CHAPTER 8.

Beech Seed Orchard Development: Identification and Propagation of Beech Bark Disease-Resistant American Beech Trees

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INTRODUCTION

Beech bark disease (BBD) has long been negatively impacting the American beech (*Fagus grandifolia*), an important component of hardwood and mixed hardwood forests throughout eastern North America that provides food and habitat for over 40 species of birds and mammals (McCullough and others 2001). BBD is initiated by feeding activities of the beech scale insect (*Cryptococcus fagisuga*), which create wounds that act as entry points for the *Neonectria* spp. fungi. It is the fungal component of the disease complex that weakens and kills the tree. Mortality levels in the first wave of the disease can be as high as 50 percent (Miller-Weeks 1983). Surviving beech trees are often severely deformed, and their tendency to produce root sprouts can result in the formation of "thickets" that prevent regeneration of resistant beech or other species. The deformed trees offer no economic value and severely reduced ecological value as the disease continues to kill susceptible beech over time (Morin and others 2007). Fortunately, there are American beech trees that remain healthy despite intense BBD pressure. Studies have shown that when eggs are directly affixed to the bark of such trees, scale insects fail to establish, indicating that these trees are resistant to the scale insect (Houston 1983, Koch and others 2010). In the absence of feeding by the beech scale insect, there is little opportunity for *Neonectria* to invade, minimizing impact of the fungus. Large-scale mortality levels in beech due to *Neonectria* have never been reported in the absence of the insect, so resistance to the beech scale insect equates to resistance to beech bark disease.

Genetic studies have confirmed that resistance to the scale insect can be successfully selected and bred for because it is a heritable trait (Koch and others 2010). In a single generation, the proportion of resistant progeny can be increased from the 1 to 5 percent estimated to occur in natural stands to 50 percent by using two resistant parents. Genetic improvement of stands can be accomplished either through traditional tree improvement (seedling development and planting), through silvicultural methods designed to manipulate stand genetics by favoring resistant trees (remove susceptible beech), or a combination of both (Koch and others 2010). Both State and National Forest managers have been including beech bark disease-related silvicultural treatments as well as plans for restoration/regeneration of beech as part of their resource management plans. However, there is a lack of genetically diverse, regionally adapted, disease-resistant planting stock for forest managers to use to carry out such plans. The goal of this study was to survey for healthy beech trees in heavily BBD-infested areas, then test them by applying scale eggs to confirm their resistance. Scion would then be collected for grafting from these validated scale-resistant beech trees for inclusion in ongoing efforts to establish seed orchards. The long-term goal of this work, which builds off previously funded projects, is the completion of four interagency BBD-resistant regional beech seed orchards in the following locations: the Hardwood Tree

Regeneration and Improvement Center in Indiana, the Oconto River Seed Orchard in Wisconsin, the Monongahela National Forest, and the Pennsylvania State Nursery. Each seed orchard will consist of scale-resistant beech trees collected regionally, within an approximate 200-mile radius, from both National forest and State lands.

METHODS

Survey for Candidate Resistant Beech

Surveys for resistant beech were carried out in sites where the beech component was at least 20 percent of the basal area and there was a long history of significant BBD infestation. To be considered a candidate BBD-resistant tree, the following criteria had to be met: diameter at breast height >9 inches; located within 50 feet of an infested beech tree; healthy crown; and no signs of scale infestation (rough, cracked bark) or fungal infection (cankers, tarry spots, fruiting structures). Surveys were conducted across 73 stands on 19 sites (over 5,300 acres) on the Hiawatha National Forest, Michigan. In West Virginia, four sites were surveyed on the Monongahela National Forest and five sites on State forest land. In Pennsylvania, four sites on State forest land were surveyed (table 8.1).

