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Questioning a plant pathogen in your greenhouse crop?

Getting a quick and accurate diagnosis is essential during the rush of spring production, so that cultural changes or plant protection/corrective products may be used in a timely manner for best efficacy. As such, growers may want to consider in-house testing kits that can provide a quick preliminary diagnosis.



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This time of year, growers need reliable diagnosis in a timely manner for disease management and decision-making. In-house plant pathogen diagnostic kits can give results in as little as a few minutes and have become more user-friendly in the last few years. These

kits can help growers detect specific pathogens that produce similar symptoms. For instance, root rots of greenhouse crops are typically caused by *Pythium*, *Phytophthora*, or *Rhizoctonia*. All three pathogens may cause plant wilt, stunting, chlorosis, or death. Getting

a quick and accurate diagnosis is essential during the rush of spring production, so that cultural changes or plant protection or corrective products may be used in a timely manner for best efficacy. While plant diagnostic labs are very concerned with a rapid diagnosis of plant



Figure 1. Examples of some pathogens for which to test using rapid response in-house detection kits. Left: *Phytophthora*. Middle: Impatiens necrotic spot virus (INSV). Right: *Pythium*. Photo credit: Dr. Jan Byrne, Michigan State University.

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samples sent to them, shipping and timing of formal testing procedures, as well as case load may cause the receipt of a formal diagnosis to be several days to a week out. Therefore, growers may want to consider in-house testing kits that can provide a quick preliminary diagnosis.

Some companies that sell field-based tests include Agdia (www.agdia.com), Hydros, Inc. (www.hydros.cc/planttest.htm), and Neogen (plant.neogeneurope.com). Examples of in-house testing kits available for purchase include viral, fungal, and bacterial pathogens for ornamental crops (Figure 1). Each manufacturer has slightly different methods for collecting and displaying the results, but all operate on the same principles of using pathogen-specific antibodies to detect the pathogen in question. At-home pregnancy test kits use this same technology.

Proper collection of diseased material is the first step in performing an in-house test. For pathogens that affect the above-ground portion of the plant, collect tissue exhibiting the symptoms and the apparently healthy area immediately surrounding it from several different leaves or stems. For root pathogens, remove the plant from

the container and inspect the root system, removing soil with your hands as you go. If possible, try to collect major roots for your sample as they provide more reliable pathogen detection. If the root system is very small, as in plug production, then use the entire root system. Wash the collected roots under running water and blot dry with paper towel. For all samples, try to collect only tissues that have recently been infected and sample both apparently healthy tissue and adjoining dying tissue. In this way, you maximize your chance of detecting the primary pathogen (not a secondary pathogen that moved in after the initial infection).

After collection of suspect plant material, most tests will have you put those samples into a buffer solution (that is specific for and stabilizes the pathogen in



Figure 2. An example of a species-specific extraction buffer (in this case for *Pythium*).

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question; Figure 2) and gently crush or grind the plant material in a vessel containing the buffer to extract the potential pathogen (Figure 3). Then, the liquid mixture (of buffer extract and ground tissue) is collected (usually through a screen; Figure 4) and placed onto a collection device (a horizontal sandwich strip, a vertical immunostrip, or a test-tube; Figure 5) which contains the recognition element.

Most tests have a control indicator to indicate that the test is working properly, which usually appears within a minute or so. If the control indicator does not appear, it generally means the test has been administered improperly, so start over with a new test and make sure to



Figure 4. Collecting the plant tissue extract.

precisely follow the manufacturer's instructions. The result indicator (if the result is positive) will appear within five to 30 minutes, depending on the manufacturer.

While a positive test result is very accurate, a negative result may occur for multiple reasons. First and most obviously, the pathogen for which you are testing is indeed not present in your sample or in the crop itself (i.e. - an accurate negative result). In this case, if you still suspect a pathogen is causing the symptoms it is advisable to test for other pathogens that produce similar symptoms. If there is no in-house testing kit available or if you aren't sure what other pathogens may have caused the symptoms, then consider sending



Figure 3. Gently grinding a plant tissue sample in an extraction buffer with a blunt object.

