Indexing for Dasheen Mosaic Virus in *Zantedeschia* Species

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INTRODUCTION

Over the past few years we have become increasingly aware of virus infection in summer flowering *Zantedeschia* hybrids in New Zealand. The only virus that has been positively identified in commercial crops is dasheen mosaic virus (DMV), a potyvirus that is widespread in aroids around the world. Although it has been possible to eliminate this virus from selected clonal material using meristem-tip culture (Cohen, unpublished data), indexing for the presence of DMV has been difficult because of low virus titre and uneven distribution in the plant.

This paper will describe some of the characters used to classify and name plant viruses, outline some of the methods used to detect (index) and identify viruses, review the literature on viruses reported to infect *Zantedeschia* species, and conclude with some comments on the production and maintenance of *Zantedeschia* cultivars free of DMV.

PLANT VIRUS NAMES

Growers are often confused by the names that are given to viruses and, in particular, to virus group names such as potyvirus or potexvirus. Viruses are classified using a number of characteristics including:

- The shape and size of the virus particle
- The type and number of strands of nucleic acid in the virus particle (RNA or DNA)
- The mode of transmission, i.e. egg, by aphids, thrips, sap, etc.
- The host range of the virus.

On the basis of these characteristics, plant viruses are placed in one of more than 20 groups. Knowledge of these groups tells us about many of the properties to anticipate when we begin to research new virus—plant combinations. For example, DMV is a potyvirus and it belongs to a large group of viruses infecting many important crop and ornamental species. This group includes potato virus Y, bean yellow mosaic virus, iris severe mosaic virus, narcissus yellow stripe virus, and tulip breaking virus. Viruses in this group have rod-shaped particles about 750 nm long, they usually have a narrow host range, and they are usually transmitted by aphids in, what is called, a non-persistent manner. This means that aphids probing the crop with its mouth parts can acquire the virus within minutes or even seconds and then transmit it to a new plant, but the virus does not persist on the aphid for more than a few hours. This information is very helpful in devising ways to reduce virus spread.

VIRUS INDEXING

Viruses are usually first detected on plants by the presence of symptoms, but symptomless infection may occur in some hosts and/or at some times of the year. To index for the presence of virus, we employ a variety of methods such as transfer of the virus to indicator plants, i.e. plants which produce characteristic symptoms following infection. We usually rub plant sap onto the surface of a young leaf on the indicator plant and wait for symptoms to appear one or two weeks later. In some cases we use aphids to transfer the virus, or diseased tissue is grafted onto a healthy indicator plant. The electron microscope (EM) can be used to detect viruses in plant sap and this is a very useful method for rod-shaped viruses. Different groups of viruses have different shapes of particles, but we cannot usually distinguish between viruses within a group, such as the potyviruses, using the EM alone. A variety of serological methods are available but the ELISA procedure is the most widely used for diagnostic purposes. Kits for detection of many plant viruses are now available commercially. Some of these kits will only detect specific strains of a virus, but in one case a kit is claimed to detect all the aphid-spread viruses in the potyvirus group (Jordan and Hammond, 1991). Another serological technique that has recently been described is the 'squash blot' (Lin et al., 1990). In this procedure cut plant surfaces are pressed against a nitrocellulose or nylon membrane. The presence of virus is then detected using enzyme-labelled antiserum followed by a reagent that gives a colour reaction in the presence of the virus/antiserum/enzyme complex. A number of molecular procedures are also available, some of which can detect extremely low levels of virus (Langefeld et al., 1991).

The ability to detect a virus depends on the sensitivity of the assay used, the titre (concentration) of the virus in the plant tissues, and the distribution of the virus in the plant tissues. Where virus titre is high and where the virus is evenly distributed in the plant, detecting the virus is usually simple and reliable. However, when virus titre is low and/or there is uneven distribution, it is very easy to miss the presence of virus (i.e. false negatives). For this reason we try to find ways of raising virus titre and to understand the factors affecting virus distribution in order to improve index reliability (Cohen et al., 1986).

VIRUSES IN *ZANTEDESCHIA*

DMV is widespread in a number of aroid plants including *Zantedeschia* (callas) in many countries (Table 1). It has been reported on *Z. aethiopica*, *Z. elliottiana,* and other species from U.S.A. (Zettler et al., 1970), South Africa (Van der Meer, 1985), and Italy (Rana et al., 1983). In New Zealand, it has been detected in many summer flowering *Zantedeschia* hybrids (Balasingam, pers. comm.; Cohen, unpublished data), but has not been detected on *Z. aethiopica*. DMV is a potyvirus and is transmitted by aphids. It has rod-shaped virus particles, which can be easily recognised using an electron microscope.

