VIRUSES INFECTING DAPHNE IN NEW ZEALAND

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by

Richard Llewellyn Forster

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ERRATA

p. 15 and 16 Table 2 : substitute under "free of detectable viruses", "0" for "1" in Leucanthe Alba,
"41" for "42" in Sub totals,
"0" for "2" in D. x dauphinii,
"68" for "71" in Totals;
substitute under "DVY (FM)", "3" for "1" in
D. x dauphinii,
"516" for "514" in Totals.

p. 35 line 19 : substitute "Dr. J.K. Uyemoto (tomato ringspot virus and tobRSV)" for "Dr. J.K. Uyemoto (tomato ringspot virus)".

p. 56 line 5 : substitute "Poth were found in Leucanthe" for "Poth were confined to Leucanthe".

p. 56 line 8 : add "Arabis mosaic virus was also found in D. x dauphinii."

p. 88 line 6 : substitute "detected in 65" of specimens" for "detected in all specimens".

p. 88 line 20 : substitute "large (4 - 5mm) or small (0.5 - 1mm) chlorotic local lesions" for "0.5 - 1mm chlorotic local lesions".

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PREFACE

The genus Daphne belongs to the Thymelaeaceae and contains both evergreen and deciduous shrubs which produce attractive, highly fragrant flowers. Plants are neat and shapely and seldom grow more than 3-4 feet high, fitting well into rock gardens or herbaceous borders.

A large number of species of this very popular ornamental are grown in New Zealand, the most common of which is Daphne odora Thunb. This latter species contains several cultivars and one (Daphne odora 'Leucanthe') is almost ubiquitous at least in the North Island. Its importance is illustrated by the fact that several nurseries visited during the study cited production figures for Leucanthe of between 5,000 and 12,000 specimens per annum and one firm has also entered the export trade. Daphne burkwoodii Turrill and Daphne cneorum L. are two other species which are also commonly grown in N.Z.

Most species of daphne are vegetatively propagated, prefer well drained, sunny situations and tolerate a range of soil pH. However, they are intolerant to dry conditions and numerous authors reflect on the often unexplained sudden collapse and death of many daphne specimens. Furthermore, cultivars of D. odora frequently exhibit severe leaf mottling and accompanying lack of vigour. Severely diseased plants are unsaleable due to their unsightly appearance and their acknowledged short life.

These factors have undoubtedly contributed to a lessening in popularity of daphnes and a recent rejection of an export consignment on the basis of foliar mosaic

symptoms has stimulated the concern of nurserymen. In view of the existence of a disease problem, considered at least in part to be caused by virus, a study on the virus aspects was commenced.

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ABSTRACT

Ten viruses were detected in one or more of 20 Daphne species and cultivars in an extensive national survey. Four of these viruses were identified as alfalfa mosaic (AMV), arabis mosaic virus (ArMV), cucumber mosaic virus (CMV) and tobacco ringspot virus (TobRSV), by a variety of methods including serology, host range and symptoms, particle morphology and vector transmission. Purification methods were also developed for AMV and CMV. Three rod viruses, daphne-tobacco mosaic virus (D-TMV), daphne virus X (DVX) and daphne virus Y (DVY), with normal lengths of 300nm, 499nm and 733 nm respectively, were also detected. Daphne virus Y was widespread in several Daphne species while D-TMV and DVX were only found in Daphne cneorum L. and Daphne odora Thunb. Daphne virus X and DVY were characterized by host range and reactions, physical properties, vector studies and a purification method was developed for DVY.

Three other viruses were partially characterized by host range and reactions. Two of these, Leucanthe Variegata virus - 1 (LVV-1) and Leucanthe Variegata virus - 2 (LVV-2), were confined to Daphne odora 'Leucanthe Variegata', while the third, Burkwoodii sphere virus (BurkSV), was only found in Daphne burkwoodii Turrill 'Variegata'. Icosahedral particles were detected in squash homogenates from plants infected with both BurkSV and LVV-1, but particles were not detected in plants infected with LVV-2.

Alfalfa mosaic virus, ArMV and CMV have been reported previously infecting Daphne species but this is the first record of TobRSV and the six other tentatively identified viruses in this genus.

Specimens of Daphne odora 'Leucanthe' and Daphne odora 'Rubra', in which no viruses could be detected, were located during the survey and these are currently being increased for distribution to the nursery industry.

CHAPTER 1

SURVEY OF VIRUSES INFECTING DAPHNE

INTRODUCTION

Although many different species and cultivars of Daphne are known (2), virus infections and 'virus-like' symptoms have been reported for only 2 species, viz., Daphne odora Thunb and Daphne mezereum L. Chamberlain and Matthews (8) reported a mosaic disease of D. odora in New Zealand which was later attributed to cucumber mosaic virus (CMV/7). Both CMV and alfalfa mosaic virus (AMV) are reported to occur in D. odora in the United States of America (20) and Europe (22,24).

In England D. mezereum is regarded as particularly susceptible to CMV (26) while recent reports refer to arabis mosaic virus (ArMV/16,18) and a soil and seed-borne virus (30) occurring in Daphne species. An uncharacterized virus (hardy primrose virus) with a wide host range has also been found in D. mezereum in Germany (17,33).

