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Detection, Diagnosis and Control Strategies of Viral Diseases of Ornamental Crops

Roger-H Lawson and Hei-Ti Hsu

The United State Department of Agriculture, Agriculture Research Service, Florist and Nursery Crops Laboratory, Beltsville, Maryland 20705-2350, U.S.A.
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ABSTRACT

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Many important horticultural crops are vegetatively propagated. Production of these crops begins with a single plant of a few progeny that are selected for superior horticultural value. Production of healthy nuclear stock has been achieved in several major crops such as carnation, chrysanthemum, orchids, roses, freesias, bulbous iris, lilies and several minor bulb crops. In some instances, commercial selections have been maintained as clean stock by producing plants in a protected environment. In other instances, when the newly introduced clutivars have become infected, it has been possible to free plants of infection from viruses and other pathogens through the use of heat or chemotherapy and tissue culture. The current methods for virus detection and disease diagnosis, and strategies for management of virus diseases are discussed. The virus detection methods discussed here includ enzyme-linked immunosorbent assay (ELISA), dot blot immunoassay (DBIA), tissue blot immunoassay (TBIA), & immune specific electron microscopy (ISEM).

Key words: viral disease control, virus detection, virus diagnosis, ornamental crops.

INTRODUCTION

Many of the most important horticultural crops including floral and nursery crops are vegetatively propagated. Production of these crops involves a scale-up in production that often begins with a single plant or a few progeny that are selected for superior horticultural value. Many years ago, producers of nuclear stock realized the importance of certifying the foundation plants free of all known pests and disease organisms. Production of healthy nuclear stock has been achieved in several major crops such as carnation, chrysanthemum, orchids, roses, freesias, bulbous iris, lilies and several minor bulb crops. In some instances, commercial selections have been maintained as clean stock by producing plants in a protected environment. In other instances, when the newly introduced cultivars have become infected, it has been possible to free plants of infection from viruses and other pathogens through the use of heat or chemotherapy and tissue culture.

The purpose of this article is to review current methods of virus detection and disease diagnosis. In the 1930's and 40's bioassay was the most commonly used procedure (23). Serological tests for detection have also been utilized for many years (43). New reagents and procedures have increased the sensitivity and reliability of immunological diagnosis. During this period procedures for virus detection have moved from the laboratory to use by growers.

Immunological procedures utilized in the 1940's were developed to detect virus or virus related products of infection. Many improvements have been made in serological methods. Serology is the single most important diagnostic method in use today (60). Detection of nucleic acids in diseased plants has also become important in disease diagnosis. Procedures involving detection of double-stranded RNA have been used for detection of some viruses (45,53) and dot blot hybridization also became important (46,47). Most recently, the use of the polymerase chain reaction (PCR) for the amplification of nucleic acid has been adopted as a new tool for detection of plant viruses (49, 58). PCR is a highly sensitive procedure for virus and viroid detection.

IMMUNOLOGICAL DETECTION

Principle of serology

Antibodies are proteins that are produced in sera of animals to combat invasions of foreign substances. Plant virus antisera are generally produced by injecting rabbits with "purified virus" (21,54). The purified virus preparation used for immunization of animals should be free of contaminating plant materials. Animal virus antisera free from host contaminating protein antibodies may be produced by immunizing with virus preparations propagated in homologous host systems. It is, however, sometimes difficult to obtain immunizing viral antigens free from contaminating host plant proteins that also elicit the formation of antibodies.

Polyclonal antisera have been produced to many different viruses infecting both woody and herbaceous ornamental crops. Antisera are available not only from research laboratories but have been marketed commercially by several companies. Many polyclonal antisera are broad-spectrum reagents that react with strains of the same virus and related viruses.

