Quarantine Viruses, Viroids and Phytoplasmas that Affect Movement of Ornamental Plants

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INTRODUCTION

Some viruses, viroids and phytoplasmas infecting ornamental crops have been widely distributed around the world and have caused significant damage and economic losses. When trade in ornamentals was initiated many years ago, the threat of distributing viruses and viruslike agents was minimized because much of the germplasm was exchanged as seed. In the last fifty years the transport of vegetatively propagated clonal material has resulted in the dissemination of many viral and other graft-transmissible agents.

Some of the first viruses transported across national and international borders were those infecting cut flower crops. Infected cuttings of carnations and chrysanthemums carried disease agents over long distances. More recently, vegetative propagation of Petunia and perennial crops such as Hosta and seedlings of Eustoma served as virus carriers. In some instances the agents were spread before it was recognized the material was diseased. In other instances, methods of detection and diagnosis for a specific disease agent were not developed for certification purposes.

Quarantine regulations are designed primarily to exclude the movement of pathogens into geographic areas where they were not previously known to occur. Regulations have also been developed to restrict the movement of disease agents into areas where they may occur but distribution is limited. Practical procedures of certification depend on adequate methods of detection. Certification also ensures that standards of quality are maintained for optimum productivity of each of the major crops in international commerce.

The purpose of this review is to examine the differences in quarantine regulations between the European Union and the U.S. and how the application of these regulations affects the movement of plant material. Virus and viruslike agents of quarantine importance are presented. In addition, the potential of some "new" viruses, viroids and phytoplasmas to limit distribution of ornamentals are discussed.

EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION (EPPO)

Quarantine Regulations

Virus, viruslike pathogens and phytoplasmas of ornamental plants are the subject of quarantine rules established for a group of several European and Mediterranean countries. The European and Mediterranean Plant Protection Organization (EPPO) is a regional organization operating under guidelines of the International Plant Protection Convention. Today, the EPPO includes 44 member states in the European and Mediterranean region (http://www.eppo.org/). EPPO sets regional standards for phytosanitary protection of plant products. In the EPPO system the A1 list includes a quarantine pest not present in that area and the A2 list identifies a quarantine pest in the area that is not widely distributed and is officially controlled (EPPO, 2004). Watermelon silver mottle virus and Chrysanthemum stem necrosis virus are the only two viruses, out of 24 listed on the A1 list, that infect ornamentals. Apple mosaic virus, Chrysanthemum

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stunt viroid, Impatiens necrotic spot virus, Tobacco ringspot virus, Tomato ringspot virus, Tomato yellow leaf curl virus, Tomato spotted wilt virus and Cherry leaf roll virus (in Rubus) are the eight agents out of eighteen that affect ornamentals on the A2 list. Elm phloem necrosis phytoplasma and palm lethal yellowing phytoplasma are on the A1 list.

Quarantine Regulations Administered by the U.S. Department of Agriculture

The Regulated Pest List is the document published by the Animal and Plant Health Inspection Service (APHIS) of the U.S. Department of Agriculture. The Pest List (http://www.invasivespecies.org.Newlnitiatives.html) provides a guide for trading partners and alerts them to pests of quarantine concern in the U.S. APHIS activities include an inspection at ports-of-entry, surveys of exotic pests, methods of risk mitigation and eradication programs. APHIS also oversees a post-entry program for vegetatively propagated materials entering the U.S. and a pre-clearance program from specific countries that allows products to enter without additional inspection following importation. The Pest List currently includes seventy viruses but only six of them infect ornamentals. Included are Arabis mosaic virus, Elm mottle virus, Raspberry ringspot virus, Tobacco ringspot virus (Andean potato calico strain), Tomato blackring virus, and Columbian datura virus. During the last two years Raspberry ringspot virus, Tomato blackring virus, and Arabis mosaic virus were detected in samples sent to Agdia, Inc., (http://www.agdia.com).

There are 93 genera and families of plants in the prohibited quarantine category in the U.S. regulations. Most are propagated vegetatively. There are very few restrictions on import of seeds. Prohibitions may apply to certain countries or to all countries depending on the risk assessment (United States Department of Agriculture (USDA) Title 7--Code of Federal Regulations-Part 319). Only four genera of cut flowers, including Rhododendron and azaleas, Camellia, Gardenia, Rosa and Syringa vulgaris are required to have written permits issued by the Animal and Plant Health Inspection Service of the USDA for entry into the U.S. Importers must also obtain phytosanitary certificates from the exporting country.