In view of the concern expressed by nurserymen in N.Z. about the 'virus-like' symptoms frequently exhibited by many daphne plants, and in particular cultivars of D. odora, a national survey was undertaken. The principle objectives of this survey were to determine:

(i) the identity and characteristics of viruses occurring in Daphne species and cultivars;

(ii) the prevalence of these viruses and their influence on plant vigour and appearance;

(iii) whether any plants free of virus were available for the establishment of a 'high-health' nucleus stock.

VIRUSES RECOGNIZED IN THIS STUDY

By the completion of this research four previously described viruses (AMV, ArMV, CMV and tobacco ringspot virus / TobRSV), and six apparently uncharacterized viruses (Burkwoodii sphere virus / BurkSV; daphne - tobacco mosaic virus / D-TMV; daphne virus X / DVX; daphne virus Y / DVY; Leucanthe Variegata virus -1 / LVV-1; Leucanthe Variegata virus -2 / LVV-2 were recognized. Two forms of DVY were recognized, namely a sap transmissible form (DVY-1) and a non sap transmissible form (DVY-2).

MATERIALS AND METHODS

Plants were propagated in a modified UC IIC mix (19) in fan-cooled glasshouses. The temperature was maintained between 17 and 24C with shading provided in summer by means of a white-wash and interior shade cloth. Plants were sprayed 3 - 4 times per month for pest and disease control using lannate and benlate.

Virus inoculum was prepared by grinding selected tissue with a pestle in a mortar containing a small quantity of celite and buffer. Inoculations to test plants were made by gently rubbing leaves with either the pestle or cotton wool budsticks dipped in virus-containing solution. Local lesions from half-leaf assays were counted within 1 - 3 days of appearing.

Electron microscopy. For indexing of plant material by electron microscopy the squash homogenate method of grid preparation (34) was used in this study in preference to the leaf dip (4) or epidermal strip (13) techniques. A small piece of tissue was homogenized in a drop of negative stain and then a 300 mesh copper grid placed face down on the solution for 1 - 2 sec before draining off excess liquid on filter paper. This technique, besides being simple and rapid, was suitable for use with both leaf and flower tissue. Grids were routinely scanned at a magnification of approximately 20,000x using a 'Phillips EM-200' electron microscope.

Particle measurements were taken from micro - graphs using 100 - 200 particles of tobacco mosaic virus (TMV) as a standard (assuming a normal length for the latter of 300 nm).

The pH's of the negative stains, phosphotungstic acid (PTA) and ammonium molybdate (AmMo), were adjusted using 2M KOH and 1M NH_4OH respectively. Single-distilled water was used for these stains but double glass-distilled water was used with uranyl acetate (UrAc).

Serology. The Ouchterlony agar double-diffusion method was used for serology. The medium consisted of Oxoid Ionagar No. 2 (0.75%) in 0.1M K_2HPO_4, with 0.01M sodium azide added as a preservative. Test patterns normally comprised 6 peripheral wells (3 mm diameter) and a central well (4 mm diameter) with 4 mm between central and surrounding wells. Leaf tissue from healthy and virus infected plants was crushed in 0.1M K-K_2 phosphate buffer, pH 7.0 (1g : 2ml). Plates were placed in a large petri dish lined with moist

filter paper and incubated at either 12 or 24 C. The development of precipitin patterns was recorded over several days.

DEVELOPMENT OF TRANSMISSION AND ELECTRON MICROSCOPY METHODS

Because the success of a virus survey is largely dependent on sensitive and reliable detection methods, efforts were made to improve transmission from daphne leaves to differential hosts following inconsistent preliminary results. Specific efforts were also made to improve detection of AMV, rod and icosahedral viruses occurring in daphne.

Transmission

Difficulty was noted transmitting viruses from daphne leaves although flowers always provided an excellent source. A series of trials revealed that reliable transmission of CMV and DVY-1 from young soft leaves was possible using 0.01M K-K_2 phosphate pH 7.0 or Yarwood's bentonite solution (0.5% bentonite plus 0.5% K_2HPO_4, pH 8.7 / 35) respectively. The Chenopodium species, Chenopodium amaranticolor Coste & Reyn. and Chenopodium quinoa Willd., were found to be the most reliable indicators for CMV when inoculated directly from daphne leaves.

Electron microscopy

The electron microscope is useful for the detection of both rod and icosahedral viruses in crude sap (13). Variable results have been recorded in the liter-

ature however on the ease of detection of viruses and their stability in various negative stains. A series of trials was therefore undertaken to compare the effect of commonly used negative stains on several viruses found infecting daphne. Standardization for comparison of different stains was achieved by grinding 0.5 cm square of tissue in a single drop of stain.

Firstly AMV was considered, as a method of detecting this virus in plant sap would enable immediate recognition on the basis of its unique particle morphology. Alfalfa mosaic virus particles have been detected in leaf dips using PTA of an unspecified pH (25) or lithium phosphotungstate and several other stains (15). Hitchborn and Hills (13) however, using neutral PTA, reported great difficulty in detecting AMV from Nicotiana glutinosa L. and Gibbs (11) could only find AMV in purified preparations after fixation with formaldehyde.

