IMPROVING INSV DIAGNOSIS

If you've received a "false negative" while thrips or infected plant material spread INSV throughout your impatiens crop, The Ohio State University's ongoing research to pinpoint virus movement and replication may help you improve diagnostic accuracy and stop infections before they start.

By Stephen G.P. Nameth

Gloxinia infected with INSV. (All photos courtesy of Stephen Nameth.)

Virus-induced diseases of greenhouse-grown flowers have the potential to be some of the most economically devastating diseases associated with the production of these crops, and some plant viruses can be more of a problem than others. Common viruses such as cucumber mosaic virus (CMV), tobacco mosaic virus (TMV) and tobacco ringspot virus (TRSV) can cause disease in floral crops, but they are relatively easy to control. In most cases, they result in little or no economic loss.

Impatiens necrotic spot virus (INSV), on the other hand, is the most important virus associated with the production of greenhouse-grown flowers and herbaceous perennials. Unlike CMV, TMV and other viruses, INSV can cause serious economic damage to many different species and varieties of floral crops and perennials. The virus usually enters the greenhouse via infected plant material, such as propagation stock, or it can come in via thrips, its insect vector.

Once in the greenhouse, thrips can spread the virus from plant to plant; if thrips populations are left unchecked, a virus epidemic will ensue. INSV can affect an entire crop and render it unsalable. This is particularly the case with impatiens, both regular and New Guinea types. Impatiens have, at best, very little or no resistance to INSV infection, so the best way to control the disease in impatiens is to prevent the initial infection. Knowing when a plant is infected at the earliest possible time is critical to successfully controlling this virus.

THE PROBLEM

One of the greatest challenges to plant health care professionals is the timely diagnosis and accurate identification of plant viruses that infect greenhouse-grown floral crops and perennials. Diagnosis is necessary so the grower and/or plant propagator can initiate the proper management strategies. If the diagnosis of infected material in the greenhouse is not timely, the virus can continue to spread and cause more damage. And if the identification of the virus is not accurate, the proper management strategies cannot be initiated for that particular virus.

Management strategies will vary depending on the virus. It is the nature of some plant

Leafspots and dieback of New Guinea Impatiens infected with INSV. Symptoms in both New Guinea Impatiens and Gloxinia could be mistaken for other pathogens.

viruses that little or no controls are needed. These viruses have a narrow host range — infecting only one species — and the method of their movement from plant to plant is restricted by the specific insect vector or by the virus' unique biology. Some viruses may be highly unstable outside of their living host, thus reducing their ability to be transmitted during routine greenhouse production applications. INSV and the closely related Tomato Spotted Wilt Virus (TSWV infects mostly non-ornamental hosts but does cross over to some floral crops), however, can be spread rapidly from plant to plant by thrips. With these two viruses, a rapid and accurate diagnosis is essential to head-off disaster.

Some viruses can produce symptoms in their hosts that are very characteristic, and these symptoms can be used as a relatively accurate method of virus identification. INSV and TSWV, however, induce a wide variety of symptoms in their hosts, many of which appear to be symptoms that a grower or plant health professional could mistakenly associate with other pathogens, such as fungi and bacteria. This makes an accurate diagnosis even more essential, since the control procedures initiated for a fungal or bacterial disease will greatly differ from those initiated for plant viruses.

Research has shown that when INSV infects both regular and New Guinea impatiens, the virus forms localized areas of low virus concentration within the plant. When plants infected with INSV are sampled for enzyme-linked immunosorbent assay (ELISA) testing, the standard assay used in most private and university labs for the detection of plant viruses, "false negatives" can occur. This is because the test is not sensitive enough to detect the INSV in these areas of low concentration.

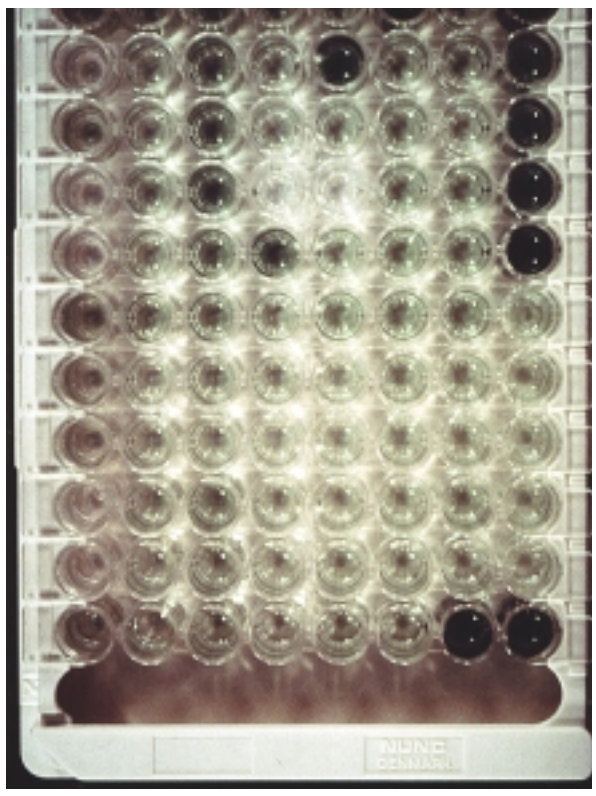
A "false negative" result has the potential to be very devastating because the grower may be mistakenly assured that the subject plant is virus-free. If allowed to remain in the production facility, the "false negative" plant will continue to be a source of virus infection for the entire greenhouse. Worse yet, the grower may use this plant as propagation stock, and every cutting taken from this infected plant will be infected with the same virus. For plant propagators, stock plants are tested multiple times over many months in an effort to approach 100 percent confidence that the material they release is virus-free. However, "false negatives" with INSV and impatiens continue to be a problem.

