DEVELOPMENT OF MOLECULAR TOOLS FOR USE IN BEECH BARK DISEASE MANAGEMENT

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ABSTRACT

Beech bark disease (BBD) has been killing American beech trees in eastern North America since the late 1890s. The disease is initiated by feeding of the beech scale insect, *Cryptococcus fagisuga,* which leads to the development of small fissures in the bark. Over time, as the population of scale insects builds on the bark, the small wounds provide entryway for fungal infection by one of the species of *Neonectria*. As the fungus invades, it kills the inner bark tissue and may completely girdle the tree, leading to death. Cankers may form as the tree attempts to stop the infection from spreading, resulting in wood defects. Often trees are weakened to the point that they are susceptible to splitting or snap during windy conditions. Large numbers of severely deformed American beech persist in long-affected stands, and their propensity for root-sprouting can result in the dense beech "thickets" that prevent other species from establishing, while offering little economic or ecological value. As a result, BBD has the potential to severely degrade and alter the species composition of the forests it occupies.

Damage from this disease complex has been significant as it has spread through areas throughout the eastern United States where American beech is an important component of mixed hardwood stands. Presumably BBD will continue to spread throughout the natural range of American beech in the United States. Current forest inventory data suggest that BBD has already invaded most of the area with relatively high densities of beech, but has yet to invade the bulk of the range of beech (Morin et al. 2007). This means there is still time to develop management strategies aimed at minimizing the impacts of the disease ahead of the disease front, in addition to developing strategies for restoration of aftermath forests.

Fortunately, some beech trees remain disease-free even in heavily affected areas. Testing has shown that they are resistant to the insect portion of the disease complex (Houston 1983), which we have confirmed in recent genetic experiments. In these studies, families of beech seedlings were tested for resistance to the scale insect by artificially applying insect eggs to their stems (Koch and Carey 2005). One family that was tested came from a resistant tree, ME(R), located in a stand in Sebois County, ME, that had been managed for BBD through the removal of all diseased American beech trees in 1991 (Farrar and Ostrofsky 2006). The only possible paternal parents (i.e., pollen donors) were the remaining resistant trees, so this family can be considered as having two

resistant parents. The families that had the highest proportion of resistant seedlings were those with two resistant parents, including the open-pollinated family from ME(R), providing evidence that management directed at the removal of diseased trees can lead to stand improvement. Comparisons between the different families in this study demonstrated that the degree of genetic influence (versus environmental influence) on resistance to the beech scale insect is sufficient to realize genetic gain (Koch et al. in press).

Genetic improvement of stands can be realized either through traditional tree improvement programs (seedling development and planting) or through silvicultural methods designed to manipulate stand genetic composition by favoring resistant trees (and their natural regeneration), or a combination of both. The deployment of any of these strategies could be expedited through the application of molecular technologies to identify marker(s) for resistance to BBD. We have initiated a framework for the development of such molecular tools through the establishment of a linkage map in American beech. Two full-sib families were used to construct four linkage maps and perform QTL analysis of beech scale resistance/susceptibility. Fifty AFLP primer pairs generated 550 markers heterozygous in both parents segregating in a 3:1 ratio and 285 markers heterozygous in one parent segregating in a 1:1 ratio for a total of 1,130 markers for each family on average. The linkage maps ranged from 12 to 16 linkage groups spanning 547 to 750 centimorgans (cM) with an average of 210 markers mapped for each map. Two loci were identified that were linked to beech scale resistance, and two markers closely associated with one of these loci predict the correct phenotype of individuals within the families 86 percent of the time. Our current goal is to increase the map density to identify markers that are more closely linked to the resistance phenotype so that they may be used for marker-aided selection (MAS). Such an approach would replace the current labor intensive method of screening for resistant seedlings, which relies on artificially infesting the seedlings with eggs from the beech scale insect.

An American beech genomics project has been initiated using ultra high-throughput DNA sequencing of the American beech transcriptome. The resulting sequences (more than 150 million bases) will be aligned and analyzed with bioinformatics software to identify SNPs (single nucleotide polymorphism), a type of DNA-based marker. The goal is to identify as many markers as possible, so that a large enough portion of the genome is covered to maximize the probability of identifying a marker that is very closely linked to disease resistance. Priority will be given to SNPs that are identified in sequences homologous to known defense response genes and in genes differentially expressed between resistant and susceptible American beech. The selected SNPs will be used to prepare a high-throughput genotyping assay for beech. The beech mapping population will be genotyped using this assay, and a minimum of 200 SNPs will be added to the beech map, greatly increasing the likelihood of identifying marker(s) that are sufficiently closely associated with the resistance phenotype to be useful for MAS.

The selected SNPs will also be used to genotype 250 resistant beech and 250 susceptible beech that have been collected from five stands in Maine, West Virginia, Massachusetts, Michigan, and Nova Scotia (Houston and Houston 1994, 2001). Genetic association tests will be performed to determine which of the many SNPs are most predictive for BBD resistance in natural populations. Such a marker will be a useful tool for carrying out silvicultural management strategies ahead of the disease front by allowing forest managers to identify and retain BBD-resistant American beech trees while removing the susceptible beech trees.

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