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DEVELOPMENT OF A PROPAGATION PROGRAM FOR BEECH BARK DISEASE-RESISTANT AMERICAN BEECH (FAGUS GRANDIFOLIA) AND AN APPLIED RESTORATION PLAN FOR MITIGATION OF BEECH BARK DISEASE

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DEVELOPMENT OF A PROPAGATION PROGRAM FOR BEECH BARK DISEASE-RESISTANT AMERICAN BEECH (FAGUS GRANDIFOLIA) AND AN APPLIED RESTORATION PLAN FOR MITIGATION OF BEECH BARK DISEASE

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Once again, dedicated to Mitch Grogg.

Through your stalwart support, you make everything herein possible.

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Preface

General Preface

All chapters, outside the introduction (1) and general conclusions (6) presented are intended for submission as manuscripts to peer-review outlets, as described below. The manuscript contained in chapter two has been reviewed and the presented version is revised per reviewer comments. The authorship on all manuscripts is intended to be the same, i.e., Andrea L. Myers, Yvette L. Dickinson, Andrew J. Storer, and Tara L. Bal. Primary manuscript preparation was conducted by Andrea Myers, the author of this dissertation. Review and editing was provided by Yvette L. Dickinson, Andrew J. Storer and Tara L. Bal; funding acquisition was due to Yvette L. Dickinson and Andrew J. Storer as co-PIs of a GLNF CESU grant, Task Agreement Number P16AC01398, Beech Reintroduction at Pictured Rocks National Lakeshore and Sleeping Bear Dunes National Lakeshore; project administration was conducted by Yvette L. Dickinson and Tara L. Bal; supervision of the project was conducted by Yvette L. Dickinson and Tara L. Bal. All field work and data analyses presented within were completed by Andrea Myers, with the exception of wandering surveys presented in chapter 3, as credited within the manuscript. These authors and contributions are anticipated to continue for all manuscripts presented within this dissertation. The above outlined author contributions were credited for the submission of the manuscript presented in chapter 2.

Recent Advances in Propagation of American Beech and their Application in Mitigation of Beech Bark Disease (Chapter 2) is the presentation in whole of a manuscript submitted for peer-reviewed publication. The manuscript has been submitted to the journal *Forest Ecology and Management* and is currently in review. This manuscript was prepared as a review of propagation techniques for American beech, and is targeted at applied scientists and forest health or restoration ecology professionals. The co-authors for the manuscript include members of the dissertation committee, Yvette L. Dickinson, Andrew J. Storer, and Tara L. Bal.

Beech Bark Disease in Northern Michigan and Site Selection for a Resistant Tree Restoration Plan (Chapter 3) is intended for submission to *Restoration Ecology*, the journal of the *Society for Ecological Restoration*, as a Case-Based Article (Technical Articles + Policy/Practical Articles) (<4000 words) that describes pioneer techniques likely to be of use to other practicing restoration ecologists. This type of article is best suited where the focus is on a smaller number of case studies or single but unique or large-scale case study; they may be technique-driven, methodology focused, or may be an in-depth examination of decision-making, monitoring, planning, implementation or policy relevance of the case study or studies. These have a briefer introduction and less focus on theoretical frameworks in favor of a focus on technical approaches and outcomes. Tables 4, 5, and Figure 3 are presented at the end of this chapter because they are intended for submission as supplemental materials for submission. Appendix C presents supplemental maps which were presented in a detailed report of findings to the National Park Service, but will not be included in any manuscript preparation.

Development and Refinement of a Grafting Program for the Propagation of BBD-Resistant American Beech (Chapter 4) is prepared for submission as a Natural Resource Report, which will be submitted through the National Park Service's internal, peer-reviewed publishing process. The appendices associated with Chapter 3 are illustrated manuals which are prepared with the intent of serving as guides for internal use. These illustrated manuals will be included with the general technical report, and for use as internal process guides. These general technical reports do not provide style guides, nor word limits. They are intended to serve as institutional memory of processes used on research projects beyond the timeline of the initial endeavor.

A Pilot Study in Transplanting Methods for Wildling American beech (*Fagus grandifolia*) (Chapter 5) is prepared as a Brief Communication manuscript (<2500 words). It will be submitted to *the Journal of Forestry*. This journal publishes preliminary results or novel applications of techniques with limited data sets. This journal is read by a variety of foresters and land managing personnel, and the manuscript is presented as results of a pilot study to inform future work on the restoration of American beech.

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I want to thank field technicians Elisabeth Stimmel, Kaydi Picard, Joslyn Knox, Matthew VanderMolen, Samuel Knapp, Liam Krause, Alexander Rice and Mitchell Grogg for assistance in the field.

List of abbreviations

BBD: Beech Bark Disease

NPS: The National Park Service

PIRO: Pictured Rocks National Lakeshore

SLBE: Sleeping Bear Dunes National Lakeshore

Abstract

This dissertation describes the work accomplished towards mitigation of beech bark disease (BBD) through a joint venture by Michigan Technological University and the National Park Service. American beech is an ecologically important species that is threatened throughout its range by beech bark disease and other newer, emergent pressures such as climate change and beech leaf disease. A literature review is included to synthesize recent advances in American beech (*Fagus grandifolia* Ehrh.) propagation and their application in mitigation of BBD (Chapter 2). These concepts are examined in an applied restoration framework to outline the importance of understanding ecological and technological context of the proposed project. It was determined that the target properties, Pictured Rocks and Sleeping Bear Dunes National Lakeshores, both in northern Michigan, are in differing phases of the progression of beech bark disease, making a restoration plan more complex. Planting sites and site preparation activities are proposed for the applied restoration project meeting ecological context and stakeholder's objectives (Chapter 3). Development and refinement of methods for successfully grafting BBD-resistant American beech are described as knowledge transfer (Chapter 4). Furthermore, plain language, illustrated manuals were created to maintain institutional knowledge of the process to collect resistant scions and graft them to create resistant trees (Appendix A, B). Finally, a pilot study exploring methods for transplanting wildling American beech is described (Chapter 5) that confirms survivability for potentially a more cost-effective way to obtain grafting materials. This will also inform future work examining the potential transplanting of naturally resistant beech root sprouts, which may significantly reduce the monetary cost and increase long-term survival of fully resistant trees. Overall, the work described here details a holistic approach to the mitigation of beech bark disease through creation and planting of resistant American beech using local provenance genetic sources and considering a public agency's objectives.

1 Introduction

1.2 Beech Bark Disease and Impacts on an Ecologically Important Species

American beech (*Fagus grandifolia* Ehrh.) is an ecologically important species which occurs through much of the eastern United States (Tubbs and Houston, 1990). American beech serves important roles while living as a driver of forest food webs through its hard mast production which is associated with impacts from rodents to black bears (Faison and Houston, 2004; Rosemier and Storer, 2010; Jensen et al., 2012; Seger et al., 2013; Conrad and Reitsma, 2015; Stephens et al., 2019). It also serves as a preferred foraging substrate for many bird species, and snags of American beech provide important nesting cavities (Maurer and Whitmore, 1981; Robb and Brookhout 1995; Lemaître and Villard, 2005; Kahler and Anderson, 2006; Holloway and Malcolm, 2007; Tozer et al., 2012). The hard wood of beech can be utilized as fuel wood, lumber or veneer logs, railroad ties, and pulpwood, among other uses (Carpenter, 1974). American beech serves an important ecological role, occurring as a component in 20 eastern forest types, and three of the dominant forest types in the Great Lakes region (Sugar Maple-Beech-Yellow Birch, Red Spruce-Sugar Maple-Beech, and Beech-Sugar Maple) (Eyre, 1980).

Beech bark disease (BBD) is an invasive disease complex which affects American beech. The disease complex is made up of two parts, an insect (beech scale, *Cryptococcus fagisuga* Lind.; Erricoccidae) and a fungus (genus *Neonectria*) (Ehrhlich, 1934). The beech scale is an invasive exotic insect which infests American beech. Beech scale is wingless, and only mobile in its first instar. The phloem-feeding damage caused by the minute insects is not separately enough to cause mortality alone. *Neonectria* fungi are endemic in northern hardwood forests, although the two major fungi associated with beech bark disease includes the potentially exotic *Neonectria faginata* (Castlebury), and endemic *N. ditissima* (Samuels and Rossman), which have extremely similar pathology

(Houston 1994; Mahoney et al., 1999; Castlebury et al., 2006). For a review of further taxonomic or phylogenetic information regarding the classification of *Neonectria* see Chaverri et al. (2011) and associated literature.

Beech bark disease causes extensive mortality in mature American beech by initiating a decline in overstory vigor through repeated annual infections, where *Neonectria* enter through feeding damage sites from the scale insect and infect the bark and inner bark. Scale insects produce one generation per year and are most commonly spread via wind, as all life stages but the first instar nymph are immobile (Wainhouse and Gate, 1988). BBD may be visually identified from a distance by the signs of the scale insect infestation, i.e., the presence of white waxy chaff on the bole of an American beech. *Neonectria* produce conidia (imperfect spores) annually and ascospores (perfect spores) less frequently from old lesions. Both spore types are spread by rain splash, and ascospores may spread via wind dispersal (Gómez-Cortecero et al., 2016). The occurrence of annual necrotic cankers is a major symptom of the disease. Trees may also produce a black bark exudate, referred to as "tarry spots," and sunken or raised lesions on the bole may occur (Ehrhlich, 1934; Koch 2010).

