Optimizing detection and management of virus diseases of plants

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Introduction

Viruses and viroids are common pathogens of many ornamental plants (unless otherwise noted, "virus" information presented here is also applicable to viroids). They are many times overlooked since symptoms may be absent or mild in certain hosts. There is no cure for plants already infected, so for many growers viral infections are just tolerated or ignored unless obvious symptoms arise, at which time affected plants are discarded. While these asymptomatic combinations of host and pathogen may not be of concern, the problem arises when the virus is transmitted to another more susceptible host where severe symptoms or even death may occur. The asymptomatic hosts therefore act as pathogen reservoirs. Knowledge about prevention, transmission, hygiene, proper testing and susceptible hosts are all important to a good virus management program.

Prevention

In today's industry which almost exclusively uses vegetatively propagated plant materials, it is important to start with virus free plants. If the mother plant is infected, all cuttings will also be infected. Tissue culture of shoot tip meristems and heat therapy are used to eliminate viruses from infected plants. Due to the growth rate and cell structure of the apical meristems, viruses cannot fully function there and heat suppresses virus multiplication in most cases (viroids however are not always reduced by heat). By removing a small (0.2-0.5mm) piece of tissue from this area it is many times possible to derive a healthy plant from a previously infected one. However, extensive testing must be done throughout this procedure to ensure proper detection of infections that are not eliminated. These procedures work well to produce virus free materials most of the time, but my research has shown that virus levels may be temporarily reduced below the level of detection resulting in a negative virus test, but after some time (several weeks), as the plant matures, virus levels return to normal allowing a positive test result. This time frame means that initial screening will declare a plant as virus free, it is sold to growers and just about the time plants are ready for propagation they test positive and responsible growers eliminate them from the production cycle. This can cause huge losses in time and money especially if large scale propagation of cuttings has already occurred. Another pitfall is the presence of new, unknown viruses for which there are no available detection methods. If there are no symptoms and no appropriate tests, virus infections can be missed. Tissue culture however is the only method currently available to "rescue" desirable varieties from viral infections. Growers should buy from sources that utilize the best virus elimination and testing protocols and advertise that their products are virus tested. This doesn't always guarantee that completely virus free plants will result, but it will be the best method available for obtaining healthy stock plants.

Transmission and Hygiene

Viruses do not have self movement or entry mechanisms like many fungi and bacteria do. They require a wound and direct delivery into that wound in order to infect a plant. Only once inside a plant cell, can the virus begin to multiply, highjacking the host machinery for its own uses, thus causing a variety of disruptions to normal plant physiology. Virus transmission can occur mechanically by general handling, pruning and propagation or by insects such as aphids, whiteflies and thrips and sometimes by nematodes or seed (some viroids are readily seed transmitted, but are rarely transmitted by insects, if at all). All such agents are graft transmissible even when none of the other methods work. Microscopic wounds to epidermal cells or breakage of leaf hairs can be all it takes to produce

an available wound, a large cut caused by clippers etc., is not always necessary. Insect or nematode vectors usually inject the virus directly into the plant during feeding activities. Thrips transmit two of the most important viruses, *Tomato spotted wilt virus* (TSWV) and *Impatiens necrotic spot virus* (INSV), two closely related *Tospoviruses,* and once they obtain the virus at the larval stage, adults are able to transmit it for the rest of their life. These two viruses have the widest host range of any known plant virus, with over 1,000 plant species being susceptible (Table 1). Seed transmission (relatively rare, about 14% of known viruses) can occur either as a surface contaminant of the seed coat or by actual infection of the embryonic cells (= true seed transmission). In greenhouse operations mechanical transmission is of the most concern. Some of the most severe viruses are readily moved simply by touching an infected plant with the hands, a watering apparatus or pesticide spray nozzle, thereby picking up virus particles which are moved to a healthy plant with the next touch. *Tobamoviruses* (e.g. *Tobacco mosaic virus* [TMV] and *Tomato mosaic virus* [ToMV]) are the major culprit in these cases due to their stability and high levels in plants. Once tobamoviruses become established in a greenhouse it can take weeks and months to completely eliminate. All mechanically transmitted viruses should be considered threats however and proper hygiene must be used to avoid spread.

Since most greenhouse operations practice good insect control, aphids and whiteflies generally don't present much of a virus vector problem. They are more of an issue in outdoor settings where viruliferous insects can land on a crop and transmit the virus before they are killed by systemic pesticides or subsequent contact sprays. Thrips many times do become established and as mentioned above transmit some of the most important viruses. Extra effort should be taken to prevent or eradicate thrips infestations. For avoiding mechanical transmission, several steps can be used to prevent moving viruses to other healthy plants.