The prohibited category includes many genera of nursery stock, plants, roots, bulbs and other plant products. Prohibited does not mean that all the materials classified as prohibited are restricted from entering the country (USDA Title 7--Code of Federal Regulations-Part 319). Prohibition is a function of both host and country of origin. Some articles that are intended for propagation may be in a restricted category that allows entry when specific requirements are fulfilled. For example, Chrysanthemum vegetative material can enter the country with a phytosanitary certificate from the nation of origin and a declaration that the greenhouse where the plants were grown was free of white rust based on visual inspection monthly for a period of four months prior to shipment. Imported Dianthus from Great Britain must be accompanied by a phytosanitary certificate and an additional declaration verifying that the propagated material was grown in a greenhouse free of Carnation etched ring virus, Carnation "streak" virus and Carnation "fleck" virus.

In addition to the requirements imposed on the exporter, the importer may have to grow designated restricted articles in post-entry quarantine. Imported Chrysanthemum, Crocosmia, Dianthus, Gladiolus, Hibiscus, Hydrangea, Jasminum, Rosa, Syringa, and Watonina must all be grown in a protected environment that meets State regulations and must be periodically inspected by state phytosanitary officials to monitor and enforce postentry quarantine rules during the period of evaluation.

The list of restricted articles also contains a section that includes woody ornamentals. In addition to several ornamental trees and shrubs, the list includes Hibiscus, Hydrangea, and Jasminum. The regulations are less specific in these instances since they only state Chlorotic ringspot agent (associated with Jasminum spp), Jasmine variegation agents, Yellow ring mosaic agent (associated with Jasminum spp), and Hibiscus leafcurl agent are conditions that prevent importation. Also included is Rose wilt. In most cases, a phytosanitary certificate is required from the plant protection service of the country of
origin that specifies that a particular virus or agent does not occur in the country of origin or that visual examination and indexing have been performed on the material grown for export. Viruses of woody material mentioned in this section of the regulations, that may also infect herbaceous ornamental plants, include *Arabis mosaic virus*, *Raspberry ringspot virus* and *Tomato black ring virus*, *Plum pox virus*, *Cherry leaf roll virus*, and *Strawberry latent ringspot virus*.

Plum pox virus was recently detected in the U.S. Although the virus infects many Prunus species, including ornamental Prunus, it is not considered an important virus in ornamentals. Experimental hosts include *Campanula*, *Lamium*, *Lupinus*, *Petunia*, *Ranunculus*, *Silene* and *Zinnia* species. These genera have not been identified as natural hosts. They could, however, possibly serve as reservoirs of the virus (Levy et al., 2000).

**Quarantine Regulations for Australia, the Republic of China and Singapore**

These countries are primarily interested in regulating plant products and the viruses that are associated with tropical crops. The Australian Quarantine and Inspection Service maintains a list of "Targeted Plant Pathogens". The list currently identifies two viruses each in sugarcane and banana, and one each in citrus, papaya and sorghum of quarantine importance on their website. The Republic of China has specific requirements for the importation of *lily*, *gladiolus* and *dahlia* bulb and *corn* material. In addition, however, there are a total of 83 viruses infecting ornamentals that are included in the list of regulated pathogens. In Singapore the emphasis is also on viruses in tropical plant hosts. *Arabis mosaic virus* and *Tobacco ringspot virus* are the two pathogens infecting ornamentals that are of quarantine importance.

**EPPO and APHIS Quarantine Standards and Regulations--A Comparison**

We have briefly presented a summary of standards and regulations to compare and contrast the differences. For example, the EPPO A1 and A2 lists of quarantine pests are recommendations to Member governments. The status of quarantine pests has to be technically justified by the process of pest risk analysis. Although the pest lists are drawn up to cover the entire geographical region, the A1 and A2 lists may not be of concern to all countries and they may elect not to take protective measures for a particular pathogen. But for each pest on the A1 and A2 list, EPPO recommends EPPO Standards "Specific Quarantine Requirements" to Member Governments. Included is a recommendation on the listing of the quarantine pest in national phytosanitary regulations and on the requirements to be made to exporting countries in relation to commodities which may carry that pest in international trade.

