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Controlling Virus Diseases in Major International Flower and Bulb Crops

During the past 10 years, flower and bulb crop production has expanded into geographic areas that allow growing and marketing at less cost than in traditional growing areas. Central and South America, Africa, and the Middle East grow a diverse range of species that are vegetatively propagated as cuttings or bulbs and grown into a finished product that is then exported to traditional consumer markets in Europe or the United States. Plants of more than 60 genera in 30 families are shipped to more than 35 countries through distribution centers like the United Aalsmeer Flower Auction in the Netherlands (Fig. 1).

Rapid vegetative propagation of diseased plants has increased the risk of spreading viruses in cuttings, bulbs, and corms. Control of virus diseases depends on establishing and maintaining healthy propagation stock. Methods of virus indexing of protected stock plants utilizing improved techniques of bioassay, serology, and electron microscopy in research diagnosis or routine indexing are described in this paper. Other approaches to control include meristem-tip culture and reducing spread of insect-borne viruses.

Twelve ornamental crops and 33 of the most economically important viruses, viroids, and mycoplasma organisms are listed in Table 1. Identification methods, diagnostic host species, mode of transmission, and geographic distribution are specified for each virus. The choice of a test procedure depends on many factors, including the availability of specific reagents and instruments.

Plant Quality

Many of the viruses listed in Table 1 affect foliage and flower size, number, and color. Improved plant quality has been associated with eliminating one or more viruses from many ornamental species, and selecting healthy plants for vegetative propagation is an important approach to control. For example, aspermy virus in chrysanthemum (tomato aspermy), possibly introduced into the United States from Japan or Europe, induces small, abnormally colored flowers with distorted florets (Fig. 2). This virus is controlled using bioassay and serological test procedures.

Some virus diseases described as symptomless are improperly named. For example, lily symptomless virus reduces vegetative growth and both flower size and number compared with virus-free plants derived from tissue culture (Fig. 3). This virus is controlled using serological diagnosis and electron microscopy.

Nuclear Stock Propagation

A tested nuclear stock is a source of propagation material indexed for known viruses and certified to be free from those viruses within the limitations of sensitivity and reliability of the test procedures. Development of nuclear stocks depends on: 1) recognition and characterization of the virus diseases in each crop, 2) development of reliable indexing methods to identify healthy plants and detect any symptomless carriers, and 3) establishment, maintenance, and distribution of virus-free cloned foundation stocks (9). Nuclear chrysanthemum and carnation stocks were established many years ago. These stocks may include only 100–300 plants in a major foundation stock greenhouse maintained free from insects. Several million cuttings may be produced over a 3–4 year cycle of vegetative propagation in a buildup of the nuclear plants. The risk of introducing pathogens into the nuclear stock can be reduced by taking precautions to prevent introduction and subsequent spread of the disease agents at each stage of vegetative multiplication.

Virus Therapy

Establishment of nuclear stocks in the production of virus-free bulb and flower crops has often been based on the recovery of healthy plants from known infected sources. A variety of therapeutic procedures have been used, including heat treatment and a combination of heat treatment and meristem-tip culture. Virus-infected stock plants are treated at 35–40 C constant temperature, and meristem tips are removed from vegetative branches after a few weeks or several months. The tips are transferred to a sterile medium where some of the resulting plantlets are usually virus-free. Many important ornamentals have been freed from one or more viruses using these techniques (9,19).

Virus Spread

There are three classes of viruses of ornamentals based on ecological and phytosanitary considerations (10). The first class includes viruses that have wide host ranges, usually have efficient vectors, and are already widespread. Examples are the cucumoviruses (cucumber mosaic virus group), including strains of cucumber mosaic virus and related viruses, and the nepoviruses (nematode-transmitted group), including tomato and tobacco

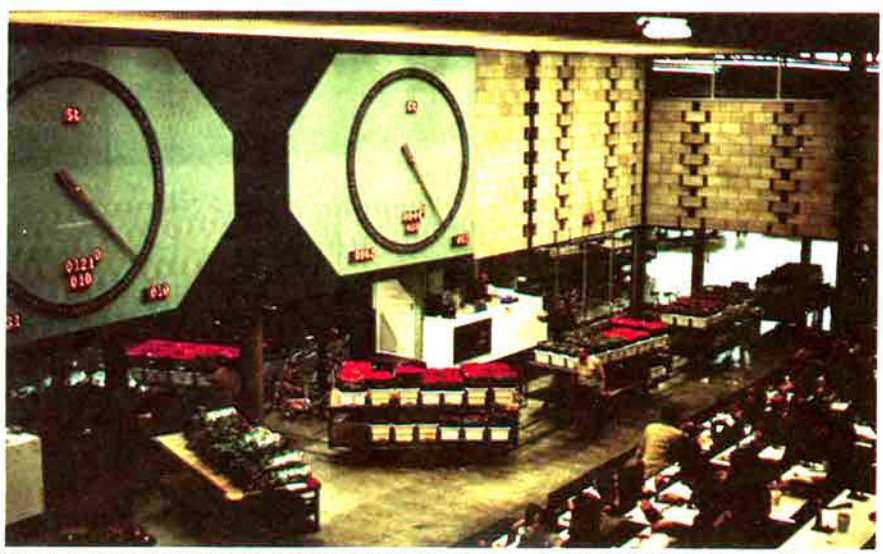

Fig. 1. Auction hall with plant material from South America, South Africa, Israel, the United States, and many other countries purchased by more than 300 exporters and wholesalers at the United Aalsmeer Flower Auction in the Netherlands. More than $270 million in cut flowers and $80 million in pot plants were auctioned in 1978.

