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DNA Testing: An Application to Armillaria Root Disease

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Strategic Importance

DNA testing is fast, accurate and inexpensive. The use of DNA diagnostics has become widespread over the past decade for identifying genetically different individuals and measuring the degree of relatedness among them. This approach is particularly relevant for positively identifying forest pathogens of commercially important trees and for studying their population genetics. In British Columbia (B.C.) alone, forest pests damage between 6 million and 12 million m^3 of timber every year. The primary goal of DNA diagnostics is to improve understanding of the distribution and dynamics of forest pests, thereby assisting development of tools for disease management.

An important reason for the increased prevalence of DNA diagnostics is the development of faster and more efficient tools for examining DNA sequence variation. Molecular markers are being developed for assessing genetic variability in pathogens and trees. One excellent example of the application is a DNA diagnostic for identifying species of *Armillaria*, the fungi that cause Armillaria root disease. This DNA tool is being used in studies of *Armillaria* species distribution in different biogeoclimatic zones. It provides the fastest, cheapest and most accurate method of identifying the *Armillaria* species on a site.

Armillaria root disease

Foresters, managers, and researchers have identified root diseases as a high priority for forestry research because they cause growth loss and mortality of between 1.4 and 3.8 million m^3 annually in B.C. alone. A more specific

Armillaria ostoyae ***is a major cause of growth reduction and mortality -*** **A. sinapina** ***is not. DNA testing will greatly improve the study and management of forest pathogens in British Columbia.***

example is in the Nelson Forest Region of B.C. where areas infected with *Armillaria ostoyae* may yield up to 20% less merchantable volume than uninfected areas. To further complicate forest management, the susceptibility of different tree species to root disease varies. Consequently, over time, stand composition will be altered by root disease as the most resistant trees are favoured.

There are seven species of *Armillaria* in western Canada, the most damaging of which is *Armillaria ostoyae*. *Armillaria ostoyae* occurs over the southern one-third of British Columbia, and the range of another *Armillaria*

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species, *A. sinapina*, overlaps its range. Three facts make it necessary to distinguish *Armillaria* species:

- The ranges of damaging and weakly pathogenic species (*A. ostoyae* and *A. sinapina*) overlap, and they are difficult to distinguish in the field. They are both found in stumps of cut trees, on suppressed trees and as rhizomorphs on root surfaces. It is necessary to identify *A. ostoyae* infestation since it is worsened by clearcutting, partial cutting, thinning, and brushing. This means that regeneration of conifers on infested sites will cause additional losses if the prescription is inappropriate.
- *A. ostoyae* is difficult to detect from above-ground symptoms. For example, in the Interior Cedar/Hemlock (ICH) zone of British Columbia as many as 80% of the trees in mature stands may be infected, yet the above-ground symptoms may not indicate this level of infection.
- Hazard rating of stands using environmental factors may be possible, but a database for hazard rating must first be collected with unequivocal species identifications. There are indications that environmental factors, especially moisture and temperature, limit the distribution of *Armillaria* species. However species distributions within biogeoclimatic subzones and variants, and gradients in elevation, are not well understood. Unequivocal species identification is essential for effective prescriptions.

The development of a rapid and reliable test for distinguishing these species is therefore the first step towards developing guidelines for managing Armillaria root disease.

DNA Species Identification Methodology

A DNA-based test for differentiating between *A. ostoyae* and *A. sinapina* takes advantage of variation in the intergenic spacer regions (IGS-1 and IGS-2) between the ribosomal genes of the fungus (Figure 1). There is sequence variability in these regions and inter-species differences are maintained by the reproductive isolation of the species. Restriction endonuclease digestions of the DNA of the two species therefore produce different patterns of DNA fragment sizes. The variability, however, is not so high that the DNA of different isolates of the same species show completely different restriction fragment patterns. There is enough conservation of DNA sequences within the species that characteristic patterns can be recognized for each species. The chances of misidentifying a species with the test are extremely small.

The analysis process starts with polymerase chain reaction (PCR) amplification of the IGS-1 region, followed by digestion of the DNA with the restriction enzyme AluI. Differences in the sizes of the fragments can then be used to distinquish the species. In some cases, a pattern emerges that fails to distinguish *A. sinapina* from *A. gallica*, another species of *Armillaria* that poses no threat to timber. If necessary, a second PCR amplification, this time of IGS-2, followed by an AluI digestion, provides a positive identification. The entire procedure takes as little as 24 hours and is therefore the method of choice for species determinations in the laboratory. The restriction site map of the IGS-1 is shown in Figure 2.