The standards and regulations developed by the EPPO are both similar and different from the USDA regulations. For example, the quarantine regulations are similar in that both organizations list viruses that do not occur in the EPPO region or within the borders of the U.S. In the EPPO certification programs have been established that are recommended for implementation by Member Governments in an effort to prevent the introduction of pathogens of quarantine significance. The U.S requires that exporters of certain specified ornamentals make both a visual inspection as well as conduct appropriate tests to certify that the plant material is free of specified known viruses. For example, viruses listed in the U.S. regulation on *Nursery Stock, Plants, Roots, Bulbs, Seeds, and Other Plant Products* include some infecting *Dianthus* that are already known to occur in the U.S. The distribution of these viruses is thought, however, to be limited. Certification by the exporter is required to avoid further distribution in the U.S. and a postentry growing out period is an added safeguard for many restricted articles. This regulation is similar to the EPPO recommendation that specifies the use of certification methods to limit spread in areas of the EPPO region where the virus is already known to occur.
**Certification Standards for Ornamentals in the EPPO**

In Europe, the European Community (EC) health regime, originally established in 1976, was modified in 1993 to account for the "Single Market" principle. This new rule transferred phytosanitary evaluation from the internal borders of member countries to places of production in the EC and to external borders of the EC for third country products (Krczal, 1998). The rule also provided for establishing a system of plant passports that attest to compliance with Community standards. The passport ensures free movement of plant material within the Community and replaces plant health certificates in intra-EC trade. An EC health inspection activity was established to ensure uniform application of rules by inspection when needed to check imported plants from third countries.

Several EPPO Member governments developed certification schemes for certain ornamental species to increase health standards and promote trade (Krczal, 1998). Certification schemes vary, however, among countries due to differences in testing methods. Under the new system it is assumed that Member governments cooperate. The EPPO has, therefore, developed certification schemes that are recommended, but not compulsory, for Member governments.

The EPPO Panel on "Certification of Ornamentals" developed procedures for carnation, pelargonium, lily, narcissus, chrysanthemum, tulip, crocus, iris, begonia, impatiens, rose, freesia, hyacinth, kalanchoe and petunia (http://www.eppo.org/Standards/certitication.html). The EPPO certification standards for production of healthy plants are examples of the development of practical and useful guidelines that will help ensure the production of virus-free planting stock. The guidelines specify the selection of candidate nuclear stock, and maintenance of nuclear stock that includes growing conditions and testing requirements. The standards include the use of visual inspection combined with biological testing and ELISA where appropriate. The highest priority is placed on viruses that are efficiently vector transmitted.

**Pest and Diseases of Potential Quarantine Significance**

The EPPO has also adopted an "Alert List". The list includes pests selected by the EPPO Secretariat from the literature and from suggestions of member countries. All the pests on the list are selected because they may present a phytosanitary risk for the EPPO region. The pests included in the alert list are not included in a quarantine list and are not immediately recommended for the A1 or A2 lists. The list is an attempt by the Secretariat to identify the main elements of risk. If the risk is high the pest may be added to the quarantine list; but if further evaluation determines the risk is low it will be removed from the "Alert List". This is an example of an excellent early warning system to help predict the future status of pests and pathogens that were not previously considered a threat to the EPPO region and member states. The list published in December 2003 includes Iris yellow spot virus, Tomato chlorosis virus and Tomato infectious chlorosis virus. The latter two viruses may infect ornamentals (EPPO, 2004).

The North American Plant Protection Organization (NAPPO) is an organization composed of Canada, Mexico and the U.S. The purpose of the organization is to disseminate information on emerging plant pests of significance to plant protection services and member countries. The organization publishes the Phytosanitary Alert System and provides updates on emerging pests and diseases (www.pestalert.org). In the U.S. there is also a National Agricultural Pest Information System. Every State Department of Agriculture maintains a system of surveillance that provides information on any new pest or disease outbreaks that cause previously unrecognized plant damage. In addition, there is a Cooperative Agricultural Pest Survey that reports the movement of damaging foreign organisms and determines the nature and extent of the pest or disease distribution. This information is included in a national database that is accessible to plant health officials in every state. Fact sheets are published that describe the nature and extent of the problem and the methods employed to contain or eradicate the causal organisms.
Every two years the American Phytopathological Society conducts a survey of each of the fifty states to determine which viruses occur in each State. This list is useful because it provides information on which viruses have recently become established and will give an indication of which viruses have recently been introduced. On this basis it is possible to trace the introduction of viruses that may have been recently introduced from abroad and may alert other states of the need to test incoming plant material for the presence of the virus. Another important component of the surveillance system is the information gathered by Agdia, Inc. Plant samples from many different states that are suspected of virus infection are tested by the company. A review of the records reveals that samples were submitted from every state during the last two years. This information can be very important in determining the presence of viruses of quarantine importance.

Viruses of Limited Distribution in the Importing Country

Arabis mosaic nepovirus--A Case Study in the U.S.

