



Refinement of a Grafting Program for the Propagation of BBD-Resistant American Beech



Volunteers assisting with a transplanting trial in Alberta, Michigan, to inform planting of grafted beech in forest understories within National Parks in Michigan.

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Refinement of a grafting program for the propagation of BBD-resistant American beech

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Abstract

A grafting program for creation of beech bark disease-resistant American beech (*Fagus grandifolia*) has been established in a cooperative agreement between Michigan Technological University and the National Park Service (NPS). The resistant trees are being used in a restoration project at Pictured Rocks National Lakeshore and Sleeping Bear Dunes National Lakeshore. This report provides an overview of the methods for propagation and planting of American beech, with suggestions for refinements including a forest planting trial. Rates of resistance are quantified for target NPS properties based on survey methods. Resistant trees were located and confirmed through field challenge tests to serve as scion donors for grafting. Resistant tree locations and conditions are not presented to maintain security of valuable individuals but are maintained by each property and revisited annually. This report will be used to inform continuing work towards restoring and preserving American beech in the landscape within NPS properties.

List of Terms and Acronyms

BBD: Beech bark disease. A disease complex that arises from collective impacts of a non-native scale (*Cryptococcus fagisuga*) and two introduced fungal pathogens (*Neonectria ditissitima* and *N. faginata*).

BLD: Beech leaf disease. Caused by the foliar nematode *Litylenchus crenatae* ssp. *mcannii* (Lcm) and believed to be non-native in North America.

HNF: Hiawatha National Forest (Michigan).

PIRO: Pictured Rocks National Lakeshore (Munising, Michigan).

Putatively Resistant Tree: A tree that displays no or little annual scale accumulation over successive years. These trees may or may not be resistant and should be monitored and subjected to resistance testing. Between 3% and 13% of trees generally fall in this category, but this number is dependent on many factors such as annual population dynamics, spatial patterning, and other factors.

Scale: An insect (commonly known as beech scale) that infests beech trees and feeds on them by boring a hole into the bark. The hole it creates allows fungal pathogens to enter and infect the tree.

Scion: Branches cut from a desirable donor tree for grafting. Scions are attached to rootstock and make up the upper portion of a grafted tree.

SLBE: Sleeping Bear Dunes National Lakeshore (Empire, Michigan).

Resistant Tree: A tree that has been subjected to a field resistance challenge test and allowed no or few scale to establish on the tree. About 1% of trees are resistant.

Rootstock: The below-ground portion of a grafted tree that a scion is attached to.

Introduction

American beech is an ecologically important hardwood tree in northeastern forests in the United States (Cale et al. 2017). This species has been threatened by an invasive disease complex, beech bark disease (BBD), for over a century. BBD has been present in Michigan since at least 2001 (O'Brien et al. 2001), and efforts to mitigate the disease have been undertaken by many agencies. American beech can be naturally resistant to the scale insect necessary for the disease, and mitigation efforts have largely focused on exploitation of the natural resistance of a small percentage of mature trees (Carey et al. 2013, Houston 1982, Koch and Heyd 2013, Ramirez et al. 2007). The U.S. Forest Service has created resistant seed orchards through grafting of resistant trees and planting of resistant seed orchards in Ludington, Michigan, in cooperation with the Michigan DNR (Koch et al. 2015, Koch and Heyd 2013). Seeds developed from these orchards are intended for future beech restoration efforts for both state and federal land managers. Restoration efforts for landowners in Michigan have focused on mitigation of the disease through retention of naturally resistant American beech (*Fagus grandifolia* Ehrh.) trees, with guidance provided by the Michigan Department of Natural Resources (DNR) and the Michigan State University Extension Office (Heyd 2005, McCullough et al. 2001).

To enhance the proportion of American beech that are resistant to scale infestation, some type of propagation must occur to create trees for restoration activities, whether these are seeds, plantlets, or mature plants. American beech is regarded as difficult to propagate through cuttings, but grafting is an acceptable method of propagation (Doran 1957). Regular bench grafting methods have been done, though they are reported to lead to low success rates, between 12% and 30% (Carey et al. 2013, Ramirez et al. 2007). The application of a hot callus apparatus while the grafted trees heal (in which the entire plant is kept cold dormant except for the graft union, which is gently heated in an insulated chamber to stimulate growth in that region) has shown to increase success rates up to 57% (Carey et al. 2013).

Grafting success rates can vary widely. Genotype of scion donor tree or rootstock family can affect success, with some trees being particularly good or particularly bad donors (Carey et al. 2013). The type of graft used and the diameter of the rootstock can also have dramatic effects on success (Carey et al. 2013). The highest success rates are achieved by skilled grafters working with healthy, vigorous donor plants who have experience specifically grafting American beech. American beech wood is very hard and stiff when dry, which is challenging to cut through cleanly (Carpenter 1974). Additionally, the bark and cambium are very thin. While cambium thickness of American beech is unknown, the related species European beech (*Fagus sylvatica*) displays only about a three- to five-cell thickness of cambium layers in the dormant season (Oladi et al. 2011). This necessitates a very precise layering of wood for successful unions. Grafting is a technically challenging process and requires skilled technicians, even in applications that use specialized tools to simplify the process.

Beech bark disease-resistant trees must be identified and field-challenge tested to serve as scion donors for the grafting process. About 1% to 3% of American beech are expected to display resistance to beech scale (*Cryptococcus fagisuga* L.) infestation (Houston 1982). In the northeast, where BBD has been present since before 1934, as many as 12–15 trees per hectare (ha) may be

resistant to BBD (Houston 1983). The rate of resistance in the western range of American beech has not been reported. We expect the rate of resistance to be about the same in PIRO and SLBE but due to relatively lower dominance of beech, we expect to find fewer resistant trees in total.