Serodiagnostic assay

Serological assay has become the most powerful single testing procedure for detection and diagnosis of a wide range of viruses infecting plants (56,60) since it is specific, sensitive, reliable and rapid. Serological techniques have evolved from simple precipitin tests in a liquid phase and gel diffusion tests in semisolid media to highly sophisticated enzyme-linked immunosorbent assay and immune-specific electron microscopy (2,10,17,41,50,55,57). A recent introduction of tissue blot immunoassay further simplified the diagnostic procedures (42).

Traditionally antibodies have been produced in rabbits (54), but monoclonal antibodies to plant viruses are becoming more important in serological testing (24,39). Initial generation of specific antibody secreting hybrid cells is time consuming but production of antibodies by hybridoma cell lines is technically no more demanding than normal *in vitro* cell culture. High concentrations of monoclonal antibodies to some important viruses in ornamentals have been produced in ascitic fluids (28,30,33).

Preparation of antiserum

Polyclonal antisera Rabbits are routinely used in production of polyclonal antisera because of their ability to respond to a variety of plant virus antigens, the quantity of available serum and the relatively modest space required to house them. Experience shows that individual rabbits respond differently to immunization with the same antigen preparation. In most laboratories several animals must be immunized in order to obtain one good responder. If larger volumes of serum are required, goats, sheep and even horses have been employed (1,52,55). Serological procedures that require antibodies from different animal sources have utilized other species of animals such as guinea pigs, mice, rats or chickens (30,35). In chickens, egg yolks are the primary source of antibodies (51).

Systematic investigations of the immunization procedures to obtain high titered antisera have not usually been reported. It is difficult to standardize the immunization protocol such as antigen dose, route and schedules of immunization as well as the use of adjuvant. In the authors experience, initial intravenous injection with a sub-milligram quantity (usually 0.2–0.5 mg) of plant virus usually provides adequate sensitization. After a 3 to 4 week resting period this is followed by a subcutaneous and 2 to 3 intramuscular injections at 2–3 week intervals, of 0.5 to 1.0 mg virus in Freund's complete adjuvant. Injections with an excess quantity of "purified virus" may stimulate production of antibodies to minor contaminants of plant constituents which may be present in the immunogen.

Monoclonal antibodies Monoclonal antibodies provide a level of standardization that is not possible with polyclonal antibodies. Unlike the heterogenous mixtures of immune sera which differ not only from individual animals but also from each bleed of the same animal, the monoclonal antibody produced by a selected hybridoma cell line is a well defined chemical. Because monoclonal antibodies are so specific they may be used to differentiate closely related strains of a virus (19,27). In addition, a monoclonal antibody that reacts with a portion of the coat protein that is shared by all strains of a virus, or a group of viruses, should be useful for broad spectrum testing (38). Hybrid cell cultures that produce monoclonal antibodies can be stored in cryogenic freezers and can be revived and cultured to produce monoclonal antibodies as required.

Construction of hybridomas secreting antibodies to plant viruses is a well established procedure and monoclonal antibodies have been produced to numerous viruses including those infecting ornamental and nursery crops (24,36,39,48). Although the hybridoma technique does not require purified virus for production of virus-specific monoclonal antibodies, incubation of the immunogen with an antiserum prepared to normal plant constituents prior to immunization will reduce the contaminating plant antigens (16). Tolerance to plant antigens can be induced immunologically in mice by injecting excess amounts of plant extracts into newborns. Using this method, it was possible to enhance the proportion of hybridomas secreting antibodies specific to tomato spotted wilt virus (34). A similar procedure of establishing immunological tolerance in the neonatal

mice was utilized to enhance the production of mouse hybridomas secreting antibodies to a plant mycoplasmalike organism that is difficult to obtain in pure form (32).

Serological differentiation of related viruses Depending on the purpose of the assay, serological tests may be utilized for either broad or narrow spectrum detection of a specific serotype of an individual virus or a group of viruses. Polyclonal antibodies have, for example, been used for many years to detect individual viruses and closely related strains of the same virus. Polyclonal antisera have frequently been used to establish the degree of relationship among different isolates of the same or different viruses and to distinguish among different groups of plant viruses (54).