In a series of tests AMV was detected in high concentrations in local lesions from C. quinoa and Vigna cylindrica (L.) Skeels and in lower concentrations in several Nicotiana species (Nicotiana clevelandii Gray, N. glutinosa and Nicotiana tabacum L.). Neutral 2% PTA proved the poorest stain giving little contrast with plant material and at magnifications used for routine detection very few particles were found. Ammonium molybdate (2% ; pH 5.6 and 7.0) gave better results with high concentrations of AMV particles being found, although still with some difficulty (Figure 35). Sharp contrast was not achieved with AmMo but the spreading of cellular material

over the grid was always excellent. Particles were more clearly defined in pH 4.0 uranyl acetate but this stain was not favoured because of inconsistent spreading results. Furthermore, with this stain grids could only be prepared by the slower process of crushing tissue in water, draining and then staining and, as with neutral PTA, high concentrations of virus particles were not found (Figure 35). Low pH PTA (pH 3.0 and 4.0) and sodium silicotungstate (pH 6.5) gave the best results, the latter being slightly superior at low magnifications but often giving particles a woolly appearance.

The value of low pH PTA was noted by Uyemoto (pers. comm., 1973) who could not detect AMV from purified preparations stained in neutral PTA whereas in pH 4.0 PTA, particles were readily identified. Finlay and Teakle (10) also noted the value of low pH, compared to neutral, PTA finding both tobacco necrosis virus and sowbane mosaic virus were more stable in PTA at pH 3.0 and pH 4.0 than at pH 7.0.

Arabis mosaic virus and TobRSV were both detected in squash homogenates. High concentrations were found in local lesions of C. quinoa, but Vigna sinensis (Torner) Savi. (cowpea) and N. tabacum also proved useful sources. Considerable variability in the quality of staining was noted with preparations of these viruses but PTA at all values tested gave excellent contrast (Figure 22). In neutral AmMo however TobRSV was rarely observed, most particles being electron dense and only found in areas of high concentrations (Figure 22).

Daphne viruses X and Y were readily observed in

squash homogenates from daphne plants, with very high concentrations of particles being found, especially in flowers. Virus particles however were often poorly defined in preparations from this source. Results with stains were variable but 2% PTA, pH 4.0 usually gave the poorest results (Figure 44). Particles were more clearly defined in neutral 2% PTA and 2% AmMo (Figure 44). Uranyl acetate was unsatisfactory with preparations from flowers due to the formation of a black precipitate immediately upon crushing with tissue. Virus particles were also readily detected in squash homogenates of flowers which had been shadow-cast with platinum (Figure 44), a process by which rod-shaped potato viruses could be detected at greater dilutions than with negative staining (23). In the daphne study however shadow-casting was not favoured because of the time involved.

In daphne leaves rod viruses were readily detected with all stains tested.

As recently as 1966, Brandes (5) alluded to the general thinking that icosahedral viruses could not be differentiated from host plant material. Since then however, there have been many reports of the detection of icosahedral viruses in crude sap and in this study icosahedral (ArMV, BurkSV, CMV, LVV-1, TobRSV), rod (D-TMV, DVX, DVY) and bacilliform (AMV) viruses were readily detected in this source, although the type of negative stain was shown to markedly affect particle definition and spreading of material over the grid. The source plant, nature of tissue and age of infection also influenced

the detection of viruses infecting daphne. The effect of negative stains on particle definition has also been noted by other workers (3, 29).

Alfalfa mosaic virus was readily identified by electron microscopy on the basis of particle morphology but the specific identity of the icosahedral viruses found in daphne was only obtained by applying other definitive tests such as serology, vector transmission and differential host reactions.

SURVEY METHOD

An extensive survey was conducted during late winter and spring to coincide with the flowering periods of most Daphne species. Where possible unopened flowers were indexed with young soft leaves being used only as an alternative.

Representative samples of all available symptom types were collected from each site in separate plastic bags and stored at 4 C until required. Samples from more distant nurseries were mailed and invariably arrived in fresh condition.

Inoculations from daphne flowers were made using 0.01M K-K_2 phosphate buffer pH 7.0 while Yarwood's bentonite solution (35) was used with leaves. Grids for electron microscopy were prepared using neutral 2% AmMo with flower tissue and 2% PTA, pH 4.0 with all leaf tissue.

Each daphne sample was inoculated to C. quinoa and an electron microscope grid prepared concomitantly. Grids were scanned for viruses within 1 to 2 days of

preparation, and enabled identification of D-TMV, DVX and DVY (1 and 2).

Cucumber mosaic virus was detected by the appearance of characteristic rusty necrotic local lesions after 2 - 3 days in C. quinoa (Figure 4). Burkwoodii sphere virus was detected in C. quinoa by the appearance of 4 - 5mm chlorotic local lesions 7 - 12 days after inoculation, but no systemic reaction was produced. Upon reinoculation to a second C. quinoa 1mm chlorotic local lesions were produced after three days followed by systemic chlorotic blotches after 10 - 14 days. All other sap-transmissible viruses from daphne produced systemic symptoms in the primary C. quinoa, but of these only DVX (Figure 42) and DVY (Figure 39) could be identified by their characteristic reactions. The systemic symptoms of AMV, ArMV, LVV-2 and TobRSV appeared initially as chlorotic netting at the base of expanding terminal leaves or over the entire lamina of the smallest leaves. Netted areas subsequently developed a bright-yellow appearance and sometimes necrosis (Figures 6 and 7). From each C. quinoa showing the latter symptoms, systemically infected tissue was separately inoculated to a second C. quinoa with Yarwood's bentonite solution as this worker reported improved transmission with bentonite from another Chenopodium species (C. amaranticolor). High concentrations of local lesions were produced on the second C. quinoa (Figure 5) and these were used as a source of virus for electron microscopy, inoculation to further hosts and serology.