The first description of BBD in the United States was around 1920, after the disease arrived from Halifax, Nova Scotia, Canada and the disease spread to much of the northeastern US shortly thereafter (Ehrhlich, 1934). The first description of the disease in Michigan was published in 2001 (O'Brien et al.). Although American beech suffers extensive mortality, forested stands in the Northeast have seen an increase in beech abundance in the recent past (roughly 20 years) (Bose et al., 2017). This increase in abundance of American beech is a documented consequence of BBD.

The regeneration regime of American beech can change in response to BBD. Beech thickets may form in the area affected by BBD (Cale et al., 2013), but not always (Roy and Nolet, 2018). When there is an increase in regeneration, the dominance of beech can increase, and floristic diversity in these areas is reduced (Cale et al., 2013), and other desirable species can experience interference, especially after cutting (Bohn and

Nyland 2003; Nyland et al., 2006). These regeneration increases occur in spatial clusters around dead and diseased American beech (Geinecke et al., 2014). Beech poles may dominate the taller seedling strata when cutting is used to control or mitigate damages by BBD in stands with high levels of beech dominance pre-cutting (Elenitsky et al., 2020).

About 3% of beech trees in the Northeast may escape infestation by scale, and when subjected to field challenge tests, about 1 to 3% of American beech trees are resistant to scale insect infestation, and therefore considered resistant to BBD (Houston 1982; Houston 1983; Taylor et al., 2013). Putatively resistant, disease-free trees may be identified in the field by conspicuous lack of scale infestation (still rather grey bark). While the exact mechanism of resistance is not described, a major gene linked to resistance of BBD has been identified, and bark protein profiles of resistant beech differ between BBD resistant and susceptible beech (Mason et al., 2011; Ćalić et al., 2017). Location of resistant American beech trees have been recorded across the state of Michigan by a number of agencies for use in propagation of BBD-resistant American beech.

The progression of BBD has been described as occurring in three fronts: the advance front, the killing front, and the aftermath front. In the advance front, the pathogenic scale insect is first arriving in a new range. The number of apparent scale insects is high, while levels of *Neonectria* presence and American beech mortality continue at normal levels. In the killing front, the scale insects have established and *Neonectria* levels rise, so levels of both pathogenic organisms are high, and American beech mortality increases drastically. As BBD kills high levels of American beech, the disease enters the aftermath front. Here, numbers of the host organism have lowered due to mortality caused by the disease, resulting in a decreased amount of apparent pathogenic organisms (Shigo, 1972).

Significant mortality occurs by the time the disease enters the aftermath front, often accompanied by excessive root sprouting as trees are extremely stressed and more light is coming through the canopy. As time progresses and small American beech recruit

into higher size classes, the disease re-establishes into these new cohorts, and successive waves of the disease occur (Cale et al., 2012). After many successive waves occur, root sprouts are exhausted and all but the small portion of resistant trees are expected to die. The mortality caused by BBD is slow, due to the repeated annual infestations. Some trees may take as long as 30 years to die from complications of BBD, even while showing symptoms of the disease (Cale and McNulty, 2018).

Stress stimulates root suckering in American beech (Del Tredici, 2001). Logging, or other root damage to American beech in particular can increase the amount of regeneration present in the understory in BBD-affected forests (Houston, 2001). This often results in increased clonal reproduction by trees which are susceptible to BBD. The predisposition hypothesis refers to the state of a BBD-affected forest with increased susceptible beech regeneration. The predisposition hypothesis supposes that this increased regeneration creates a positive feedback loop, where infestation by scale, and increase in human activity to remove diseased beech, creates a forest with decreased amounts of large-diameter beech, but increased frequency of BBD-susceptible beech (Cale et al., 2012). As this small susceptible beech recruits into gaps left by dying clonal parent beech, scale and *Neonectria* are able to reproduce on the new hosts, repeating the cycle of infestation, stress, regeneration, mortality and recruitment indefinitely (Geinecke et al., 2014).

Restoration is necessary to retain beech as an ecologically important species. Although dominance may change, the loss of mature beech negatively impacts forest communities through the loss of the physical characteristics, i.e., its importance as foraging substrate and nesting cavities. The impacts of changes in regeneration, i.e., the loss of biodiversity associated with beech thickets, also pose challenges to natural areas which are affected by BBD. Restoration of resistant trees may fill the niche left by the mortality of mature American beech in response to BBD.

1.3 Propagation Considerations of Resistant American Beech for Use in Restoration

Resistant American beech can be propagated by a number of means. Retention of naturally resistant trees in the landscape allows natural regeneration to occur (Wisconsin DNR, 2014). Somatic embryogenesis can be used to create plantlets from root or shoot tissue, although with low transfer rates to soil (Barker et al., 1997; Cuenca and Vieitez, 1999; Cuenca et al., 2000; Pijuit et al., 2010). Grafting can be used to create entire resistant trees when a resistant scion is joined to a non-resistant rootstock. Grafting rates are highest with application of a hot-callus chamber to heal the trees (Carey et al., 2013). Because of the relatively high success rates, much restoration is focused on the creation of resistant trees through grafting (Ramirez et al., 2007; Carey et al., 2013; Koch and Heyd, 2013).

Grafted trees can be utilized in a number of ways. Resistant seed orchards consisting of grafted, BBD-resistant trees have been established in Michigan by the United States Forest Service, though they are not yet producing large amounts of available seed (Koch and Heyd, 2013). In this project, grafted trees from confirmed resistant scions are proposed to be used to create nucleated seed orchards within the National Park Lakeshores. The decision-making for selection of grafting is explained in Chapters 2 and 3 of this dissertation.

Nucleated seed orchards differ from traditional orchards. In traditional seed orchards, desired species are planted in a remote location and bred to create seed of known parentage, and seed is then taken from the orchard and sown in the property targeted for restoration. In nucleation, the desired species is planted in the degraded landscape, so that seed is released into a predefined niche (Corbin and Holl, 2012). Because there are natural vectors (wildlife in the case of beech) and potentially pollen sources, diverse seed is released and spread via vectors preexisting in the area (Wright, 1952).

The creation of nucleated seed orchards still requires some site preparation to create suitable planting sites. Above and belowground resources must be available to provide growing space for the planted trees. Competing vegetation should be removed (Löf et al., 2012; Traux et al., 2018) and appropriate light or canopy openings should be available for above-ground resources. American beech dominates in moderate to low light, as it is matched in shade tolerance in the western edge of its range only by sugar maple and Eastern hemlock (Burger and Kotar, 2003). Although beech can survive in the understory in light levels as low as 1% relative light intensity (RLI), and grow best with full light availability (RLI=100%), they can grow equally well from RLI 30% to 50% (Genmel et al., 1996; Modrý et al., 2004; Kunsler et al., 2005; In: Wagner et al., 2010).

1.4 Overall Objectives

This project was planned as a cooperative agreement between Michigan Technological University (MTU) and the National Park Service (NPS). The objectives and research were jointly developed with NPS and all data and results are co-owned and shared between both entities.

In order to identify gaps in the knowledge necessary for successful restoration, a literature review relating to the establishment of resistant American beech plantings, and identifying fundamental knowledge gaps relating to the creation of resistant American beech seed orchards was prepared, presented in chapter 2. To make successful plans for restoration, the state of Beech Bark Disease in the target properties was described, and specific restoration activities were identified as appropriate for the stakeholder agency (National Park Service), as presented in chapter 3. Also in chapter 3, specific locations for seed orchards in the target properties are suggested. Methods for creation of resistant American beech trees through grafting to be planted in Sleeping Bear Dunes National Lakeshore and Pictured Rocks National Lakeshore are presented in chapter 4. In order to support the continued success of the project, MTU identified the additional objective of developing methods to transplant wilding American beech successfully to a greenhouse,

which are presented in chapter 5. In the overall conclusion, chapter 6, future work is identified which will be necessary for continuance of the restoration activities.

1.5 Literature Cited

Barker, M.J., Pijut, P.M., Ostry, M.E., Houston, D.R. 1997. Micropropagation of juvenile and mature American beech. *Plant Cell, Tissue and Organ Culture*. 51, 209-213.

Burger, T.L., Kotar, J. 2003. A guide to forest communities and habitat types of Michigan. University of Wisconsin--Madison, Department of Forestry Ecology and Management.

Castlebury, L., Rossman, A., Hyten, A. 2006. Phylogenetic relationships of *Neonectria/Cylindrocarpon* on *Fagus* in North America. *Canadian Journal of Botany*. 84(9), 1417–1433.

Cale, J.A., Letkowski, S.K., Teale, S.A., Castello, J.D. 2012. Beech bark disease: an evaluation of the predisposition hypothesis in an aftermath forest: Evaluating the beech bark disease predisposition hypothesis. *Forest Pathology (J. de pathologie forestière)*. 42, 52-56.