With the introduction of hybridoma technology, it has been possible to select panels of monoclonal antibodies with both narrow and broad spectrum reactivity. A monoclonal antibody has been produced that reacts with most potyviruses (37,38). In addition, other monoclonal antibodies have been produced that react with a more limited group of viruses. These reagents may be useful where a plant is known to be susceptible to more than one potyvirus and it is important to determine that a potyvirus is present without regard to the specific identification of the virus (38). For example, bulbous iris may be infected by at least 3 different potyviruses that include iris mild mosaic, iris severe mosaic and bearded iris mosaic. The panel of monoclonals can be used to distinguish between iris mild and severe mosaic.

Monoclonal antibodies produced to TSWV have been used to distinguish the two distinct serogroups that comprise the Tospovirus genus (12,34). Although polyclonal antibodies have also been used to distinguish these two serogroups, the monoclonal antibodies have been used to further distinguish the isolates comprising a serogroup (4).

Test methods

Enzyme-linked immunosorbent assay The enzyme-labelled immuno-sorbent assay (ELISA) has become a standard procedure for detection of many plant viruses. It allows a quantitative estimation of virus antigens in infected plants with high sensitivity and specificity. Detection of nanogram amounts of viral antigens has been achieved. The most commonly used method is the double antibody sandwich ELISA (DAS-ELISA) procedure in which viral antigens are trapped by virus-specific antibody in sensitized microtiter plates (10). Following the incubation with enzyme-labelled anti-virus immunoglobulins, a chromogenic substrate solution is added to the wells. The presence of viral antigens in the wells of the microtiter plate is revealed by the development of a colored product after enzymatic degradation of the substrate. The intensity of the reaction is proportional to the concentration of the enzyme molecules which in turn is related to the presence of viral antigens in each well. Accurate measurements of the viral antigens can be made spectrophotometrically.

Variations in ELISA procedures that are equally applicable for virus detection are the use of enzyme-labelled protein A instead of enzyme-labelled antibodies (5,13,25,26). The major feature of the protein A molecule in serology is its extraordinary affinity for binding immunoglobulins, notably IgG (15). In the intact molecule, at least two sites are accessible for binding of IgG. Enzyme (either alkaline phosphatase or horseradish peroxidase) labelled protein A conjugates are readily available from a number of suppliers. The protein A procedure offers the advantage that one form of conjugate can be used for detection of different viruses. A specific antibody-enzyme conjugate is not required for each virus to be tested.

The strong interaction between avidin and biotin molecules has drawn the attention of immunologists (40). The system is quickly adapted to virus detection. The biotin molecule can be easily activated and coupled to antibodies; whereas avidin can be conjugated to an enzyme with an increase in the sensitivity of an assay. As with the enzyme-labelled protein A ELISA system, only one conjugate preparation is required for many different assays. The biotin-avidin system is very attractive for use in virus detection.

Dot blot immunoassay (DBIA) Dot blot immunoassay is similar to ELISA except that a membrane matrix rather than a plastic plate is employed as a solid phase supporting material (14). Microliter quantities of antigen solution can be spotted in a grid pattern on the membrane by hand. Larger volumes of diluted antigen can be applied to the membrane matrix with the aid of a manifold under vacuum.

A comparison of ELISA and DBIA in tests with tomato spotted wilt and cymbidium mosaic viruses showed that DBIA is about 8 times more sensitive than ELISA (31,33). In tests with lily symptomless virus, a 30 fold increase of sensitivity was achieved by dot blot immunoassay (29). The increased sensitivity of the detection limit with DBIA on nitrocellulose membranes over the ELISA procedure in microtiter plates is a definite advantage. In ELISA tests, about one third of antigens applied were actually trapped by specific antibodies immobilized in plastic wells in which no more than 200 μl was added (31).