1) Routinely scout plants for common virus symptoms such as mosaic, ringspots, stunting, leaf deformation, irregular flower color or shape (see descriptions below). Discard immediately and increase testing in that area (see below for testing protocols).

2) Have staff wear disposable gloves and aprons when handling plants. Gloves should be changed frequently during pruning/propagation and always when moving to a new variety or species. Since many infections come in undetected on original stock plants, many individuals of a given variety or species may all be infected, risking cross contamination with others in the greenhouse. Changing gloves between them will at least limit infection to that block of plants and will remove the risk of seeing alternate symptoms on the other host.

3) Disinfect tools frequently and again, always between species/varieties. There are several anti-viral materials available with variable efficacy (quaternary ammonium, chlorohexidine, bleach). Since the recommended contact time is usually 5-10 minutes for efficient disinfection, several sets of tools should be used to avoid work delays. Many such chemicals are harsh on metal and care must be used for worker safety. An old favorite, non-fat dry milk, is being revisited by researchers and may be recommended in the near future as a non-toxic, efficient virucide.

4) After removal of plants, whether infected or not, benches and walls where plants may have touched should be washed well, at least with soapy water, but ideally with one of the virucide products above.

5) Remove plant litter on and under benches, viruses can survive for varying amounts of time on inert solid surfaces. TMV can survive in dried leaves for 10 years and still be infectious. While all mechanically transmissible viruses do not have such spectacular longevity, it is always best to remove all potentially infected material.

6) Disinfect pots and water emitters before reuse.

Common Virus Symptoms

Viruses in general cause a disruption of the plant's metabolism which usually results in changes in the pigmentation or shape of the leaves, flowers or fruit, stunting, and/or reduction in the number or size of fruit. By far the most common visible symptom is "mosaic" or "mottle" which is a mixture of light and dark green areas on the leaves where uninfected leaves would be uniform in color. Blisters can also be associated with the dark green areas. Many times this is the only symptom seen in infected plants and the plant otherwise may seem fine. Ringspots and line patterns are also common and range from small circles to multiple concentric rings (like a target pattern), to lines that randomly run along the leaf surface, usually yellow or light brown in color (common in roses). A few viruses are able to cause necrotic (dead) patches to appear on leaves and stems, and may kill growing shoots, or eventually the entire plant. Flowers can become spotted or striped with either a darker or lighter shade of the normal flower color, or with a completely different color. Overall growth may be affected and plants will be noticeably shorter with fewer branches than normal. Viroids typically cause bleaching of leaf color (to yellow or white, from normal green), and spotting, cracking or color changes in fruit. The same virus may cause dramatically different symptoms in different hosts so it is important to be aware that what is a minor issue in one plant, may be dramatic or deadly in another species. This can affect management decisions when considering removal of infected plants.

Sometimes other factors can cause symptoms similar to those caused by viruses and viriods. Abiotic disorders caused by environmental factors (mainly spray damage, but occasionally also cold, heat, air pollution, fertilizer toxicity) may mimic virus symptoms since they can also disrupt plant metabolism. Things to consider when distinguishing between biotic and abiotic causes are:

Abiotic	**Biotic**
Symptoms occur rapidly (1-3 days)	Symptoms gradually appear (days-weeks)
Uniform spots, mottling or lesion size/shape	Variable sizes and shapes
All leaves or those exposed affected	May be confined to one leaf stage, as new leaves develop, they begin to show also
No progression, symptoms stay the same	Symptoms change over time:size, color, shape
Plants may recover, new growth normal	Symptoms persist, get worse

Testing Methods

The most common detection method for viruses is the enzyme linked immunosorbent assay (ELISA) which uses virus specific antibodies. ELISA cannot be used for viroid detection since they do not possess a protein coat like viruses do. ELISA is quite sensitive and reliable, with commercial kits available for dozens of common viruses. It is a quick (usually 2 days) and moderately priced detection method, especially if done in-house (less than $1 per sample, per virus for reagents), but can be costly if large numbers are sent to a commercial lab ($50-75 per sample for first virus, less for each additional virus test). ELISA can be done with minor laboratory space and supplies (sink, refrigerator,

distilled water), several companies sell all other materials required. Personnel need to have the ability to follow detailed instructions and use care in preparing samples to avoid cross contamination and false positives. Another alternative for routine detection are "dipsticks", a test that uses the same basic antibody system of ELISA, similar to a home pregnancy test. These can be used in the greenhouse and take 5-30 minutes to obtain a result. The number of available virus dipsticks is small compared to ELISA, but are increasing yearly. Since these methods depend on testing for a specific virus, or in some cases virus group, negative results only reflect the absence of those viruses tested for, but new unknowns or other viruses could still be present. It is important to know which viruses are able to infect specific hosts so appropriate tests can be selected. It is also important not to rule out the presence of mixed infections, most plants can easily become infected with more than one virus.