Cale, J.A., McNulty, S.A. 2018. Not dead yet: Beech trees can survive nearly three decades in the aftermath phase of a deadly forest disease complex. *Forest Ecology and Management*. 409, 372-377.

Ćalić, I., Koch, J., Carey, D., Addo-Quaye, C., Carlson, J.E., Neale, D.B. 2017. Genome-wide association study identifies a major gene for beech bark disease resistance in American beech (*Fagus grandifolia* Ehrh.). *BMC Genomics*. 18, 547-514.

Carey, D.W., Mason, M.E., Bloese, P., Koch, J.L. 2013. Hot callusing for propagation of American beech by grafting. *HortScience*. 48, 620-624.

Carpenter, R.D. 1974. American beech (*Fagus grandifolia*). FS-220

Chaverri, P., Salgado, C., Hirooka, Y., Rossman, A.Y., Samuels, G.J. 2011. Delimitation of *Neonectria* and *Cylindrocarpon* (Nectriaceae, Hypocreales, Ascomycota) and related genera with *Cylindrocarpon*-like anamorphs. *Studies in Mycology*. 68, 57-78.

Conrad C.A., Reitsma, L. 2015. Demographic responses if myomorph rodents to mast production in a beech- and birch-dominated northern hardwood forest. *Northeastern Naturalist*. 22, 746-761.

Corbin, J.D., Holl, K.D. 2012. Applied nucleation as a forest restoration strategy. *Forest Ecology and Management*. 265, 37-46.

Cuenca, B., Vieitez, A.M. 1999. Histological Study of In vitro Development of Adventitious Buds on Leaf Explants of Oriental Beech (*Fagus orientalis* Lipsky). *In Vitro Cellular & Developmental Biology*. Plant, 35, 326-332.

Cuenca, B., Ballester, A., Vieitez, A.M. 2000. In vitro adventitious bud regeneration from internode segments of beech. *Plant Cell, Tissue and Organ Culture*. 60, 213-220.

Del Tredici, P. 2001. Sprouting in temperate trees: a morphological and ecological review. *The Botanical Review*. 67(2), 121-140.

Ehrhlich, J. 1934. The beech bark disease: a *Nectria* disease of *Fagus*, following *Cryptococcus fagi* (Baer.). *Canadian Journal of Research*. 10, 593-692.

Elenitsky, L., Walters, M., Farinosi, E. 2020. Tree Regeneration Structure Following Beech Bark Disease-Motivated Harvests: Factors Associated with Patterns and Management Implications. *Forests*. 11(2), 180–206.

Eyre, F.H. 1980. Forest Cover Types. Washington, DC: Society of American Foresters.

Faison E.K., Houston D.R. 2004. Black bear foraging in response to beech bark disease in northern Vermont. *Northeastern Naturalist*. 11, 387-394.

Gemmel, P., Nilsson, U., Welander, T. 1996. Development of oak and beech seedlings planted under varying shelterwood densities and with different site preparation methods in southern Sweden. *New Forests*. 12(2), 141–161.

Giencke, L.M., Dovčiak, M., Mountrakis, G., Cale, J.A., Mitchell, M.J. 2014. Beech bark disease: spatial patterns of thicket formation and disease spread in an aftermath forest in the northeastern United States. *Canadian Journal of Forest Research*. 44(9), 1042-1050.

Gómez-Cortecero, A., Saville, R., Scheper, R., Bowen, J., Agripino De Medeiros, H., Kingsnorth, J., Xu, X., Harrison, R. 2016. Variation in Host and Pathogen in the *Neonectria/Malus* Interaction; toward an Understanding of the Genetic Basis of Resistance to European Canker. *Frontiers in Plant Science*. 7, 1365–1365.

Holloway G.L., Malcom J.M. 2007. Nest-tree use by northern and southern flying squirrels in central Ontario. *Journal of Mammalogy*. 88, 226-233.

Houston, D.R. 1982. A technique to artificially infest beech bark with beech scale, *Cryptococcus fagisuga* (Lindinger). Res. Pap. NE-507. Broomall, PA: U.S. Department of Agriculture, Forest Service, Northeastern Forest Experiment Station. 8p.

Houston, D.R. 1983. American beech resistance to *Cryptococcus fagisuga*. In: Proceedings, I.U.F.R.O. Beech Bark Disease Working Party Conference; 1982 September 26-October 8; Hamden, CT. Sponsored by the USDA Forest Service, Northeastern Forest Experiment Station. Gen. Tech. Rep. WO-37. [Washington, DC]: U.S. Department of Agriculture, Forest Service: 38-42.

Houston, D.R. 1994. Temporal and spatial shift within the *Nectria* pathogen complex associated with beech bark disease of *Fagus grandifolia*. *Canadian Journal of Forest Research*. 24(5), 960-968.

Houston, D.R. 2001. Effect of harvesting regime on beech root sprouts and seedlings in a north-central Maine forest long affected by beech bark disease. Res. Pap. NE-717. Newtown Square, PA: U.S. Department of Agriculture, Forest Service, Northeastern Research Station. 20 p.

Jensen, P.G., Demers, C.L., Mcnulty, S.A., Jakubas, W.J., Humphries, M.M. 2012 Marten and fisher responses to fluctuations in prey populations and mast crops in the northern hardwood forest. *The Journal of Wildlife Management.* 76, 489-502.

Kahler, H.A., Anderson, J.T. 2006. Tree cavity resources for dependent cavity-using wildlife in West Virginia forests. *Northern Journal of Applied Forestry*. 23, 114-121.

Koch, J.L., Heyd, R.L. 2013. Battling beech bark disease: establishment of beech seed orchards in Michigan. *Newsletter of the Michigan Entomological Society*. 58 (1 & 2), 11-14.

Koch, J. 2010. Beech Bark Disease: The Oldest “New” Threat to American Beech in the United States. *Outlooks on Pest Management*. 21(2), 64–68.

Kunstler, G., Curt, T., Bouchaud, M., Lepart, J. 2005. Growth, mortality, and morphological response of European beech and downy oak along a light gradient in sub-Mediterranean forest. *Canadian Journal of Forest Research*. 35(7), 1657–1668.

Lemaître, J., Villard, M.A. 2005. Foraging patterns of pileated woodpeckers in a managed Acadian forest: a resource selection function. *Canadian Journal of Forest Research.* 3510: 2387-2393.

Löf, M., Dey, D.C., Navarro, R.M., Jacobs, D.F. 2012. Mechanical site preparation for forest restoration. *New Forests*. 43, 825-848.

Mason, M.E., Koch, J., Krasowski, M., Loo, J. 2013. Comparisons of protein profiles of beech bark disease resistant and susceptible American beech (*Fagus grandifolia*). *Proteome Science*. 11, 2.

Mahoney, E., Milgroom, M., Sinclair, W., Houston, D. 1999. Origin, Genetic Diversity, and Population Structure of *Nectria coccinea* var. faginata in North America. *Mycologia*. 91(4), 583.

Maurer, B.A., Whitmore, R.C. 1981. Foraging of five bird species in two forests with different vegetation structure. *The Wilson Bulletin*. 93: 478-490.

Modrý, M., Hubený, D., Rejšek, K. 2004. Differential response of naturally regenerated European shade tolerant tree species to soil type and light availability. *Forest Ecology and Management*. 188(1), 185–195.

Nyland, R.D., Bashant, A.L., Bohn, K.K., & Verostek, J.M. 2006. Interference to hardwood regeneration in northeastern North America: Controlling effects of American beech, striped maple, and hobblebush. *Northern Journal of Applied Forestry*. 23(2), 122-132.

Pijut, P.M., Woeste, K.E., Michler, C.H., Janick, J. 2010. Promotion of adventitious root formation of difficult-to-root hardwood tree species. Pages 213-251 In: *Horticultural Review*. Hoboken, NJ, USA.

Ramirez, M., Krasowski, M., Loo, J. 2007. Vegetative propagation of American beech resistant to beech bark disease. *HortScience*. 42, 320-324.

Robb, J.R., Brookhout, T.A. 1995. Factors influencing wood duck use of natural cavities. *The Journal of Wildlife Management*. 59: 372-383.

Rosemier JN, Storer AJ (2011) Assessing native small mammals' responses to an incipient invasion of beech bark disease through changes in seed production of American beech. In: Fei, Songlin; Lhotka, John M.; Stringer, Jeffrey W.;

Gottschalk, Kurt W.; Miller, Gary W., eds. Proceedings, 17th central hardwood forest conference; 2010 April 5-7; Lexington, KY; Gen. Tech. Rep. NRS-P-78. Newtown Square, PA: US Department of Agriculture, Forest Service, Northern Research Station: 675-676. Vol. 78. 2011.

Seger, R.L., Servello, F.A., Cross, R.A., Keisler, D.H. 2013. Body mass and mast abundance influence foraging ecology of the American black bear (*Ursus americanus*) in Maine. *Canadian Journal of Zoology*. 91: 512-522.

Shigo, A.L. 1972. The beech bark disease today in the northeastern US. *Journal of Forestry*. 70, 286-289.