ringspot viruses. Although viruses in these classes are sap-transmitted, the greatest danger results from aphid transmission of the cucumoviruses and nematode transmission of the nepoviruses. Viruses in the second class infect only one or a few ornamental species and are restricted to the geographic areas where the crop is grown. Some carlaviruses (carnation latent virus group) and potyviruses (potato virus Y group) are in this category; other examples are carnation mottle virus, carnation ringspot virus, and chrysanthemum stunt viroid. The third class includes viruses that are host-specific and cause symptomless infections. They are not efficiently spread and may be geographically restricted. Detection and diagnosis of these agents are not routinely applied in commercial production. The risk posed by spread of viruses from ornamental species to other economic crops is often minimal. Host specificity, mode of spread related to current distribution of a specific virus, and virus strains must all be considered when assessing the risk.

Controlling Insect Transmission

The application of insecticides to reduce vector populations has not usually been successful in controlling the spread of insect-borne viruses. Application of mineral oil sprays, however, has effectively reduced field spread of viruses in lilies and is used commercially. Aphid-borne tulip breaking virus, lily symptomless virus, and cucumber mosaic virus can spread quickly because lilies grow rapidly at the time flying aphids migrate. In the Netherlands, transmission of the persistent tulip breaking virus and the nonpersistent cucumber mosaic virus is reduced in lilies sprayed with oil. In Israel, tests are in progress to adapt the use of oil sprays to control virus spread in bulbous iris. Most transmissions among crop plants in the field result from secondary spread, and oil treatments that interfere with introduction of the virus into the crop can be very effective (20).

One disadvantage of the use of oil in bulb crops is possible phytotoxic damage, with a resulting reduction in bulb or flower size. Explicit formulations, methods, and rates of application under specific environmental conditions must be established before large-scale treatments can be successful. Oils will be more widely used as additional information is obtained on the effectiveness of oils applied under different insect population pressures and improved data become available on the number of plants primarily infected in relation to planting density of the crop and extent of oil coverage required on the plant surface.

Using repellent mulches of aluminum foil or plastic to control insects has prevented virus spread in some bulb and corm crops. A description of these procedures is beyond the scope of this article.

Diagnosis and Indexing

In virus disease control, distinguishing between research diagnosis and routine indexing is important. Research diagnosis is used to determine if a virus is present and to identify it. This often involves laboratory research in virus purification, identification, and strain differentiation in a small number of samples. Routine indexing is the detection of known viruses and usually involves testing a large number of samples. Indexing methods should be rapid, inexpensive, and easy to perform but still be reliable, sensitive, and specific.

The choice of indexing procedures may depend on the type of ornamental crop and the stage of growth when a test is performed. If tests are applied to a limited number of nucleus plants to be used for large-scale propagation, research diagnosis at a high unit cost per test may be justified. Testing larger numbers of samples may involve routine indexing procedures at lower unit cost.

Virus Indexing Procedures

Bioassay, serology, and electron microscopy are used for virus indexing. Several viruses in carnations, chrysanthemums, geraniums, roses, lilies, gladiolus, tulips, bulbous iris, and freesia can be mechanically transmitted, and infectivity tests have been used to detect viruses in these crops. Serological tests are usually used to confirm visual diagnosis or results of mechanical transmission tests. Recently, the use of serology in routine indexing has expanded with the use of the enzyme-linked immunosorbent assay (ELISA) procedure. By electron microscopy, virus particles can be observed in crude sap extracts from some infected species. The virus can be recognized and tentatively identified on the basis of its size and shape. A recent advance combines serology and electron microscopy using the technique of immune electron microscopy (18). This method greatly increases the sensitivity of electron microscope detection.

Bioassay. Bioassay is the primary method used in routine indexing for several of the economically important viruses in ornamentals. The choice of a particular test procedure is determined by both the virus and the host. One host may have inhibitors that prevent reliable transmission without the use of chemical additives. The same virus may be more readily transmitted from another host. Some other factors that influence results of a bioassay are the effects of light and temperature on symptom expression and virus content of the naturally infected host and on the susceptibility of the bioassay species.

One of the most important diseases of ornamentals, chrysanthemum stunt, is still detected commercially by graft indexing. A method using polyacrylamide gel electrophoresis, however, can detect the stunt agent in as little as 50 mg of infected chrysanthemum tissue (11). The reliability of the test method is similar to that of the bioassay.