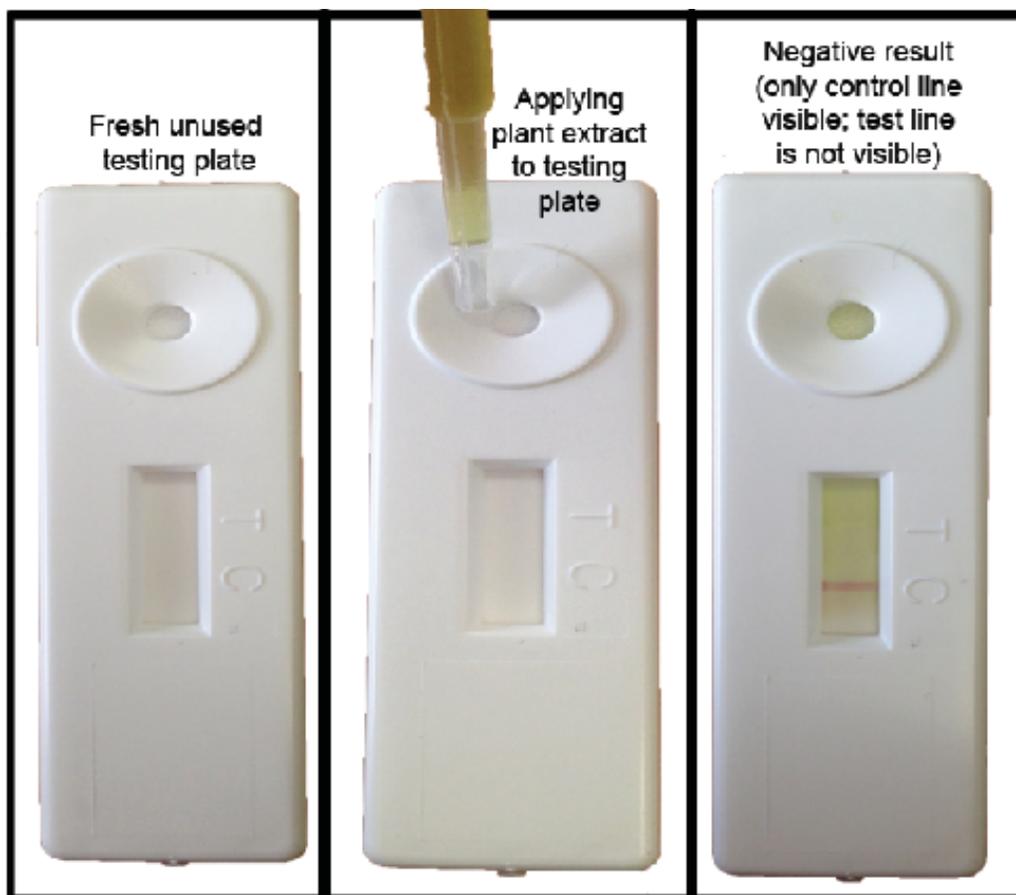


Figure 5. Applying the plant-buffer extracted sample to a test plate, resulting in a negative result (i.e. - no disease detected).

a sample into a plant diagnostic lab. Plant sampling procedures and a listing of plant diagnosis labs are available in a previous e-gro alert titled 'Sampling and Submitting Greenhouse Substrate, Irrigation Water and Tissue for Analysis' (www.e-gro.org/pdf/E_GRO_Bulletin_1_13.pdf). There is also the possibility that the pathogen is indeed the cause of the disease symptoms, but the pathogen did not occur in your particular sample of plant tissue (i.e. - a false negative). In this case, it is best to test more than one plant using

separate kits before you base treatment actions on test results because of the potential for false negatives.

When performing in-house testing, it is important to practice good sanitation. Be sure to either wash your hands thoroughly or wear and discard disposable gloves after handling diseased plants to avoid cross-contaminating other tests you may be performing at the same time and to avoid spreading the pathogen within the greenhouse.

Be sure to follow all manu-

facturers' instructions including how to properly store the tests when not in use as some need refrigeration. In addition, some tests have limited shelf life, so use caution in purchasing in bulk and check expiration dates prior to use.