Tissue blot immunoassay (TBIA) Direct blotting of newly cut tissues on nitrocellulose membrane adds a new dimension to the diagnosis and detection of plant viruses (42). Virus antigens in tissue blots on the membranes can be detected by enzyme-labelled immunological probes. Indirectly, it allows visualization

of the distribution of specific antigens in plant tissues. In addition, tissue specialization of luteoviruses or mycoplasmalike organisms has been demonstrated (42). One of the advantages that the TBIA offers is that it does not require extraction of materials for testing, thus providing simplicity and convenience for the assay of larger numbers of samples. The technique still retains all the advantages of specificity, sensitivity, reliability and rapidity that ELISA and DBIA provide (31,33). The virus antigens that are irregularly distributed with only a few cells infected will show up clearly in tissue blots that would give only a weak positive reaction or even go undetected if tested in sap extracts (31,33).

Immune specific electron microscopy (ISEM) Immune specific electron microscopy offers different types of advantages that allow direct observation of virus particles and the antigen-antibody interaction with an electron microscope (2,44). Although the procedure was first introduced in the investigation of tobacco mosaic virus, the technique has not been fully utilized in the routine assay of plant viruses. The sensitivity of quantitative immune specific electron microscopy can exceed ELISA tests (33).

Commercial sources of serological reagents for diagnosis

During the past several years, serological diagnosis has expanded rapidly and new companies have emerged to meet the needs of this new market. In th United States, the company Agdia has expanded its product line that now includes antisera to more than 50 different viruses infecting ornamentals. Twenty of these viruses are detected by the broad-spectrum reacting, monoclonal antibody that is known to react with a large number of potyviruses (37,38). Agdia also offers a testing service where samples are processed on a fee per sample basis.

In addition to the firm Agdia, the American Type Culture Collection sells antisera that may be used to detect a range of viruses infecting ornamentals. The address and companies that offer antiserum for plant virus disease diagnosis are listed below:

Agdia, Inc.
30380 County Road 6
Elkhart, Indiana 46514 USA

American Type Culture Collection
12301 Parklawn Drive
Rockville, MD 20852 USA

Agrailab
Science Park
Kiryat Weizmann
Rehovot, Israel

Bioreba AG
CH-4008 Basel/Switzerland
Bempenstrasse 8
Switzerland

Boehringer Mannheim Corp.
Biochemical Products
9115 Hague Road
P. O. Box 50414
Indianapolis, IN 46250 USA

Sanofi Phyto-Diagnostics
Z. I. de la Ballastiere
B. P. 126
F-33501 Libourne cedex
France

Detection of nucleic acids

Polymerase chain reaction (PCR) The polymerase chain reaction is an important new technology for the amplification of nucleic acid. Using this procedure, very low amounts of nucleic acid can be amplified. In theory, a single copy of nucleic acid can be amplified to whatever level is required for detection. It is possible to amplify a fragment that can be separated in an agarose or polyacrylamide gel and stained so the amplified product can be visualized.

An essential first step in performing PCR is the necessity to have data on the sequence of the viral, viroid or other nucleic acid structure that is to be amplified. Since the genome of most plant viruses is RNA, a cDNA must first be synthesized from the RNA with reverse transcriptase. This cDNA is used as a template for PCR. In the PCR process amplification of the DNA involves two oligonucleotide primers which hybridize to opposite strands of the target sequence. The enzyme DNA polymerase makes copies of the DNA that has been supplied with the specific primers. Repeated cycles of heat denaturation of the DNA annealing of the primers to their complementary sequences, and extension of the annealed primers with the DNA polymerase result in the exponential increase in the target portion of the viral nucleic acid. There is an exponential increase in the number of copies formed since in each cycle the number of copies is doubled. This technique allows the selective enrichment of a particular DNA sequence by a factor of 10^6 to 10^7 (58).

Viroids can be detected in nucleic acid extracts that contain 1-100 pg of total nucleic acids in tissue extracts. This procedure is 10-100 fold more sensitive than detection by hybridization and 2,500 fold more sensitive than detection by gel electrophoresis (18). Reverse transcription PCR can detect a concentration of plum pox virus of 10 fg of purified RNA which is equivalent to about 2,000 virus particles (61) and is

more sensitive than the use of ^{32}p labelled cRNA probes.