In collaboration with the National Park Service (NPS), a grafting program to create BBD-resistant American beech was developed for restoring impacted forests at Sleeping Bear Dunes National Lakeshore (SLBE) and Pictured Rocks National Lakeshore (PIRO), both in Michigan. The goals of this project were to locate BBD-resistant beech trees via field-challenge tests, quantify resistance levels at each park, collect and graft scions from resistant local provenance genotypes, improve propagation methods for both grafting and container tree maintenance, and develop planting methods to improve outplanting success at both parks. Refinement of these propagation techniques is intended to assist with restoration in BBD impacted areas within these national lakeshores. Alterations and additions to methods reported in literature were identified through trial-and-error and a working partnership was developed with the U.S. Forest Service Oconto River Seed Orchard. These methods will result in the creation of grafted trees suitable for use in restoration activities and allow continuation through detailed documentation.

Methods

Sourcing Donor Material

Identification and testing of resistant trees

Straight transects were walked from random starting points throughout both SLBE and PIRO, beginning at roadsides and trail edges. Lengths of transects were variable, and putatively resistant American beech were identified within 12.5 meters (m) of the transect. Putatively resistant trees can be seen from a distance by the complete absence of scale infestation signs and confirmed up close by careful visual inspection of the entire bole. All putatively resistant trees identified along transects had GPS coordinates recorded.

Some putatively resistant trees were identified by NPS personnel in Pictured Rocks National Lakeshore before 2017. These trees were included in the list of putatively resistant trees, and GPS coordinates were combined for all trees identified prior to 2017 and for trees identified during transect surveys at both properties in 2017–2019. Additional wandering surveys of variable length were performed by NPS personnel in SLBE. In wandering surveys, technicians entered from roadsides and walked at random with a GPS tracklog of their location recorded. If a beech tree was visible while walking these wandering surveys, it was approached and visibly assessed for presence of scale using binoculars to examine the entire bole. To quantify the proportion of resistant American beech in the target properties, the number of putative resistant trees located was divided by the amount of area covered to estimate the number of resistant trees present per area in the parks.

Putatively resistant trees were subjected to field resistance testing to confirm resistance to scale infestation (Houston 1982, Koch and Carey 2014). Scale eggs were collected from beech trees in August 2019. To collect eggs, the white mass was collected from the boles of infested beech trees. A two-inch paintbrush was used to gently brush the bole of a tree with a visibly heavy infestation of scale. The wax, insects, and eggs were collected in a plastic bag and stored in cool conditions for no more than two weeks. During these two weeks, the mass was gently pressed through 250-micron fine-mesh sieves to separate the eggs into a fresh container. A volumetric estimate standard was used to count out 500 eggs. This quantity (about 500 eggs) was then brushed onto the bottom of a damp polyethylene sponge.

The damp sponge was applied to a putatively resistant tree with the egg-covered surface flush against the bole of the tree. Plastic-coated wire was used to hold the sponge in place and vapor-permeable house wrap was affixed over the sponge and sealed on three sides with waterproof silicone caulk. A “Do Not Remove” message, the scientific permit number, and contact information was clearly written on each house wrap. Pads remained in place for 52–57 weeks. After this time, the pads were carefully removed and the number of scale insects and egg clusters present on trees were counted. Trees with low scale infestations were retained in the database but not considered suitable as scion donors. Trees were considered resistant if no or very few egg clusters (three or fewer) were present on the bole of the tree or the foam pad, and the nearby control showed intense scale infestation.

Five additional control field challenges on additional beech were placed in each geographic cluster of resistant trees. Control trees had a visible presence of scale infestation but were not heavily infested. The bole was brushed clean before scale eggs were applied in the same manner as test trees. Area controls were considered a successful challenge if many scale egg clusters and multiple adult scales were present.

Trees were considered resistant if they harbored no scale egg clusters 52–57 weeks after application of the artificial infestation. GPS coordinates of resistant trees have been recorded and shared for internal use but are not published to protect the location of resistant trees. Surveys were performed with a variety of methods, so area assessed from transect lines was used to account for survey efforts.

Collecting from resistant trees

The National Park Service indicated in the initial conceptualization of the project that only locally constrained genetic material was appropriate for restoration in their properties. In order to meet stakeholder objectives, any healthy rootstock could be used, but only scions from trees originating within the park boundaries could be planted within the boundaries of the parks. Rootstock could originate from elsewhere in the region (i.e., still within the upper Great Lakes region), but we removed the root stock or monitored for root sprouting in the case of mortality or graft failure after planting. Root sprouting does occur on trees that are unaffected by disease if roots are disturbed (Jones and Raynal, 1988). Because the rootstock is still susceptible to BBD, sprouting of non-resistant root suckers could occur in the future if roots are naturally disturbed by wildlife or weather. However, grafted trees utilizing scions from reproductively mature donor trees can begin flowering and creating seed within their first growing season following grafting (Koch et al., 2012). These resistant grafted trees can begin positively contributing to park genetics following outplanting, while seed origin resistant trees would take around 40 years before they mature and begin flowering (Tubbs and Houston, 1990). Outplanted grafted trees should be monitored long-term to assess non-resistant sucker development.

