

Detecting Virus in Orchids

A New Field Test that's Easy to Use at Home/Text and Photographs by Loren Batchman



FEW THINGS BOTHER SERIOUS orchid growers more than the dreaded V word — virus. Most, if not all, orchids are susceptible to one or more types of viral infection. There is no currently known cure for an orchid virus, other than discarding the plant. Some techniques have been reported that may be able to eliminate a viral infection in the meristem tissue-culture process, but these are tedious, costly and time consuming at best, take five to seven years before the plant may bloom again and are certainly not going to be economically effective for Aunt Gertrude's 70-year-old division of *Cymbidium Alexanderi* (Eburneolowianum × *insigne*).

The most common viruses in cymbidiums and other outdoor orchids are CymMV or cymbidium mosaic virus and ORSV or odontoglossum ringspot virus (also referred to as TMV-O or tobacco mosaic virus — orchid strain). Several other types of viruses, such as the orchid fleck virus, cucumber mosaic virus, bean yellow mosaic virus, various *Potyvirus* strains, and others can also infect orchids, but are much less common in collections and nurseries today than CymMV and ORSV, and seldom represent a problem for most growers.

Several methods of testing for the presence of the more common viruses have been available to the amateur

orchid grower for a number of years: the Enzyme Linked Immunosorbent Assay (ELISA) test that is performed by several laboratories across the country, the Double Radial Diffusion test, and, of course, the indicator plant that shows the presence of virus by the development of necrotic lesions when inoculated by a virus. Of these, the ELISA test has been the most popular and is widely used by commercial laboratories. The time required to take a sample, send it to the lab and get results back is not practical for doing a spot test on a suspect plant in the nursery, as is waiting on the results from an indicator plant or the Double Radial Diffusion test.

NEW OPTIONS Several companies have recently developed tests for ORSV and CyMV that can be performed in the field and provide results in five to 10 minutes. These include Mukoyama Orchids in Japan, Forsite Diagnostics in Great Britain and Agdia, Inc., in the United States. Each of these tests requires a small sample of a leaf or flower to be ground up in a sample extraction buffer to provide a liquid test sample. Each then uses a test strip, broadly termed a lateral flow device that detects viruses by means of specific antibodies and antibodies tagged to color particles, to combine the sample with a viral antibody and dye and move the solution over a narrow line or stripe primed with a viral antibody to attach to the virus particles. The liquid sample is wicked up through the strip where it combines with specific detection antibodies

- [1] Symptoms of ORSV (odontoglossum ringspot virus) on a cymbidium leaf.
 [2] A cymbidium flower infected with both CyMV (cymbidium mosaic virus) and ORSV (odontoglossum ringspot virus). Very few cymbidium flowers show any color break with just ORSV, so the color break may possibly be due to CyMV (cymbidium mosaic virus).
 [3] ORSV on a *Cattleya*-type flower.

tagged with a color particle. This sample-color antibody solution then flows over a line of specific capture antibodies. The virus is sandwiched between the line of capture antibodies and the detection antibodies that are tagged with a color particle forming a visible test line. The Agdia ImmunoStrip® and Forsite Diagnostics Pocket Diagnostic™ test strips include a positive control line as well to assure the validity of the test.

An ImmunoStrip® test for several different strains of tobacco mosaic virus has been available for several years from Agdia, and was effective in determining the presence of ORSV as well, since ORSV is in the tobacco mosaic family. An evaluation of the effectiveness of the generic TMV ImmunoStrip® test for ORSV published in the Sept–Oct 2004 *Cymbidium Society of America Journal* found it to be effective. This test suited a number of California cymbidium growers well, since CyMV is relatively uncommon to nonexistent in many cymbidium nurseries and collections. However, a similar test for CyMV was desired to complement the TMV test for screening

SOURCES

Agdia, Inc. (Web site www.agdia.com)

Forsite Diagnostics (Web site www.forsitediagnostics.com)

more mixed genera collections, and several California growers worked with Agdia to accelerate the development of a CyMV test.

The Agdia test has combined tests for both CyMV and ORSV in a single test strip, unlike the Mukoyama or Pocket Diagnostic tests, which require a separate test strip for each virus. Agdia also has developed similar test strips available for a number of other virus types also found in orchids, so that a particular plant can be tested for several of the other virus types with the same test sample, if desired.

HOW IT WORKS The Agdia test is available as a simple kit consisting of a sample extraction bag containing a grinding mesh with a buffer solution, and a single test strip for a combined CyMV and ORSV test. The sample bag is cut open, and a sample of the most recently mature or nearly mature leaf or flower weighing about .01 ounce (150 mg) is placed between the grinding mesh layers in the bag. The sample is ground up in the bag using a blunt stick, knife handle or other tool till the sample is thoroughly ground and the buffer solution takes on a green color, in the case of a leaf sample. The test strip is placed in the sample bag so the bottom 1/4 inch (about 5 mm) is in the buffer.

The buffer solution wicks up through the pad, picking up detection antibodies tagged with color particles before passing over the capture antibody stripes and the control stripes until the absorption pad is filled. The presence of either ORSV or CyMV, or both, is shown by a purple test line(s) in addition to the control line developed on the strip. The test strip takes about 10 to 15 minutes to completely fill the absorption pad. The control line usually shows up shortly after the solution reaches it, and in the case of a strong virus presence, the virus test line(s) may be visible even before the control line appears. Any appearance of a test line the same color as the control line indicates the presence of virus in the sample, so long as the control line is also present.

The development of these simple, relatively inexpensive field test kits provides a time and cost-effective way to quickly verify the presence or absence of the common virus types in an orchid collection. Now, the commercial seller can offer to test any plant prior to sale, and to certify that plants are free from a detectable virus

level at the time of sale or shipment with minimal effort and at a reasonable cost. Likewise, the orchid collector can test suspect plants in the greenhouse or new acquisitions with results available in 10 to 15 minutes. The availability of such test capability may help put pressure on sellers with suspect stock, especially if their customers begin to request testing before shipment, or complain when a plant tests positive when received. However, certification to a detectable level can only be for CyMV and ORSV.

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How to Use the Agdia ImmunoStrip® Test



Step 1 Open the ImmunoStrip sample extraction bag.



Step 2 Place sample into the bag and crush the sample.



Step 3 Insert the test strip into the bag.



Step 4 Developing test strip.



Step 5 Read the strips. A = CyMV indicator line; B = ORSV indicator line; C = control line.