Grower 101:

Rapid Diagnostic Kit

Using rapid diagnostic kits to detect viral diseases in the greenhouse is a good way to prevent wide-scale infestation and crop loss.

By Mike Tiffany

Using rapid diagnostic kits for detecting viral diseases in greenhouse crops can be very beneficial. These kits produce results in a short period of time and are reliable, sensitive, cost effective and user friendly. In fact, tests are usually as sensitive as their comparable ELISA test counterparts that are used in diagnostic labs. Results from rapid diagnostic kit tests are ready in about 10-30 minutes and each test costs between $3.00 and $7.00.

BEFORE STARTING

Before using these kits, it is important that you know your crops and what pathogens can infect them. You also need to know if the crop is a local host for the virus, meaning the virus does not spread throughout the plant, or a systemic host, meaning the virus moves through the entire plant. Finally, you need to know what symptoms the virus produces in the crop or if it produces any symptoms at all.

Viruses often cause diseases that have visual manifestations in ornamental plants. However, there are viruses that do not produce symptoms at all and often appear to have no effect on the plant. These are called latent viruses, and the most common are ring spot viruses. But a word of caution, these viruses will often produce strong symptoms if the plant becomes stressed or is infected with another virus.

Viral infection often reduces the number of cuttings that a stock plant can produce and can affect the flower color and quality of the plant. Viral infected plants are often unmarketable because of poor flower quality and the presence of symptoms. All of these problems combined lead to lost profits.

COMMON PLANT VIRUSES

The four most common plant viruses that infect ornamental plants and other crops are cucumber mosaic virus (CMV), impatiens necrotic spot virus (INSV), tobacco mosaic virus (TMV) and tomato spotted wilt virus (TSWV). The following are some of the most common virus symptoms.

Lesions — are localized spots that may be chlorotic (yellow) or necrotic (brown). The virus does not go systemic in the plant.

Line patterns — a series of chlorotic lines or patterns.

Mottle — a diffuse pattern of various shades of green, see calibrachoa mottle virus (CbMV) bottom left.

Mosaic — a definite pattern of light green/dark green areas, see CMV left.

Ringspots — concentric ring patterns that may be chlorotic or necrotic, see pelargonium flower break virus (PFBV) and tomato ringspot virus (ToRSV) bottom right.

Calibrachoas present an interesting situation. Many cultivars develop virus-like symptoms when the plants are infected with CbMV. There are other calibrachoa cultivars that do not produce any virus-like symptoms when infected with CbMV or TMV. However, when these plants are tested with the rapid diagnostic kit, they do produce a positive result for CbMV or TMV. If you inoculate sap from these asymptomatic calibrachoas onto bioindicator plants, virus-like symptoms are produced, and these plants test positive for CbMV or TMV.

USING RAPID DIAGNOSTIC KITS

All of the current rapid diagnostic kits are designed to detect the pathogen in symptomatic plant tissue. You might obtain a positive result in asymptomatic tissue, but you cannot predict what tissue to sample to ensure a positive result. Also a negative result does not assure that the tested plant does not contain the pathogen; it only ➧

Top: Anthurium showing strong mosaic symptoms of CMV; Bottom left: Calibrachoa showing mottling symptoms of CbMV; Bottom right: Geranium showing ringspot symptoms of PFBV/ToRSV. (Photos courtesy of Agdia)

means that the plant tested negative at that point in time. This problem is due to latent infections.

Follow the instructions. It is important that you follow the specific instructions provided by the manufacturer for the kit. Even though instructions might be similar between kits, it has been observed that mixing the instructions or components from multiple kits could result in false results.

Extraction method. All of the kits have a basic format for use. There is an extraction method followed by strip development and reading the results. There are two methods of extraction: The sample can be ground in a plastic mesh bag containing the extraction buffer or placed in a vial that contains the extraction buffer and some small beads and shaken.

Follow the manufacturer's test instructions to ensure correct strip development.

Strip development. There are also two formats for strip development. The ImmunoStrip is placed vertically within the mesh bag with the tip of the strip immersed in the extract. Development time is 30 minutes. The second method uses a cassette that remains in a horizontal position. The extract is pipetted into a well at one end of the cassette. Development time is 5-10 minutes. Neither test should be removed and read until the recommended time is over. The reason for this is that during development of the sample test line the line might be difficult to see because the test area has a background color. The test area becomes a clear white zone after development is complete. This enhances the ability to observe faint positive results that might occur because of low titer.

All of the rapid diagnostic kits employ the same principal in their strip development. All rely on a strip control line that appears at the top of the test zone. The appearance of this line ensures that the strip is working properly. The failure of this line to develop indicates an invalid test, and the results should be ignored. If this happens, repeat the test and/or call the manufacturer to help trouble shoot the test.

If the pathogen is present in the extract at detectable levels, a second line will appear below the control line. The intensity of the line will increase as the titer of the pathogen increases. The presence of just the control line indicates a negative result. The presence of two lines indicates a positive result, see above.

IN ADDITION TO TESTING

Testing is only one facet of virus prevention. Testing could result in the early detection of pathogens within your greenhouse, thus protecting other plants from infection, but to ensure a clean greenhouse, you should also:

- Check the health of incoming plants.
- Monitor disease as part of a scouting program.
- Protect healthy plants from infection.
- Assure plant health quality before plants are shipped.

When you receive a shipment of impatiens and you notice that some have necrotic ring spots, check them for the presence of INSV. If they test positive for INSV, this will protect your green-

house from introducing the disease into your production areas. It will also allow you to contact your broker faster and reach a favorable solution sooner.

If when walking the benches looking for insects or disease problems you spot a plant, such as a petunia, with mosaic symptoms, remove it from the area and check it for the presence of TMV. By using a rapid diagnostic kit in these two scenarios, you can protect your healthy plants from becoming infected.

Also, just as you checked your incoming plant material, you can use rapid diagnostic kits to spot-check the material that you ship to your customers. This way you can reduce the chance of shipping infected plants. Remember, just because a plant tested negative on receipt, does not mean it will not test positive at some point in the future.

WHEN TO TEST

Test anything that has symptoms. These symptoms should correlate to the symptoms of the pathogens for which you have test kits available. Remember, there is no correlation between severity of symptoms and viral titer. You could have a negative test result in the presence of severe symptoms.

Listen to the industry grapevine. If a particular crop is having a problem with a specific pathogen, watch that crop carefully and test when symptoms first appear. Strict sanitation practices should always be used, but more so with a problem crop. If the crop is one that might not produce any symptoms, then test on a frequent basis.

SUMMARY

In order for you to get the best results from your rapid diagnostic kit, adhere to the following steps:

- Use only symptomatic tissue.
- Isolate any suspect plant material.
- Confirm the test result by sending samples to a diagnostic lab.
- If the results are confirmed, the infected plants should be destroyed.
- Disinfect and clean the infected area before moving in clean stock.

Following these steps should help you detect viral pathogens in your ornamental crops and allow you to solve the problem in a timely manner. Remember, there is no one course of action to prevent disease in the greenhouse. Always purchase certified stock; establish, follow and enforce strict sanitation methods; and above all, know and watch your plants. GPN

Mike Tiffany is a senior plant pathologist for Agdia. He can be reached by phone at (574) 264-2014 or E-mail at miket@agdia.com.

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