Stephens, R.B., Hobbie, E.A., Lee, T.D., Rowe R.J. 2019. Pulsed resource availability changes dietary niche breadth and partitioning between generalist rodent consumers. *Ecology and Evolution*. 9, 10681-10693.

Taylor, A.R., McPhee, D.A., Loo, J.A. 2013. Incidence of beech bark disease resistance in the eastern Acadian forest of North America. *The Forestry Chronicle*. 89(5), 690-695.

Tozer, D.C., Burke, D.M., Nol, E., Elliott, K.A. 2012. Managing ecological traps: logging and sapsucker nest predation by bears. *The Journal of Wildlife Management*. 76, 887-898.

Traux, B., Gagnon, D., Fortier, J., Lambert, F., Pétrin, M.A. 2018. Ecological factors affecting white pine, red oak, bitternut hickory and black walnut underplanting success in a northern temperate post-agricultural forest. *Forests*. 9, 499.

Wagner, S., Collet, C., Madsen, P., Nakashizuka, T., Nyland, R., Sagheb-Talebi, K. 2010. Beech regeneration research: From ecological to silvicultural aspects. *Forest Ecology and Management*. 259(11), 2172–2182.

Wainhouse, D., Gate, I.M. 1988. The Beech Scale. Pages 67-85 In: Dynamics of Forest Insect Populations. Springer, Boston, MA.

Wright, S. 1952. The genetics of quantitative variability. Quantitative Inheritance. Her Majesty’s Stationery Office, London.

Wisconsin Department of Natural Resources (DNR). 2014. Management of beech bark disease in Wisconsin. Website. https://dnr.wisconsin.gov/sites/default/files/topic/ForestHealth/beechBarkManagement.pdf Accessed 12/7/2020.

2 Recent Advances in Propagation of American Beech and their Application in Mitigation of Beech Bark Disease

2.1 Abstract

American beech (*Fagus grandifolia* Ehrh.) has been impacted by the beech bark disease (BBD) complex throughout the northeastern United States for over 100 years, but the disease has been present in the Great Lakes region only since 2000. This disease threatens to remove a foundational tree species from Great Lakes forests as American beech is especially important ecologically, for wildlife habitat, mast, and a climax successional species. We review recent advances in propagation techniques of American beech, with the goal of addressing their use in the mitigation of BBD. Natural regeneration and artificial methods of propagation are addressed, along with how they may be applied for mitigation. An existing restoration framework is used to define likely methods for restoration. Nucleated seed orchards of grafted resistant trees may currently be the most effective and practical method for introduction of BBD-resistant American beech into affected northern hardwood forests.

2.2 Key words

Grafting; Restoration; Tree Breeding; Invasive species; *Neonectria*; *Cryptococcus fagisuga*

2.3 Introduction

In response to global changes over the last century, forest diseases have dramatically altered the composition of forests in the United States. Examples include the almost complete losses of American chestnut (*Castanea dentata* Michx.) due to the exotic disease chestnut blight (*Cryphonectria parasitica* Murr. Barr) and of mature elms

(*Ulmaceae*) due to Dutch elm disease. Newly emergent diseases present challenges to modern forests, and land managers rely on a constantly evolving suite of forest health management tools to combat these diseases and prevent the loss of future species (Flachowsky et al., 2009; Sniezko et al., 2017). American beech (*Fagus grandifolia* Ehrh.) has been impacted by beech bark disease (BBD), an exotic disease complex, in the northeastern part of its range for over a century. Recent advances in propagation methods have allowed for a new suite of techniques to combat BBD using resistant trees. Renewed attention is being placed on BBD mitigation, as it expands its range into the northern Great Lakes area (McCullough et al., 2001).

Retaining genetically diverse, healthy American beech in the landscape is important because BBD is not the only forest health issue exerting pressure on the species. Beech leaf disease (BLD), an emergent tree disease present in Ontario, Canada, Ohio, Pennsylvania, and to the east in Connecticut, and Long Island, NY, (with an expanding range as new populations continue to be discovered), is placing additional pressure on the species (Ewing et al., 2018; Reed et al., 2020, Burke et al., 2020; Marra and LaMondia, 2020). Beech leaf mining weevil is an introduced defoliating pest affecting beech in the Nova Scotia, Canada area for at least 15 years (Sweeney et al., 2020). Beech leaf mining weevil, BLD and BBD are all known to co-occur in the Nova Scotia province of Canada. Climate change is expected to reduce the southern extent of the range for this species (Iverson et al., 2008). Other unknown challenges are sure to emerge for American beech and all other species in the future, and retaining resilient, healthy populations is going to be critical for conservation of ecosystem health.

There is a need to carefully evaluate management decisions made for current control of BBD in areas where it is newly arrived because much of the existing literature on beech bark disease in the United States was generated in the northeast. BBD has been present in the northeastern United States for over a century, but was only described in Michigan in 2001 (Ehrhlich, 1934; Heyd, 2005; O’Brien et al., 2001). Because of the longer history in the northeast, much of the fundamental literature about BBD has

originated east of the Appalachian Mountains (Houston, 1994a; Houston, 2005; Cale et al., 2017).

The objective of this review is to summarize current efforts to propagate American beech, and synthesize the relative benefits and drawbacks of different methods in their application in mitigation of BBD. Recent reviews have been completed detailing the ecology and impacts of BBD (Cale et al., 2017; Stephanson and Coe, 2017) and information about American beech and its habitat requirements is available (Tubbs and Houston, 1990; Bonner and Leak, 2008). Therefore, we will not seek to repeat these efforts, rather focusing on information relevant to propagating American beech and with the goal of ultimately enhancing the proportion of BBD-resistant trees in landscapes. This provides a foundation which can be used to develop management recommendations to sustain beech as a component of forests. Here, we review BBD management, and discuss 1) the impacts of BBD on regeneration, 2) methods of management utilizing natural beech regeneration and identification of naturally BBD-resistant trees in the landscape, 3) techniques currently being utilized to artificially propagate BBD-resistant beech, and 4) how these techniques can be applied towards beech restoration.

The field of forest health is inherently interdisciplinary. Interdisciplinary science requires professionals from many natural resource fields to work closely together. In order to effectively synthesize knowledge into a restoration plan, terminology from multiple disciplines will be used throughout the course of this review. We have supplied a list of terminology in this review for clarity, and marked the first appearance of terminology(*) in the text of the review (Table 1).

2.3.1 American Beech Ecology and Natural Reproduction

American beech is an ecologically important species in northern hardwood forests and throughout its range in the eastern United States. It serves as a major source of forage for wildlife, as the nuts are consumed by a diverse suite of wildlife. American beech is also ecologically important as a late-successional, shade-tolerant “climax” species in the

northern Great Lakes region (Burger and Kotar, 2003). In this area, sugar maple (*Acer saccharum* Marsh.) and Eastern hemlock (*Tsuga canadensis* L.) fill similar successional roles, but neither creates hard mast that can compete for quantity and quality (Rosemier and Storer, 2011). The challenges posed to American beech by beech bark disease stands to radically alter ecologies of forests in regions newly invaded by the disease.

American beech is widely distributed throughout the eastern United States and is present in all states along the Atlantic Coast, west to parts of Michigan, Wisconsin, Illinois, Missouri, Arkansas, Louisiana and eastern Texas (Tubbs and Houston, 1990). American beech is present as an associate or major component in five of the six northern hardwoods forest types of the eastern region of the United States. It is a major component in three of the dominant forest types in the Great Lakes region, the western extent of the range of American beech (West of about 82°W, North of about 40°N, to the far Western and Northern edges of the range) (i.e., Sugar Maple-Beech- Yellow Birch, Red Spruce-Sugar Maple- Beech, and Beech-Sugar Maple) and a component in 20 eastern forest types (Eyre, 1980). Beech occurs on podzolic and laterite soils, and rarely occurs on limestone-rich soils except in the western edge of its range (Tubbs and Houston, 1990). The tree is exceptionally slow-growing and very long-lived, upwards of 300-400 years (Tubbs and Houston, 1990). Beech is easily identified by its unique thin, gray bark, which makes the tree especially susceptible to damage by piercing- sucking insects, fire, and diseases entering through the bark (Tubbs and Houston, 1990).

Beechnuts may be collected, stored and planted. The nut matures and drops from the tree after the first frost at the end of the growing season (Rushmore, 1961). Nuts may be collected in seed traps, raked from the ground, or picked from the crown before falling. The green nuts should be allowed to mature if collected from the crown, which will be clear as the green spiked shell will turn from green to brown, indicating that they are mature and ready to harvest (Bonner and Leak, 2008). After collection, the nuts can be dried at temperatures below 20°C (Poulsen, 1993) and then placed in cold storage. Cold stratification is required for germination. To determine the time required, fresh seeds can be placed in moist medium and stored at 3°C until 10% of the seeds have

germinated. The remaining chilled seeds can then be mixed in damp media (Oconto Seed Orchard Personnel, pers. comm. 2019) with a moisture content of about 29% and stored at 4°C for that amount of time plus two weeks (Bonner and Leak, 2008). The seeds can then be brought out of dormancy as described in the woody plant seed manual (Bonner and Leak, 2008).