Grafting BBD-resistant scions is severely limited by availability of commercial bare-root American beech seedlings, in Michigan. Therefore, a variety of rootstocks were initially sourced. Bare-root seedlings (45- to 60-cm tall) were purchased commercially prior to 2020. Seedlings were purchased from an in-state grower, as all efforts were made to keep the rootstock of a known, relatively local provenance. However, supply issues led to shipments from unknown provenance to be mixed with the local rootstock. In addition, beech scale arrived on a mixed shipment of rootstock. Trees with visible scale infestations were destroyed, and trees without scale present, but packaged in the same shipment, were scrubbed gently with dish soap and a stiff brush, treated with diatomaceous earth to prevent reinfestation by other pests, and monitored in the greenhouse.

Rootstock was also excavated from the Hiawatha National Forest (HNF) in Michigan's Upper Peninsula under a non-commercial use permit (FS-2400-08) (Figure 1). Survival and vigor of excavated rootstock equaled or surpassed commercially available rootstock (Myers et al. 2022). Additional rootstock of mixed Wisconsin and Michigan provenance was donated to the project by the Oconto River Seed Orchard (United States Forest Service). The lack of commercial beech seed or

seedlings and recent detections of DNA from the nematode related to beech leaf disease (BLD) in southeast Michigan (Martin and McIntire 2024) caused us to shift entirely to wildling rootstock, sourced from the HNF.



Figure 1. Excavating beech rootstock at the Hiawatha National Forest, Munising, Michigan.

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Scions were collected from tested resistant trees in March 2020, November 2020, March 2021 and 2022, March 2023, and April 2024. Scion branches were removed from the outer canopy of resistant American beech trees using an arborist slingshot and manual rope saw (Figure 2). In grafting manuals, fall collection is often recommended, however no grafted scions collected in fall survived in our trials, thus, we did not continue fall scion collection. More details on scion collection procedures can be found in Myers (2021).



Figure 2. An arborist slingshot is used to set a line around the desired scion (A). Once the line is set, a saw chain is hoisted to the scion for removal (B).

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Grafting

American beech were grafted primarily using a modified side graft, but veneer or top cleft grafts were used if appropriate for size and shape of rootstock. The modified side graft was taught at an in-person grafting blitz hosted by the U.S. Forest Service Oconto River Seed Orchard. The modified side graft is preferred because the diameter match between scion and rootstock does not have to be exact, so long as the scion base diameter is no larger than the rootstock. The scion should display healthy, full buds. The scion, rootstock, and all grafting tools were sanitized prior to proceeding with each individual graft. Detailed grafting procedures used in this program can be found in Myers (2021).

Immediately after grafting, trees were placed in a hot callus apparatus, housed in a modified refrigerated trailer with an ambient temperature of 4°C (40°F). The trees were placed so that the graft union was inside an insulated chamber heated to 27°C (80°F). The trees were allowed to heal in the hot callus apparatus for 4–8 weeks and removed at the first sign of callus tissue formation. After graft healing, trees were moved to an indoor greenhouse under a light shade net. Trees were lightly watered at this time and allowed to continue to heal.

Success of grafts were determined by leaf-out, survival of the scion tissue and growth above the graft. Codes were assigned to failed grafts to aid in diagnosing of failures (Table 1). Trends in failure codes were analyzed to provide guidance towards refinement of methods.

Table 1. Codes of graft failure attribution.

Code	Failure
0	No failure; survival
1	Graft union failure: No callus tissue formation
2	Mechanical failure; callus tissue present but signs of splitting or lifting of graft union
3	Scion failure; callus tissue present, but no leaf out or growth from above-graft portion
4	Pathogen; callus tissue present, but clear signs of pathogen on scion or rootstock
5	Rootstock failure; callus tissue present, but signs of root decay, signs of death above root collar but below graft union

Containerized Beech Maintenance in the Greenhouse and Outdoors

Prior to use as rootstock, beech were maintained in a mixed-use greenhouse from late winter to mid-summer (Figure 3). In mid-summer, trees were moved to an outdoor space with partial sun to prevent scorching from high greenhouse temperatures. In the mixed-use greenhouse, trees were watered twice a day for three minutes with an automatic, drip watering system until spring of 2023. Under the drip watering system, due to different sized containers and various stages of growth, some trees would remain saturated while others would dry out between waterings. In response, since the spring of 2023, all greenhouse trees have been individually hand-watered as needed to maintain moderately moist soils. When outdoors, trees were watered to saturation two to three times a week to maintain moderately moist soils.



Figure 3. Containerized seedlings of wild origin from the Hiawatha National Forest growing under shade cloth in the greenhouse. These plants will be suitable for rootstock once dormant.

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Beech rootstock was treated several times in different growing seasons to reduce the impact of common greenhouse pests such as spider mites, thrips, armored scale, and wooly aphids. Pest treatments included neem oil, diatomaceous earth, and the commercial products Organocide, Avid, and Ovation. Outdoor beech were placed in a sheltered area with moderate shade, watered deeply, and allowed to drain between rain events to maintain damp soils but prevent waterlogging. From 2018 through 2022, grafted trees were moved indoors before the first projected frost event and allowed to go dormant in the greenhouse. However, we saw an overall decline in grafted tree vigor that was not seen in outdoor wintered rootstock, and through discussions with researchers experienced with grafting beech, it was determined that an extensive chilling period of at least four months is necessary for long-term health and survival of grafted trees (D. Carey, personal communication, 27 February 2024). Beginning in the fall of 2023, all grafted trees were moved to a cold storage trailer in late October and kept at an ambient temperature of 4°C (40°F) until March.