Sap inoculation procedures are used to detect chrysanthemum aspermy, chrysanthemum B, carnation mottle, ringspot, latent, etched ring, vein mottle, cymbidium mosaic, and orchid tobacco mosaic viruses. Tomato and tobacco ringspot and several other viruses from geranium can all be transmitted to *Chenopodium amaranticolor*. Prunus necrotic ringspot and apple mosaic are also detected from rose by bioassay. Improvements in bioassay procedures include addition of polyethylene glycol to geranium extracts and 2-mercaptoethanol to rose leaf extracts. Sampling the species to be indexed when new growth first appears in the spring is usually desirable and sometimes essential.

Environmental factors are difficult to control in a routine indexing program when a large number of plants are tested. In a nuclear stock, some control of light and temperature may be possible when a smaller number of plants are sampled. Advantages of environmental control may be to induce symptoms that are diagnostic for a particular virus in the naturally infected host or to increase the sensitivity of the bioassay species when tests are performed in the greenhouse. Because tests must often be conducted in the greenhouse, they can be performed only at certain times of the year, usually in early spring when new growth begins and

Fig. 2. Reduced size of aspermy-infected chrysanthemum flower (right) compared with healthy chrysanthemum flower (left). (Courtesy R. K. Horst)

Fig. 3. Reduced height and smaller flowers of lily cultivar Enchantment infected with lily symptomless virus (right) compared with virus-free plant (left). (Courtesy T. C. Allen)

plants are most susceptible. In winter, supplemental illumination may be used to induce growth of the host or to increase the susceptibility of test plants.

Light and temperature may significantly influence local lesion formation on some indicator hosts. For example, greenhouse-grown *Saponaria vaccaria* 'Pink Beauty' mechanically inoculated with carnation etched ring virus may show concentric red rings, yellow spots, rings, and leaf curling, but not predictably. In a controlled environment, concentric red rings developed consistently at 27 C with either 12–16 hours of fluorescent (2,000 ft-c) or 12 hours of fluorescent (2,000 ft-c) plus incandescent (100 ft-c) illumination (8). At 32 C, chlorotic spots or concentric ring local lesions, some with red borders, were predominant. Plants grown at 16 hours of fluorescent illumination are induced to flower with added incandescent light. Local lesion development is greatly suppressed in plants induced to flower (8).

'Pink Beauty' is susceptible to at least four other carnation viruses. The broad-spectrum susceptibility of *S. vaccaria* could be an advantage in a program of routine indexing but a disadvantage in research diagnosis for specific identification of carnation etched ring virus from carnation infected with more than one virus (15).

Detached leaves may be used for detecting some viruses. Carnation mottle virus incites local lesions on *Chenopodium amaranticolor* and *C. quinoa*, and assays can be performed on detached leaves in large trays on moist paper under low light conditions in an incubator. The procedure requires fewer plants and thus less greenhouse space. An isolate inducing local lesions incited symptoms on inoculated leaves of *C. amaranticolor* and *C. quinoa* at 21, 27, and 32 C under fluorescent illumination with or without incandescent light (R. H. Lawson, *unpublished*). A disadvantage of using *Chenopodium* for detecting carnation mottle virus is the occurrence of attenuated strains that may produce only a few or no local lesions on *Chenopodium* leaves on the initial transfer from carnation (16). Carnation ringspot induces many local lesions on *C. amaranticolor* and *C. quinoa* grown at 21 or 27 C but only a few at 32 C (Fig. 4).

Symptom suppression at high temperature was also observed on *Gomphrena globosa* inoculated with the orchid strain of tobacco mosaic virus (14). Local lesions were induced on plants grown at 21 or 27 C but not on those grown at 32 C.

Several viruses in bulbs are sap-transmissible. For example, bean yellow mosaic virus can be detected using crude sap extracts of leaves and flowers of gladiolus, but cucumber mosaic virus can be detected routinely only with the extracts of flowers with severe color break. Neither virus can be recovered from corms. Some of the most important viruses in bulbs cannot be reliably detected routinely by bioassay in extracts of crude sap from leaves or bulbs, although the viruses are mechanically transmissible. Lily symptomless and tulip breaking viruses in lily and tulip are examples. Iris mild mosaic, iris severe mosaic, and narcissus latent viruses cannot be detected in a bioassay in routine indexing, but detection of bean yellow mosaic virus in iris may be possible by this method. Freesia mosaic and bean yellow mosaic viruses can be detected in *Freesia* by bioassay, but serological test procedures are used for routine indexing.

Serology. Serological test procedures for research diagnosis have been used for many years to detect and identify several viruses in ornamentals. In the Netherlands, antisera were prepared to chrysanthemum aspermy, chrysanthemum virus B, carnation ringspot, carnation mottle, iris severe mosaic, iris mild mosaic, bean yellow mosaic, freesia mosaic, tulip breaking, narcissus mosaic, narcissus yellow stripe, hyacinth mosaic, lily symptomless, hippeastrum mosaic, galtonia mosaic, scilla mosaic, and nerine mosaic viruses. Many of these viruses have limited host ranges, and it is remarkable that antisera to several of them were prepared more than 20 years ago at the Laboratorium voor Bloembollenonderzoek in Lisse. Antisera were also prepared at the Glasshouse Crops Research Institute in Great Britain and the Biologische Bundesanstalt in West Germany. Antisera to some viruses in flower crops were also produced in the United States during this period. With an expansion of bulb production, interest in serological test methods renewed in the United States.