In addition to prodigious seed production, American beech readily reproduces vegetatively via root suckering (Held 1983, Tubbs and Houston, 1990). American beech produces suckers in response to senescence or injury, which triggers the formation of reparative root buds, from which suckers arise (Del Tredici, 2001). Dense thickets of American beech regeneration can establish around injured or dying American beech trees (Gienecke et al., 2014). It is even possible for sucker-originated young trees to outcompete seed-originated young trees to comprise the majority of regeneration in patches (Nyland, 2008). For a more detailed description of these mechanics, a recent review of regeneration characteristics of the genus *Fagus* is available (Wagner et al., 2010).

When considering the value of American beech in eastern forests, its use in wood products is sometimes overlooked or discounted. In the northeast, American beech regeneration was historically undesirable, and considered a nuisance preventing the establishment of other species (Nyland et al., 2006). However, beech is being explored as an option for flooring timber in Canada (Bernard et al., 2018) and trees felled in salvage cuttings in response to Beech Bark Disease are being utilized for pulp, railroad ties or fuel wood where possible. BBD can cause sunken lesions and these timber defects reduce economic value of timber forests affected by BBD (Burns and Houston, 1987). Beech is good quality firewood, ranking below hickory and white oak in heating value (Carpenter, 1974).

Economic value aside, American beech also holds great importance throughout its range as a foundation* species. Many birds prefer large American beech stems as a foraging substrate, including pileated woodpecker (*Dryocopus pileatus* L.), Acadian

flycatcher (*Empidonax virescens* (Vieillot)), and scarlet tanagers (*Piranga olivacea* (Gmelin)) (Maurer and Whitmore, 1981; Lemaître and Villard, 2005). American beech trees and snags offer cavities in abundance across a range of forest types, creating appropriate nesting sites for diverse species such as yellow-bellied sapsuckers (*Sphyrapicus varius* L.), southern flying squirrels (*Glaucomys volans* L.), and wood ducks (*Aix sponsa* L.) (Robb and Brookhout, 1995; Kahler and Anderson, 2006; Holloway and Malcolm, 2007; Tozer et al., 2012). American beech mast is a critical component in many forest food webs. It serves as forage for mammals at many trophic levels, from myomorphic rodents to black bears (*Ursus americanus* Pallas), and increased beechnut crops are associated with increased predatory animals (Faison and Houston, 2004; Jensen et al., 2012; Seger et al., 2013; Conrad and Reitsma, 2015; Stephens et al., 2019). These specific roles cannot be filled by other species with similar life histories, so American beech is a foundational forest species.

2.3.2 Beech Bark Disease Ecology

The pathology of beech bark disease (BBD) is well understood and is described in two parts: the insect portion of the disease complex, and the fungal portion of the disease. In BBD, a scale insect infests American beech trees and creates feeding damage sites on the bark, which are subsequently infected by a fungus from the genus *Neonectria*, causing a slow decline and eventual death as the tree is slowly girdled (Ehrhlich, 1934). The insect component of the disease is usually the non-native *Cryptococcus fagisuga* Lind. (Hemiptera: Eriococcidae), the felted beech scale or beech scale, a wingless, parthenogenetic insect with piercing-sucking mouthparts which it uses to feed on the phloem of trees (Wainhouse, 1980). A second native insect with a similar life history, *Xylococcus betulae* Perg., may also create the infection court* necessary for the disease, through a similar feeding habit (Shigo, 1962; Ćalić et al., 2017). Parthenogenetic insects do not require additional genetic material to create offspring, so a single healthy adult scale insect arriving on a tree may be enough to fully infest that tree (Wainhouse, 1980). The two commonly identified fungal species associated with the disease are *Neonectria*

faginata Castlebury and *Neonectria ditissima* Samuels and Rossman (Kasson and Livingston, 2009). Both fungi apparently cause mortality through the same mechanism: slow annual accumulation of necrotic cankers on the above-ground portion of the tree.

Based on easily measurable differences, stands affected by BBD can be sorted into three phases of disease progression: the advance front phase, killing phase, and aftermath phase (Shigo, 1972). In the advance front, beech scale has not yet established in large numbers and *Neonectria* fungi are not present on beech. In the killing front, large numbers of scale have established on beech, and aboveground mortality occurs. In the aftermath phase, numbers of scale and *Neonectria* are reduced, as is aboveground mortality of trees. In addition, this phase is typically marked by increased regeneration of beech in the understory (Cale et al., 2017).

While fundamental research from the early invasion of BBD in the US is still referred to, some facets of the disease have become better understood over time. One example of this change in understanding is the shift from high levels of *N. ditissima* to high levels of *N. faginata* as the pathogenic organism (Houston, 1994b; Kasson and Livingston, 2009). While the mortality associated with BBD was first described as very rapid, we now know that BBD may cause a slow decline of overstory trees via girdling, and may take as long as 30 years to kill individual trees (Houston, 1994a; Cale et al., 2015). In the northeastern range of the disease, a strong suckering response is frequently described in literature (Garnas et al., 2011; Cale et al., 2012a; Gienecke et al., 2014). Recent literature from the expanded range of the disease has described differing regeneration responses, without the suckering response (Roy and Nolet, 2018). It is necessary to continue exploring BBD, both to fill gaps in fundamental knowledge, and to describe novel dynamics, such as those seen in regions newly affected by the disease.

Some American beech are resistant to the disease, and this small portion of trees are the focus for most restoration efforts. While as much as 13%, but an average of 3% of trees may naturally escape scale, when subjected to field challenge tests, about 1 to 3% of trees in the landscape are truly resistant to the insect portion of the disease (Houston,

1982; Taylor et al., 2013). Research is ongoing to determine the cause of the resistance, but the increased expression of a major gene, and the increased profile of certain bark proteins have been separately identified to be associated with increased levels of resistance (Mason et al., 2013; Ćalić et al., 2017). Even when challenged in the field with protected scale egg inoculations, these trees display resistance to establishment of scale (Houston 1982; Koch and Carey, 2014). This supports the notion that the trees are truly resistant to the disease.

Beech bark disease causes a major alteration of disturbance regimes*, including indirect effects which can alter regimes beyond the mortality caused directly by the disease. Canopy disturbances are directly created by declining crowns. As beech mortality increases, the amount of coarse woody debris increases (McGee, 2000). Beech snap occurs in higher frequency as windthrow resistance is reduced (Papaik et al., 2005). Light levels in lower strata are reduced by the thicket response (Cale et al., 2012b). The species composition shift triggered in affected forests can change soil chemistry through altered litter and woody debris input (Arthur et al., 2017). Because pure beech forests do not occur widely throughout the majority of the range of American beech, the disturbance caused by BBD does not create stand-replacing disturbances. While beech occurs as a dominant species in the northeastern part of its range, comprising 20% to 51% of total forest basal area in the northeastern United States near New York and Quebec, Canada, it accounts for about 17% of total forest basal area in the Great Lakes region of the United States. The species occurs less frequently in the Appalachian and midwestern regions, near the southern extent of its range (around 1% of total basal area) (Cogbill, 2005). In some stands, beech is the dominant overstory species, however, the indirect and direct effects combined classify BBD as a major to moderate disturbance severity*.

2.3.3 Direct Control of BBD

Few direct controls are recommended for beech bark disease, and the methods that are described are primarily only for retention of individuals, such as landscape trees. This is partially due to the endemic nature of the fungal component. In addition, shortly

after it has arrived in an area, scale infestations are ubiquitous because the mobile and wind-dispersed first instars so readily disperse (Wainhouse and Gate, 1980; Wainhouse, 1988; Garnas et al., 2013). This combination of factors makes individual-tree control costly and time- and labor-intensive.

Biological control* has been explored for BBD, with a number of predators identified as feeding on beech scale, though none have been described as effective in the broader control of BBD (Wiggins et al., 2001, Mayer and Allen, 1983, Houston, 2005). The twice-stabbed lady beetle, (*Chilochorus stigma* (Say)) dispersed ineffectively and did not feed on all stages of the scale insect (Mayor and Allen, 1983). A velvet mite, (*Allothrombium mitchelli* Davis) has also been noted, but little is known about the species (Wiggins et al., 2001).

Chemical controls* have been used for control of scale. While scale can be treated with a number of insecticides, treatments must be continuous. Scale insects are minute enough to escape into bark crevices, making bark sprays ineffective. Chemical controls are only recommended for individual ornamental trees where repeated applications are possible (Houston and O'Brien, 1983; McCullough et al., 2001). *N. ditissima* (under syn. *N. galligena*) has been controlled in apple species (*Rosaceae*) with copper oxychloride and copper oxide, and with apple-specific fungal control compounds, but no reports of the fungicide efficacy on *Fagus* species are available (Weber, 2014; Walter et al., 2015). Prophylactic control of *Neonectria* is also difficult due to the endemic, rain- and wind-dispersed life history of the fungus.