Grids were prepared using 2% PTA, pH 4.0 and examined for the presence of AMV and icosahedral viruses. As well all isolates were inoculated to a series of differential hosts to group the icosahedral viruses and separate any virus mixtures which may have been present.

Details of the differential hosts and their reactions are provided in Table 1.

Isolates reacting on differential hosts in a manner typical for ArMV were run against ArMV antisera kindly supplied by Drs. P. Fry, B. Harrison and M. Hollings. Similarly TobRSV isolates were checked against TobRSV antisera from Dr. J. Uyemoto. Leucanthe Variegata virus -1 was masked by the presence of LVV-2 with which it always occurred. The method to separate these two viruses involved reinoculating infected systemic C. quinoa tissue to a further C. quinoa as soon as net symptoms appeared. This eliminated LVV-1 which systemically invades C. quinoa more slowly. The latter virus was separated by inoculation to tobacco species in which hosts LVV-2 does not produce a systemic reaction. The survey method is summarized in Figure 1.

SURVEY RESULTS AND DISCUSSION

The results of the survey of viruses infecting daphne are illustrated in Table 2.

The results show that D. odora contains a greater number of viruses than any of the other species tested, but this may be biased by the fact that a far greater number of specimens of D. odora were indexed. Viruses only found in this species include AMV, ArMV, LVV-1, LVV-2 and TobRSV. Leucanthe Variegata viruses -1 and -2 were only found in specimens of D. odora 'Leucanthe Variegata'.

Daphne virus Y was common in several Daphne species, DVX was prevalent in D. cneorum while BurkSV was

TABLE 1 Differential hosts for daphne viruses producing systemic net symptoms in *C. quinoa*

Host	Virus			
	AMV	ArMV	LVV-2	TobRSV
Cowpea	necrotic local lesions with irregular outline; no systemic infection	chlorotic local lesions; systemic chlorosis then necrosis	no infection	ditto ArMV
Havana 423	chlorotic local lesions with etched necrotic flecks; systemic mosaic	local deep-green or yellow ring-spots; no systemic infection	symptomless local infection; no systemic infection	chlorotic local lesions; systemic chlorotic blotches or 'oak-leaf'
Nicotiana clevelandii	local chlorotic blotches; systemic vein-net	symptomless or mild systemic chlorosis	———	necrotic local lesions; systemic chlorosis and severe necrosis