Tomato spotted wilt virus (TSWV) was the first virus to be detected on callas (Tompkins and Severin, 1950). Chamberlain (1954) reported the presence of TSWV in *Zantedeschia* in New Zealand. In Europe, it is possible that mosaic symptoms attributed to TSWV may, in some cases, have been caused by DMV (Kolbasina and Protsenko, 1973).

A number of other viruses have been reported to infect callas. Both Salinger (1985) and the new Royal Horticultural Society 'Dictionary of Gardening' report

that callas are infected by CMV but no references were cited. Chamberlain (1954) reported CMV on *Zantedeschia* species in New Zealand and Fletcher (1987) detected CMV in a sample of *Z. albomaculata* from a garden in Christchurch, New Zealand.

Arabis mosaic virus (AMV) and potato virus X (PVX) have been reported on *Z. aethiopica* in Poland and Japan respectively (Okuyama and Saka, 1976; Kaminska, 1985), and tobacco mosaic virus (TMV) has been found on *Zantedeschia* spp. in South Africa (Gorter, 1981), but it is unclear whether these infections are widespread even in these countries.

Table 1. Viruses that have been reported to infect *Zantedeschia* species.

Virus	Shape	Host range	Vector	Reference
Dasheen mosaic virus (DMV)	rod-shaped	aroids	aphids	Zettler et al. (1970) Rana et al. (1983) Kolbasina and Protsenko (1973) Van der Meer (1985)
Cucumber mosaic virus (CMV)	spherical	wide	aphids	Chamberlain (1954) Fletcher (1987)
Tomato spotted wilt virus (TSWV)	spherical	wide	thrips	Tompkins and Severin (1950) Chamberlain (1954)
Potato virus X (PVX)	rod-shaped	wide	non insect	Okuyama and Saka (1976)
Arabis mosaic virus (AMV)	spherical	wide	aphids	Kaminska (1985)
Tobacco mosaic virus (TMV)	rod-shaped	wide	non insect	Gorter (1981)

It appears that DMV is the major viral disease of hybrid *Zantedeschia* in New Zealand, but there have been no experiments to quantify the effects of DMV infection on tuber or flower production. Nor have there been any studies on the effects of other factors on the severity of infection. However, comparison of the growth of micropropagated plants of several cultivars that had been freed of DMV with the growth of the original stock showed enhanced vigour and delayed senescence. Plants with severe mosaic symptoms on leaves, often show streaks and/or spots on the spathe of the inflorescence. I have found that these symptoms are associated with zones of higher virus titre.

This raises the issue of the distribution of virus within the plant. The assay for DMV employed in this laboratory uses the broad spectrum potyvirus monoclonal antibody produced by Jordan and Hammond (1991) and commercially available from Agdia Inc. (Elkhart, Indiana, U.S.A.). This assay procedure has been found to be sensitive and reproducible for replicates taken from the same leaf. A sample

size of 20 to 50 mg is collected and this is ground in 2.0 to 5.0 ml of the recommended extraction buffer. This represents a 1/100 dilution of the plant tissue. The sample can be further diluted to determine the titre of the virus in the tissue. For a leaf sample with severe mosaic symptoms, positive reactions can be obtained when the tissue is diluted down to 1/7000. In contrast, for tuber samples from the same plants, virus has only been detected in samples diluted to 1/800. DMV was often undetectable from other samples taken from the same tuber.

The squash blot technique has been used to detect DMV in tuber sections (Lin et al., 1990). Sections were cut longitudinally through the tuber and pressed on to a nitrocellulose membrane. The virus was detected after soaking the membrane in the Agdia potyvirus antiserum followed by a detection reagent. Zones with high titre were clearly seen in the basal part of the tuber. Virus was either not detected or was present at much lower titre in the upper and central parts of the same tuber.

Further evidence for uneven virus distribution can also be seen from the expression of virus symptoms. Sometimes virus symptoms on different shoots on the same tuber or even different leaves on the same shoots will vary from symptomless to severe. The titre of virus in leaf tissues seems to closely reflect the symptoms observed. This result suggests that leaves might be susceptible to infection only at an early stage of leaf development.

To distinguish between samples which are free of DMV and samples with a very low virus titre, it will be necessary either to develop a more sensitive assay system or to learn how to raise virus titre. It may be possible to raise the titre of the virus in leaves by defining a temperature range which promotes virus replication in the tissues and growing the plants within this temperature range. This approach would be particularly useful when we are trying to index for virus following meristem-tip culture. If we can reliably index shoots in vitro, virus-free shoots could be micropropagated immediately, saving considerable time. This approach worked very well with lilies (Cohen et al., 1985; Cohen, 1986), but we do not yet know whether this approach will work for *Zantedeschia* hybrids.

