline bodies were observed in some epidermal strips, but similar crystals were also seen in epidermis from apparently virus-free plants.

1.5.5 Serology

Serology was used to confirm the identity of ArMV and CRSV whenever their presence was suspected. Initially, the identity of CarMV was also checked by serology. The serological tests were generally made with inoculated leaves of C. quinoa containing a high concentration of virus.

1.5.6 Survey method

Carnation cuttings or samples were obtained from cut-flower growers, in both the North and South Islands, a major supplier of carnation cuttings and from public and home gardens. One of the cut-flower producers imported his stock plants from Holland.

Virus indexing (Figure 1) involved mechanical inoculation to C. guinoa and one or more of the following hosts: G. elegans, Saponaria vaccaria 'Pink Beauty', Spinacia oleracea L. (spinach); concomitantly grids were prepared for electron microscopy and epidermal strips stained and observed by light microscopy. If the tissue could not be examined immediately, it was held in a refrigerator. Subsequently, tissue from the primary hosts (both inoculated and systemic leaves) was used for sap transmission to the differential hosts (Table 1). The initial host plants were used because the viruses could not be transmitted directly from carnation to some of the differential indicators because of the presence of inhibitors in the carnation sap (Ragetli, 1958; Ragetli & Weintraub, 1962; Want, 1951).

In some cases the symptoms induced by the viruses on the initial hosts were sufficient for identification, for example the reaction of CarMV on C. guinoa, but the reaction of CarMV frequently obscured the reaction of other viruses. The presence of viruses in the primary and differential hosts could be monitored by electron microscopy but the actual identity of most of the icosahedral viruses could not be resolved by this method, and the reactions on differential hosts and serological tests were required to characterize these viruses.

1.5.7 Results and discussion

The results of the present survey are summarized in Table 2. Further details of the incidence of viruses in commercial carnations of which 33 varieties were tested are presented in Appendix 2.

Commercial carnations were almost totally infected with CarMV but the incidence of CERV-50 (75%) may be inflated to some extent as many samples were selected from plants that appeared to have virus symptoms. Not all plants containing CERV-50, however, had obvious symptoms. In particular, plants with light coloured flowers (yellow, salmon and pink) generally expressed fewer leaf symptoms than did darker flowered varieties such as 'Joker' and 'Orchid Beauty'. Also many samples were of very young tissue in which it was more difficult to detect CERV-50 owing to the lower concentration of virus. This negates to some extent the bias in sampling referred to previously. The high incidence of CarMV and CERV-50 is, in all probability, primarily the result of vegetative propagation without an adequate clean stock program. The low incidence of CarMV in garden carnations and Dianthus species, however, may be attributed to propagation of many of them, particularly the species, from seed. Evidence for the likelihood of seed propagation