DIFFERENTIATION OF VIRUSES
INFECTING DAPHNE

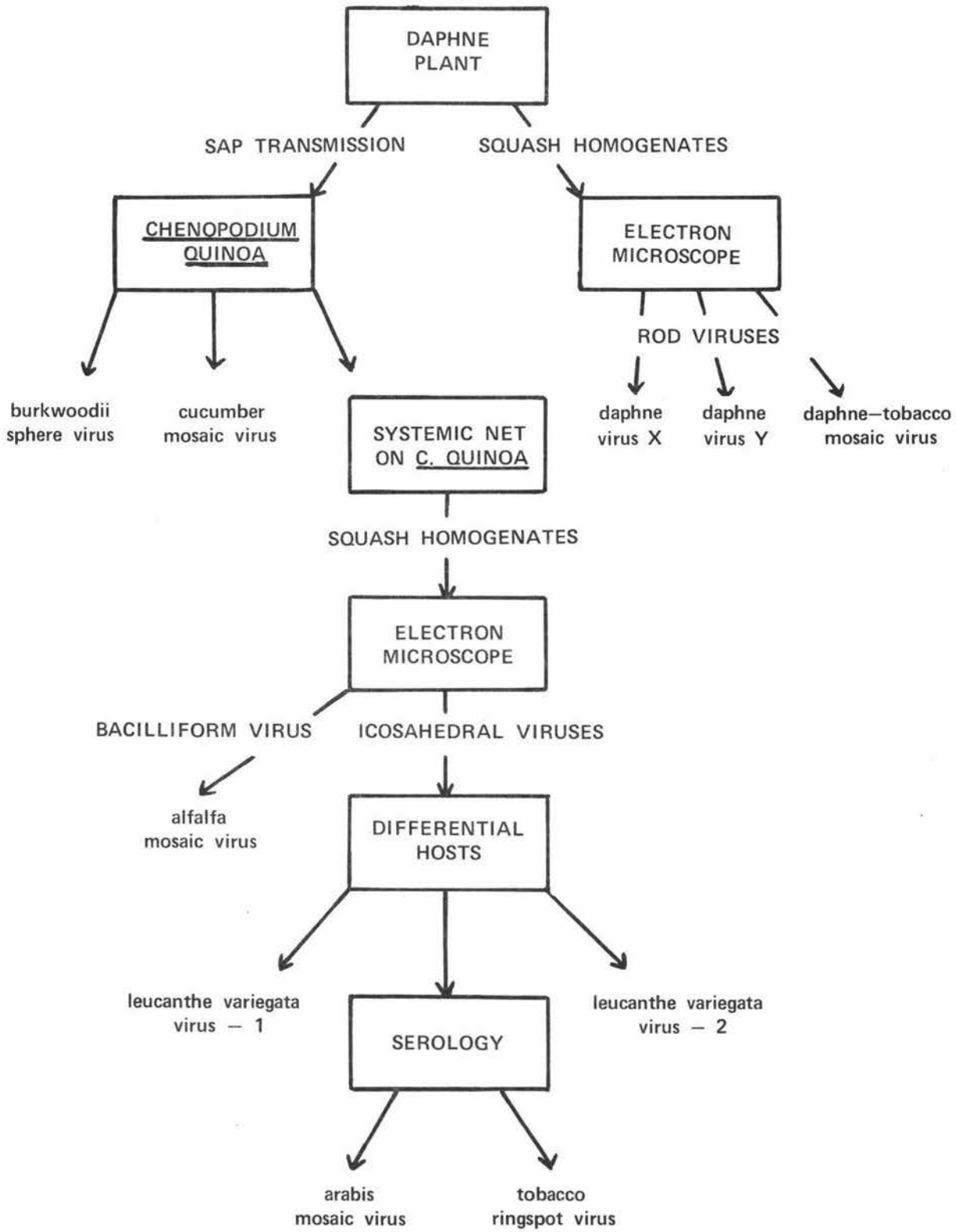

Figure 1 Flow diagram of the procedure used to differentiate viruses infecting daphne.

TABLE 2 Viruses detected in *Daphne* species and cultivars

Daphne species or cultivar	Number tested	Virus											Free of detectable viruses
		AMV	ArMV	Burk SV	CMV	D-TMV	DVX	DVY (EM)*	DVY-1	LVV-1	LVV-2	Tob RSV	
Daphne odora Thunb.													
'Leucanthe'	356	1	33		59	8	2	336	136			3	11
'Leucanthe Alba'	7				1			7					1
'Leucanthe Variegata'	59				5			27	1	3	43		8
'Rubra'	72				5			47	11				21
'Rubra Variegata'	6							5					1
Sub totals	500	1	33		70	8	2	422	148	3	43	3	42
Daphne burkwoodii Turrill													
'Somerset'	37				1			37					
'Variegata'	20			13				20					
Daphne cneorum L.													
'Eximia'	3						3						
'Major'	30					1	30	16	1				
'Variegata'	19				1		19	2					

continued over.....

TABLE 2 Viruses detected in Daphne species and cultivars - continued

Daphne species or cultivar	Number tested	Virus											Free of detectable viruses
		AMV	ArMV	Burk SV	CMV	D-TMV	DVX	DVY (EM)*	DVY-1	LVV-1	LVV-2	Tob RSV	
Daphne blagayana Freyer	2												2
D. collina Smith	1												1
D. x dauphinii Hort.	3		1					1					2
D. genkwa Sieb. & Zucc.	14												14
D. giraldii Nitsche	1												1
D. laureola L.	1							1					
D. mezereum L.	2												2
D. mezereum 'Album'	1												1
D. x neopolitana Lodd.	11							11					
D. retusa Hemsl.	7							4					3
D. tangutica Maxim.	3												3
Totals	655	1	34	13	72	9	54	514	149	3	43	3	71

*DVY(EM) - detected with electron microscope

only detected in D. burkwoodii. Cucumber mosaic virus was found in D. odora and also in single specimens of D. cneorum and D. burkwoodii. The low incidence of CMV in Rubra compared with Leucanthe suggests that Leucanthe is more susceptible than Rubra to CMV and that even with the use of 'clean' planting stock infections of this virus will still present some problems to gardeners.

Species from which virus could not be detected include D. genkwa, D. collina, D. mezereum and D. tangutica, although only a small number of samples of each were tested. A few specimens of other genera of the Thymelaeaceae, Edgworthia and Pimelia, were also free of detectable viruses.

Milbrath and Young (20) conducted a survey of 45 specimens of D. odora. They reported 100% infection with CMV and at least 33% with AMV. These results are in contrast with those from the present study, CMV occurring in only 14% of D. odora and AMV being found only once. Furthermore these authors did not detect viruses in D. cneorum.

During the survey samples were collected from both nurseries and home gardens as it was believed that a comparison of the incidence of viruses in specimens from the two sources could provide information on the origin of viruses in daphne. The results of the survey revealed a difference in virus incidence between nursery and home garden specimens of Leucanthe. No other species or cultivars were considered because of insufficient numbers. Results are presented in Table 3.

TABLE 3 Incidence of viruses in home garden and nursery specimens of Leucanthe

Source	No. of specimens tested	Virus					Free of detectable viruses
		AMV	ArMV	CMV	DVY	TobRSV	
Home gardens	63	2%	12%	57%	94%	0%	5%
Nurseries	293	0%	8%	9%	94%	1%	3%

The results in Table 3 reveal that CMV was the only virus in Leucanthe which increased appreciably in incidence (48%) in garden specimens. This result is probably not surprising considering the many sources of CMV and number of vector aphid species. On the other hand no appreciable difference in the incidence of the nepoviruses ArMV and TobRSV was detected between nurseries and home gardens suggesting that the use of 'clean' stock would provide effective control of these viruses. The high incidence of DVY in both nursery and home garden specimens of Leucanthe precludes any conclusion as to whether this virus has a major reservoir in nature, other than daphne.