Serological tests have the advantage of speed and specificity in disease diagnosis. Chloroplast agglutination and micro-precipitin tests have been employed to detect flexuous rod-shaped viruses, such as carnation latent and vein mottle in carnation and B virus in chrysanthemum. Lily symptomless and tulip breaking viruses have been diagnosed with micro-precipitin tests. Immunodiffusion and ELISA tests are now being used, however.

Immunodiffusion tests were first adapted for detection of small polyhedral viruses. Although never adapted for large-scale routine indexing, chrysanthemum aspermy virus and carnation mottle and ringspot viruses were among the first detected in laboratory tests using agar gel double diffusion tests. The advantage of the gel diffusion test in detection of aspermy was demonstrated in comparative bioassay tests on tobacco. Serological tests were more reliable than biological tests for aspermy detection during the summer months, regardless of the age of the tissue sampled (12).

Carnation mottle virus can be successfully detected in crude sap of carnation using agar double diffusion tests. The virus occurs in high concentration in carnation grown in a wide range of light and temperature conditions. Narcissus tip necrosis virus can be detected directly in crude sap from narcissus in agar diffusion tests. Other polyhedral viruses, such as carnation ringspot, cucumber mosaic, tomato and tobacco ringspot, prunus necrotic ringspot, and apple mosaic, can usually be detected in agar diffusion tests only after the virus is concentrated from the naturally infected host. These viruses may also be transmitted to an herbaceous indicator species and tested in crude sap or concentrated from crude sap for serological testing.

Double and single diffusion methods in agar gel are used in tests for flexuous rod viruses. Diffusion of the virus in the gel is improved by first degrading the virus with chemicals. Cymbidium mosaic and odontoglossum ringspot virus (orchid tobacco mosaic) have been detected in orchids (6) and carnation latent virus in carnation (17).

The single immunodiffusion drop

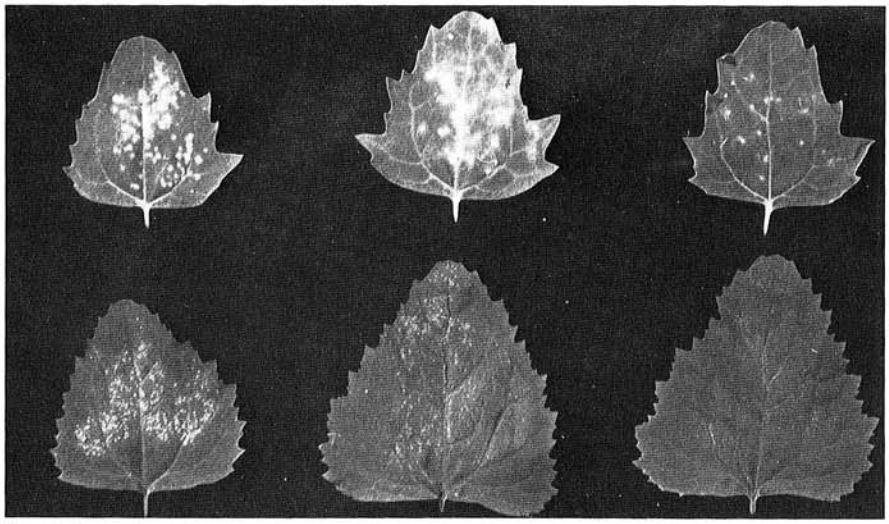

Fig. 4. Carnation ringspot virus local lesions on *Chenopodium quinoa* (top row) and *C. amaranticolor* (bottom row) grown at (left to right) 21, 27, and 32 C. Note absence of lesions on *C. amaranticolor* at 32 C.

(IDD) test was first developed for detection of lily symptomless virus (LSV) (21). Antiserum to pyrrolidine-degraded virus was prepared, and leaf extracts to be tested for virus were extracted in pyrrolidine. The test required about 12 times less antiserum than the radial diffusion test. Antiserum was also prepared against intact virus (7). Intact and degraded LSV have very few, if any, antigenic determinants in common. The sensitivities of the microprecipitin and IDD tests were about the same but were lower than that of electron microscopy (7). In lilies, the most reliable results were from leaves obtained 2 weeks after flowering until the end of the growing season. In tulips, LSV was detected more readily in flowers than in leaves.

The ELISA procedure has significantly extended the use of routine serological indexing. This method offers great promise for detection of viruses in bulb tissue as well as in leaves. It would be an advantage if the routine indexing carried out with the IDD test on lily leaf samples in the summer could be substituted by testing the bulbs during the storage period (21). Although LSV was readily detected in bulb extracts of the cultivar Enchantment, the virus could be detected in bulbs of the cultivar Destiny only after the extract was preincubated with cellulase and hemicellulase to break down the gumlike substances (3). This work showed that lily cultivars differ in content of the inhibitory substance and that individual bulbs within the cultivar Destiny may also differ.

