Landscape Diagnostics: DNA and RNA Testing for Landscape Pests

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At last, the high-tech methods that are widely used in crime scene investigations are now playing a useful role in landscape diagnostics. Just as in criminal and legal cases, analysis of genetic material (i.e., DNA or RNA, the unique genetic material contained by all organisms, where DNA is a double-stranded molecule that stores the genetic code and RNA is single stranded and directly codes for amino acids) from samples of trees and shrubs can help in solving landscape diagnostic cases. It can be as simple as case open, sample tested, case closed.

Although molecular analysis of certain pathogens and insects from trees has been available for some time, development of commercially viable testing is only now becoming a reality for a wider range of pests (Photograph 1). In time, arborists will become comfortable with testing protocols, and given the unchallengeable accuracy of DNA testing, these methods can greatly improve the reliability of pest identification in the landscape. At the center of all pest management is accurate diagnosis.

Advantages of DNA/RNA Testing

DNA and RNA testing has several advantages and potential uses in the landscape that are currently not easily achieved using traditional culturing or diagnostic testing methods. These include:

- Detection of the slightest level of insects, mites, or pathogens in a sample.
- Detection of pests in samples that have dried or deteriorated, been contaminated, or been altered in other ways that limit the reliability of results derived from or preclude them from being tested using traditional methods.
- Detection of pests in very small-sized samples (if a pest was in the sample, it likely can be detected).
- For insects, the ability to detect pests in tissues after the pest has emerged or left the host by testing frass (insect parts without the whole insect) or swabs of borer galleries (Photograph 2).
- Rapid turnaround time, as samples can be processed and an answer gained the same day. There is no need to culture or wait for an expert in the field to identify the specimen.
- The ability to test both infected or infested samples and fruiting bodies/body parts of the pathogen or insect. Any life stage of the pest can be tested.
- Unequivocal diagnostic confirmation of pathogen or pest presence.
- Low cost. DNA/RNA testing often costs a fraction of conventional diagnostic lab charges, with single tests for DNA/RNA costing about half ($20) standard laboratory analysis.

Photograph 1. DNA/RNA testing for a wider range of pathogens and insect pests is becoming available commercially. A limited number of molecular tests have been available in the past for some pathogens, such as Phytophthora bleeding canker shown here.

- Flexible, as tests for new pathogens and insects can be added if there is demand or need, as long as standards are available.

Testing Details

DNA and RNA testing uses unique pieces of the genetic code for plant pests to verify the presence of an organism in or on a sample. To avoid being overly

Photograph 2. DNA testing can be developed for insect pests such as emerald ash borer (shown here), even in absence of the insect. Exit holes, frass, or insect parts can be tested once the processing test is created.

technical, the technique uses polymerase chain reaction (PCR), which is a method that multiplies a very specific portion of a DNA sequence into potentially trillions of copies for testing against known samples. Essentially, it has the ability to identify the presence of an organism in a sample even if only a single cell of the organism is present. PCR relies on custom-designed beginning and ending points for DNA replication. Known as primers, these specifically created attachment points target a section of the genetic code that is unique to the organism being sought out. If the target organism's DNA is present in the sample, the primers bond chemically to the organism's DNA. The DNA between the primers can then be replicated. This bonding and replication process is repeated 30 to 50 times, each time doubling the amount of targeted DNA present in the reaction. Once the replication process is complete, upwards of 1 trillion copies of the targeted DNA are produced.

The latest PCR protocols involve quantitative PCR (qPCR). Traditional PCR techniques only replicate targeted DNA. With qPCR, fluorescent dyes are incorporated into the reaction. These dyes, in conjunction with the latest replication equipment, can quantify the amount of DNA that has been produced. Multiple dyes and primers can also be incorporated into the same reaction by a process known as multiplexing. With this technology, it is possible to test for multiple organisms in each sample. By associating a different dye with each target organism's DNA, costs can be kept extremely low, and the detection of a variety of organisms is achieved simultaneously.

