CSI Landscape Diagnostics:
DNA and RNA Testing for Landscape Pests

By Christopher Luley, PhD.

The time has come finally that the high tech methods that are widely known from crime scene investigations become a common part of landscape diagnostics. Just as in criminal and legal cases, analysis of genetic material (DNA or RNA) from samples of trees and shrubs can solve landscape diagnostic cases. Case open, sample tested, case closed.

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

Advantages of RNA/DNA Tests

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

- Ability to detect even the slightest amount of an insect, mite or pathogen in a sample.
- Ability to detect pests in samples that have dried, deteriorated or been altered in other ways.
- Ability to detect pests even in the smallest samples, or samples that have been stored for long periods or contaminated. If the 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 even swabs of borer galleries.
- Rapid turn around 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.

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 for Phytophthora bleeding canker, shown here.

Photo Courtesy: Chris Luley

- Ability to test both infected or infested samples, and fruiting bodies or the insect. Any life stage of the pest can be tested.
- Unequivocal diagnostic confirmation of pathogen or pest presence.
- Low cost. DNA/RNA testing is often a fraction of conventional diagnostic lab charges.

Details and Sampling Protocols

RNA and DNA testing uses unique pieces of the genetic code of plant pests to verify the presence of an organism in or on a sample. With avoiding being overly technical, the technique uses Polymerase Chain Reaction (PCR), which is a method that multiplies a portion of DNA into millions of copies for testing against known samples.

Theoretically, it has the ability to identify even a single cell of an organism in a sample if genetic material is present. 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 is likely to be present.

For example, testing for Verticillium wilt requires stems sections with outer vessels where the fungus is present. Or for bacterial leaf scorch, petioles are the best sampled when the foliar symptoms are present but the pathogen (*Xylella fastidiosa*) can be tested for within tissues that might contain the pathogen in branches, trunks or roots but results may be more variable. Further, some sampling protocols may require development to gain experience on the limits of testing, such as with bacterial leaf scorch and others diseases.

Application in Landscape Diagnostics

DNA/RNA testing has several distinct uses in the landscape and broader scale diagnosis of pests of trees and shrubs. Clearly there is no need for use of this testing technique for common pests that are generally easy to identify based on field symptoms or signs (presence of fruiting or the insect on the sample). But, this testing method will be extremely useful where diseases or insects are challenging to diagnose based on field symptoms and with traditional diagnostic methods.

For example, oak wilt (*Ceratocystis fagacearum*) can be difficult to identify in the field, and culturing from 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 of molecular testing to identify the pathogen in the sample. There are a number of other examples like oak wilt, such as Verticillium wilt, where positive field identification can be challenging and the diagnosis has important treatment or management implications.

DNA testing can be developed for insect pests. Exit holes, frass, or insect parts can be tested once the processing test is created. Asian long-horned beetle shown here is a potential candidate for DNA testing.

Photo Courtesy: Chris Luley

Further, diseases and pathogens with regulatory implications such as Sudden Oak Death caused by *Phytophthora ramorum*, or Thousand Canker disease (*Geosmithia morbida*) of black walnut, are a good place where DNA testing can provide definitive identification needed to make regulatory decisions. Pathogens such as *P. ramorum* are difficult at best to identify to the species level using standard methods and DNA testing can provide definitive results.

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. These pathogens can also be identified by species if needed. In many cases, identification by species is not critical because the potential treatment is the same no matter what the species turns out to be.