When a virus has been identified in a country, but has very limited distribution, should it be considered of quarantine significance? An example in the U.S. is Arabis mosaic virus (ArMV). The virus has been detected in 13 States. Although ArMV may be localized in each State, it has been detected in California and Connecticut on the west and east coasts and in several mid-western states (Data from Agdia collected from July, 2001 to July, 2003). Based on this information, it could be argued that the quarantine should be removed. Infected genera included Aconitum, Ajuga, Artemisia, Astilbe, Bacopa, Begonia, Calibrachoa, Campanula, Cheiranthus, Cosmos, Dianthus, Diascia, Erysimum, Fuchsia, Pelargonium, Helenium, Helichrysum, Heliopsis, Hosta, Lavandula, Lobelia, Lysimachia, Impatiens, Nierembergia, Oenothera, Osteospermum, Petunia, Sedum, Verbena, Vinca major and Vinca minor. Several of these genera are grown as perennials and could serve as reservoirs for further distribution of the virus.

Possible removal of ArMV from the quarantine list must be carefully evaluated. Because ArMV is present in herbaceous perennials as well as woody species, it may be spread rapidly in propagation material if the plant material is not accurately evaluated for the presence of the virus. It is, therefore, essential to strengthen detection procedures in countries that harbor ArMV. If the virus was removed from the U.S. list of quarantine viruses, exporters would no longer be required to certify material free of ArMV. Although the nematode vector, Xiphinema diversicaudatum, has been found only sporadically in the U.S., it has been identified in 13 States according to USDA records. The risk of accidental introduction and possible spread of the virus by vegetative means and by the vector nematode cannot be discounted. This is an example of issues that must be considered when determining whether a specific agent should be considered of quarantine importance.

Viruses with Limited Distribution Now Widespread in the EPPO Tospoviruses--A Case Study in Europe

Tomato spotted wilt virus (TSWV) has been known in Europe since the 1930's. In the late 1980s and early 1990s the virus became one of the most important viruses in Europe. Until the mid 1980s the virus was found in tomato and various ornamentals, but only occasionally. Spread of the virus was apparently limited because Thrips tabaci was not an efficient vector and could be controlled with available insecticides (Verhoeven and Roenhorst, 1994). The situation changed rapidly with the introduction of the western flower thrips, Frankliniella occidentalis in the late 1980s.

In 1990-91, Impatiens necrotic spot virus (INSV) was detected three times in begonia in the Netherlands. The virus was found when companies appeared to carry on an active exchange of plant material from abroad. At the time the virus was not considered established in the Netherlands (Verhoeven and Roenhorst, 1994). Since 1995 TSWV has been identified in 66 species of ornamental crops and INSV was found in 71 species, including ornamentals and several other crops tested from both domestic sources and samples from abroad (Verhoeven and Roenhorst, personal communication). Today, the
number of growers in the Netherlands with significant TSWV and INSV disease problems has been greatly reduced because of thrips management programs.

In Italy tospoviruses have been detected in Alstroemeria, Kalanchoe, Columnea, Primula obconica and Valeriana officinalis since 1991 (Bellardi, personal communication, 2003). The rapid expansion of tospovirus host range has been exacerbated by the multiplication of the virus in the thrips vector with subsequent persistent transmission to a large number of susceptible hosts.

At the meeting of our Working Group in 1992 seven papers were presented on the expanded host range of TSWV and INSV and methods of detection. It is interesting to note that an INSV isolate from anemone was shown to be either defective or represented a recombinant isolate that evolved from a mixed infection of INSV and TSWV by exchange of the M RNA segments. This interpretation was based on the unique structure and serological reactivity of the isolate (Adam and Lesemann, 1994). The "new" virus reacted serologically with TSWV on the glycoprotein level and with INSV on the nucleoprotein level, but TSWV did not react on the nucleoprotein level and INSV did not react on the glycoprotein level.

Temperature influenced the ultrastructure and serological reactivity of two tospovirus isolates (Lawson et al., 1994). Results of these experiments indicate that tospoviruses have the capacity to change in form and serological reactivity. This is of particular importance in assessing the reliability of serological methods used in any certification system designed to restrict movement of infected plant material.

The importance of using a serological test procedure that will specifically identify the tospovirus that is being diagnosed is emphasized in the report of Verhoeven et al. in 1996. They found that antisera prepared to isolates of TSWV and INSV did not react to the chrysanthemum stem necrosis tospovirus. The only positive reaction occurred with the homologous antiserum. Differences in patterns of reactivity of tospovirus isolates with different antisera should be considered when designing a screening test for disease diagnosis. Possible recombination of viruses or the influence of environment on altering the reactivity of an isolate should be considered in evaluating test results.

Molecular tools using degenerate primer pairs designed from conserved regions of the L genes will provide a wider application for detection of tospoviruses. In reverse transcription-polymerase chain reaction (RT-PCR), the regenerate primer pairs were able to amplify the corresponding regions of the L genes from total RNAs extracted from plant tissues individually infected with five serologically distinct tospoviruses (Chu et al., 2001).