Sensitivity of methods. Viruses in this survey were detected and identified using indicator plants, serology and electron microscopy.

(i) Indicator plants. These proved to be a very sensitive method for virus detection. Daphne virus Y and ArMV could be detected in C. quinoa at dilutions of 10^{-6} and 10^{-5}

respectively from daphne flowers. Chenopodium amaranticolor and C. quinoa were both found to be sensitive primary indicator hosts of CMV from daphne leaves and were among the few hosts of DVY-1. For virus identification however, C. amaranticolor does not react characteristically with DVY-1 or CMV, local lesions of the latter taking 3 to 5 days to appear. In contrast both viruses produced characteristic local lesions on C. quinoa, those of CMV appearing 2 to 3 days after inoculation. This was the only virus to react in less than 4 days thus enabling separation on time to react as well as type of reaction. Chenopodium quinoa was also favoured because it is easy to grow, has a relatively long period of sensitivity and is susceptible to a large number of 'woody plant' viruses (in this survey five viruses previously unreported on daphne were first detected on C. quinoa). Hollings (14) noted that C. amaranticolor is susceptible to a wider range of viruses than C. quinoa but that the latter contains less inhibitory material. This was an important consideration enabling direct transmission from C. quinoa to additional hosts for further characterization.

(ii) Electron microscopy. The electron microscope was essential for detection of rod viruses, as isolates of DVY-2 and D-TMV are not sap transmissible and the reactions of both DVX and DVY-1 are masked on C. quinoa in the presence of other viruses causing chlorotic local lesions. The method was very rapid, scanning of each grid requiring only 1 to 2 minutes.

In experiments with potato viruses, Sampson and

Taylor (23) reported that although test plants were the most sensitive virus indicators, rod viruses could be detected by electron microscopy in high concentrations in field material. Using their technique DVY could be observed in daphne flower sap at dilutions of 1:20,000 and the fact that this virus was detected in the great majority of Leucanthe plants indexed also suggests the method is both sensitive and reliable. Besides the sensitivity of electron microscopy for virus detection, it is also considered an accurate method for separation of rod virus groups. Brandes (5) stated that he was able to distinguish between elongated viruses whose normal lengths differed by no more than 10 - 20 nm. Also Sampson and Taylor (23) were able to distinguish potato viruses X, Y, and M on size and morphology as could Brunt and Atkey (6) with 3 filamentous viruses from narcissus. More recently however environmental conditions have been shown to affect the length and morphology of rod viruses. Moore and Guthrie (21) found that the normal length of potato virus X was influenced by air temperature with virus particles in several plants grown at 40 C 2 to 5 times longer than those in plants grown at 20 C. Govier and Woods (12) noted a marked 'environmental' influence on the potyviruses henbane mosaic virus (HMV) and bean yellow mosaic virus (BYMV). Particles of the former in extracted sap were 900 nm long and straight in the presence of magnesium but were shorter (800 nm) and flexuous when exposed to EDTA. A host-dependant variation in length of BYMV has also been noted (31).

The possibility of such length variations

accentuate the need to approach the electron microscopic identification of flexuous rod viruses with care.

Daphne viruses X and Y however, differ sufficiently in length and morphology to be confidently distinguished using electron microscopy (Figure 46). This was illustrated when fresh electron microscope grids were prepared from D. cneorum specimens which, during normal surveying, had been noted to contain DVY alone, DVX alone or a mixture of the two. One hundred and sixty-five particles from the first had a single normal length of 491 nm. Similarily 62 particles from the second had a single normal length of 751 nm while in the specimen noted to contain both viruses two modal peaks 503 nm (131 particles measured) and 739 nm (73 particles measured) occurred representing the 2 distinct virus groups. This separation was confirmed on the basis of other definitive tests (summarized in Table 8).

Antisera to AMV was not available and the virus was identified by electron microscopy from crude sap rather than by differential hosts as different strains vary widely in their host range and reactions.

Limitations of survey method. Although sensitive detection methods were used in this survey there are several limitations affecting the results obtained. Firstly the methods were originally designed for detection of AMV, ArMV, CMV and DVY these being the viruses previously reported in Daphne species or initially found in this study. However as other viruses were detected the method was altered to finally include BurkSV, D-TMV, DVX, LVV-1, LVV-2 and

TobRSV. Daphne virus X was recognised late in the survey and prior to this, electron microscope grids were scanned only for 750 nm rods, shorter particles being considered as fragments. Daphne-TMV was also found late in the survey and occurred in such low concentrations that it was undoubtedly overlooked many times, as was the complex of LVV-1 and LVV-2 which was elucidated after the survey with only a few specimens being rechecked for the presence of either or both viruses. A similar situation occurred for BurkSV.

Secondly the use of single primary indicator hosts and electron microscopy would not necessarily detect non sap-transmissible viruses, other microorganisms or icosahedral viruses or strains of such viruses not infectious to C. quinoa. For example isolates of ArMV from Cyphomandra betacae (Cav.) Sendt. (tamarillo) were not infectious to C. quinoa (32) and TobRSV is reported to be rarely systemic in C. quinoa (27, 28) and thus may be more prevalent than the figures in Table 2 indicate. Isolates of AMV, ArMV, LVV-1 and LVV-2 not systemic in C. quinoa would also have been overlooked.

