Is there a genetic basis for disease resistance in wild populations of *Juglans cinerea*?

[1]Nicholas R. LaBonte and [2]Keith E. Woeste
[1]Purdue University, Dept. of Forestry and Natural Resources, Hardwood Tree Improvement and Regeneration Center (HTIRC), 715 West State St., West Lafayette, IN 47907, [2]USDA Forest Service, Northern Research Station, HTIRC.

Background and overview

Butternut, *Juglans cinerea*, is a walnut tree native to the eastern U.S (Figure 1). Never common, it has been historically valued for its edible nuts and valuable hardwood lumber. In 1967, a canker-forming fungus (*Ophiognomonia clavigignenti-juglandacearum; Ocj*) was discovered killing butternuts in southwestern Wisconsin, and today butternut has been decimated across most of its range. *Ocj* cankers are not the result of a systemic infection, but rather kill when cankers coalesce and girdle the host (Figure 2). Infected butternuts survive for varying amounts of time in the wild. Some trees in areas of high disease pressure have remained apparently healthy for more than 20 years of monitoring while neighboring trees have died. This pattern has also been observed in other North American forest trees facing introduced pathogens, including American beech (beech bark disease) and eastern white pine (white pine blister rust). It is unknown whether the observed resistance is due to genetics or to local environmental factors, chance escape, or other variables.

Figure 1: Map of native range of butternut showing (a.) Slocum's Woods and (b.) Great Smoky Mountains National Park.

Project Objectives

- ◆Develop additional microsatellite markers for butternut and Japanese walnut
- ◆Extract DNA from archived samples to increase sample size and range
- ◆Score wild butternuts in Slocum's woods for disease severity and bark color
- ◆Assess relatedness of study trees with microsatellites
- ◆Use regression analysis to estimate heritability of disease resistance in wild populations (Castellanos et al., 2011)

Figure 2:Mature butternut showing *Ocj* stem cankers.

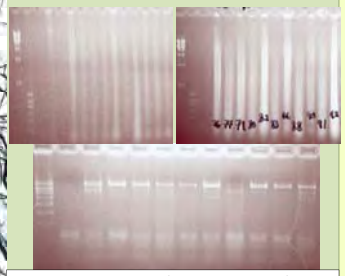

Figure 3: Agarose gels of DNA extractions from ten-year-old leaf samples. Top left is isolation with 1 phenol treatment and 4 with chloroform: ladder tops are 12 kb (lane 1) and 700 bp (lane 2). Lane 4 is a control of DNA from fresh leaves. To the right is a gel of isolations with 2 phenol treatments and improved yield and quality. Bottom picture is an amplified ITS fragment using old leaf isolations as template: the third lane is from fresh leaves, and quality of template declines from left to right.

Study site

The study population, known as Slocum's Woods, is located near the town of Whitewater in southern Wisconsin (Figure 1). The butternut population there, numbering in the hundreds, is believed to have originated from a small number of female founder trees. Some butternuts are still in very good health, and many of these display a rare, dark-barked phenotype that seems to correspond to greater disease resistance. The site has a valuable history of monitoring and sampling, including archived tissue samples from trees now dead. We began extracting DNA from these 10-year-old samples and obtained high enough quantity and quality to amplify ITS markers in all the samples tested. Another well-characterized population in the Great Smoky Mountains National Park may be included if time permits (Figure 1).

Approach for analyzing heritability of traits in a wild species

If the observed phenotypic variance for resistance to butternut canker disease has a genetic component, it would be expected that closely related trees would have similar levels of disease resistance. We will use an expanded microsatellite library to assess relatedness among butternuts in Slocum's woods, and plot these values in a regression against scored observations of disease resistance. This technique was first proposed by Ritland (2000) and has been used to find rough estimates of heritability for traits in wild plant populations. Using the relatedness dataset, we will also be able to assess the heritability of the dark-bark phenotype and its correlation with disease resistance. Currently, we are developing new microsatellites in butternut and Japanese walnut, a congener with which butternut frequently hybridizes to produce F1 offspring called "buartnuts," or "buarts". Japanese walnut markers would be useful in future research as a tool for genetic mapping in the two species using a buart x buart cross along with detection of hybrids or backcrossed hybrids in wild butternut populations (Figure).

Figure 3:
Leaves of (left to right) Japanese walnut, buartnut, butternut, and black walnut. Note the morphological similarities between Japanese walnut, butternut, and their F1 hybrid.

DNA extraction from archived samples

In the summer of 2002, leaves were collected from nearly 300 living butternut trees in Slocum's Woods. Many of these trees are now dead. We have been able to extract DNA from these desiccated samples and successfully amplify a 700 base-pair ITS locus from samples of low, intermediate, and high DNA quality. The quality and yield of our extractions has been improved by modifying the HTIRC DNA extraction protocol for walnut, which uses CTAB buffer and phenol/chloroform purification. The modified protocol includes an extraction buffer with increased PVP and added sodium sulfite, 18 hours of incubation in CTAB buffer at 50° C before grinding with beads in a FastPrep, and using 2 phenol:chloroform purifications instead of 1.

Figure 4: Two mature butternuts in the Slocum's Woods population. The tree on the right displays the rare dark-barked phenotype; most butternuts have pale gray bark like the tree on the left.

References

Ritland, K. (2000) Marker-inferred relatedness as a tool for detecting heritability in nature. Molecular Ecology, 9, 1195-1204.

Castellanos MC, Alcantara JM, Rey PJ, Bastida JM. (2011) Intra-population estimates of vegetative and floral trait heritabilities estimated from molecular markers in wild *Aquilegia* populations. Molecular Ecology, 20, 3513-3524.

Acknowledgement

Nick LaBonte's work is funded in part through the Partnership for Research and Education in Plant Breeding and Genetics at Purdue University: USDA NIFA, Purdue University, Industry (Ag Alumni Seed, AgReliant Genetics, ConAgra Foods, DOW AgroSciences, KWS Cereals USA LLC, Indiana Crop Improvement Association, Pioneer Hi-Bred International a DuPont Company, United Soybean Board, China Scholarship Council)