PCR has been used to detect bean yellow mosaic virus in gladiolus leaves. Detectable amounts of virus RNA in BYMV-infected plant tissue by PCR were about 3 to 4 orders of magnitude lower when compared with those detectable by ELISA and molecular hybridization (58). Combining PCR with molecular hybridization (using a ^{32}p-labelled transcript of viral sequences as a probe) increased the sensitivity of the method with a further improvement of 4 to 5 orders of magnitude compared with direct molecular hybridization. Using this method a concentration as low as 1 picogram of virus was detected. Extracts of RNA from corm tissue inhibit amplification of viral sequences when added to a PCR reaction (59). An additional step in purification of the RNA extracts with a Sephadex G-50 column eliminated the inhibitory effect and enabled the PCR to amplify and detect the viral RNA in corm preparations.

Riboprobes in viroid detection Viroids are previously detected by bioassays on suitable hosts or by polyacrylamide gel electrophoresis of extracted nucleic acids. A nucleic acid hybridization technique employing cloned DNA complementary to viroid or RNA probes (riboprobes), a transcript from cDNA, is a sensitive method for detection of viroid infection (47). Detection of chrysanthemum stunt viroid using an RNA probe is now routinely performed at Agdia, Inc. Samples are prepared and applied at the growers location and the membrane is shipped to Agdia for processing.

DNA probes in pathogen detection Mycoplasmalike organisms (MLOs) were detected in naturally infected plants of *Gladiolus, Ranunculus, Brassica* and *Hydrangea* by dot hybridizations using six different biotin-labelled cloned DNA probes (6). The results indicated similarities in nucleotide sequences homologies among the MLOs in four genera of naturally diseased plants and between these MLOs and some in North America. The probes could also be used to distinguish the MLOs from one another. Dot hybridization has also been used to show that MLOs causing ash yellows and lilac witches broom are similar and possibly identical (22). This research has demonstrated that nonradioactive probes can be used to detect MLOs in field-grown plants and may be useful in indexing florist and nursery crops for the presence of these pathogens (6). Probes have also been used to detect chrysanthemum yellows mycoplasmalike organism (7) and the MLO associated with virescence in periwinkle (11) by both dot hybridizations and Southern hybrid analysis.

Comparative sensitivity of detection of four viruses in ornamentals

Cymbidium mosaic virus (CyMV) Orchids are frequently infected with CyMV. In a parallel test of 155 orchid samples using mouse monoclonal antibodies, 30 infected plants were identified by TBIA and only 24 of the 30 samples tested positively by ELISA (33). Three of the remaining TBIA-identified infected plants gave ELISA A_{405} values between 0.15 and 0.2; the other three plants were tested negatively (A_{405} less than 0.05) by ELISA.

Tomato spotted wilt virus (TSWV) DAS-ELISA based on polyclonal antisera produced against tomato spotted wilt virus and Impatiens necrotic spot virus are specific for homologous virus and show little cross reaction with heterologous virus. Anti-TSWV monoclonal antibodies were found to be highly specific for TSWV and were effective in DAS-ELISA for capture of TSWV and for detection of TSWV captured by anti-TSWV polyclonal antibodies. An improved TSWV test utilizing polyclonal antibodies for capture and monoclonal antibodies for detection resulted in lower background reactions to healthy tissue and greater detection endpoints of sap from virus-infected plants (12). Dot blot immunoassay (DBIA) proved to be a sensitive method for detection of TSWV infection in plants. Using biotin-labelled mouse monoclonal antibodies followed by avidin-enzyme conjugate, DBIA was nearly 8 times more sensitive than ELISA for detection of TSWV in extracts from infected *N. benthamiana* leaves. Leaf and stem samples from *Eustoma* (Lisiant-hus) and Impatiens plants showing virus-like symptoms tested positively by direct tissue blottings (31).