The ELISA test is also used to detect cucumber mosaic and bean yellow mosiac viruses in gladiolus (22), narcissus mosaic and tip necrosis viruses in narcissus (5), nerine latent virus in nerine, and freesia mosaic and bean yellow mosaic viruses in freesia.

Routine indexing by ELISA is now performed with viruses in several flower crops, including aspermy (17) and chrysanthemum B viruses (13) in chrysanthemum, carnation cryptic (17) and carnation necrotic fleck viruses in carnation, prunus necrotic ringspot and strawberry latent ringspot viruses in rose (23), and cymbidium mosaic and orchid tobacco mosaic viruses in orchids.

The comparative use of bioassay, electron microscopy, and serological procedures proved the increased reliability of the ELISA test method. For example, in detection of chrysanthemum virus B, reliable results were obtained with the drop precipitin test only until March under the growing environment in West Germany (13). After March, the concentration of virus dropped below the level of detection. During late spring and summer, infectivity tests, as well as single and double diffusion tests with pyrrolidine-degraded virus and antiserum to the degraded virus, also gave unreliable results. The latex test was only about 16 times more sensitive than the drop precipitin test, but ELISA was about 2,000 times more sensitive. In summer, only the ELISA test and electron microscopy gave reliable results with infected mature chrysanthemum plants.

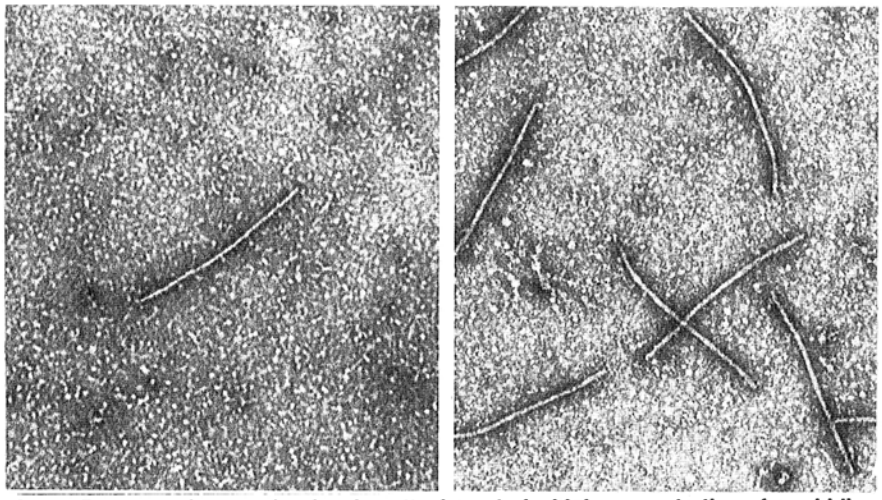

Fig. 5. Electron micrographs showing attachment of a high concentration of cymbidium mosaic virus particles on a grid coated with antiserum (right) and a low number of particles on a grid coated with normal serum (left).

Prunus necrotic ringspot and strawberry latent ringspot viruses were reliably detected by ELISA in leaf extracts from infected rose. However, detection of these viruses using an antiserum-sensitized electron microscope grid was twice as sensitive as ELISA for detecting these viruses in rose extracts (23).

Although the use of ELISA testing is likely to increase rapidly in routine indexing for many additional viruses, certain aspects of the method should be carefully considered before large-scale testing is adopted. For example, ELISA testing may be more specific, and serological cross-reactions that show relatedness among strains of a single virus in gel double-diffusion may not show similar cross-reactivity in ELISA. Thus, the natural occurrence of strain mixtures may be a limiting factor in routine indexing where antiserum is prepared to a single isolate or strain of virus.

Electron microscopy. A method commonly used to identify a virus is to observe the particles in the electron microscope. Rod-shaped virions can often be observed in crude sap preparations on an electron microscope grid in the presence of an electron-dense stain. This procedure, known as negative staining, has been used to detect cymbidium mosaic virus in orchids (14). The method was slightly more sensitive than the bioassay procedure for detecting this virus.

Immune electron microscopy (IEM) is a method of virus detection and identification that is receiving increased attention (18). The technique involves observation of specific binding of antigen and antibody in the electron microscope. This procedure has been applied in several ways. The simplest method is to apply a drop of antiserum to an electron microscope grid and place the freshly cut surface of a leaf in the drop for 1 or 2 seconds. The antiserum clumps the virus particles and coats them with antibody if the antibody in the antiserum is related to the virus. The virus particles are visualized by negatively staining the preparation with an electron-dense stain before the sample is examined. Virus particles can be visualized in samples where the virion concentration is below the level of detection by conventional negative staining alone.

Virus particles may also be attached to antiserum-coated electron microscope support films. With this method, known as the Derrick technique, the increase in particle number over untreated control grids was up to 30,000, usually 5,000–10,000 for carnation cryptic virus and 100-fold for tobacco rattle and tomato bushy stunt viruses. A comparison of the number of virus particles attached to a grid treated with cymbidium mosaic antiserum and a grid treated with normal serum shows a large increase in the particles attached to the grid treated with the specific antibody (Fig. 5). The method has been modified (18) to include a second application of antiserum after attachment of the virions. In this procedure, a "decoration" or coating of the antigenic sites with antibody occurs, and specific attachment can be observed when the homologous or a related antibody is applied.