Planting Trial in Alberta, Michigan

With the continued impact of BBD and growing threat of BLD across the range of American beech, best practices for artificially regenerating resistant beech are increasingly important but are scarcely documented (Wagner et al. 2010). To determine methods suitable for American beech, (particularly in our target properties that occur in this species' western range limit and feature heavy annual snowfall), a series of trials examining site preparation intensity, seasonality, and stem protection began in 2021 and will continue through the 2025 growing season. Four planting sites using 30 trees each of transplanted rootstock were installed in Alberta, Michigan, outside the range of American beech and associated BBD (Figure 4). Two of the four plantings were installed in fall of 2021 and two of the four in the spring of 2022. One of each of the seasonal plots utilized heavy site preparation (all competing understory removed in the 10-m² plots) and the other featured light site preparation (only raking and removing competing vegetation 30 cm around individual planted trees) (Figure 5). Fifty percent of all trial trees featured tree guards constructed of hardware cloth to primarily protect stems from being damaged by small mammal herbivory. We have observed girdling by rodents and lagomorphs to be an issue on trees being staged for outplantings.

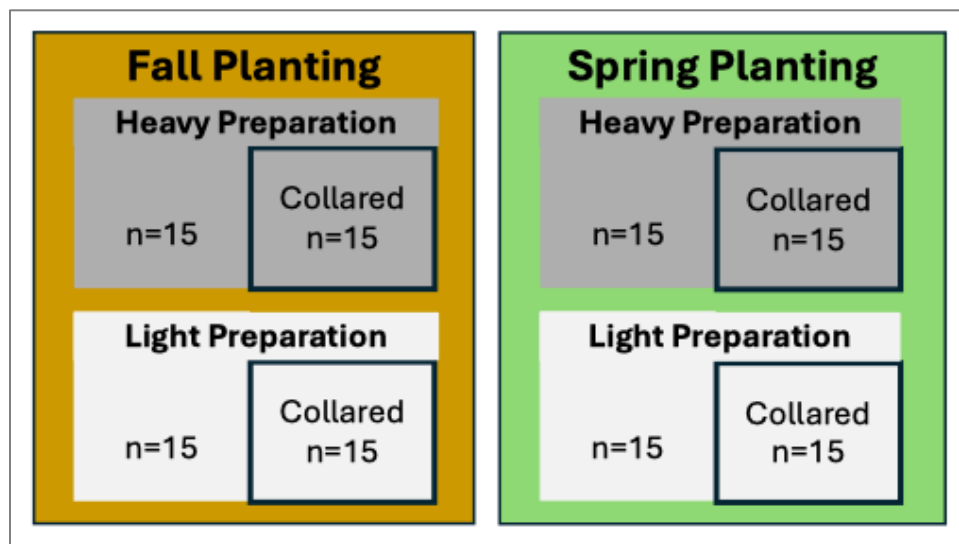


Figure 4. Block trial planting design located in Alberta, Michigan (Baraga County). A total of 120 transplanted wildling rootstock trees were utilized for this trial.

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Figure 5. A prepared planting microsite outside of NPS property (Alberta, Michigan). Holes were dug to the minimum size needed to accommodate trees, no more than 30 cm deep. Each hole was flagged for visibility but flags were removed after planting. Leaf litter is raked away, but only to a distance necessary to clear the planting site. Ground was disturbed and vegetation removed about 30 cm around each hole.

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Outplanting Grafted American Beech in Parks

In the fall of 2022, ten trees were planted at PIRO and eight trees were planted at SLBE. Planting occurred after full senescence and before snow began to accumulate at both PIRO and SLBE. We used low site preparation for both park plantings. Tree collars were initially installed on all outplanted grafted trees. Collars were removed in fall of 2023. Trees were planted no less than two meters apart and minimum of two meters distance from existing trees (Figure 6). Site selection was based on previous spatial analysis and in consultation with park managers, and especially considered maintaining a limiting distance of 100 meters of apparently healthy, mature BBD-resistant American beech (Myers 2021). Monitoring of planted trees occurred multiple times throughout subsequent growing seasons after snow melt.



Figure 6. Installed trees at Pictured Rocks National Lakeshore. Hardware cloth with ¼-inch openings was installed as a tree collar, and existing leaf litter was placed as mulch around the tree.

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Results

Resistant Tree Results: 2017–2021

Trees were considered resistant if they harbored no scale egg clusters 52–57 weeks after application of the artificial infestation. While resistant trees had no egg clusters present, a few had three or fewer scale egg clusters present. These intermediate trees were neither classified as resistant or completely as non-resistant. No scions were collected from these trees however they should be monitored and retested in future years (i.e., it may have been difficult to discern if egg clusters were dead). The controls in the Grand Sable Lake area of Pictured Rocks National Lakeshore failed, so these putatively resistant trees have been excluded from resistance testing results (eggs may not have been viable). However, there is multi-year data provided by NPS of scale absence, so the trees are still being retained in the records as putatively resistant.

Wandering surveys performed in 2017 covered 242 hectares (ha) of both National Park Service properties (SLBE and PIRO), where collaborators identified 68 putatively resistant trees after an initial survey, but only 24 trees had escaped scale infestation upon second visits between 2019 and 2022 (0.09 disease-free trees per ha). Although the area covered is known, the amount of diseased beech was not recorded during wandering surveys, so rates of disease-free trees per total trees are unknown for these surveys.