Tulip breaking virus (TBV) Mouse monoclonal antibodies were successfully utilized in detecting TBV and monitoring aphid transmission of the virus in tulip growing fields in Washington State (20). The indirect ELISA with antigen coated plates was employed in the survey. The antigen coated indirect ELISA procedure has the advantage of simplicity since it allows many antibodies or antisera to be compared simultaneously using a universal conjugate. Monoclonal antibodies were superior to the polyclonal antisera in detecting TBV (20).

Lily symptomless virus (LSV) In comparative studies, the sensitivity and reliability of ELISA, DBIA and TBIA were evaluated for detection of LSV. Using purified virus, DBIA was shown to be about 16 to 30 times as sensitive as ELISA (29). Similar orders of magnitude were attained for detection of LSV from

infected *Lilium longiflorum* or *L. elegans*. In another experiment when 26 bulbs were analyzed for LSV infection, ELISA showed that 8 samples were infected with LSV-whereas DBIA revealed 19 samples including those tested positively by ELISA, were infected with LSV. Analysis by TBIA revealed the presence of LSV antigens in tissue blots of 20 samples.

STRATEGIES FOR MANAGEMENT OF VIRUS DISEASES

The first principle of control of virus diseases of florist and nursery crops is the development and application of reliable methods of pathogen detection. Protection of clean stock through sanitation is essential in the maintenance of virus-free stock. Protection may also involve protecting plants with spray materials that reduce transmission by insect vectors. Finally, if stocks become infected, a combination of tissue culture and heat therapy may be used to recover healthy stock from infected plants.

Nuclear stock propagation

Nuclear stock maintenance is the most critical phase in virus disease control. Nuclear stock plants are usually multiplied in small numbers in a protected environment. It is essential to maintain plants free of potential insect vectors that may transmit some viruses. This may be achieved by the use of chemicals to control insects and fine mesh screening in the glasshouse. Plants should also be adequately spaced so they are not in contact with one another. Care should be taken to avoid mechanical transfer of viruses by minimizing plant handling and through the use of pathogen-free propagation cutting tools. Water should also be clean and efforts should be made to avoid splashing or dripping water from plant to plant.

Testing for known viruses should be conducted on a regular schedule in the first phase of production. In many instances, a single test is inadequate and repeated testing must be performed before a plant can be certified to be free of a particular virus. The nuclear stock phase is also the phase of production in which test methods can be most economically applied since the number of plants that must be tested is limited.

Secondary propagation

Methods of virus detection described in this article are usually applied to plants utilized in the primary but not the secondary stage of propagation. This is due mainly to the cost associated with testing large numbers of samples as the number of plant units rapidly increases. Secondary propagation is, however, the stage of buildup that may present the greatest opportunity for virus spread in vegetatively propagated crops. Often growers believe that virus diseases can be effectively controlled if only the nuclear stock is certified. In those instances in which viruses are efficiently spread by either mechanical means or by insect vectors, a false sense of security may prevail. Although many factors may influence the introduction of virus and virus spread in the secondary stage of propagation, failure to enforce strict rules of sanitation may be most important. Introduction of uncertified material into a nuclear stock block may lead to breakdown of the entire certification system. Even when stringent sanitary practices are observed, virus infections may become established from no identifiable source. Limited sampling during secondary stock buildup is an essential step that should be incorporated in a stock buildup program since viruses may pose a major threat to both quality and productivity of the final product.

Cost effectiveness

Pay now or pay later is a principle that should be recognized and understood by producers who grow crops that are adversely affected by viral pathogens. The presence of a virus may limit the value of a crop and make a crop unacceptable for both domestic consumption and export. For example, economic unification in Europe may result in adoption of a higher standard of health for products moving between and among countries. Certification standards will likely become more stringent as larger quantities of plant material and a greater diversity of new kinds of material enter international commerce.