These procedures have been used to detect chrysanthemum virus B, carnation vein mottle and carnation necrotic fleck viruses, orchid tobacco mosaic and cymbidium mosaic viruses, and narcissus latent virus (5). Flexuous rod-shaped viruses can be detected in bulbous iris with direct negative staining of the sap, and the IEM method should be applicable for detecting and identifying iris mild and severe mosaic, bean yellow mosaic, and other rod-shaped viruses in iris, gladiolus, tulip, lily, and other bulb species and to many rod-shaped viruses in flower crops.

Table 1. Viruses of primary importance infecting major flower and bulb crops

Crop	Virus name	Identification methods	Diagnostic test plants	Mode of transmission
Carnation	Carnation mottle virus	Test plants, serology, electron microscopy	*Chenopodium quinoa*, *C. amaranticolor*, *Dianthus barbatus*	Mechanically in sap
	Carnation vein mottle virus	Test plants, serology, electron microscopy	*C. amaranticolor*, *C. quinoa*, *D. barbatus*	Mechanically in sap, aphid
	Carnation etched ring virus	Test plants, electron microscopy	*Saponaria vaccaria*, *Silene armeria* 'Jocker'	Mechanically in sap, aphid
	Carnation necrotic fleck and yellow fleck viruses	Test plants, electron microscopy	*D. barbatus*	Mechanically in sap, aphid
	Carnation latent virus	Test plants, serology, electron microscopy	*C. amaranticolor*, *C. quinoa*	Mechanically in sap, aphid
	Carnation ringspot virus	Test plants, serology, electron microscopy	*C. amaranticolor*, *D. barbatus*, *Phaseolus vulgaris*	Mechanically in sap
Chrysanthemum	Chrysanthemum aspermy virus (tomato aspermy)[a]	Test plants, serology	*Nicotiana glutinosa*, *N. tabacum*, *C. amaranticolor*, *C. quinoa*	Mechanically in sap, aphid
	Chrysanthemum virus B	Test plants, serology	*Petunia hybrida*, *N. glutinosa*	Mechanically in sap, aphid
	Chrysanthemum stunt viroid	Test plants, polyacrylamide gel electrophoresis	*Chrysanthemum morifolium* 'Mistletoe' (graft), 'Bonnie Jean'[b] (tissue implant)	Mechanically in sap
	Chrysanthemum chlorotic mottle viroid	Test plants	*C. morifolium* 'Velvet Ridge' or 'Bonnie Jean'[b] (tissue implant or graft)	Mechanically in sap
Freesia	Freesia mosaic virus	Test plants, serology, electron microscopy	*C. amaranticolor*, *C. quinoa*	Mechanically in sap, aphid
	Bean yellow mosaic virus	Test plants, serology, electron microscopy	*P. vulgaris*	Mechanically in sap, aphid
Gladiolus	Bean yellow mosaic virus	Test plants, serology, electron microscopy	*P. vulgaris*, *Pisum sativum*	Mechanically in sap, aphid
	Cucumber mosaic virus	Test plants, serology	*Cucumis sativus*, *Vigna unguiculata*	Mechanically in sap, aphid
	Tomato ringspot virus	Test plants, serology	*C. quinoa*, *C. amaranticolor*	Mechanically in sap, nematode
	Tobacco ringspot virus	Test plants, serology	*C. quinoa*, *C. amaranticolor*	Mechanically in sap, nematode
Hydrangea	Hydrangea ringspot virus	Test plants, serology, electron microscopy	*Gomphrena globosa*, *C. quinoa*, *C. amaranticolor*	Mechanically in sap
	Hydrangea virescence (mycoplasma induced)	Electron microscopy	None	Grafting
Iris (bulbous)	Iris mild mosaic virus	Test plants, serology	*Iris* spp., *C. amaranticolor*, *Nicotiana* spp.[c]	Mechanically in sap,[d] aphid
	Iris severe mosaic virus	Serology	*Crocus* spp., *C. quinoa*[c]	Mechanically in sap,[d] aphid
	Bean yellow mosaic virus	Test plants, serology, electron microscopy	*P. vulgaris*, *P. sativum*	Mechanically in sap, aphid
	Narcissus latent virus	Test plants, serology, electron microscopy	*Tetragonia expansa*, *N. clevelandii*	Mechanically in sap, aphid
	Iris mild yellow mosaic virus[e]	Test plants	*Iris* spp.	Aphid
Lily	Lily symptomless virus	Serology, electron microscopy	*Lilium longiflorum*	Mechanically in sap, aphid
	Cucumber mosaic virus	Test plants, serology	*C. sativus*, *V. unguiculata*	Mechanically in sap, aphid
	Tulip breaking virus	Test plants, serology, electron microscopy	*L. formosanum*	Mechanically in sap and by bulb grafting, aphid
Orchid	Cymbidium mosaic virus	Test plants, serology, electron microscopy	*Cassia occidentalis*, *C. amaranticolor*	Mechanically in sap
	Orchid tobacco mosaic virus	Test plants, serology, electron microscopy	*C. amaranticolor*, *G. globosa*	Mechanically in sap
	Bacilliform viruses	Test plants[d]	*Chenopodium* spp., *Nicotiana* spp.	Mechanically in sap
Pelargonium	Tomato ringspot virus	Test plants	*C. quinoa*, *C. amaranticolor*	Mechanically in sap, nematode
	Tobacco ringspot virus	Test plants, serology	*C. quinoa*, *C. amaranticolor*	Mechanically in sap, nematode
	Pelargonium leaf curl virus (tomato bushy stunt)	Test plants	*C. quinoa*, *C. amaranticolor*	Mechanically in sap (probably through soil)
Poinsettia	Poinsettia mosaic virus	Test plants, serology	*Euphorbia cyathophora*	Mechanically in sap
	Poinsettia cryptic virus	Serology	None	Unknown
Rose	Prunus necrotic ringspot virus	Test plants, serology, electron microscopy	*C. sativus*, *Momordica balsamina*	Mechanically in sap, pollen
	Apple mosaic virus	Test plants, serology	*C. sativus*	Mechanically in sap
	Strawberry latent ringspot virus	Test plants, serology, electron microscopy	*C. amaranticolor*, *C. quinoa*, *C. sativus*	Mechanically in sap, nematode
	Arabis mosaic virus	Test plants, serology	*C. amaranticolor*, *C. quinoa*, *C. sativus*	Mechanically in sap, nematode
Tulip	Tulip breaking virus	Test plants, serology, electron microscopy	*L. formosanum*	Mechanically in sap and by bulb grafting, aphid
	Lily symptomless virus	Test plants, serology, electron microscopy	*L. formosanum*	Mechanically in sap, aphid