Transect surveys performed by personnel from Michigan Technological University in 2017, 2018, and 2019 covered an additional 13.8 ha. This effort identified four additional putatively resistant trees, or 0.28 disease-free trees per ha in PIRO in the first year. Of the 29 trees tested that had multi-year absence of scale reported in PIRO, 27 were confirmed resistant. In Sleeping Bear Dunes, 91 American beech were encountered during transect surveys, and five were putatively resistant (5.49%). None of the trees identified during transect surveys had escaped scale infestation upon revisiting (0%). In Pictured Rocks National Lakeshore, 186 American beech were encountered during transect surveys, and four were disease-free (2.1%). After revisiting the sites, four trees were tested and three were confirmed resistant (1.61%). A summary of resistance findings is compiled in Table 2 and compared to published rates reported from the northeastern United States and Canada (Houston 1983).

Table 2. Rates of resistance reported by Houston (1983) from sites in New England (USA) and Nova Scotia (Canada) compared with those found in PIRO and SLBE.

Location	Putative Resistance	Resistant	% Confirmed	Beech Stems/ha	Disease Free Trees/ha	Resistance Rates/ha
Maine and New Hampshire, USA (tested trees)	17	17	100	N/A	N/A	N/A
Riversdale, Nova Scotia, Canada	75	N/A	N/A	2,129	15	3.5
Brookside, Nova Scotia Canada	32	N/A	N/A	1,872	12	1.7

Table 2 (continued). Rates of resistance reported by Houston (1983) from sites in New England (USA) and Nova Scotia (Canada) compared with those found in PIRO and SLBE.

Location	Putative Resistance	Resistant	% Confirmed	Beech Stems/ha	Disease Free Trees/ha	Resistance Rates/ha
SLBE, Michigan, USA (transect surveys)	5	0	0	86.8	0.6	N/A
SLBE, Michigan, USA (wandering surveys)	24	16	66	N/A	0.6	N/A
PIRO, Michigan, USA (transect surveys)	4	3	75	82.0	0.3	1.1
PIRO, Michigan, USA (multi-year surveys)	25	24	96	N/A	N/A	N/A

Grafting Success Rates

Grafting has been attempted for 35 resistant donor trees, each representing an individual genotype, and 28 are represented (Table 3). Two genotypes (FAGR 1701 and FAGR 109) were grafted and are healing, with initial success assessed in July 2024, but have not yet completed one year of growth.

Table 3. Grafting results of resistant trees at PIRO and SLBE across all years. Genotype indicates the name for individual trees assigned by NPS personnel. Associated GPS coordinates are available internally. Initial take is the number of trees that formed callus tissue or leafed out after grafting. Survival number is the number of healed grafts that survived to the following year after grafting.

Park	Genotype	Year	Attempts	Initial Take Number	Initial Take Rate (%)	One-Year Survival Number	One-Year Survival Rate (%)
PIRO	FAGR108	2020	5	2	40	1	20
PIRO	FAGR116	2020	30	20	66.7	12	40
PIRO	FAGR118	2020	30	27	90	8	26.7
PIRO	FAGR119	2020	30	18	60	3	10
PIRO	FAGR128	2020	30	22	73.3	10	33.3
PIRO	FAGR31	2020	5	3	60	1	20
PIRO	FAGR34	2020	30	16	53.3	8	26.7
PIRO	FAGR85	2020	5	4	80	0	0
PIRO	F105	2021	10	6	60	3	30
PIRO	FAGR1715	2021	10	7	70	2	20
PIRO	FAGR1715	2021	11	10	90.9	10	90.9
PIRO	FAGR 104	2022	20	4	20	4	20
PIRO	FAGR1703	2022	11	7	63.6	7	63.6
PIRO	FAGR85	2022	20	1	5	0	0
PIRO	FAGR1701	2024	46	31	67.4	N/A	N/A

Table 3 (continued). Grafting results of resistant trees at PIRO and SLBE across all years. Genotype indicates the name for individual trees assigned by NPS personnel. Associated GPS coordinates are available internally. Initial take is the number of trees that formed callus tissue or leafed out after grafting. Survival number is the number of healed grafts that survived to the following year after grafting.

Park	Genotype	Year	Attempts	Initial Take Number	Initial Take Rate (%)	One-Year Survival Number	One-Year Survival Rate (%)
PIRO	FAGR109	2024	24	7	29.2	N/A	N/A
SLBE	faggra016	2020	20	10	50	8	40
SLBE	faggra041	2020	30	14	46.7	3	10
SLBE	faggra043	2020	30	24	80	6	20
SLBE	faggra047	2020	30	16	53.3	1	3.3
SLBE	faggra075	2020	20	1	5	0	0
SLBE	faggra102	2020	30	7	23.3	3	10
SLBE	faggra104	2020	30	26	86.7	6	20
SLBE	FAGR030	2020	20	1	5	0	0
SLBE	FAGR039	2020	21	3	14.3	0	0
SLBE	FAGR129	2020	8	2	25	0	0
SLBE	FAGR133	2020	16	1	6.3	0	0
SLBE	faggra 016	2022	20	8	40	7	35
SLBE	faggra 038	2022	20	6	30	6	30
SLBE	faggra 096	2022	20	4	20	4	20
SLBE	faggra 106	2023	30	12	40	10	33.3
SLBE	faggra 130	2023	30	7	23.3	6	20