Reports of "New" Viruses and New Diseases—1985-2003

During the past 25 years many "new" viruses, viroids and phytoplasmas have been isolated and characterized from diseased ornamental plants. In some instances these disease agents have been described only once in the literature. In other cases reports of rapid spread of agents have followed the initial report of the newly described agent. The rate of spread has differed depending on the method of propagation, geographical distribution of clonally propagated plants and the emergence of insect vectors in geographical areas where they were previously unknown or of limited distribution.

Between the years 1980 and 1985 nearly 80 incidences of new diseases were reported (Koenig, 1985). About one-half of the disease agents were reported for the first time during this period. Koenig noted that a number of virus or viruslike pathogens, which under certain conditions cause no symptoms or only mild symptoms have spread unnoticed in ornamental plants. Under different conditions, however, these plants may show obvious disease symptoms or may serve as potential sources of inoculum for plants that manifest more severe disease symptoms. None of the "new" or ungrouped viruses that she described as unrecorded prior to 1984 currently are on the A1 or A2 lists with the exception of Tomato ringspot virus and Tobacco ringspot virus. There were several nepoviruses, including Tomato ringspot virus, Strawberry latent ringspot virus and Arabis mosaic virus reported in new hosts from the period of 1980 to 1984. Also,
previously unrecorded during this period were potyviruses, carlavirus, potexviruses, closterovirus, bacilliform viruses, and rhabdovirus. None of these agents known to infect ornamentals are on the A1 or A2 lists of the EPPO.

A later report (Lovisolo, 1988) listed 48 virus and phytoplasma associated diseases that reportedly occurred outside of Europe. Although 11 of these viruses were potyviruses, none are on the current A1 or A2 lists. There were no tospoviruses on the original list. Perhaps none of these agents listed by Lovisolo were considered for quarantine status because the distribution of the host plants was limited. Or perhaps the occurrence of the agents in the diseased plants was limited in the absence of wide-scale propagation and an efficient vector. Several of the reports (Lovisolo, 1988) were from Japan, Brazil, Australia and New Zealand. Reports from the U.S. included Bidens mottle virus, Commelina mosaic virus, Dendrobium rhabdovirus, Iris fulva mosaic virus, Mimosa striped chlorosis virus, Nandina mosaic virus, Palm mosaic virus, Ramunculus mottle virus, Rhododendron necrotic ringspot virus, Sycamore bacterial leafscorch (Xylella), and Spring beauty latent virus.

At the Eighth International Symposium six viruses were reported in Alstroemeria, including Arabis mosaic virus in Italy (Bellardi et al., 1994). Lisianthus line pattern virus, related to Tobacco streak virus, was also reported from Italy (Lisa et al., 1994). A carlavirus isolated from Dimorphotheca simula that was related to Carnation latent virus, Cactus virus X, Helium virus 5 and Red clover vein mosaic virus was reported (Dellavalle et al., 1994). A potyvirus isolated from Chrysanthemum frutescens, Chrysanthemum spot virus, was also reported from Italy (Bertaccini et al., 1994). At this meeting, a new soilborne virus isolated from Limonium tartareum (syn. Goniolium tartareum) (statice), was identified and provisionally named Statice decline virus (Krčzal and Beutel, 1994). The economic importance of these viruses and their further spread is not known with the exception of Arabis mosaic virus that is now more widespread.