Finally it is possible that viruses could have been missed in this survey due to variability in sensitivity of individual C. quinoa plants or because distribution of virus in the source plant was incomplete (a phenomenon known for other woody plants).

VIRUS SYMPTOMS IN DAPHNE

Because no virus-free plants were available for inoculation with a known virus or virus combination, all

comments on symptoms are based on the correlation between indexing results and the symptoms of the plants in question. During the survey symptom types were recorded from each source together with details of whether the plant was growing in full sun or shade.

With few exceptions virus disease symptoms were only noted on cultivars of D. odora. A range of leaf symptoms was noted on virus-infected Leucanthe plants and these were categorized into four types, namely:

(i) symptomless - no obvious symptoms, leaves long and straight;

(ii) mild mosaic - leaves with a few pale-green streaks or blotches;

(iii) severe mosaic - light-green areas over most of each leaf blade giving a regular mosaic and in some cases scattered orange blotches. Usually 1 - 2mm sunken spots, chlorotic streaks (sometimes between normal-green lateral veins and at other times as 1 - 2mm wide lateral vein bands) or irregular chlorotic blotches and rings on diseased leaves. (Figures 2 - 3).

(iv) necrosis - necrotic flecks occasionally found, usually in centre of light-green or orange blotches (Figure 3).

Flower symptoms on severely affected plants occurred as a considerable reduction in flower size together with a loss of flower brilliance and unevenness of colour in the form of off-white or grey blotches on the petals.

Plants often displayed varying degrees of decline including a general reduction in leaf size and thin leathery leaves (generally associated with severe mosaic), leaf curling, excessive defoliation during autumn and winter and death of single limbs or whole plants. Flowering on declining plants was often delayed with production of small pallid flowers which failed to open completely.

Often symptoms were most striking in growth associated with the spring flush or in other cases during the hot summer months. In both cases symptoms were noted to recede partially or completely during winter.

Daphne odora 'Rubra' plants usually showed symptoms similar to those on Leucanthe. Slight twisting and rugosity of leaves was common on this cultivar and leaves were often small and narrow with irregular margins.

A similar range of symptoms was noted by other workers. Milbrath and Young (20) found virus in symptomless and diseased plants while Chamberlain and Matthews (8) reported virus only in plants displaying symptoms. The latter authors also noted that symptoms were associated with young growth and Chamberlain (7) recorded stunting of plants, rosetting near the shoot apices and twisting and distortion of the leaves. In the present study Rubra specimens free of detectable viruses also displayed twisting which was usually associated with the presence of aphids. The severe symptoms recorded on D. mezereum by Smith (26) which included distortion and necrosis were not found but single branchlets of only 3 specimens were observed.

An attempt to correlate symptom types on D. odora

with particular viruses was restricted as DVY was the only virus commonly occurring alone. Other viruses almost always occurred in combinations as illustrated in Table 4.

TABLE 4 Virus combinations in Leucanthe and Rubra

Cultivar	Number tested	Virus combinations						Free of detectable viruses
		CMV	DVY	ArMV DVY	CMV DVY	ArMV CMV DVY	CMV DVY TobRSV	
Leucanthe	354	3	263	25	43	10	2	11
Rubra	72	4	40	3	1	0	0	24

The predominance of plants infected with only one virus (DVY) possibly reflects roguing or death of the most severely diseased. Plants infected with DVY-1 alone and grown in an unshaded environment often showed a light green mosaic, blotches and streaks (Figure 2). Plants infected with DVY-2 alone however usually displayed only mild symptoms or were symptomless (most species other than D. odora only contained DVY-2 and lacked symptoms). Often plants with the most severe mosaic symptoms and those plants in the state of decline were infected with CMV (Figure 3). In one instance, for example, where 8 D. odora Leucanthe plants were growing in close proximity to each other, 6 plants infected with CMV and DVY-1 were much more severely diseased than two plants infected with DVY-1 alone.

Necrotic flecks and orange blotches on leaves

and blotches on flowers could not be associated consistently with any particular virus and a totally consistent correlation between virus and symptoms was not found. Furthermore in one instance a single D. odora plant had light-green mosaic on leaves of one limb but no virus could be detected.

While recognising that decline symptoms in daphne could be caused by other factors such as fungal root rots (e.g. phytophthora), agents such as mycoplasmas or poor cultural conditions (e.g. water stress), it is highly likely that virus does play an important part in the development of these conditions.

Environmental influence on symptoms

Daphne species are considered by some writers to prefer cool or shaded conditions (1, 9) and it was in this habitat that plants often exhibited mild symptoms or were symptomless. For example, in one nursery Leucanthe plants infected with DVY-1 alone and maintained in a permanently shaded greenhcuse, produced large symptomless leaves and flowers. However cuttings propagated from this source and subsequently grown in open fields exhibited severe leaf mosaic and indexing again only revealed DVY-1.

A comparison of the degree of severity of foliar symptoms in Leucanthe plants grown in shade with those in full sun is presented in Table 5.

