Lower Michigan. As of 2012, three advancing fronts were delineated; one in the central Upper Peninsula and two in Lower Michigan. Recent data indicate spread rates from 2008-2012 were extremely variable, ranging from less than 1 km to more than 6 km per year. Several satellite populations of beech scale have become established on islands in Lake Michigan and Lake Huron or in areas of Lower Michigan that are more than 20 km from the nearest known infestation. This likely reflects long distance dispersal of beech scale eggs or crawlers by birds or humans. The most recent infestation in Isabella County, discovered in 2010, remains relatively localized at this point. Overall, in 2012, beech scale infestations were present in 30 Michigan counties, encompassing a total area of roughly 16,400 km^2 in Lower Michigan and 17,000 km^2 in Upper Michigan. In comparison, in 2005, beech scale was present in only 12 Michigan counties covering an area of about 2,667 km^2 in Lower Michigan and 6,214 km^2 in Upper Michigan.

Impact of BBD: In 2002-2003, we established permanent plots on 62 sites located in forests in Upper and Lower Michigan, in cooperation with forest health specialists at the University of Michigan and Michigan Department of Natural Resources. Sites were selected to represent three levels of beech basal area (low, moderate, high) and three levels of beech scale infestation (absent, low, heavy). Overstory and understory vegetation and coarse woody material (CWM – logs over 7.6 cm in diameter) were intensively surveyed. There was little evidence of beech mortality or other BBD impacts in 2003. These plots, however, provided baseline data, enabling us to quantify the extent, progression and rate of change in composition and structure, including CWM, as BBD advances. The absence of such baseline data has largely precluded any detailed assessment of the impact of the BBD complex in beech forests of the Northeast.

In 2003, beech scale was present in only 23 of the 62 sites, including 9 sites in Lower Michigan and 14 sites in the Upper Peninsula. As of 2012, beech scale is now present in 55 of the 62 sites, including 22 of the 28 sites in Lower Michigan and 33 of the 34 sites in the Upper Peninsula. Survey results from 2012 indicate that less than 6% of the beech trees in lower Michigan have been killed, while more than 25% of the beech trees in the Upper Peninsula have died. Composition of CWM in these sites, particularly those most affected by BBD, is shifting. In 2003, CWD was dominated by moderately or severely decayed logs, while in 2012, freshly downed, large diameter beech logs, have become more common. Tree species composition and abundance of saplings and seedlings remains similar to that observed in 2003. As BBD progresses, at least 50% of the beech trees are expected to eventually die and another 25% may survive as infected “cull” trees. So be sure to appreciate those big gnarly beech trees now, while you still can.

Battling Beech Bark Disease: Establishment of Beech Seed Orchards in Michigan

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Amidst the dead, dying, and deformed beech trees left in the wake of beech bark disease (BBD; see Wieferich and McCullough , this issue), we are fortunate to find beech trees that remain healthy even in heavily infested areas. In stands across several US states it has been reported that disease-free beech trees are often found in clusters, providing evidence that resistance could be a genetic trait. Trees located in close proximity are likely to be closely related - either clonally through root-sprouting or as full- or half-sib seedlings (Houston and Houston 1994, 2000). BBD is initiated by feeding activities of the beech scale insect (*Cryptococcus fagisuga* Lind.), which create wounds that serve as entry points for *Neonectria* spp. of fungi. It is the fungal component of the disease complex that weakens and kills the tree. However, David Houston, retired US Forest Service plant pathologist and BBD research pioneer, demonstrated that beech trees that remained healthy despite intense BBD pressure failed to allow beech scale insects to establish even when eggs were directly affixed to the bark. Using the same technique, susceptible trees were readily infested (Houston 1983). 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 on Beech Scale-Resistance

In 2002, research collaboration between Michigan Department of Natural Resources (MI DNR) personnel and researchers at the US Forest Service’s

Figure 1. Pollination bags placed on mature BBD-resistant American beech tree in Ludington State Park, MI, to prevent pollen contamination during controlled cross-pollinations. (photo by DW Carey)

Northern Research Station laboratory in Delaware, OH, was initiated. The goals were to 1) demonstrate that resistance to the scale insect was a (heritable) genetic trait and 2) study the genetic basis/mode of inheritance of this trait in order to develop a beech tree-improvement program. As a starting point, full- and half-sibling families had to be developed for genetic studies. Resistant trees were identified along a campground road in Michigan's Ludington State Park that allowed a 70-ft-tall bucket truck access to the canopy of these trees. Certified tree climbers also assisted in these efforts that resulted in the production of two full-sib families and three half-sib families (Fig.1). The full-sib families were generated from the cross-pollination of two separate mother trees, one resistant and one susceptible, with pollen that had been collected from a second resistant tree. The half-sib families were obtained by collecting open-pollinated seed from the susceptible mother tree and the resistant mother tree as well as an additional susceptible tree.