To solve the "false negative" problem with the INSV and impatiens pathogen/host system, most university and private testing facilities take multiple samples from the individual plant. The more samples taken from a plant, the lower the likelihood of a "false negative" result. In most cases, the multiple sample procedure solves the problem for the testing labs; however, it does not completely eliminate the problem. In some situations, the grower or propagator submitting the sample may not be able to send the entire plant and may only send enough tissue for one test. Also, conducting multiple tests on every plant can be very time consuming for the lab, and it doubles the cost for the grower who pays the lab on a per sample basis.

There are two possible solutions to this problem. The first is to develop a test that is sensitive enough to detect the presence of the virus in plant tissue even if the virus concentration is very low (a polymerase chain reaction-based test [PCR] may work well in this application). The second is to develop a method or conditions that would guarantee that the tissue sampled would have a virus concentration high enough to be consistently detected with ELISA.

PLANT VIRUS BACKGROUND

For many common plant viruses, much is known about how they infect, how they multiply and how they move throughout their host. Plant viruses enter the plant cell via a wound or with help from an insect vector. Once inside the cell, the virus uses the biological mechanisms of the cell to replicate (reproduce). Due to this infection, the plant becomes "sick." In most cases, the plant doesn't die because the virus needs a living host



End result of ELISA test. The darker the blue color, the more virus in the sample. In most cases, no color indicates no virus.

Modifying the levels of light and temperature in which the host plant is growing can profoundly influence INSV's progress, either slowing or increasing replication.

to carry out its life cycle. Once the virus has infected the host and has started to replicate, the virus begins to move from the original infected cell to a new cell, and the whole replication process starts again. This cell to cell movement continues until the virus reaches the host plant's vascular tissue.

Once in the vascular tissue, the virus can move relatively quickly throughout the entire plant — this is termed a systemic movement or systemic infection. At this point, the plant reacts to this systemic infection by expressing symptoms. With INSV, however, symptoms vary greatly, with all symptoms indicating a systemic infection. The time it takes the virus to move throughout the entire plant and the eventual concentration it reaches in the infected tissue, depend on many factors, including temperature and light. Modifying the levels of light and temperature in which the host plant is growing can profoundly influence the virus' progress, either slowing or increasing replication.

Most plant viruses eventually move systemically through the plant in a relatively even distribution and practically any tissue that is sampled will contain significant and detectable levels of the virus. With INSV and impatiens, the distribution and concentration of the virus in various parts of the plant can vary greatly. This complicates the testing process and results in the "false negative."

THE SOLUTION

Research is being conducted at The Ohio State University that will hopefully shed a greater light on what INSV does once it is inside the plant. How the virus moves from cell to cell, which tissue is most likely to have a detectable concentration of INSV and what environmental conditions can enhance these factors are all questions that will be explored. Impatiens plants infected with INSV will be subjected to a variety of temperatures to determine the temperatures at which virus replication and movement is optimum.

Symptoms typical of INSV infection in regular impatiens (opposite top), cyclamen (opposite bottom), gloxinia (left) and dahlia (above).

The virus concentration will be monitored using the ELISA test; under our ideal conditions for virus replication, this widely used test will assure the industry that it is still applicable. INSV-infected impatiens will also be subjected to a variety of light intensities with the same end result in mind as with the temperature studies. We hope to determine a temperature and light regime that will allow for optimum test results.

HOW GROWERS AND PROPAGATORS STAND TO BENEFIT

Growers and impatiens producers will benefit from this research in that it will allow plant health professionals greater diagnostic accuracy and will greatly reduce the possibilities of "false negatives." It will allow impatiens producers to subject plants that need to be screened for the presence of INSV to environmental conditions that will be optimum for virus replication and movement. This, in turn, will increase the accuracy of the test, and it will reduce the risk of virus-infected plant material being released to growers. It will also help reduce the cost of testing large numbers of samples.

Although this research will focus on INSV and impatiens, the conditions established for optimum virus replication may be applied to other viruses and other hosts, in particular TSWV and its wide list of hosts. GPN

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