PRODUCTION AND MAINTENANCE OF PLANTS FREE OF DMV

What are the best ways to obtain and maintain plants free of DMV? Although we can now use the ELISA method to determine whether leaves with mosaic symptoms or narrowed leaves are infected with DMV, there is currently no simple way to determine the percentage infection in a block of plants. To be certain that a plant is free of DMV, the plant should be indexed several times over preferably two growing seasons. If infected tubers are used as mother stock for micropropagation at least some of the resulting tubers will be infected. For this reason, only virus-free tubers should be used to initiate cultures for micropropagation. As mentioned above, it is possible to produce virus-free plants using meristem-tip culture and this is recommended if an alternate source of virus-free plants of the cultivar is not available.

To reduce the risk of reinfection, micropropagated plants from DMV-free cultures, should not be grown with non-indexed stock. In addition, virus-free tubers should preferably be stored separately from infected stock, particularly when the new sprouts are beginning to emerge. Aphid infection at this stage might transmit virus.

CONCLUSION

Techniques for detecting DMV in *Zantedeschia* have been improved. When combined with the use of meristem tip culture, it is possible to produce plants free of DMV. Precautions need to be taken to prevent reinfection.

LITERATURE CITED

Balasingam, G. MAF Lynfield Research Centre, Auckland, N.Z.

Chamberlain, E.E. 1954. Plant virus diseases in New Zealand. New Zealand Department of Scientific and Industrial Research. Bulletin 108.

Cohen, D. 1986. Virus eradication in vitro: Theory and practice. 6th Intl. Congress of Plant Tissue and Cell Culture. University of Minnesota, Abstract p. 305.

Cohen, D., K.S. Milne, and **M.J. Hyland**. 1985. In vitro manipulation of virus concentrations in hybrid lilies. Acta Hort. 164:319-324.

Fletcher, J.D. 1987. New plant disease records in New Zealand: Additional hosts of alfalfa mosaic virus and cucumber mosaic virus. N.Z. J. Agr. Res. 30:505-506.

Gorter, G.J.M.A. 1981. Index of pathogens II and the diseases they cause in cultivated plants in South Africa. Dept. Agr. Fish., Pretoria. Science Bulletin 398.

Hartmann, R.D. and **F.W. Zettler**. 1972. Dasheen mosaic virus infections in commercial plantings of aroids in Florida. (Abstract) Phytopathology 62:804.

Jordan, R. and **J. Hammond**. 1991. Comparison and differentiation of potyvirus isolates and identification of strain-, virus-, subgroup-specific, and potyvirus group-common epitopes using monoclonal antibodies. J. Gen. Virol. 72:25-36.

Kaminska, M. 1985. Isolation of arabis mosaic virus from *Zantedeschia aethiopica* plants. Experimental work of the Institute of Pomology and Floriculture. Ser. B.- Ornamental Plants 10:179-182.

Kolbasina, N.I. and **A.E. Protsenko**. 1973. Mosaic virus of the arum lily (*Zantedeschia aethiopica*). Byull. Gl. Bot. Bada 88:98-101.

Langefeld, S.A., J-M. Dore, J. Memelink, A.F.L.M. Derks, C.I.M. van der Vlugt, C.J. Asjes, and **J. F. Bol.** 1991. Identification of potyviruses using the polymerase chain reaction with degenerate primers. J. Gen. Virol. 72:1531-1541.

Lin, N.S., Y.H. Hsu, and **H.T. Hsu**. 1990. Immunological detection of plant viruses and a mycoplasma-like organism by direct tissue blotting on nitrocellulose membranes. Phytopathology 80:824-828.

Okuyama, S., and **H. Saka**. 1976. Potato virus X isolated from common calla (*Zantedeschia aethiopica*). Sci. Rep. Fac. Agric. Ibaraki Univ. 24:31-36.

Rana, G.L., C. Vovlas, and **F.W. Zettler**. 1983. Manual transmission of dasheen mosaic virus from *Richardia* to nonaraceous hosts. Plant Disease 67:1121-1122.

Salinger, J.P. 1985. Commercial flower growing. Butterworth, Wellington, N.Z.

Tompkins, C.M. and **H.H.P. Severin**. 1950. Spotted wilt of white, yellow, and pink callas. Hilgardia 20:207-218.

Van der Meer, F.W. 1985. Occurrence of dasheen mosaic virus in South Africa. Phytophylactica 17:95-98.

Zettler, F.W., M.J. Foxe, R.D. Hartman, J.R. Edwardson, and **R.G. Christie**. 1970. Filamentous viruses infecting dasheen and other araceous plants. Phytopathology 60:983-987.