Sampling Protocols

Because the testing method is so sensitive and only small amounts of tissue can be tested at one time, extreme care needs to be exercised when extracting samples. This requires that an arborist take a sample from the exact tissues where the pest

Photograph 3. DNA/RNA testing only requires small samples from the leading edge of diseased tissues. In fact, larger samples are less desirable, as the arborist removing the sample should be responsible for sampling the exact tissues that are being tested.

is likely to be present. Samples only need to consist of small pieces of tissue—6 x 6 mm is sufficient in size (Photograph 3). This typically means sampling on the margin or advancing edge of diseased tissues (Photographs 4A and 4B).

For example, testing for Verticillium wilt requires stem sections from discolored outer vessels where the fungus is present (Photograph 4A). Sampling foliage or small twigs for this pathogen is unlikely to yield a positive result. Furthermore, some sampling protocols may require development to gain experience on the limits of testing (e.g., bacterial leaf scorch, where petioles are usually the best sample when the foliar symptoms are present). The pathogen *(Xylella fastidiosa)* might be tested for in branches, trunks, or roots at other times of year, but results may vary.

Drill extraction of decay with a small diameter drill bit provides an adequate sample by capturing the sawdust on the drill bit if it came from the desired tissue in the tree (Photograph 5).

Photograph 4A. Sampling for Verticillium, oak wilt, or any pest, requires that the sample contain infested or infected tissues. Here, the vessels of a redbud are infected with Verticillium, and a cross section or vertical section through the discoloration should be tested. Photograph 4B. Testing requires that samples be taken from the leading edge of discolored tissues when sampling for pathogens.

Photograph 5. Drill extraction of decay with a small diameter drill bit provides an adequate sample.

Extreme care must be exercised to avoid cross contamination when using DNA/RNA testing. Sampling tools need to be flame-sterilized between samples, or a new tool used to extract the sample. A clean pair of latex gloves should be worn when handling each individual sample. Samples must be placed immediately into plastic bags at the collection site. Handling multiple samples or transporting them together for organization greatly increases the potential for cross contamination. The testing is so sensitive that failure to exercise appropriate caution when extracting samples can cause spurious results.

After extraction, samples can simply be placed in a plastic bag and mailed—typically in a letter-sized envelope. DNA/RNA testing requires the arborist to identify for which pathogens or pests to test. Research Associates Laboratories (RAL, vetdna.com), for example, has a wide selection of tests already developed that can be run. For special projects, additional pathogen- or insect-specific tests can be developed, but this would require coordination with the laboratory at potentially additional costs.

Application in Landscape Diagnostics

DNA/RNA testing has several distinct uses in the landscape and for broader scale diagnosis of pests of trees and shrubs. There is less need to use this testing technique for common pests that are generally easy to identify based on field symptoms or signs (e.g., presence of fruiting or the insect on the sample); however, DNA/RNA confirmation can be useful, even for common pests where documentation or confirmation is a requirement or helpful for an assignment, such as positive identification of likely diseases when treatment-specific decisions need to be made (e.g., specialized fungicides for Phytophthora treatment).

DNA/RNA testing can be extremely valuable when diseases or insects are challenging to diagnose based on field symptoms or with traditional diagnostic methods. For example, oak wilt *(Bretiziella fagacearum)* can be difficult to identify in the field, particularly in the months/years after primary wilting symptoms are gone. Culturing from fresh samples can be equally challenging, especially if the samples are not processed quickly or have dried or been subject to heat. These factors do not affect the ability to use molecular testing to identify the pathogen in the sample. There are a number of other examples (e.g., Verticillium wilt) where positive field identification can be challenging and the diagnosis has important treatment or management implications.

In some cases, more general application of molecular testing is useful. The testing allows identification at the genus level for pathogens such as *Armillaria* or *Phytophthora* (Photograph 6). These pathogens can also be identified at the species level if needed, but specific identification is not critical because the potential treatment is the same regardless of what species is present.

Photograph 6. DNA/RNA testing to the genus level is adequate for some pathogens, such as Phytophthora, shown here on rhododendron, because management is not dependent on specific identification.