Equipped with this powerful new DNA test, scientists can now collect and identify *Armillaria* isolates from selected

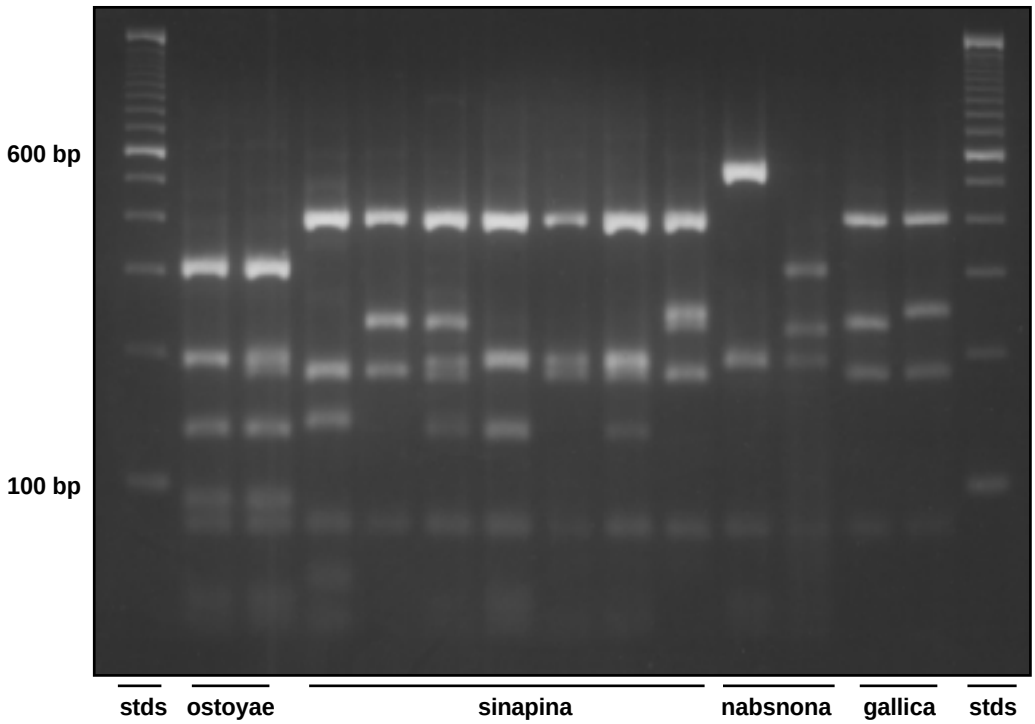

Figure 1. Variation in the ribosomal intergenic spacer regions (IGS-1) permits isolates of* A. ostoyae *and* A. sinapina *to be distinguished.

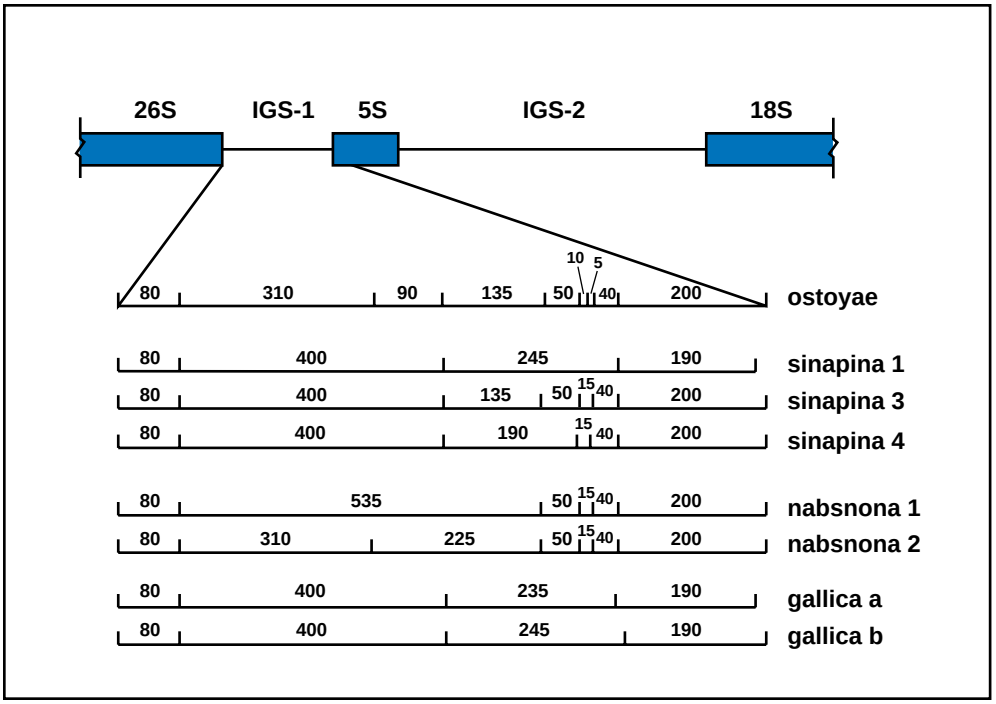

Figure 2. The map of IGS-1 in Armillaria.

sites throughout their ranges, with the aim of sampling from a spectrum of subzones and variants with different moisture and temperature regimes. The goal is to develop more accurate risk factors for Armillaria root disease.

Other Potential Applications

There are many opportunities for developing DNA diagnostic methods for other species of disease-causing fungi, such as for *Cronartium ribicola*, the cause of white pine blister rust. The accuracy of these methods is far beyond that of any method previously available.

Conclusion

DNA diagnostics will be of value to foresters and consultants who require fast pathogen identifications or similar services. The accuracy of the *Armillaria* diagnostic test has been verified, and it is currently ready to be transferred to the laboratories of interested parties.

Additional Reading

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