Seedlings were tested for scale-resistance using an adaptation of Houston's method (Houston 1982) to artificially apply insect eggs to the stems. A known number of eggs were placed on foam pads and these were affixed to the bark of the seedlings. Approximately one year later the pads were removed and the number of healthy, egg-laying adults that had established were counted as well as the number of egg clusters (Fig. 2). Highly resistant trees did not allow successful establishment of any adults. In cases where a few adults were observed, there was no evidence of reproduction (eggs or nymphs). A range of susceptible phenotypes were observed, from trees that only had a few adults and egg clusters to trees that had hundreds of adults and eggs. All scale-resistance screening was carried out in a dedicated polyhouse located on site with our partners at the Holden Arboretum in Kirtland, OH.

Analysis of the data from the scale-resistance screening indicated there were significant differences in scale infestation and egg production between families (Koch et al. 2010). The family that had the highest proportion of resistant progeny (50%) was the one with two resistant parents. The full-sib family from a resistant x susceptible cross had only a slightly higher proportion of progeny that were scale-resistant than the half-sibling (open-pollinated seed) families from the susceptible mother trees, and both were in the same range of the occurrence of resistant trees reported in natural stands, 1to 5 %. Of particular interest was a fourth open-pollinated family that had been collected and sent to us from personnel at the Maine Department of Conservation. The beechnuts for this family had been collected from a resistant mother tree located in a stand that had been managed for BBD through the removal of all diseased trees ten years earlier (Farrar and Ostrofsky 2006). The only possible paternal parents or pollen donors for this family were the remaining resistant trees, so although this family was open-pollinated, each of the progeny apparently had two resistant parents. The proportion of progeny with resistance to beech scale in this family was also about 50%, similar to what was observed in the full-sib family with two resistant parents.

These studies allowed us to estimate the heritability of the scale-resistance trait, demonstrating a significant level of genetic control versus environmental influences. Not only is scale-resistance a heritable trait that can be successfully selected and bred for, but in a single generation using two resistant parents significant improvement can be achieved, increasing the proportion of resistant progeny in the next generation from about 1-5% to 50%. These initial genetic studies provided the information that was necessary for researchers to develop a tree improvement program as a tool in the battle against BBD.

The performance of the open-pollinated half-sib family from the managed stand in Maine, indicated that silvicultural methods designed to manipulate stand genetic composition by favoring resistant trees can also lead to tree improvement in the next generation. However, these findings are based on a limited sample size (one family) and may be influenced by the density and relatedness of the remaining resistant beech trees in the stand, as studies have shown that American beech is self-incompatible and even crosses between closely related beech trees can have low success rates (Koch and Carey 2004; unpublished data). Given the propensity of beech to reproduce clonally through root-sprouting, the number of mature beech in a stand may be significantly higher than the actual number of unique genotypes, possibly resulting in a limited number of reproductively compatible combinations of resistant parents.

The Hot Callus Grafting Method for American Beech

One way to ensure sufficient genetic diversity to promote efficient reproduction of resistant beech trees is through the establishment of seed orchards. This involves the identification of select trees in native stands and the use of a method of vegetative propagation such as grafting to create clonal replicates of the desired genotypes that can be planted together in a field setting managed to encourage optimal nut-production. American beech is a hardwood species that has not been

Figure 2. Screening seedlings for resistance to the beech scale insect. Panels from left to right: Adult egg-laden scale insects laying string of eggs (in center), eggs placed on foam affixed to bark of test seedling, resistant seedling 52 weeks later when foam was removed, susceptible seedling 52 weeks later when foam was removed. (photos by DW Carey & JL Koch)

amenable to vegetative propagation methods. Methods such as rooting of cuttings and micro-propagation have been unsuccessful in beech, and traditional grafting methods have yielded low and variable success rates (Barker et al. 1997, Ramirez et al. 2007, Pond 2008).