Physical controls* do work well to eliminate scale. Horticultural oils can be applied during the dormant season, however, the mobile nymphs (the ideal target of the horticultural oils) emerge in August or September, reducing efficacy unless oils are applied with precise timing to control first-instar nymphs (Houston, 1982). Scales may be physically washed or brushed from the boles of trees (McCullough et al., 2001), however, a single adult scale remaining is enough to re-infest the tree. A combination of physical and biological control, consisting of field paint containing a strain of *Bacillus subtilis*

painted over active cankers reduced spore release from apple trees, but the entire canker must be painted shortly before spore release (Walter et al., 2017). In forests where *Neonectria* is endemic on other species, such as in Beech-Maple forests, this control method is impractical.

Few cultural controls* exist, and many programs are focused on removal of beech (Ostrofsky and McCormack, 1986). Cutting followed by herbicide application removes disease-susceptible regeneration (Bohn and Nyland, 2003; Geinecke et al., 2014). This method can be coupled with retention of disease-resistant overstory trees to affect cultural control by removing advanced regeneration which is disease-susceptible, and allowing new potentially resistant regeneration (Favjan et al., 2019). There is evidence that removal of diseased beech and the retention of disease-free trees can improve the quality of beech over long periods of time (Leak, 2006).

2.4 Natural Vegetative Propagation of BBD-Resistance

Naturally resistant American beech trees can be retained in the landscape as potential seed trees*, allowing propagules from these resistant trees to reinvade areas where healthy beech is being removed by BBD. In Wisconsin, the Department of Natural Resources has made information available to the public describing how to identify resistant beech and encouraging their retention (Wisconsin DNR, 2014). Land owners are advised to watch for trees which are not infested by scale, and if such trees are found, to retain them and if possible, implement a sanitation cut around them. The United States Department of Agriculture, Forest Service recommends similar treatment in Michigan (Heyd, 2005).

Silvicultural practices can be combined with the retention of BBD-resistant trees to enhance the rehabilitation of BBD-affected stands through natural regeneration. The removal of diseased trees may increase the quality of beech stands over long periods of time (Leak, 2006). Sprouts may survive regardless of if the parent tree is cut or retained, so silvicultural management in the form of removing non-resistant regeneration is

necessary to manage BBD through removal of diseased trees (Farrar and Ostrofsky, 2006). Broadcast herbicides may be applied to remove the regeneration surrounding susceptible beech, to allow the seedlings and sprouts surrounding retained resistant trees to dominate the regeneration layer (Favjan et al., 2019).

Putatively resistant trees may be identified in the landscape by the lack of infestation by scale. Scale infestation can be identified by the presence of white waxy chaff on the bole of the tree. If no scale signs are visible on the tree after careful visual inspection of the entire bole with the naked eye and binoculars, the tree is considered putatively resistant. Putatively resistant trees should be monitored and re-inspected over successive years. Other visible symptoms of BBD include the presence of annually increasing necrotic cankers, or less commonly tarry spots or sunken lesions (Ehrhlich, 1934; Houston, 1994a). These symptoms only develop slowly after the tree has been infected by the fungal component of the disease. The absence of scale infestation demonstrates resistance of the tree, as some lesions and other signs of decay can occur on beech trees that do not develop into the disease.

Resistant trees identified in the field can be confirmed through field challenge tests (Houston, 1982; Koch and Carey, 2014). Field challenge tests should only be performed in areas where scale has already arrived so as to prevent introduction of the disease to new areas. Scale insects are brushed from the bole of a tree at the end of summer, after adults have laid egg, from July to September. The presence of eggs can be confirmed by lifting waxy chaff from the bole and inspecting the wax and bark surface for eggs using a magnifying glass. The collected mass is sifted through a fine screen (250 micron nylon mesh) to remove mature insects and debris. The cleaned eggs must be stored at about 4C in a sealed, damp container. Viability may be reduced after a week of storage. Eggs are applied to a damp polyethylene sponge and the damp sponge is affixed to the bole of the suspected resistant tree, and affixed with flexible wire. Vapor permeable house wrap (genericized trademark "homewrap") is affixed over the damp sponge and sealed on the top and two sides with silicone caulk. Two pads should be affixed to the identified resistant tree. Pads are also affixed to non-resistant trees near the resistant tree to serve as

positive controls. Pads should be left undisturbed for at least 52 weeks. When the pad is removed after one year, carefully inspect the bark and the polyethylene pad with a hand lens for adult insects and new egg clusters (Koch and Carey, 2014). Trees without the presence of egg clusters can be considered truly resistant to BBD.

A potential source of resistant young trees is transplanting of seedlings or suckers. American beech has a popular reputation as difficult to transplant (University of Tennessee, 2015; Missouri Botanical Garden, 2020; Morton Arboretum, 2020). When resistant trees are identified, regeneration will occur through seed, however if root damage occurs incidentally to resistant beech, it should be monitored for production of clonal root suckers. Excavated young trees can be morphologically identified as root suckers by the presence of a lateral feeding root, while seedlings will display a taproot (Rushmore, 1961). Development of reliable transplant procedures could allow pursuit of this natural regeneration as a source of resistant young trees via transplanting of clonal root suckers of identified resistant trees, rather than seedlings which may have mixed resistance. Research is necessary to develop reliable transplanting methods.

The transplanting of root suckers for restoration has been successfully applied in the reforestation of arid landscapes, notably in the species *Ziziphus jujube*; Rhamnaceae, in New Mexico, *Amelanchier* spp; Roseaceae, and *Pongamia pinnata*; Fabaceae (Sapkota et al. 2019; Gough 2010; Maiti 2012, respectively). Propagation by root suckering is not widely reported in scientific literature, but a known route of propagation for many trees. The application of root suckers as a route for restoration of other species can be successful, and should be explored for American beech, which so readily produces root suckers.

2.5 Artificial Vegetative Propagation of BBD-Resistant American Beech

American beech is generally regarded as difficult to propagate vegetatively, but recent methods have improved the rates of success in many methods. Additional

pressures, including the expanded range of beech bark disease, climate change, and newly described BLD, have drastically increased the demand for propagation methods for the species in the last 30 years. Many methods are being explored for large-scale clonal propagation of the species.

2.5.1 Micropropagation

Micropropagation* methods can be used to produce a large number of clonal plantlets* *in vitro* in a short amount of time. Micropropagation techniques can be broadly classified into two methods: organogenesis*, where many shoots are cultivated from tissue of an existing tree which then form roots, and somatic embryogenesis*, where many fully-formed embryos are cultivated from tissue of an existing tree, which contain root and shoot embryonic structures. Micropropagation allows for short timelines for creation of clonal plantlets, but not all produced clones will recruit into soil media (Diner, 1999).

American beech plantlets can be produced through organogenesis (Barker et al., 1997; Cuenca and Vieitez, 1999; Cuenca et al., 2000, Pijuit et al., 2010), but no methods have been published to successfully transfer them to soil. Beech is propagated from shoots by removing root shoots and treating them with indole-3-butryic acid (IAA) and naphthalene acetic acid (NAA) (Barnes 2003, in Pijut et al., 2010). About 25% of shoots successfully root in growing media in this method. New growth shoots stripped from young beech were successfully cultured in growing media (Aspen culture media supplemented with 0.89 uM 6-benzyladenine(BA), 0.27uM NAA, 20 g 1^{-1} sucrose and 7g 1^{-1} Difco Bacto-agar), but did not survive potting in soil, resulting in no usable clones (Baker et al., 1997). Internodal segments were removed from the upper portion of young Oriental beech (*F. orientalis*) and European beech (*F. sylvatica*) and grown in bud induction medium (Woody Plant Medium supplemented with 2.9 uM indole-3-butryic acid (IBA) and 4.5uM thidiazuron(TDZ) and 30 g-1 sucrose) and transferred to proliferation medium (Woody Plant Medium supplemented with 2.2 uM BA, 9.1 uM zeatin and 2.9uM IAA), but success rates for transferring to soil were not reported

(Cuenca et al., 2000). Buds may be cultured from leaf cuttings of European beech on growth medium (Woody Plant Medium supplemented with 2.9 uM IAA, 4.5 uM TDZ, and 30g/L sucrose), but soil planting rates were not reported (Cuenca and Vieitez, 1999). Findings have indicated that the genotype of the tissue donor tree also affects success of these propagation methods (Barker et al, 1997; Cuenca et al., 2000).

American beech embryos can be created through embryogenesis, but again no process has been developed for successful transfer to soil. European beech seeds have had some limited embryonic growth from seed incubated on growth medium (Woody Plant Medium supplemented with 68uM zeatin, or 9.1uM 2,4-dichlorophenoxyacetic acid (2,4-D) and 2.2uM BA), however no complete plantlets were developed (Hazubska-Przybyt et al., 2015).

The continued development of in vitro methods of propagation is desirable because they create a large number of complete clones of resistant trees. Micropropagation methods for American beech have been explored by the Canadian Forest Service Atlantic Forestry Center and the University of New Brunswick (Loo et al., 2005), as well as the United States Forest Service (Barker et al., 1997), though no agency has reported a reliable method of successfully propagating and growing plants through micropropagation. Organogenesis and embryogenesis do not appear to be reliable methods of propagation at this time. Continued research is warranted in these methods, as development of procedures to successfully transplant these plants to soil would allow for production of a large number of clones of entire resistant trees to be produced.