The Polymerase Chain Reaction (PCR) is a highly sensitive molecular detection method that uses the nucleic acid of the virus or viroid (RT-PCR for RNA viruses and viroids, DNA viruses use standard PCR) as a target for virus specific "primers" that allow millions of copies to be made of a portion of the virus which can then be detected by gel electrophoresis or other detection methods. Each set of primers is unique to a given virus or group of viruses, so as with ELISA, new viruses will not be detected. PCR requires a more extensive laboratory set-up and well trained personnel usually found at commercial labs or universities and in some of the larger commercial grower/propagator operations. The equipment required could easily cost over $25,000 just for the basics.

Other detection methods include the use of simple mechanical inoculations to host indicator plants, nucleic acid hybridization and electron microscopy (EM) of purified virus extracts. The former can be especially useful for tobamoviruses that cause a necrotic local lesion response on some hosts within 2-3 days, and for unknown viruses that may be transmitted and cause noticeable symptoms in the alternate host alerting you to the presence of a virus that can then be followed up by other tests. Hybridization methods are particularly useful for viroids, some of which have well conserved regions across several species and a single test can be used for several individual pathogens. These tests are rather advanced and usually require a special lab. EM is usually only available at university or government labs and requires skilled technicians to make the diagnosis.

The use of one or more of these tests for routine screening of plants can help detect infections before they become a major problem. Early detection is critical so that infected plants can be removed from production, preventing further spread. Proper collection of plant tissue for use in one or more of these tests can be critical for accurate detection. While there is some variation with different virus/host combinations, there are several general tips that can be used to ensure a good sample.

1) Select young to medium age leaves, showing symptoms if available. Avoid the oldest leaves, particularly those ready to senesce.

2) Collect leaves from around the entire canopy so that leaves from several different branches or shoots are present. This is especially important for plants with many trailing shoots since the virus may not be evenly distributed throughout the entire plant. Keep in mind that many stock plants actually have several individual plants within the pot that merge to appear as a single large plant. My research has shown that only one of these plantlets may be infected and leaves from that plant may not be selected during harvest, resulting in a healthy test result from a pot with an infected component.

3) If an unknown if suspected, also collect stem tissue with young green bark. Some pathogens selectively accumulate in the phloem tissues.

4) When collecting samples, use disposable gloves for each pot to avoid cross contamination. If gloves are not available, put your hand inside of a plastic bag and use it to remove leaves. If using razor blades or clippers to harvest, disinfect or change between samples.

5) Keep samples in plastic bags, either with a zipper type lock or folded over and taped shut. Do not add moist paper towels or other wet material in with the samples, that simply encourages fungal growth. Keep samples cool, but do not freeze if possible, refrigeration temperature (40F, 4C) is best, until ready to test.

Plant Susceptibilities

Some viruses are able to infect only a small number of plant species, whereas others have huge numbers of hosts like the tospoviruses (over 1,000), TMV (350), or *Cucumber mosaic virus* (CMV, 900). Knowing what your plants are susceptible to and the types of symptoms to look out for is half the battle in developing a virus management program. Companies with a small number of plant species are at an advantage over those that grow a little bit of everything. Solanaceous plants are particularly susceptible to multiple viruses and viroids, such as *Petunia, Brugmansia,* and *Solanum jasminoides.* However, with the continual addition of new hybrid ornamental plants it is not always possible to obtain an accurate virus susceptibility list. One such list of susceptible plant families, genera and spp. is available at:

http://image.fs.uidaho.edu/vide/famindex.htm

Growers that also produce vegetable crops have an additional problem in that viruses that may produce no symptoms in an ornamental plant, may have a devastating effect on another crop. Tomatoes are a good example of a plant species with major virus and viroid susceptibilities. Food crops many times have organized commodity boards that can influence government regulations on plant pathogen quarantine issues. This brings us to our final topic.