In spite of this, we have been able to achieve consistently higher grafting success rates with beech by utilizing a hot callus system for grafting. Hot callus grafting utilizes traditional grafting techniques such as top grafting and side veneer grafting, with the difference being that the hot callus grafting system keeps the graft union heated to promote callus formation, while keeping the rootstock and scion cool and dormant. This method has been reported to significantly increase the graft success of woody plants (Langerstedt 1984, Avanzato and Tamponi 1987). The hot callus system we employ relies on thermostat controlled heating cables attached to a wooden frame to supply heat to the graft unions. The system is set up in a greenhouse or cold room kept just above freezing. Sill plate foam is used to create an enclosed "heat chamber" by wrapping it above and below the graft union and affixing it to the board that supports the heating cable, as illustrated in Figure 3 and described in Carey et al. (in press).

Utilizing the hot callus system on more than 2000 graft attempts during a 6-year period, we reported an average overall success rate of 52%, compared to previously published grafting success rates in American beech of 30% and 12% in 2 consecutive years (Ramirez et al. 2007, Carey et al. in press). In an experiment directly comparing hot callus grafting to traditional methods of grafting in beech, we demonstrated a success rate of 67% using the hot callus system compared to 13% without it (Carey et al. in press). Our method was performed on more than 74 different genotypes of American beech (27 from Michigan), and less than 10% of these genotypes had success rates less than the average. This indicates that the hot callus grafting system works across a widely diverse collection of selections, which is what is required to develop seed orchards.

Containerized Seed Orchards

In cases where scion for grafting is collected from mature, seed-producing trees whose buds are programmed to form flowers at the time of collection, viable flowers emerge post-graft. Controlled cross-pollinations can be easily performed in the greenhouse on potted grafts (Fig. 4) instead of 70 ft up in the canopy of a mature tree in the field. These types of containerized controlled-crosses have allowed us to produce multiple new full-sib families with several different pairs of resistant parents in a single season. Not only are the crosses safer and easier to make in the greenhouse, but the plants produce higher quality seed when grown under conditions where light, temperature and nutrients can be carefully controlled. The beech seed produced from containerized greenhouse crosses had germination rates about twofold higher than what was observed from field-pollinated seed (Koch et al. 2007).

Seven more full-sib families have been produced using combinations of nine additional resistant parent genotypes. Both parents were from Michigan for five of these families, the other two families had one parent from Michigan. The progeny have all been screened for beech scale-resistance, and the findings have supported the original observations that the best performing families are those whose parents are both resistant to the beech scale. Across all seven families the average proportion of progeny with scale-resistance was 56%. These results provide additional evidence for the significant genetic gain that can be expected through the development of scale-resistant American beech seed orchards.

Installation of First BBD-Resistant American Beech Seed Orchard

The MI DNR has been monitoring BBD infested areas in Michigan and tracking putatively resistant trees since 2001. MI DNR personnel relied on snowmobiles for transportation to collect dormant scion material using sling shots to get rope saws high into the crowns of the selected trees. U.S. Forest Service researchers grafted the scions and confirmed the scale-resistance of the selected trees using the artificial infestation technique described above (Fig. 5). Installation of the first resistant American beech seed orchard began in 2011 at the Tree Improvement Center in Brighton,

Figure 3. Above: Hot callus grafting system showing graft union with grafting rubber wrapped around it lined up between two heat cables prior to being sealed with sill plate foam. Below: Graft union sealed in heat chamber with sill plate foam. (photos by M Miller)

MI. To date, 74 resistant trees have been planted, but with heavy deer predation and drought conditions only 39 have survived through 2012. Installation of deer protection and improved irrigation is expected to increase survival rates as work continues to complete the seed orchard by 2014. Upon completion, the seed orchard will contain at least 15 ramets each of 20 different resistant genotypes for a total of 300 trees. It is estimated that a seed orchard consisting of 20 unrelated individuals should contain most of the genetic variation found in the native population (Johnson and Lipow 2002).

The full-sib families described from the containerized controlled crosses used nine of the genotypes destined for inclusion in the Brighton seed orchard, and so have given us a snapshot of at least a portion of the expected output from this orchard once it is mature and producing seeds (56 % of all progeny having resistance). Some of the seedlings were outplanted in November 2011 in the Michigan Upper Peninsula in an area heavily impacted by BBD. The one1-year survival rate has exceeded 95 %. These seedlings be monitored yearly for growth characteristics and scale-resistance. It is our hope that in the future the BBD-resistant

American beech seed orchard at the Brighton Tree Improvement Center will provide a valuable source of BBD-resistant beechnuts that can be used by both state and federal forest managers for restoration of healthy American beech for decades to come.

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Figure 4. Containerized beech grafts that have been pollinated and are developing beech nuts.

Figure 5. Grafted Michigan BBD-resistant American beech.

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