2.5.2 Grafting

Grafting can create clonal plants which are already established in soil. Handling time for individual plants is longer compared to micropropagation, but overall success rates are higher. Grafting is currently the leading method for clonal propagation of beech.

Bench grafting methods produce American beech with published success rates as high as 30% (Ramirez et al., 2007). In bench grafting, a scion from a resistant tree is

joined with a containerized rootstock from a non-resistant tree, and allowed to heal fully in a greenhouse before planting. Generally, top cleft grafts are used because tools are available to standardize the cut, minimizing technician error. Top cleft grafts require a very precise diameter match of the scion and rootstock. If an exact diameter match is not possible, an apical veneer graft or two staggered veneer grafts may be used (Ramirez et al., 2007; Carey et al., 2013).

The application of hot callus grafting has increased success rates to as high as 57% (Carey et al., 2013). In hot callus grafting, top cleft or apical veneer grafts are used to join scions to dormant rootstocks (stored at 4-6° C and lightly watered). Apical veneer grafts closely resemble side veneer grafts, but all of the rootstock above the graft union is removed from the plant. After trees are grafted they are moved to a cold chamber (4-6° C) while only the graft union area is placed inside an insulated chamber heated to 24° C. Trees can be checked for callus formation starting at about three weeks after grafting. Graft unions should be inspected beginning at 4 weeks by carefully removing grafted trees and checking for the formation of new callus tissue forming at the graft union site. When callus tissue has formed, the trees may be moved out of the hot callus apparatus to a standard greenhouse or shadehouse (Carey et al., 2013).

Scions may be pulled from the outer canopy of a vigorous, BBD-resistant beech in the spring, before flush occurs (February to March). For many species, scions may be stored in dry cold storage for extended periods of time, however most documented beech grafting uses freshly cut scions stored for no more than two weeks before grafting. Large scions (up to 2m in length) may be collected and trimmed to size immediately before grafting. Fresh scions, or those removed from cold storage, should have the proximal end of the branch re-cut and the scions placed cut side down in cool water.

In all grafting, knives, scions, and rootstocks should all be disinfected with a 70-80% ethanol solution to prevent contamination. Rootstocks and scions should be joined with a top cleft or apical veneer graft. A modified veneer graft with an enhanced sap drawer (to allow excess moisture in the rootstock to escape and not separate the graft

union) (Figure 1 C, D) may be used for small diameter rootstock (Oconto Seed Orchard Personnel, personal communication, 2019). For inexperienced grafters, a tool such as the Fieldcraft Topgrafter (Raggett Industries Ltd., Gisborne, New Zealand) may be used to ensure uniform cuts in the rootstock and scion. After cutting, the cambium of the rootstock and scion should be laid fully flush together, matched exactly on at least one side of the cut surface. If an exact match is not possible, a larger scion should be selected and matched on one side (Ramirez et al., 2007). Grafts should be gently, yet firmly secured with flexible materials such as grafting rubbers. Rubbers should be wrapped with space sufficient for callus expansion (Figure 1 E). The entire tree should be dipped in warm (55° C) paraffin wax past the graft union to prevent drying. If veneer grafts are used in hot callus grafting it is imperative that a sap drawer is left and the paraffin wax is partially removed from the top of the sap drawer to prevent lifting of the graft.

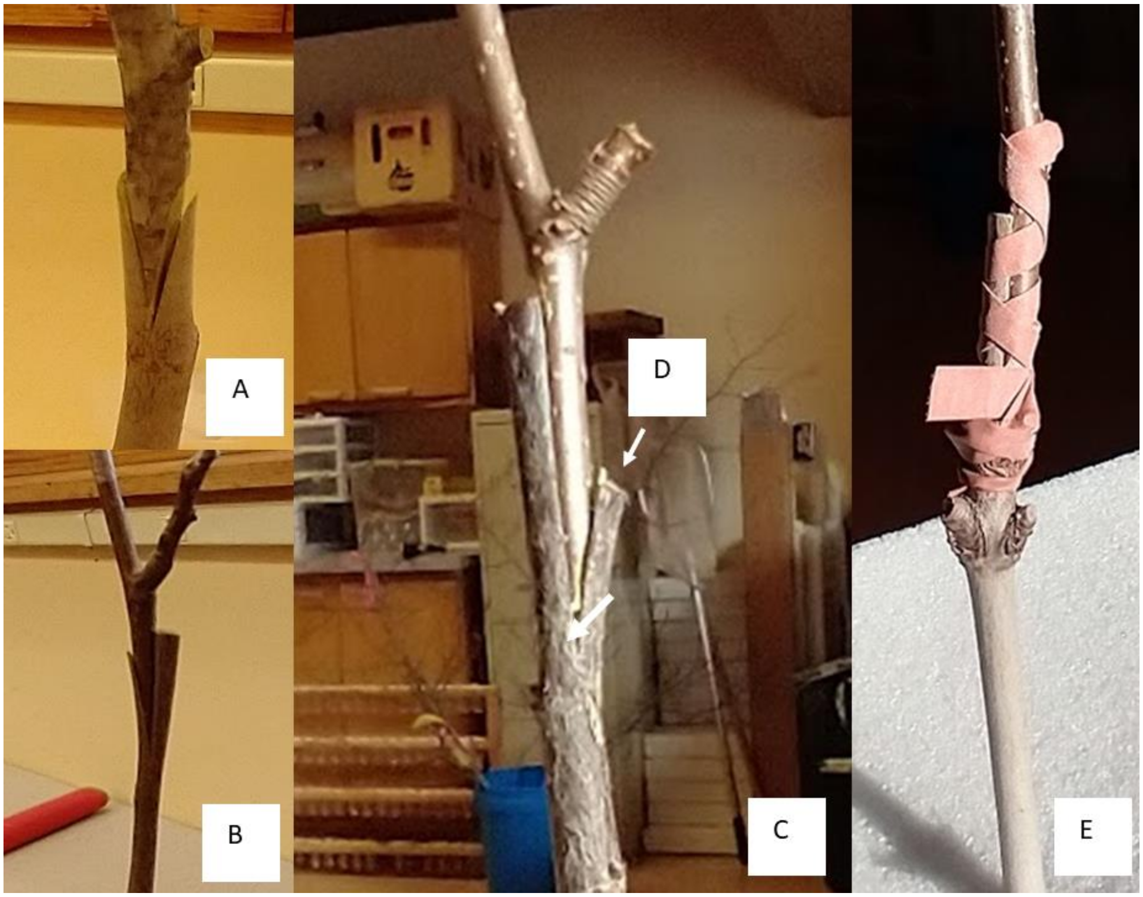

Figure 1. Grafts acceptable for use in American beech. A. Top Cleft graft, performed with a FieldCraft Top Grafter. B. Apical veneer graft. Two veneer grafts may be staggered on opposite sides of the rootstock. C. Modified veneer graft with D. enhanced sap drawer. F. A graft union wrapped with space sufficient to allow callus formation.

While success rates are certainly higher in grafting compared to micropropagation, there are additional considerations. Hot callus grafting requires cold storage and the construction of special hot chambers, limiting the amount of space for plants to be produced at one time, resulting in relatively low yield. Traditional grafting techniques (those without the aid of a tool) require practice to optimize yield. The relative difficulty to propagate American beech may lead to a shortage of rootstock availability in commercial nurseries, especially if multiple agencies in the same region are pursuing grafting while sourcing rootstock from local commercial nurseries. At this time, grafting also produces a tree which is resistant to BBD above the graft union, but is still likely susceptible to BBD below the graft union.

2.6 Applications in Restoration

Propagation can be used to create or encourage growth of resistant trees, but effective restoration requires the placement and continued survival of these materials in the field. The selection of a restoration method requires careful consideration, since restoration failures consume precious time and money, without benefit to the target ecosystem. Restoration projects are complex and can succumb at a number of points (Bayraktarov et al., 2016; Höhl et al., 2020; Theodoropoulos et al., 2020).

In complex problem solving, conceptual frameworks can help guide the creation of a focused plan of action. We have applied the conceptual framework that was developed by Jacobs et al. (2013) for the restoration of American chestnut. The authors suggest that to create an effective restoration plan, definition of goals, available inputs and limitations should be considered within the context of ecology, society, and technology spheres (Jacobs et al., 2013).

Societal context describes public perception of the species and program, governmental policies and regulations in the area for restoration, and relationships between agencies working on the disease. Ecological context should include background information of the species, as well as an accurate snapshot of the ecology of the area targeted for restoration. The level of degradation should be assessed individually for the target area for each restoration project. Accurate, timely information about the targeted area can identify ecological barriers to restoration if they exist. Technological context describes the techniques necessary for reintroduction of a species. Generally the technology of a restoration project will be dictated by constraints on time, facilities, and money. In the case of American beech, there is still much fundamental propagation knowledge missing about the species, so restoration activities should be planned understanding that emerging knowledge could change the context surrounding planned restoration activities.