TABLE 5 Effect of growth situations on the symptom expression of Leucanthe infected with DVY-1[a]

Situation	Total plants tested	Severity of foliar symptoms	
		Symptomless or mild	Severe
Shade[b]	84	93%	7%
Sun	105	0%	100%

[a] plants infected with DVY-2 were excluded as they were symptomless in all environments.

[b] less than 1 - 2 h direct sunlight per day

These results indicate a strong correlation between degree of symptom severity and growth situation and emphasize that symptoms are an unreliable guide to the virus status of a plant.

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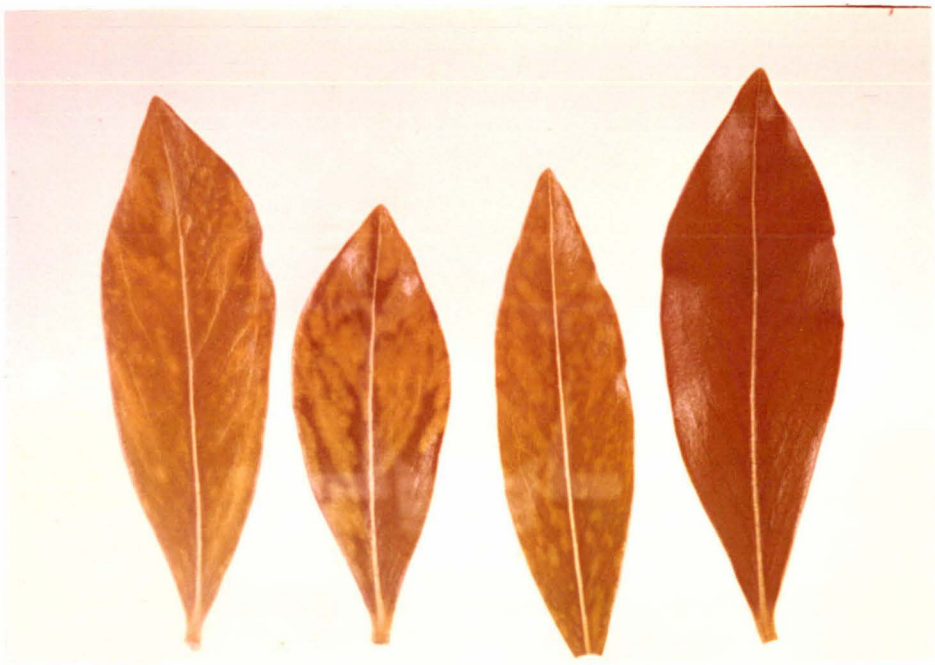

FIGURE 2: Leucanthe leaves infected with DVY-1. Symptomless leaf on right from shaded portion of same plant.

FIGURE 3: Leucanthe leaves infected with DVY-1 and CMV.

FIGURE 4: Local lesions of CMV in C. quinoa, three days after infection.

FIGURE 5: Local lesions of LVV-2 in C. quinoa, five days after infection. Identical symptoms are produced by AMV, ArMV, DVY-1 and TobRSV.

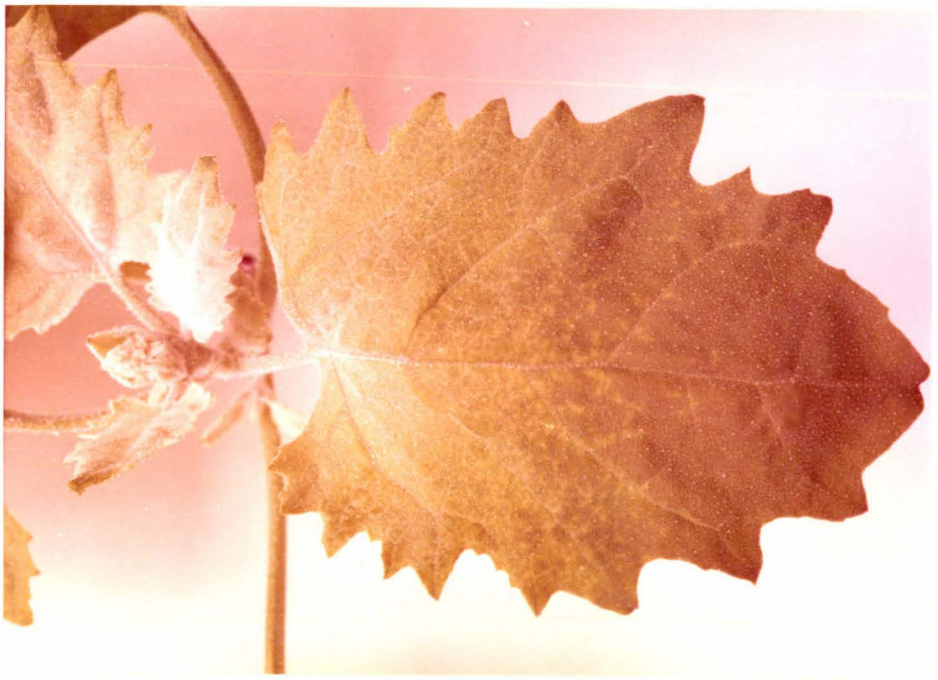

FIGURE 6: Systemic net reaction produced by LVV-2 in C. quinoa seven days after infection.

FIGURE 7: Systemic necrosis on the same plant 10 days after infection.

Identical symptoms are produced by AMV, ArMV and TobRSV.