Controlling virus vectors

Nematodes and insects Some viruses may be transmitted by nematodes and fungi in the soil. Others may be transmitted by aphids or thrips. For example, the nematode *Xiphinema* may transmit tomato ringspot virus in soil. Innovative methods for control of some nematodes may involve flooding the land, but for most nematodes, control still involves the use of synthetic pesticides.

Above ground, aphids may vector viruses in the potyvirus group including bean yellow mosaic virus and dasheen mosaic virus and other important viruses such as cucumber mosaic virus. In the potyvirus group, there are more than 400 aphid transmitted viruses and virus strains.

Thrips have become a major problem in both greenhouse and field culture in many areas of the world. Major outbreaks of this insect were first reported in the United States in 1986–87. In 1988, there was a major disease epidemic caused by the rapid spread of tomato spotted wilt virus transmitted by *Frankliniella occidentalis*, the Western flower thrips. This flower

thrips is also present in Europe and is associated with transmission of tomato spotted wilt virus in several European countries.

For many years vents and other openings in greenhouses containing nuclear stocks have been screened with 20 or 30 mesh screens to prevent entry of winged and wind blown aphids from outdoors. More recently, some growers producing crops susceptible to tomato spotted wilt virus have covered openings in nuclear stock greenhouses with a smaller nylon mesh to exclude thrips. Some new greenhouses have been designed to include an entry room where street clothing is covered with white pants and jackets before entering the area where the nuclear stock plants are grown. The use of an ultrafine mesh screen and construction of an entry area that provides a barrier between the exterior environment and the plant growing area have been successful in reducing the incidence of tomato spotted wilt virus in the glasshouse.

Crop protection

Protection of some field grown crops with mineral oil sprays has been highly effective in combating the spread of some aphid-borne viruses. In The Netherlands, lilies growing in the field are sprayed to reduce transmission of tulip break virus and lily symptomless virus (3). The benefit from oil spray depends on the seasonal buildup of aphid populations. Mineral oil was highly effective under conditions of moderate infection pressure and moderately effective under high infection pressure (3). In contrast, these authors found that vegetable oils were moderately effective under moderate infection pressure and ineffective when the infection pressure was high. Use of oils should coincide with the development of aphid populations and may require twice weekly applications during times of high infection pressure.

Application of tissue culture in production of clean stock

Tissue culture has been successfully used to eliminate many different viral pathogens, often in combination with heat therapy and with the use of chemicals. Early research emphasized the importance of applying virus testing procedures following the application of a putative therapeutic method. Unfortunately, some producers have relied on tissue culture alone and have automatically assumed that a meristemed plant is a virus-free plant. Unfortunately, this is not true. As shown in the propagation of some bulbs, tissue culture combined with chemotherapy may eliminate the presence of a virus (8). Following removal of hyacinth from tissue culture, a prolonged growing-on period may, however, be required before a pathogen reaches a detectable concentration (9). Repeated testing may be required before the plant material can be certified free of virus with sufficient confidence to allow buildup and commercialization of plants from tissue culture.

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摘　　要

Roger H. Lawson，徐惠迪．1993．花卉作物病毒病害之偵測、診斷及防治策略．植病會刊 2:177-186．(美國農部花卉及種苗作物研究室，Beltsville, Maryland 20705-2350, USA)．

很多重要的花卉作物均採用無性繁殖。康乃馨、菊花、玫瑰、鳶尾、百合及一些球根作物的無病毒健康母株已被廣泛應用。這些作物通常係由選定具良好園藝性狀的少數無病毒個體大量繁殖，而一些被感染的優良品種亦可利用熱療及組織培養技術將病毒或其他病原去除而成爲

健康母株。目前偵測花卉病毒，常用之方法則包括enzyme-linked immunosorbent assay (ELISA), dot blot immunoassay (DBIA), tissue blot immunoassay (IBIA),及 immune specific electron microscopy (ISEM)等方法。其方法在測定各病毒時各有優劣。

關鍵詞：病毒病害防治、病毒偵測、病毒診斷、花卉作物。