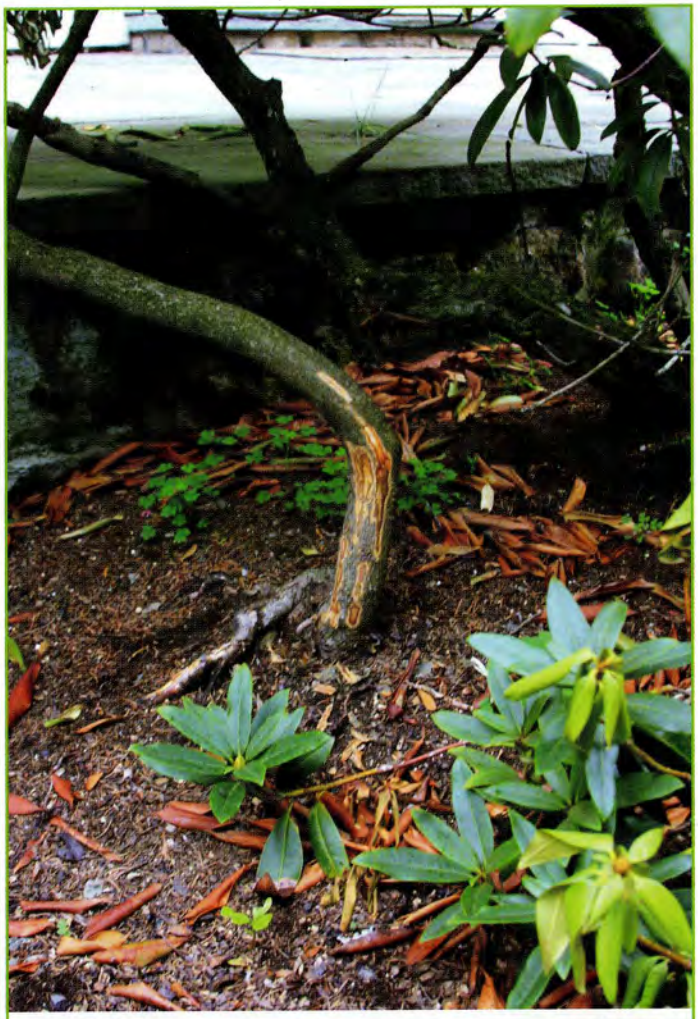
Testing of fruiting structures of fungi and insect body parts, or even frass from insects, is possible using crime scene type methods. Simple swabbing of a fungal fruiting body or an insect provides an adequate sample for testing the pathogen in question. Similarly, insertion of a swab into the exit hole of an insect can be used to identify borer pests.

Continued on Page 8

CSI Landscape Diagnostics

Continued from Page 7

Sampling for Verticillium or oak wilts, or any pathogen requires that the sample contain tissues infected by the fungus. Here the vessels of a redbud are infected with Verticillium and a cross section or vertical section through the discoloration could be tested



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

Mushrooms of suspected fungi can simply be swabbed and the swab sent in for DNA analysis. Armillaria mushrooms shown here can be tested to the genus level or for specific identification for common pathogenic species of the fungus.

Limitations

Like any diagnostic methods, 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:

- The need to sample the exact tissues where the pest is (or was) present. For example, not all vascular tissues will harbor the oak wilt or Verticillium pathogens, so care needs to be 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 not available.
- 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 disease types such as wood decay fungi (Garbelotto et al., 2008).

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. However, with time (and as we become more skilled in their use), trees and shrub pests could be tested for early in the disease process and treatments can be applied or developed to limit their impact. Clearly we still have a lot to learn about sampling methods and protocols that will allow their most efficient use in the field.

Once developed, testing for diseases such as wood decay or root pathogens will help us 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 ahead of their less progressive competitors.

Christopher Luley, Ph.D., holds TRAQ credential and is a tree pathologist and vice president at Urban Forestry LLC (www.UrbanForestryLLC.com) in Naples, New York, U.S

Literature Cited

Garbelotto, M., Gonthier, P. and Nicolotti. 2008. DNA tests identify wood decay fungi. Arborist News December: 34-36.

Looking forward, as we learn the best methods to extract samples for DNA/RNA testing of trees infected with root or trunk decay fungi, the potential to develop treatments and better prognosis will increase.

All Photos Courtesy: Chris Luley