DIFFERENTIATION OF VIRUSES
INFECTING CARNATIONS

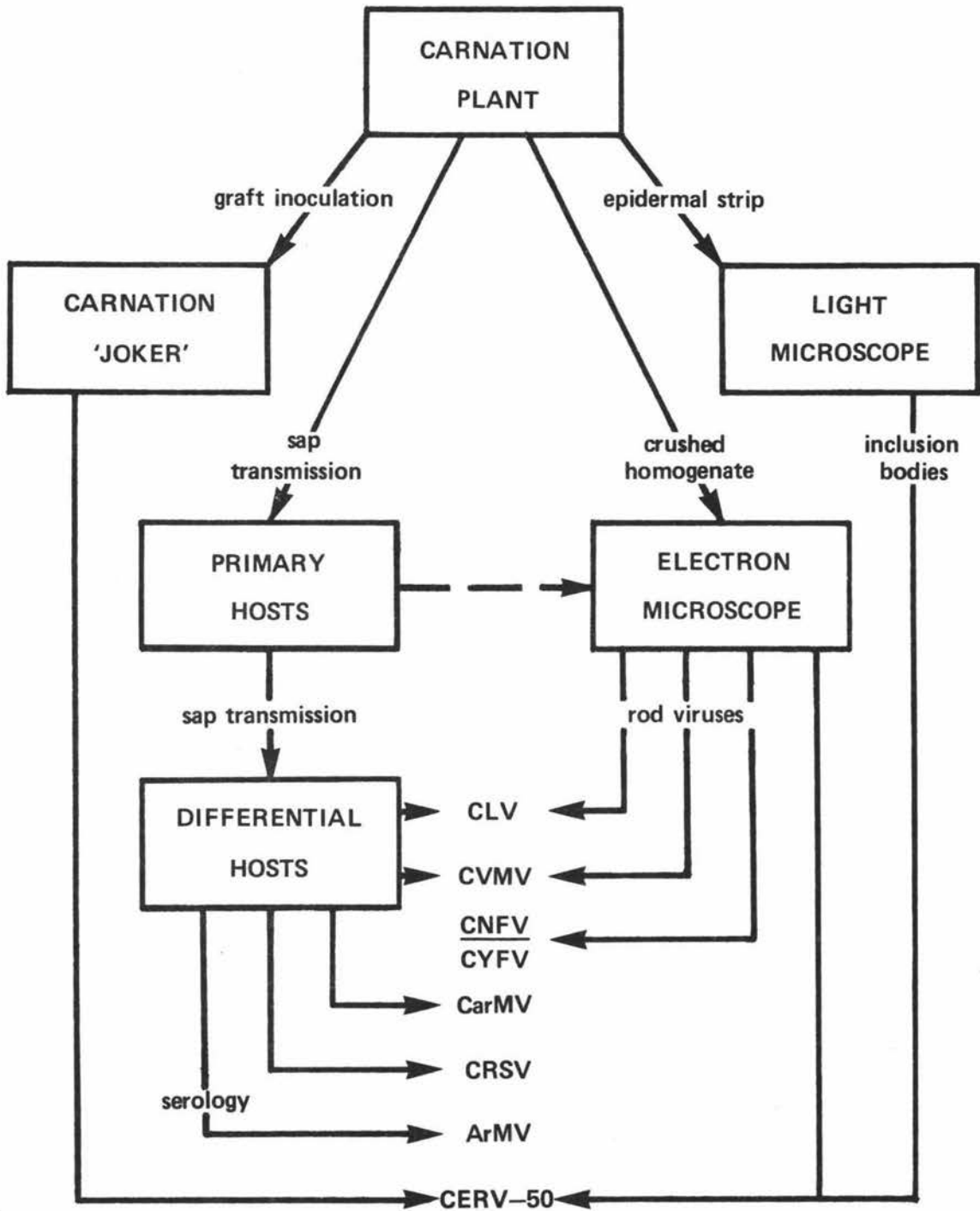


FIGURE 1. Flow chart of the procedure used to differentiate viruses infecting carnations.

TABLE 1. Differential hosts for the identification of six viruses infecting carnations.

Indicator Plants	Viruses					
	ArMV ¹	CERV	CLV	CarMV	CRSV	CVMV
<u>Celosia argentea</u> L. 'Forest Fire'	(L)	NI	NI	(L)	Ln*	NI
<u>Chenopodium amaranticolor</u> Coste & Reyn.	Lc S*	NI	S*	Lc,n	Ln	Lc
<u>Chenopodium guinoa</u> Willd.	Lc S*	NI	Lc S	Lc S	Ln	Lc S
<u>Cucumis sativus</u> L. (cucumber) 'Marketer'	Lc S*	NI	NI	NI	Ln	NI
<u>Dianthus barbatus</u> L. (sweet william)	Lc S	(S)	(S)	Ln	Ln S	S*
<u>Gomphrena globosa</u> L.	L	NI	NI	(L)	Ln S	S
<u>Gypsophila elegans</u> Bieb.	Lc S	(S)	(S)	S	Lr S*	S
<u>Nicotiana clevelandii</u> Gray.	Lr S	NI	(S)	L	Lr S	NI
<u>Nicotiana tabacum</u> L. 'White Burley'	L S	NI	NI	NI	Lr	NI
<u>Saponaria vaccaria</u> L. 'Pink Beauty'	L S	(S)	(S)	S	Lr S	S
<u>Spinacia oleracea</u> L. (spinach) 'Royal Denmark'	Ln S	NI	(L)	L (S)	Ln S	S

Symbols: c=chlorotic; L=local infection; n=necrotic; NI=no infection; r=ringspots; S=systemic infection; ()=symptomless infection.
*=important differential reactions.

¹ reactions of ArMV varied with the strain.

(Note: for details of symptoms see the relevant sections in Chapter 2)

TABLE 2. Incidence of viruses detected in carnations and species of Dianthus.