Field Assay to Confirm Scale Resistance

Workshops were conducted at each site to instruct participating personnel on the methods used to collect scale eggs and set up field assays for scale resistance (fig. 8.1A). A detailed protocol and instructional video has now been published (Koch and Carey 2014). To briefly summarize, between mid-July and early August, a paintbrush was used to gently brush the white, waxy clumps containing adult scale insects and eggs from an infested tree into a sealable collection bag. The mixture was passed through a 250-micron nylon

Table 8.1—Summary of candidate American beech trees identified and results of field assays for beech scale insect resistance

Location	No. of sites	No. of candidate trees tested	Resistant[a]	Susceptible[b]	Inconclusive[c]	Total no. resistant genotypes[d]
MI-Hiawatha National Forest	19	52	19	9	24	19
WV-State lands	5	17	9	7	1	9
WV-Monangahela National Forest	4	11	1	6	4	2
PA-State lands	4	24	9	14	1	22

[a] No egg clusters on foam pad or tree, <2 adults.

[b] One or more egg clusters on foam and ≥2 adults.

[c] Tests were inconclusive if both pads were missing on either the test or control tree.

[d] Includes trees tested in previous years.

mesh to separate the eggs from the adults and debris. Approximately 500 eggs were counted out using a dissecting microscope and sprinkled across a piece of moistened polyethylene foam. The foam was then tied to the candidate tree, with the eggs facing the bark, and covered with a synthetic home barrier wrap (Tyvek®). A minimum of two egg-containing pads were placed on each candidate tree, and additional pads were placed on visibly susceptible trees as a control. A year later, the pads were carefully removed, and each tree and pad was inspected with a hand lens or dissecting microscope to determine the number of adult scale insects and egg clusters that were present (fig. 8.1B). A tree is determined to be resistant if no egg clusters and no more than two adult scale insects are found on both the tree and the pad. The control tree pad was also removed and inspected for the presence of adult scale insects and egg clusters to confirm the viability of the eggs and the validity of the test.

Scion Collection and Grafting

Branch sections 1 to 2 m long were collected from candidate trees throughout the months of January and February in 2013 and 2014 using a shotgun, pole pruner, or rope saw, or by climbing and hand pruning. The cut ends of the branches were wrapped with moistened paper towels for shipment. Hot callus grafting was carried out in winter 2013 as described in Carey and others (2013), but due to less-than-optimal grafting success rates (table 8.2) the following changes were implemented in winter of 2014. To minimize fungal contamination of

Figure 8.1—(A) Application of foam pads with scale eggs onto a beech bark disease-susceptible beech tree as a control for field assays of nearby candidate beech trees. (Photo by Paul Berrang, U.S. Department of Agriculture Forest Service)

(B) Adult beech scale insect and egg cluster established in foam test pad viewed under 10X dissecting microscope. (Photo by David Carey, U.S. Department of Agriculture Forest Service)

rootstock, beechnuts were sown in a media mix containing 40 L Fafard® aged pine bark, 40 L BFG M2 Professional Mix, 225 g horticultural grade perlite, 94 g Osmocote® Plus (15–9–12), 25 g Micromax®, 75 g gypsum, and 255 g Actino-Iron® Biological Fungicide mixed together and moistened with a solution of Subdue® Maxx® (1.25 ml/L water). Germinants were treated throughout the growing season with foliar applications of fungicides, alternating Subdue® Maxx® (.04 ml/L) with Alude™ (3.0 ml/L). A soil drench application of Subdue® Maxx® (.66 ml/L) was given in October prior to putting the germinants into winter storage. Meanwhile, to reduce sources of contamination from the scion, the branches cut in January and February were surface sterilized by spraying with a solution of ZeroTol® (4 ml/L) upon receipt. The proximal ends of the branches were given fresh cuts and placed into buckets of water for storage at 4 to 6 °C. Water was changed, buckets were cleaned, and fresh cuts were made weekly until grafting was completed. Scions were cut from the branches. Prior to making the final cut for veneer grafting, the scion pieces were dipped in molten paraffin wax (50 °C) to push out excess sap, which would otherwise flood the graft union and promote contamination. The sap was blotted away prior to placing the scion on the rootstock. Once banded, the scion was again dipped in paraffin as previously described (Carey and others 2013).