At the Ninth Symposium a new virus was reported from Ranunculus that showed properties similar to those of members of the genus Ophiovirus (Vaira et al., 1996). The virus was named Ramunculus white mottle virus (Vaira et al., 2000). A novel tospovirus was isolated from Chrysanthemum (Verhoeven et al., 1996) that was described as Chrysanthemum stem necrosis virus (Durate et al., 1995). Viruses in Hosta in the U.S. were first reported in the U.S. (Lockhart and Currier, 1996). They described Hosta virus X, a previously unreported virus, and a tobravirus that was serologically related to Pea early browning virus and Tobacco rattle virus. Three unidentified isometric viruses were also isolated from Hosta. Three potyviruses, including Lily mottle virus, Ornithogalum mosaic virus and an unidentified rhabdovirus were reported for the first time in Alstroemeria (Bouwen and van der Vlugt, 1996). Vegetatively propagated Petunia hybrids were found infected with 12 different viruses including Tomato yellow leaf curl virus (Lesemann, 1996). In addition, 18 other viruses were previously reported. Pelargonium zonale was reportedly infected with viruses not previously reported in the crop (Lisa et al., 1996). They included Cherry leafroll virus, Lilac chlorotic spot virus, Tobacco necrosis virus, and two isolates apparently related to pelargonium ringspot disease. These viruses were isolated only sporadically and apparently did not constitute a threat to the Pelargonium industry. A previously unidentified badnavirus, named Schefflera ringspot virus, was found in Schefflera and Aralia (Lockhart and Olszewski, 1996). Phytoplasmas belonging to the aster yellows group and the peach X disease group were identified in infected Alstroemeria in Italy (Bertaccini et al., 1996). Phytoplasmas were identified in Callistephus, Tagetes, Helichrysum and Limonium in Poland (Kaminska et al., 1996). Branching in poinsettia was caused by a biotic factor that was not associated with Poinsettia mosaics virus (Bech and Rasmussen, 1996). Free-branching in poinsettia was caused by a phytoplasma (Lee et al., 1997). In ornamental Physalis species, 38 new experimental host-virus interactions were described (Horvath, 1996). Some Physalis species are natural virus hosts and some are overwintering hosts. Lisianthus leaf curl disease, caused by Tomato yellow leaf curl virus, is a limiting factor in the production of the crop in Israel (Cohen et al., 1995). A new virus, Streptocarpus
flower breaking virus, produced flower breaking and occasionally chlorotic line patterns in the leaves (Verhoeven et al., 1995).

In the Tenth Symposium Proceedings evidence was provided for a mixed phytoplasma infection in three groups of lilies (Bertaccini et al., 2000). Iris yellow spot virus was described as a new disease causing leaf necrosis of lisanthus in Israel (Gera et al., 2000 and Kritzman et al., 2000). The virus was also described infecting lisanthus in Japan (Doi et al., 2003). A fabavirus, closely related to Broad bean wilt virus, serotype 2, was isolated in Taiwan from lisanthus showing yellow spots and ringspots (Chen et al., 2000a). A second virus isolated from lisanthus in Taiwan was identified as a strain of *Lisianthus necrosis virus* (Chen et al., 2000b). *Kalanchoe blossfeldiana* was for the first time reportedly infected with *Sonchus* yellow net virus (Bouwen et al., 2000). Gentiana species were commonly infected with *Broad bean wilt virus*, a commercial threat (Verhoeven et al., 2000). Impatiens necrotic spot virus was also identified for the first time in this genus. A new disease in *Tabernamontana* (Cape Jasmine) caused by Tobacco mild green mosaic virus was reported for the first time in Israel (Cohen et al., 2001). The first report of two serologically distinct potyviruses in *Lycoris aurea* (Spider Lily) were provisionally named *Lycoris mild mottle virus* and *Lycoris potyvirus* (Chang et al., 2000). Detection of several potyviruses in *Zantedeschia* spp. was achieved with a broad spectrum monoclonal antibody, DSMZ AS 0537 in Germany (Lesemann and Winter, 2000) and in the Netherlands with monoclonal antibody PTY 1 (Pham et al., 2000). Two previously undescribed aphid transmitted viruses, *Spiraea leaf spot virus* and *Spiraea leaf spot spherical virus* (thought to be a member of the Totiviridae) were associated with yellowspot in *Spiraea* (Lockhart and Geering, 2000). The role of different phytoplasmas in inducing branching in *poinsettia* was described (Pondrelli et al., 2000). A new potyvirus similar to *Tulip* top-breaking virus and related to *Turnip* mosaic virus, was isolated from *lily* in Italy (Bellardi et al., 2000). Tobacco ringspot virus in aster, *Turnip mosaic virus* in *Heuchera*, *Turnip mosaic virus* and *Cucumber mosaic virus* in *Lobelia* and *Alfalfa mosaic virus* in Physostegia were reported for the first time (Lockhart et al., 2000). Virus diseases caused by Tobacco rattle virus and *Raspberry ringspot virus* (RRSV) in Italy and RRSV in Germany were reported for the first time in Japanese anemone (Lisa et al., 2000). Exchange of plant material may cause a threat since both of these viruses are nematode transmitted. *Spiranthes cernua* is the first orchid reportedly infected with *Dasheen mosaic virus* (Jordan et al., 2000).