In the case of decay in trees where fruiting is usually absent, testing for several common decay fungi is an easy way to troubleshoot what pathogen might be causing the decay. Larger samples are also not needed to run multiple tests. For example in maple, testing for *Ganoderma (lucidum) sessile, G. applanatum, Armillaria sp.,* and *Kretzschmaria deusta* covers most of the common and important decay pathogens of urban maple species. On oak, one would shift the pallet to also include *Inonotus dryadeus, Grifola frondosa,* and *Bondarzewia berkeleyi*, and possibly drop the *K. deusta*. Knowledge of what pathogen is causing the decay affects the prognosis and recommended treatment (Schwarze 2008) and may ultimately improve the reliability and professionalism of consulting assignments.

Testing of fruiting structures of fungi and insect body parts, or even frass from insects, is possible using crime scene type methods. Simple swabbing of a fungal fruiting body or an insect provides an adequate sample for testing of a pathogen in question (Photograph 7).

Photograph 7. Mushrooms of suspected pathogenic fungi can simply be swabbed or small pieces taken and sent in for DNA analysis. Even dried mushrooms, such as the Armillaria tabescens shown here, can be tested to the genus level or for specific identification for common pathogenic species.

Similarly, insertion of a swab into the exit hole of an insect can be used to identify borer pests.

Limitations

Like any diagnostic method, molecular testing must be used wisely in the field if it is to be effective. These methods have important limitations that arborists need to be aware of, including:

- Sample contamination is a potential issue. Flame sterilization of sampling tools and care in sample handling to avoid contamination are required.
- The exact tissues where the pest is or was present must be sampled. For example, not all vascular tissues will harbor the oak wilt or Verticillium pathogens, so care needs to taken to provide samples of infected tissues.
- DNA/RNA tests are run for specific pathogens or insects, meaning a sample is only tested for one or a few common species or genera at a time. Blind or general testing of samples for many types of potential problems or organisms is possible but not cost effective or done without specific request.
- The presence of a pathogen or pest does not necessarily mean it is the cause of any particular health issue. Some pests are secondary and come in after other agents have damaged a tree. Wise use of test results is essential and requires experience and an understanding of the relationship between the host and pathogen in question.
- DNA/RNA tests have only been developed for a relatively short list of pests. The number is growing, but each test is costly to develop and need will likely determine what pest tests are developed in the future. A list from one laboratory is online at vetdna.com. Some laboratories specialize in certain disease types, such as wood decay fungi (Garbelotto et al., 2008).

Validation of tests for plant pathogens has previously been limited by the availability of positive control standards. This is no longer the case, as the USDA Agricultural Research Service Culture Collection, the American Type Culture Collection (ATCC), and the CBS-KNAW Culture Collection have many species and strains of fungal and bacterial pathogens available for purchase. Since fungal nomenclature is continually changing, it is imperative to stay up to date on the latest designations. Many of the changes in fungal and bacterial nomenclature are driven by newly discovered genetic similarities, or rather differences. Another way to stay up to date on the latest nomenclature changes is by researching the organisms through search engines such as NCBI BLAST or Q-Bank, which are the typical resources utilized by most molecular diagnostic laboratories. In many cases, DNA or RNA testing is the only method available for the differentiation of newly designated species.

Looking Forward

It is not hard to envision the more widespread use of DNA/RNA testing in the landscape and the potential for tree and shrub diagnostics to take a significant step forward through its use. These techniques offer some distinct advantages over traditional methods but must be used wisely to produce effective results. With time, however, as we become more skilled in its use, we could test for tree and shrub pests early in the disease process, and treatments could be applied or developed to limit the impact (Photograph 8).

Clearly, we still have a lot to learn about sampling methods and protocols that will allow the most efficient use in the field. Once developed, testing for diseases such as wood decay or root pathogens will help us to more effectively determine prognosis and develop and evaluate more effective treatments. Finally, these methods should allow arborists desiring the best information for their clients to take a step beyond their less progressive competitors. Continued communication between arborists and diagnostic laboratories regarding the visual identification and genetic verification of pathogens and pests will be paramount to the development and specification of testing.

Literature Cited

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Photograph 8. Early detection and identification of decay pathogens in roots or trunks using DNA/RNA testing could greatly improve our prognosis and treatment of these diseases.