Emerging Diseases

In Europe there is mounting pressure from the tomato and potato organizations to put sanctions on the ornamental industry for the distribution of infected materials, particularly those infected with members of the *Potato spindle tuber* group of viroids (Pospiviroids). Recently several ornamental plants have been reported to be hosts of pospiviroids previously only reported in tomato. It turns out that several ornamental plants can act as asymptomatic reservoirs of viroids which cause severe stunting and deformation in tomato and potato. If those ornamentals were planted in the landscape, the viroids could become established in one of the food crops in an adjacent area. The U.S. needs to be aware of these issues since many of the new plant varieties are developed in and imported from Europe. California has rather strict quarantine regulations in that any pathogen new to the state, whether well characterized elsewhere or not, is automatically assigned a quarantine status (Q rating) until its threat can be assessed. All states do not have this same policy. Two viruses which recently became established in California are *Nemesia ring necrosis virus* (NeRNV) and *Angelonia flower break virus* (AnFBV). They both have relatively narrow host ranges infecting mainly members of the *Scrophulariacae* (*Diascia, Nemesia, Angelonia*) as well as *Phlox* and *Verbena* among a few others. The California Department of Food and Agriculture (CDFA) put a Q rating on NeRNV infected plants in 2005 until my laboratory was able to show that it was already well established in CA and was not a threat to most plants, particularly any food crops. I also detected AnFBV for the first time in CA, did a statewide survey and delivered the data to CDFA in order to avoid a Q rating from being applied. Approximately 5 new viruses are being reported in the U.S. each year, more worldwide. Some new ones reported in the U.S. that have not yet been detected in CA are *Phlox virus M, Alternanthera mosaic virus*, and *Bacopa chlorosis virus*. My laboratory is keeping an eye out for these viruses and will notify CDFA if found. I expect we will also continue to discover new viroids, although at a slower pace due to their unique biology and fewer researchers that work on them. Because of the truly global nature of the ornamental industry we need to be vigilant with our virus detection and improve elimination techniques.

Table 1. Some of the many ornamental hosts of *Impatiens necrotic spot virus* and *Tomato spotted wilt virus*. Reportedly almost all ornamentals are hosts except rose and poinsettia. Note that they also infect many vegetable and weed species

ageratum	chrysanthemum	geranium	poppy
amaranthus	cineraria	gladiolus	primrose
amaryllis	coleus	gloxinia	ranunculus
anemone	columbine	hydrangea	salvia
Aster	coreopsis	impatiens	sinningia
baby's breath	cosmos	lobelia	snapdragon
begonia	cyclamen	marigold	stock
calceolaria	dahlia	nasturtium	tiger lily
calendula	delphinium	N.G. impatiens	verbena
calla lily	exacum	peony	zinnia
campanula	forget-me-not	petunia	
china aster	gaillardia	phlox	

On-line Resources for Virus/Viroid Information

Plant Viruses Online: http://image.fs.uidaho.edu/vide/

Descriptions of Plant Viruses (DPV): http://www.dpvweb.net/

International Committee on Taxonomy of Viruses (ICTV):
http://www.ictvdb.rothamsted.ac.uk/Ictv/index.htm

Select 'plants' on left side and you will get the list of plant viruses separate from those of other hosts.

References and Additional Reading

Bostan, H., Nie, X., and Singh, R. P. 2004. An RT-PCR primer pair for the detection of *Pospiviroids* and its application in surveying ornamental plants for viroids. J. Virol. Methods 116:189-103.

Hadidi, A., Flores, R., Randles, J. W., and Semancik, J. S., eds. 2003. Viroids. CSIRO Publishing, Collingwood, Australia.

Hammond, R.W. and Owens, R.A. 2006. Viroids: New and Continuing Risks for Horticultural and Agricultural Crops. APS Feature Story, November 2006, www.apsnet.org/online/feature/viroids

Lewandowski, D. J., Hayes, A. J., and Adkins, S. 2010. Surprising results from a search for effective disinfectants for *Tobacco mosaic virus*–contaminated tools. Plant Dis. 94:542-550.

Mathews, D.M. 2009. Viruses and Viroids as Invasive Plant Pathogens. In: California Ornamental Research Federation (CORF) News Vol.13, Issue 1, Winter/Spring 2009. PDF Available at:

http://ucanr.org/sites/UCNFA/newsletters/Volume_13,_Issue_121661.pdf

Mathews, D.M. and Dodds, J.A. 2006. First report of *Nemesia ring necrosis virus* in North America in ornamental plants from California. Plant Disease 90:1263

Mathews, D.M. and Dodds, J.A. 2008. First report of Angelonia flower break virus in *Nemesia* spp. and other ornamental plants in California. Plant Disease 92:651.

Verhoeven, J. Th. J., Jansen, C. C. C., Willemen, T. M., Kox, L. F. F., Owens, R. A., and Roenhorst, J. W. 2004. Natural infections of tomato by *Citrus exorcortis viroid*, *Columnea latent viroid*, *Potato spindle tuber viroid* and *Tomato chlorotic dwarf viroid*. Eur. J. Plant Path. 110:823-831.

Verhoeven, J. Th. J., Jansen, C.C.C. and Roenhorst, J.W. 2007. First report of pospiviroids infecting ornamental in the Netherlands: *Citrus exocortis viroid* in Verbena sp., *Potato spindle tuber viroid* in *Brugmansia suaveolens* and *Solanum jasminoides*, and *Tomato apical stunt viroid* in *Cestrum* sp. New Disease Reports 15. Available at:

http://www.bspp.org.uk/ndr/july 2007/2007-13.asp)

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