[a] Chrysanthemum isolates can be serologically distinguished from tomato isolates.
[b] *C. morifolium* 'Bonnie Jean' can be used to detect both stunt and chlorotic mottle viroids.
[c] A. A. Brunt (4).
[d] Bacilliform particles have been observed in orchid tissue in Europe, Japan, and the United States. There may be more than one type of virus, since mechanical transmission has been reported from Japan but not elsewhere.
[e] C. J. Asjes (2).

Geographic distribution
Worldwide
Almost everywhere carnations are grown, but especially Mediterranean region
Worldwide
Japan, New Zealand, Australia, Israel, Europe, Venezuela, North America
Europe, Australia, Japan, New Zealand
Europe, North America, New Zealand
Wherever chrysanthemums are extensively grown
Widespread in cultivated chrysanthemums
Wherever chrysanthemums are extensively grown
United States, Denmark
Wherever freesia are grown
Wherever freesia are grown
Wherever gladiolus are grown
Common in many plant genera
North America, Denmark, Netherlands, probably other areas of Western Europe
North America, probably in Western Europe
Probably worldwide in cultivated *H. macrophylla*
United States, Europe
Worldwide
Worldwide
Worldwide
United Kingdom, Netherlands, West Germany, probably worldwide
Netherlands
Wherever lilies are grown
Worldwide
Worldwide
Worldwide
Worldwide
Probably in many orchid genera
United States, United Kingdom, Denmark, Sweden
United States, United Kingdom, Canada
United States, United Kingdom, Denmark, West Germany, Belgium
United States, West Germany
West Germany
Worldwide
Worldwide
Western Europe, once reported in Canada
Europe, United States, once reported in dogwood
Worldwide
Worldwide

Small polyhedral viruses can also be detected in IEM. Negative stains other than neutral phosphotungstate must be used, however, to preserve viruses such as cucumber mosaic, prunus necrotic ringspot, and apple mosaic (18). The IEM method was nearly twice as sensitive as ELISA in detecting arabis mosaic, prunus necrotic ringspot, and strawberry latent ringspot viruses in extracts from rose (23). The high nonspecific background from healthy roses after overnight incubation of sap at 4 C made detection of arabis mosaic and prunus necrotic ringspot viruses difficult in an ELISA test (23). Results with apple mosaic and prunus necrotic ringspot viruses with an incubation of 1–2 hours at 4 C reduced the background to low levels (E. Halk, *personal communication*). The concentration of prunus necrotic ringspot virus in leaves of the same plant appeared not to be related to seasonal changes in temperature or light. Both prunus necrotic ringspot and apple mosaic virus may decrease in concentration as leaves mature, since neither was detected in mature leaves.

Clumping and the modified Derrick procedure can trap low concentrations of virus and be used to detect virus in crude sap preparations. Application of the IEM procedure may be most useful in research diagnosis involving specific virus and strain identification and in routine indexing where a limited number of samples are tested from nuclear stocks or plants from tissue culture where very high sensitivity is required.