Virus	Commercial ¹ carnations		Public/home garden carnations: <u>Dianthus</u> spp.			
	No	%	No	%	No	%
ArMV	5	1.7	4	6	5	9.6
CBV	-	-	2	3	-	-
CERV-50	$\frac{201^2}{208}$	75	$\frac{25^2}{64}$	39	30	58.7
CLV	4	1.4	9	13.4	35	67.3
CarMV	276	96	11	16.4	-	-
attenuated	3		7	10.4	1	2
CNFV/CYFV	6	2	6	9	2	3.8
CRSV	-	-	2	3	2	3.8
CVMV	-	-	2	3	-	-
No detectable virus	7	2.4	22	33	12	23
Number of samples	291		67		52	

¹Appendix 2 contains more details on commercial carnations.

²Not all samples were tested for CERV-50. Total numbers tested are indicated where necessary.

of many garden carnations and Dianthus species can be found in the relatively large number of plants, 33% and 23% respectively, having no detectable viruses in them compared with only 2.4% for commercial carnations. Accordingly the high incidence of CERV-50 (59%) and CLV (67%) in Dianthus species can only be explained on the basis of aphid transmission as neither virus has been reported to be seed-borne. Lack of adequate aphid control probably compounds the incidence of these two viruses and may help account for the high incidence of CERV-50 in commercial plants. However, the very low occurrence of CLV in glasshouse crops (1.4%) is difficult to explain, especially in relation to the high occurrence in Dianthus species (67%).

A hypothesis which needs testing is that carnations (D. caryophyllus) are less susceptible to CLV than other Dianthus species. This could also explain the intermediate levels of CLV in garden carnations (13%), which are subject to a higher infection pressure by aphids than commercial varieties. The incidence of CLV in this country is similar to that in Australia where the virus was found to be much more prevalent in field (outdoor) carnations than in glasshouse crops (Sutton & Taylor, 1971). The higher incidence of CERV-50 in carnations, particularly garden carnations, compared with that of CLV suggests that CERV-50 may be more readily aphid borne than CLV. This appears to be confirmed by results of aphid transmission experiments with these two viruses in the present study (sections 2.3 and 2.4).

The low incidence of CRSV and CVMV in garden plants may be of considerable significance, particularly for CVMV which is aphid borne. In view of the ease with which CVMV is aphid borne the reason for it being less prevalent than CLV in garden carnations is not apparent. In Britain CVMV is reported to be prevalent in sweet william in home gardens (Hollings & Stone, 1971). The two CVMV-infected carnations were from the same source and may be an isolated instance of the virus in this country, but a more extensive survey of home garden plants would help to resolve this. Plants infected with CVMV and CRSV generally exhibit obvious symptoms (sections 2.7 and 2.8) and are therefore unlikely to be used for propagation, which could explain the absence of these two viruses in commercial carnations.

The presence of ArMV in glasshouse carnations would appear to be the result of importing cuttings from overseas as explained in section 2.1, but the infestations in garden carnations and Dianthus species represent natural occurrence of the virus. The garden carnations (3

cultivars) were all from one source and were infected with mild strains of ArMV, while four of the Dianthus species originated from different localities and were infected with severe strains of the virus. These Dianthus plants appeared to be the same species although different cultivars. These plants may either be more susceptible to ArMV than other Dianthus species tested or the virus could be seed-borne as has been reported for a diverse range of plants (Lister & Murrant, 1967).

Data on the incidence of CNFV/CYFV and CBV is inconclusive as the methods for detection were not sufficiently sensitive.

Sensitivity of detection methods

The combination of electron microscopy and indexing on indicator plants was sufficiently sensitive for the detection, differentiation and separation of CLV, CRSV, CVMV, and CarMV except the attenuated strain. In this case the virus was seldom detected on C. quinoa before transfer through G. elegans or S. vaccaria. Even then detection was dubious and consequently infections with attenuated CarMV may not have been detected. Carnation latent and vein mottle viruses were differentiated by electron microscopy on the basis of particle length and conformation and could be separated by inoculation to suitable indicator plants.

Carnation etched ring virus was fairly readily detected by both electron and light microscopy but could not be differentiated or separated from other viruses by inoculations to indicator plants. Graft transmission of CERV-50 to 'Joker' did not always cause characteristic symptoms, making this method insufficiently reliable for surveys.

Transmission of ArMV from carnation to indicator plants was the major problem when studying this virus (section 2.1) although the results indicate that the method used was satisfactory. Particles of ArMV were very difficult to detect by electron microscopy in carnation sap indicating that electron microscopy was less sensitive than host inoculations for detecting this virus. This conclusion is also borne out by the results with attenuated CarMV which was rarely observed by electron microscopy.

Detection of CBV and CNFV/CYFV relied solely on electron microscopy and it is possible that both these viruses are more prevalent than indicated. With the long rod virus it was even difficult to obtain reproducible results from the same piece of tissue.