Individual trees are highly variable in their grafting take rate. This may be due to a variety of factors including individual tree anatomical differences, annual environmental conditions, or handling/storage conditions. Tracking long term survival is critical, as a high take rate of one individual does not necessarily indicate long term success of grafted trees. Delayed graft failure or conditions in the greenhouse all contribute to long term survival rates. Of the represented genotypes, 12 healed sufficiently to be considered for planting, while others are still growing. Causes of graft failure varied. Typical greenhouse pests/diseases, mechanical failure, and abiotic greenhouse stress were the most common failure points. In response to these stressors, we have increased the strength of sanitizing solution, implemented the use of fiberglass support stakes, and implemented ameliorative measures such as a 40% shade cloth inside the greenhouse, a complete fertilizer containing micronutrients, and additional watering in the greenhouse. Further greenhouse trials are planned for refining tree care methods in 2024. As genotype can affect the success of grafts, if trees fail completely after more than one round of grafting, they should be removed from the pool of donor trees but retained in the list of confirmed resistant trees (Carey et al., 2013). If a tree has very good success rates, it can be revisited for additional scion donation after 3–5 years. Care must be taken

when considering where successful grafts of individual genotypes are outplanted within parks to lessen the impact of potential genetic bottlenecks.

Grafting accomplished under supervision by experienced grafters at Oconto River Seed Orchard had greater initial success than unsupervised grafting in 2020. Technicians should receive in-person training in hardwood grafting prior to attempts, due to the high value and relative scarcity of learning materials.

Fall grafting was attempted in early December 2020. Scions were collected in November 2020 and a small round of 11 trees were grafted. Five of the 11 trees initially healed in this round (45%), but ultimately experienced delayed failure. In response to initially high success rates, two additional trees were collected in December 2021. All the grafts from this collection failed. We recommend discontinuation of fall grafting exploratory methods.

Additionally, scion material from fall grafting was placed in moist storage to attempt to hold over scion material until spring. Recommendations in literature exist for the long-term storage methods of scions from many species if refrigerated storage is available, particularly with the ability to control humidity (Humphrey 2019). In our experience, the beech scion material developed mold easily, even when packaged as recommended and was not usable in the spring. While methods are described for moist long-term storage of scions, this is not likely a successful storage method for American beech.

Grafting failures

Graft union failure was identified as the primary cause of grafting failures during the 2019 season (see code definitions in Table 1). In spring 2020, failures were attributed to a combination of mechanical failure and a black mold infection that appeared under the wax. Trees grafted in 2022 displayed scion failure, likely due to drying out for a short period of time after collection. The majority of failures that occurred in 2023 and 2024 appear to be related to scion failure mixed with a limited number of trees not forming a graft union (mechanical failure).

Planting Trials in Alberta, Michigan

Following the first growing season for these four trials, there were three mortalities each in both the spring and fall plantings (Table 4). Each seasonal planting had 60 initial trees (see Figure 4).

Table 4. Treatment mean survival and growth by seasonality, site preparation intensity, and stem protection by year at the four transplanting trials in Alberta, Michigan (n = 60 for each treatment type). Note that negative growth values are due to herbivory, breakage from snow, or other causes of dieback.

Treatment	Mean Survival Fall 2022	2022 Growth (cm)	Mean Survival Fall 2023	2023 Growth (cm)	Mean Survival Fall 2024	2024 Growth (cm)
Season: Fall 2021	95%	0.018	77%	1.78	57%	0.86
Season: Spring 2022	95%	-0.021	80%	-2.19	63%	1.51
Site Preparation: High	91%	-0.045	63%	-4.3	40%	-2.2

^A Tree collars were removed after the 2023 growing season due to damage from snow weight and were not repaired due to the lack of significant differences in collar treatments.

Table 4 (continued). Treatment mean survival and growth by seasonality, site preparation intensity, and stem protection by year at the four transplanting trials in Alberta, Michigan (n = 60 for each treatment type). Note that negative growth values are due to herbivory, breakage from snow, or other causes of dieback.

Treatment	Mean Survival Fall 2022	2022 Growth (cm)	Mean Survival Fall 2023	2023 Growth (cm)	Mean Survival Fall 2024	2024 Growth (cm)
Site Preparation: Low	98%	0.0461	93%	3.53	80%	3.04
Collar: Yes	96%	0.55	80%	-6.13	N/A ^A	N/A ^A
Collar: No	93%	-0.057	78%	1.46	N/A ^A	N/A ^A

^A Tree collars were removed after the 2023 growing season due to damage from snow weight and were not repaired due to the lack of significant differences in collar treatments.

Due to ease of access and unpredictable timing of spring snow melt, it was decided that future outplantings at both parks would take place in fall. After the first growing season, variable survival was observed between plantings using heavy site preparation and light preparation, however, light preparation consistently had lower mortality across growing seasons (Table 4). The higher survival rates of the light site preparation are potentially due to the surrounding soil around planted trees being less disturbed, leading to greater moisture retention; the increased survival could also be due to less browse pressure as light site preparation trials featured more competing vegetation (i.e., short grasses and herbaceous species) around planted trees. To best align with the NPS mission statement of leaving the natural resources unimpaired, it was decided to use light site preparation for park outplantings (NPS 2023). Survival was similar between trees using and not using tree guards after two growing seasons (see Figure 6). However, tree guards were often bent due to heavy snowpack, causing branches to bend or get stuck in the steel mesh hardware cloth. Because of this and a desire to preserve a more natural aesthetic, it was decided that tree guards would not be used in park outplantings. Negative growth values were the result of stem breakage or tip dieback, which reduced the overall height of transplanted trees.