Application of Test Methods

In the United States, virus testing and indexing programs are organized in private industry and in state-operated laboratories. In private industry, the largest nuclear stock propagators employ resident pathologists who apply bioassay procedures for detection of viruses in chrysanthemums and carnations. In some instances, the propagators have nuclear stocks tested serologically or by electron microscopy for some viruses in state-operated laboratories. For example, testing for carnation necrotic fleck virus is done by the California Department of Agriculture in Sacramento. Recently, ELISA has been adapted to test for viruses in poinsettia and pelargonium. Poinsettia mosaic virus is assayed in commercial poinsettias after heat therapy to establish clean nuclear stock (R. W. Fulton, *personal communication*). The State Department of Agriculture in Pennsylvania has established standardized test procedures for detecting tomato and tobacco ringspot viruses in pelargonium. In New Jersey, a certification scheme for producing orchids free from cymbidium mosaic and orchid tobacco mosaic viruses, using bioassay and electron microscopy, was established several years ago. In this program, growers retain qualified pathologists to conduct tests or have the tests performed at a state university laboratory.

In Europe, testing is performed in private or public laboratories. In Great Britain, for example, the Nuclear Stock Association, located adjacent to the Glasshouse Crops Research Institute, is an organization of growers of chrysanthemums, carnations, and geraniums who jointly finance the large-scale production of nuclear stocks derived from heat-treated and meristem-tip-derived plants. Tests are performed for all the major viruses described in these crops. In the Netherlands, the General Netherlands Inspection Service for Ornamental Crops performs large-scale serological tests, for example, about 150,000 per year for freesia mosiac virus and approximately 80,000 per year for chrysanthemum virus B.

Bulb production for forcing and indoor use as cut flowers is an important industry in the United States. Of the nearly 60 million bulbous iris grown annually in the state of Washington, 20% are exported to Europe, where they are forced for early spring flowering. In Great Britain, foundation stock of 24 cultivars of bulbous iris have been rid of iris mild mosaic, iris severe mosaic, and narcissus latent viruses (O. W. Stone, *personal communication*). A similar program is under way in the state of Washington.

Asiatic-type garden lilies have been popular in U.S. gardens for many years. Hybrid lily growing has become a major industry in western Oregon. Ten years ago, export of the *Lilium* Mid-Century hybrid 'Enchantment' increased rapidly. Oregon-grown lilies were exported to the Netherlands, where they were field-grown for cut flower production. During the early 1970s, methods were developed to produce virus-free 'Enchantment' and other garden lily cultivars in tissue culture, and the superior performance of clean stock was demonstrated (1). Lily production in the Netherlands increased rapidly, and now more than 2,200 acres are grown. A group of 20 Dutch growers has formed the Lily Meristem Culture Foundation Holland. These growers finance the operation of the foundation laboratory and, in return, have their stocks and newly meristemmed material tested serologically and assayed on test plants or by electron microscopy at the Laboratorium voor Bloembollenonderzoek in Lisse. In 1980, 42,250 lily plants were tested for the presence of lily symptomless virus.

Narcissus grown for bulb and cut flower production is the most important ornamental crop in Great Britain, with about 8,400 acres, 55% of the world production, and an estimated commercial value of $60 million. Virus-free stocks have been produced from 55 cultivars of narcissus by meristem-tip culture (O. W. Stone, *personal communication*). More than 24,000 acres of bulbs are grown in the

Netherlands, which has the largest acreage in tulips. Control of virus diseases of tulips has depended on visual observation of symptoms and field roguing. This procedure is rapid but unreliable because virus symptoms may be masked or latent. Serological detection of tulip breaking virus as part of a quality improvement program is currently being evaluated by the Dutch Bulb Inspection Service for possible application in a nuclear foundation stock for tulip production.

Progress and Prospects

Ornamental plant crops usually have higher unit value than many field crops, and the decorative value and longevity of the finished product are of primary importance for successful marketing. Many of the fungus and bacterial diseases of bulb and flower crops can be controlled with fungicides and sanitary practices. Virus disease control, based on indexing and nuclear stock propagation, is more difficult to achieve. Nuclear stock programs require large investments of money and personnel.

Controlling virus diseases in vegetatively propagated ornamentals has involved the development and use of a variety of techniques, including bioassay, serology, and electron microscopy. The first large-scale control program was developed as a graft-indexing procedure to detect the stunt disease in chrysanthemum that threatened survival of the industry nearly 35 years ago. During the same period of the 1940s and early 1950s, rapid progress was made in isolating and characterizing several potato viruses. The use of serology in indexing was rapidly applied as this new technology developed.

Rapid advances have recently been made in developing the new and more sensitive virus detection procedures discussed in this article. Indexing for several chrysanthemum and carnation viruses is being applied by major nuclear stock propagators both in the United States and abroad. Certification programs have reduced the risk of international transport of viruses and have improved crop quality.

The success of nuclear stock programs for carnations, chrysanthemums, freesias, lilies, and geraniums can be attributed to the close cooperation of industry, scientists, and plant protection officials. Similar improvements are possible in the production of other certified crops.

As the nuclear stock concept is expanded and used for both greenhouse and outdoor crops, more effort will be made to maintain plants free from viruses.

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