Recently, a new disease of *Bupleurum* spp. caused by *Lettuce mosaic virus* has been described from Israel (Cohen et al., 2002). A new carlavirus was reported from the cut flower crop, *Aconitum napellus*, grown in Israel (Cohen et al., 2000). A distinct carlavirus was also reported from *Verbena* spp. The virus was called *Verbena* latent virus (Cohen et al., 2003). A new virus named *Calibrachoa mottle virus*, tentatively identified as a carmovirus, was isolated from calibrachoa plants (Liu et al., 2003). The virus also infects petunia (Liu and Sears, 2003). Infected calibrachoa may be symptomless or show chlorotic spotting and mottle. Disease expression is apparently enhanced in the presence of *Tobacco mosaic virus*. Petunia plants showing severe flower-mottle and chlorotic leaves were infected with a previously undescribed potyvirus named Petunia flower mottle virus (Feldhoff et al., 1998). Petunias showing vein banding were infected with a virus that was closely related to Eggplant mosaic virus and Andean potato latent virus. The virus was named Petunia vein banding virus (Alexandre et al., 2000). A disease of peony caused by *Alfalfa mosaic virus* was first reported from Italy (Bellardi et al., 2003).

### Ornamentals Infected with Viruses that Affect Quarantine Status and Quality

Regulations that apply quarantine for a specific virus or viruslike agent may be utilized for the purpose of exclusion to prevent introduction into a given geographic area or they may be applied to limit further distribution of a disease agent. Although certification is distinct from quarantine, it is closely tied to issues related to quarantine. To successfully prohibit entry of a pathogen, it is essential to have reliable methods of detection and disease diagnosis. A quarantine assessment may also include possible risks
in the spread of a particular virus or viruses to other genera growing in the same region. It was on this basis of limiting spread and improving product quality that certification programs were developed for Dianthus, Chrysanthemum, and Pelargonium in the U.S. and Europe many years ago. More recently, other genera have been added to the list and recommendations have been made to include them in certification programs.

For example, in the Netherlands certification programs include Allium, Alstroemeria, Anemone, Araceae, Aster, Begonia elatior, Bouvardia, Crocus, Cyclamen, Dahlia, Euphorbia, Freesia, Gerbera, Gladiolus, Gypsophila, Hippeastrum, Hyacinthus, Impatiens, Iris, Kalanchoe, Lilium, Marantaceae, Musaceae, Muscari, Narcissus, Nerine, Pelargonium, Puschkinia, Rosa, Scilla, Solanaceae (Petunia), Tigridea, Tulipa and Verbena (van Ruiten, 1996). Thirteen of the most important economic crops on this list are included in a mandatory inspection system managed by the Naktuinbouw (Netherlands Inspection Service for Horticulture). In this system production companies are legally bound to meet the standards of identity, health and quality for their products.

In the U.S., certification programs are managed by the major commercial propagators. Each of the major flower crops, including Dianthus, Chrysanthemum, Pelargonium, and Lilium are tested for the presence of the most prevalent viruses. Some large companies have plant pathologists on staff that conduct certification programs. Other plant propagators depend on testing services conducted by companies such as Agdia. Many states in the U.S. also have regulations that require phytosanitary certificates on plants that are transported into the state from other states. These regulations are published and many can be found on a website that describes the agricultural policies of the State.

Genera Infected with Pathogens of Possible Quarantine Importance

It is difficult to speculate which genera of ornamentals may pose the greatest threat to future international commerce because they carry one or more disease agents that may have severe economic consequences on the specific crop or other crops. It is important, however, to list some of the ornamentals that may be important carriers of pathogens of potential quarantine importance. For example, Eustoma (lisianthus) is a relatively new crop, yet at least 10 viruses are known to cause mild to severe disease (Chen et al., 2000a, 2000b). Seed production apparently does not pose a problem of virus transport, but trade of seedling material may pose a threat. Vegetatively propagated petunias are known to be sensitive for artificial inoculation with more than 30 viruses. Although some of these viruses may rarely occur, they may still be important because they include some that are fungus-transmitted, soil-transmitted and whitefly-transmitted (Lesemann, 1996). Hosta has become a major perennial crop in the U.S. As the international interest increases and trade in this crop increases, it will be important to adopt strict standards for virus certification. The number of viruses detected in Alstroemeria continues to increase. The introduction of new breeding stock from warmer climates of South America may result in additional problems with viruses vectored by insects, such as whiteflies, that are adapted to warmer temperatures and that can be efficiently transmitted. Alstroemeria harbors viruses that include potyviruses, a carlavirus, a cucumovirus, tospoviruses, a tombavirus, a nepovirus, and a rhabdovirus. These are only a few examples of the crops that may require additional scrutiny and improved standards for virus detection and certification. The first report of a new tospovirus in chrysanthemum, Chrysanthemum stem necrosis virus, was from chrysanthemum cuttings imported into the Netherlands from Brazil. Because of the potential economic damage caused by this virus it is important to adopt specific test procedures for its detection to prevent spread in Europe and into other countries. The virus was not detected in tests using antiserum to other tospoviruses (http://www.eppo.org/Standards/certification.html). A homologous antiserum prepared in the Netherlands was used for diagnosis. The EPPO Datasheet provides detailed information describing the disease, methods of spread, and detection procedures.
We have described only a few examples of viruses in genera of plants that are currently included in certification programs as well as some plants that are not currently in existing certification programs. The possible certification of additional viruses now known to occur in these host plants should be considered. It is also important to update test procedures for those genera that are currently certified. New information regarding the identity and possible spread of new or previously described disease agents that could cause severe economic damage should be more rapidly incorporated into new test protocols and certification programs.