Outplanting Grafted American Beech in Parks

High mortalities of outplanted trees that were planted in 2023 occurred at both SLBE and PIRO that same year. At PIRO, four of the ten planted trees survived. No survival occurred at SLBE. A combination of biotic and abiotic factors likely contributed to these high mortality rates. Unexpected warm temperatures in late November following outplanting, in combination with abnormally late heavy, wet snowfall after leaves had started to emerge likely contributed significantly to injuries. The spring planted trial trees did have an overall higher survival rate, but the spring weather conditions when they were established were different. As mentioned earlier, long-term graft vigor was likely reduced prior to spring 2023 due to a lack of a chilling period during dormancy. Because outplanting success is directly related to stock vigor (Landis et al. 2010), we anticipate that outplanting success will improve in coming years with an increase in containerized grafted tree health, barring unpredictable severe weather events. Additional outplantings are planned for subsequent growing seasons at both properties based on annual grafted tree availability.

Discussion

Considerations for Testing Tree Resistance

Identifying trees that display low scale infestations but are not confirmed resistant can enhance our understanding of disease-resilient trees (Cale and McNulty 2018) and the ecology of resistant tree populations. The location of additional resistant trees will enhance our ability to mitigate BBD. Our practice of considering trees truly resistant only if no egg clusters are present agrees with methods presented elsewhere (Koch and Carey 2014). Because infestation levels can vary over years or across environmental gradients, only trees that seemingly offer no suitable feeding sites for scale are desirable for propagation.

More trees were identified as putatively resistant by NPS personnel in multi-year surveys, but a higher percentage of the trees identified during transect surveys were confirmed resistant. This may be a result of the length of time of infestation in the property studied. In the property where NPS staff identified resistant trees at the time the challenge test occurred, we assessed the disease to be in an earlier phase according to Shigo's (1972) three-phase hypothesis (Myers 2021). The trees NPS staff identified may have incidentally escaped infestation in this area but will develop scale infestations when they are inoculated over time. In addition, NPS personnel identified 45 additional trees that had low levels of scale. These trees were not considered putatively resistant but should be recorded and revisited to monitor for infestation in subsequent years; the absence of long-term breeding populations of mature scale can potentially confirm resistance or partial resistance.

Multi-year scale absence may be a stronger indicator of resistance prior to testing. While trees may escape scale infestation in a single year by chance, it is unlikely that trees will escape scale infestation over successive years as wind-dispersed scale will likely arrive at the trees. Resistant trees are difficult to access, and the testing process is time and labor intensive, so focusing on testing trees with multi-year absence of scale is the most economically efficient option.

The apparent percentage of resistant trees in the target properties is consistent with early findings (1% of trees) (Houston 1983). Our estimate of one resistant tree per about three hectares is lower than reported rates, but overstory American beech stems per hectare is lower in the target properties than in the reported rates, resulting in lower trees per hectare with the same rates. This is consistent with the established hypothesis of genetic heritability of resistance, with about 1% of trees genetically resistant to scale insects (Čalić et al. 2017, Carpenter 1974, Mason et al. 2013). Note also that many of the studies regarding resistance were conducted in the eastern range of beech, whereas the target properties considered here are at the western extent of American beech. Extreme range limits may feature trees occurring in habitats or population densities that differ from much of a species range. These differences may impact disease trajectories and associated beech population dynamics.

Refinement of Grafting Methods

Assigning graft failure was instrumental in the refinement of grafting methods and evaluating the efficacy of changes made in the grafting process. Many of the changes implemented were the result of personal communication with experienced grafters. Records and publication are crucial to preserving this knowledge formally.

In response to the failure of graft unions in 2019, a training workshop was organized at the U.S. Forest Service Oconto River Seed Orchard. Technicians at this facility have successfully established tree orchards of grafted American beech. The training workshop resulted in decreased graft failure resulting from careful supervision and quality control of individual grafts, and the ability to troubleshoot with experienced grafters.

In fall 2020, the sanitizing solution was increased from 70% ethanol to 80% ethanol solution, and a more aggressive scrubbing motion was used to sanitize scion and rootstock surfaces (Carey et al. 2013). Fiberglass plant stakes were purchased to support the tree as the graft union healed in the greenhouse to reduce slight twisting of the graft union site which causes mechanical failure.

In response to the drying that led to scion failure in 2022, we retrained technicians in scion collection and temporary storage methods. The success rates of our program increased over years in response to gaining experience specifically in grafting American beech.

Budgeting Considerations

Under ideal conditions (with appropriate diameter scions and rootstocks, experienced technicians, and a mix of genotypes that respond well to grafting), about 57% of grafted trees survive initial graft healing. With our methods, just under half of grafted trees experienced mortality after the first year post-grafting. More trees succumb to environmental stress in the time between graft union healing and reaching a suitable size for planting. In our study, about 40% of grafted seedlings died between 2020 and 2022. In outplanting, trees will also face additional stresses, likely leading to mortality in the field. Activities should be planned with this in mind: ideally grafting and planting should occur in sufficient numbers to absorb losses. In the case of beginning a new program, extra materials should be available to learn the art of grafting and other lab techniques. Under these circumstances, one could expect the purchase of 100 bare root seedlings to yield about 30 trees, possibly lower in a new location or with new technicians.

When budgeting for this work in the national parks, extra time to access remote areas is needed to account for the nature of the properties. Utilizing more than one technician for scion collection and outplanting can help expediate field work and potentially save on travel costs.