RESULTS

The number of candidate trees identified and field tested at each location is listed in table 8.1,

Table 8.2—Summary of American beech grafting attempts and success rates in 2013 and 2014

Location	Total number of genotypes grafted	Number of grafts attempted, 2013	2013 percent success	Number of grafts attempted, 2014	2014 percent success[a]
MI—Hiawatha National Forest[b]	8	0	—	240	NA[e]
WV—State lands[c]	5	216	21	0	—
PA—Allegheny National Forest[d]	8	19	26	70	84
PA—State lands[d]	10	28	82	245	80
Total	**31**	**263**	**28**	**555**	**81**

— = Not applicable (zero grafts attempted).

[a] This represents a preliminary estimate, final success rates will be determined based on 1-year survival rates.

[b] Grafting done at the Oconto River Seed Orchard, White River, WI.

[c] Grafting done at the Northern Research Station Forestry Laboratory, Delaware, OH.

[d] A portion of grafting done at each facility.

[e] Success rates not yet available.

along with the number of trees confirmed to be scale resistant. In some sites, a large number of the field tests were inconclusive due to the loss of both test pads on either the test tree or the control tree. Often this appeared to be the result of bear activity. Of the 74 candidate trees that were successfully tested, just over half (38) were confirmed to be resistant. Grafting results are listed in table 8.2. In 2013, the overall success rate on the 263 total grafts attempted was only 28 percent based on 1-year survival rates, well below the average success rate of 52 percent previously reported (Carey and others 2013). The preliminary estimate of the overall success rate in 2014, based on the 315 total grafts attempted for which grafting results were available, is 81 percent. This indicates that the changes in 2014 to the potting media and grafting protocol, along with the addition of regular treatments with fungicides, contributed to improved graft success.

DISCUSSION AND CONCLUSIONS

Surveying stands with a beech component of at least 20 percent in areas long infested with beech bark disease was an effective approach for identifying candidate trees with resistance to the beech scale insect. The proportion of the candidate trees that was confirmed to be resistant through field testing varied across sites. It is possible that in areas that are considered more of a "killing front," characterized by high beech scale populations, that candidate trees can be selected with better efficiency compared to sites that are "aftermath forest," where the beech scale populations are much lower. Given that about 50 percent of the candidate trees tested demonstrated some level of susceptibility (at least one egg cluster and one adult), field testing prior to investing resources into scion collection and grafting is a more cost-effective approach to identifying scale-resistant parent trees for inclusion in seed orchards.

To capture a significant portion (>90 percent) of the genetic variation in each beech population, a minimum of 20 to 25 unrelated scale-resistant trees (8 to 10 grafted ramets of each) are needed for each regional seed orchard (Johnson and Lipow 2002). With the addition of the scale-resistant trees identified as part of this project, there are now 17 genotypes from State lands in the Upper Peninsula of Michigan and 8 from the Hiawatha National Forest that will be installed in a seed orchard at the Hardwood Tree Regeneration and Improvement Center in Lafayette, IN, in the spring of 2016. A separate seed orchard is also slated for installation at the Oconto River Seed Orchard that will include the 19 resistant trees identified at the Hiawatha National Forest, with a target of identifying 6 additional genotypes, possibly from re-assaying the trees that were inconclusive due to the loss of the test pads. Installation of the seed orchard at the Pennsylvania State Nursery was initiated in 2012 with the planting of 20 grafted ramets that represented 9 resistant genotypes. With the addition of the 9 new resistant trees identified in Pennsylvania, there are now 22 scale-resistant trees that will be added to this orchard. Efforts to identify three additional resistant trees will continue. There are currently 11 resistant trees identified in West Virginia, only 2 of which are from the Monongahela National Forest. However,

the Monongahela National Forest recently conducted additional surveys and located over 100 additional candidate trees that were slated to undergo field testing in the summer of 2014. Once fully established, these regional seed orchards will provide a source of beechnuts enriched for resistance to beech bark disease that can be used by State and Federal forest managers for restoration of healthy American beech for decades to come.

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