The Quarantine Dilemma

When should a specific quarantine be initiated? In the U.S. the rules often change slowly since a thorough review of risk is the first stage of evaluation. This process often requires not months, but years. The exception may be a rapid development of a severe disease outbreak with unmistakable severe economic consequences over a short period of time. These situations are more likely to occur where fungal or bacterial diseases are involved since the visible symptoms may appear more rapidly than in instances where a virus is the causal agent. An exception to this situation may occur where there is an efficient insect vector or in vegetatively propagated ornamentals when the virus or virus-like agent is easily mechanically transmitted.

A quarantine may also not be imposed quickly, if at all, in situations when a virus is found infrequently, even though it may be reported to have an important economic affect. Factors limiting distribution may result from the difficulty of mechanically transmitting the agent or possibly the absence of efficient natural vectors. In some cases viruses are reported as new and previously undescribed agents and are never again reported.

For the reasons mentioned, and because of other factors such as the influence of light, temperature and nutrition, the expression of disease may be greatly altered from one climatic zone to another. These factors do not preclude imposing a quarantine, but they do greatly complicate the task of certification and may make it very difficult to accurately diagnose the presence of a specific virus disease.

Successful Quarantine Depends on Reliable Certification

Quarantines are only as successful as the methods used to enforce them. In many instances this depends on measures employed at the country of origin as well as the importing country. Several years ago this system was dependent on the limited tools available that were employed for plant health certification. Visual inspection combined with a bioassay was often the only method available. For some pathogens, particularly those listed in quarantine regulations as disease "agents", this remains the primary method of certification. For example, in the U.S. regulations reference is made to 12 different syndromes as "variegation, mosaic, leaf curl, variegation and phyllody" agents associated with perennial vines, shrubs and trees. The causal agent of these conditions is unknown.

Certification methods employing biochemical methods are now widely used in many countries to detect some of the most important pathogens of quarantine significance. In these tests a single sample can be subjected to a variety of tests that will allow detection of a range of different viruses and virus-like agents. When a new virus is discovered, it is then possible to include a specific detection method along with those procedures used to detect other pathogens. This, of course, assumes that a suitable serological test reagent or nucleic acid method is available for diagnostic purposes.

Tests that have a wide spectrum of reactivity for a specific group of viruses are an important goal. For example, a wide spectrum reacting monoclonal antibody, PTY 1, reacts with most, but not all, potyviruses (Jordan, 1992). The PTY 1 monoclonal antibody is marketed by Agdia. Molecular biological methods for detecting groups of viruses have been investigated by Agdia, among others. Group tests for tobamoviruses and carlaviruses have been developed that have the advantage of overcoming the limitations imposed by serological procedures (Maroon and Zavriev, 2000).
CONCLUSIONS

Quarantine regulations applied to virus diseases that are imposed by different countries do not generally affect the movement of ornamental plants. For example, it is interesting to note that only a few known viruses, viruslike agents and phytoplasma infecting ornamentals are mentioned in the U.S. quarantine regulations. A review of the EPPO regulations results in the same conclusion as noted earlier in the pathogens mentioned previously in this article. Very few pathogens infecting ornamentals are currently included in the quarantine regulations. The most dangerous agents are probably those that are easily mechanically transmitted or transmitted by insect vectors, nematodes or soil fungi. Among the emerging diseases those agents that are whitefly transmitted may pose a major problem in the future because the vectors are widespread through both tropical and temperate regions.

Maintenance of high standards of plant health in ornamental crops may require initiatives on the part of exporters and importers of ornamentals to be aware of the most recent reports of new disease agents and the methods required to detect and eliminate them from the infected material. These steps may be necessary since official quarantine regulations may lag behind the need to update them.

Over the past thirty years great advances have been made to increase the health status of ornamentals in the developed countries. Further improvements are being made as information and methods of detection are shared by cooperating scientists, commercial producers, exporters, importers and quarantine authorities responsible for the health of ornamental crops. This working group will continue to play an important role in advancing these goals.

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Literature Cited