Grafting of trees is a costly and labor-intensive process (Table 5), but worth pursuing because of the resistance of donor trees leading to known resistant stock when successfully grafted. Grafted trees may take time to heal, but a program with vigorous stock derived from reproductively mature donor trees will be able to produce resistant seeds sooner than resistant seedlings produced from seeds, which would have either unknown resistance levels or additional costs coming from controlled seed orchards. However, when establishing a grafting program, extra time should be built into the front

end of the timeline to allow adequate sourcing of rootstock, identification and testing of resistant trees, and to allow grafters to become familiar with grafting this challenging species. Additionally, some grafted trees will fail after initial success, particularly because beech trees can be stressed in a greenhouse setting, which is not ideal for propagation. Extra care should be exercised when determining appropriate numbers of stock for grafting, taking the difficulty of propagating beech into account. The overall scale of production has a significant influence on cost and time.

Table 5. Cost and labor considerations at each stage of the process to graft BBD-resistant beech trees. All stages also include human time and effort as a cost consideration.

Stage	Cost Considerations
Scouting for resistant trees, field trials to confirm resistance level to scale insects	Travel, mapping and record keeping; resistance trials take 1+ year in forest setting.
Collecting scion material	Travel sometimes inaccessible by roads, timely planning; must be done in spring, packaged correctly, and transported to a cooler/refrigerator ^A .
Sourcing rootstock	Purchasing and growing seedlings or seed ahead of time or collecting wild seedlings by hand (travel). Potting and maintenance in greenhouse or outdoor setting. ^A
Grafting	Trained personnel, timing soon after collecting scions; one trained person may be able to do 30–50 trees per day, depending on size and space they are working in. Grafts transferred to hot-callus apparatus in a cooler ^A until healed (approx. 2 months).
Maintenance and growth	Grafted trees cared for in greenhouse or outdoor setting ^A for 2–3 years until strong enough for planting.
Outplanting	Travel, safe transportation of grafted trees to forest location identified and approved, planted and monitored 3+ years.

^A Indicates infrastructure needs.

The timeline for production of resistant trees can potentially be shortened by utilizing multi-year resistance data of trees before implementation (if such records exist) to filter down to trees that stand the highest chance of being proven resistant through field tests. Implementing quality checks and improving on maintenance processes will facilitate the quickest turnaround on restoration materials in the form of grafted trees ready for planting in as short a time as possible.

Challenges To Be Addressed

Due to challenges we encountered in maintaining containerized American beech, we initiated a trial in the spring of 2024 to assess best soil and fertilizer types. With temperatures in the greenhouse during summer likely above what is desirable for beech and considering the lack of greenhouse infrastructure at both PIRO and SLBE, this trial is taking place outdoors without protection from rain. Open grown containerized trees receive unpredictable amounts of precipitation, which can disrupt fertilizer regimes and impact soil drainage (Landis et al. 1989). This trial will examine the impacts of fertilizing solely using a slow-release dry fertilizer amended in the potting media compared to more commonly used liquid fertilizers. The trial will also assess the survival and health

impacts of using different ratios of peat, pine bark, and perlite in potting media specifically related to drainage.

Greenhouse care is difficult for American beech in a mixed-use setting, as beech trees should not be allowed to dry out or overheat. Shade cloth can be used to reduce scorching if the greenhouse becomes very hot in midsummer. Take care to water in a way that does not leave water on foliage in mid-day. Watering regimes will change over time as these trees undergo grafting and healing. Production standard watering and fertilizing may be appropriate in the beginning to encourage rapid growth of rootstock, but water and nutrients should both be reduced in the fall and winter before grafting occurs. Watering should be as light as possible while still providing adequate water until the graft union is fully healed. After the union is healed, watering and fertilizing regimes can be gradually increased to a level sufficient to encourage growth. Refinement of greenhouse methods is ongoing and their successful application will likely vary across different settings.

Planting Grafted American Beech

Planting events should occur after full senescence and before snow begins to accumulate on the ground, typically in late October to mid-November. Planting dates should be coordinated with the officer at the park where the planting will occur. Outplantings of nursery stock for restoration in the continental U.S. occur more frequently in late winter or early spring (Landis et al. 2010). However, in high altitude and latitude areas where late snow cover persists, hindering access and ease of planting, late summer and fall outplantings may be more appropriate.

Because outplanting success is directly related to stock vigor (Landis et al. 2010), we anticipate the changes identified to improve container stock health will improve outplanting success. Grafted trees are extremely valuable resistant clones. The goal is that resistant beech outplanted into these sites will thrive and produce seed that can naturally regenerate a resistant population. In addition, the outplantings are spaced in a natural arrangement and not directly next to each other as they are in a seed orchard. The clustered plantings throughout the parks will ensure more successful reproduction. The outplantings are also adjacent to nearby reproductively mature resistant beech trees of a different genotype. The planted trees themselves are a mix of local genotypes from within each park, ultimately planted back into the same park property they came from, which will increase natural cross pollination between resistant trees (e.g., Koch et al. 2007).

This method of outplanting is following a strategy of nucleated seed orchards for restoration in targeted forests (Corbin and Holl 2012, Myers et al. 2023). Over time, these plantings are meant to increase the proportion of resistant genotypes within the parks. Applied nucleation strikes a balance between passive restoration (letting nature run its course) and a more heavy-handed active restoration approach relying on large scale clearing and planting that would not align with National Park Service's mission statement focusing on preserving the resources of the National Park System unimpaired (NPS 2023). Applied nucleation of outplanted resistant American beech grafts ensures that reproductively mature trees of local provenance can reproduce and positively impact resistant genetics as quickly as the following growing season after outplanting. This approach requires upfront labor in the form of grafting and outplanting but requires low long-term costs after outplanting. The

refinement of the methods utilized for restoring beech at both PIRO and SLBE may not only benefit these target areas, but can provide a framework for land managers with similar constraints or objectives.

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