When identifying goals for restoration of BBD-affected forests, it is important to recognize that there are large amounts of desirable overstory (both a small number of resistant American beech and the remaining co-occurring species), so transformative restoration techniques are appropriate in these areas. In transformation*, partial removal of competing vegetation creates availability of growing resources, such as light, water and soil nutrients for the newly-planted individuals of the target species. In BBD-affected forests a combination of techniques can be used to accomplish the goals of transformative restoration. Cutting and removal of the existing vegetation may be necessary to remove both diseased and dying overstory American beech trees and non-resistant American beech regeneration, as well as other competing understory vegetation (e.g., invasive grasses, shrub species (Wagner et al., 2010)) before restoration plantings can occur in BBD-affected forests. The silvics for American beech are known, so thorough surveying enables site selection that is likely to provide the growing space and resources necessary for success of young plants. Information on severity of BBD and scale infestations can inform decisions for where to focus restoration efforts. Selecting a site with high mortality and low regeneration, if possible, could eliminate the need for site preparation. If these sites are not available, removal of overstory diseased beech, coarse woody debris, or regeneration may be necessary. Mechanical and chemical control of competing vegetation as described by Ostrofsky and McCormack (1986) would be appropriate site preparation.

Enrichment plantings* increase the proportion of the desired species by planting trees in the area targeted for restoration. American beech seedlings would likely be well-suited to interplanting* below disturbed beech canopy because of their tolerance of moderate shade in their early regeneration niche (Tubbs and Houston, 1990). Interplanting has been utilized to enhance regeneration of other challenged species in the Fagaceae family, but careful site preparation is necessary to meet the light demands of these seedlings (Löf et al., 2012; Traux et al., 2018). The same site preparations may not be necessary if natural gaps from BBD mortality can be utilized as planting sites.

Research is necessary to quantify the amount of cover that would best support beech seedling growth in interplantings.

Seed orchards* allow long term maintenance of grafted trees. The graft union will remain fragile for an extended period of time, so the trees should be monitored until the tree is robust enough to resist damage at the union site. Seed orchards allow production of high volumes of seed of known parentage by limiting the parents to the known grafted individuals (locations are in areas with little to no external pollen sources). While this limited breeding stock is desirable for creating crosses of two known resistant parents, it comes at the cost of an inherent genetic bottleneck when sufficiently genetically diverse parent trees are unavailable.

With careful selection of parent trees, a high proportion of genetic diversity can be retained (Johnson and Lipow, 2000). Within the western range of American beech, the limited number of resistant parent trees occurring in the landscape will inherently limit the genetic diversity of seed orchards, particularly if parent trees of local provenance are selected for creation of orchards. When combined with a desire to retain yet-unknown genetic traits that may hold the key to combating future health challenges (e.g., no resistance has been identified for BLD in American beech), the importance of propagating a suite of genetically diverse parent trees increases. Wild American beech displays lower than expected genetic diversity in both susceptible and non-susceptible trees, so careful consideration should be paid to selection of genetically diverse scion donor trees in creation of seed orchards (Houston and Houston, 1994a; Houston and Houston, 2000).

Large, robust young trees may be underplanted in areas where American beech has died, leaving openings in the canopy, to serve as nucleation centers for restoration (Figure 2). In nucleation, the species of interest is cluster planted*, with the goal of drawing natural vectors and associated species toward the cluster, accelerating the pace at which the desired species releases seed into the surrounding ecosystem (Corbin and Holl, 2012). While this strategy allows for immediate release of propagules in the target

forests, it is impossible to control the parentage of seed as is possible in traditional seed orchards. American beech seedlings with only one resistant parent are about as susceptible to scale as seedling with no resistant parents (Koch et al., 2010), however as American beech is wind-pollinated, planting many grafted resistant young trees in a patch near multiple known resistant overstory trees would increase the likelihood of resistant crosses occurring over time. Nucleation has largely been used in tropical forest restoration, and little literature exists examining efficacy in temperate deciduous forest (Boanares and de Azevedo, 2014), so research into the efficacy of this method in temperate forests is necessary. Overall, it is more difficult to maintain the health of individual seedlings in this method than in an orchard; however, each seed produced is released into an ecosystem in which it has a predefined niche, by the nature of planting the tree in disturbed areas which previously contained the species, making this a technique worth exploring as a long-term restoration strategy.

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Enrichment plantings, in the form of nucleated seed orchards or interplanting of greenhouse-raised resistant stock, may be used to increase genetic diversity of a population. While resistant trees exist in the landscape, it is not guaranteed that all have been identified and quantified. By interplanting resistant young trees within affected forests, uncontrolled pollination could occur between undiscovered resistant trees and known resistant crosses, allowing for persistence of resistant trees undiscovered by humans (Wright, 1952). Focusing on planting within forests that have retained any mature American beech, rather than converting areas that have lost all or the majority of their beech component, may allow for the retention of genetic lineages that would otherwise not be preserved, due to the remote and patchy nature of the distribution of the resistant trees.

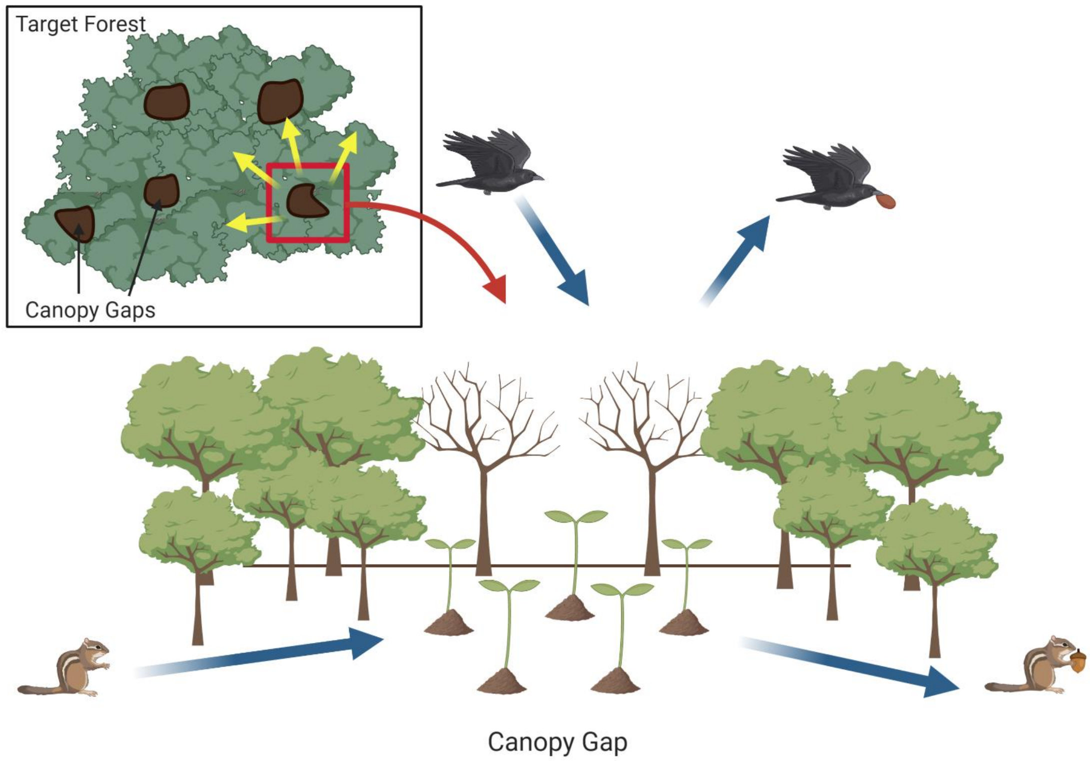

Figure 2. Nucleated beech seed orchards are an option for restoration in targeted forests. A canopy gap created by beech bark disease is chosen as a nucleation site and resistant young trees are cluster-planted (competing plants in the understory must also be removed by mechanical methods prior to planting). Seed produced by the planted young trees attracts wildlife to the nucleated seed orchard. Wildlife aid in seed dispersal. In this way, nucleated seed orchards serve as a propagule for reintroduction into the entire target forest by natural seed dispersal pathways. Image created with Biorender.com.

2.7 Recommendations

Restoration project decisions must be made on a contextualized, individual basis. In a typical BBD scenario, a transformative restoration, with an emphasis on enrichment plantings to enhance the proportion of resistant genotypes in the landscape would be recommended. If cost is a limiting factor, simple retention of resistant trees will maximize the proportion of resistant seed entering the landscape. If possible, hot callus grafting trees to create clones of resistant American beech for planting as orchards allows

for the most efficient creation of resistant trees until further technologies are developed. Flexibility and creativity can be exercised in choice of methods to affect restoration.

Much is unknown about the interactions of BBD and BLD, even as the ranges of these diseases overlap. The ranges of these diseases overlap. Although the diseases have entirely different mechanisms of mortality, both lead to a decline in vigor of overstory foliage, and eventual mortality. The impacts of the two diseases combined, which each have the potential to radically alter successional trajectories through removal of a foundational species, have not been studied to describe their compounded effects when present together. We do not expect resistance to one disease to have any bearing on the resistance to the other, due to the distinctly different pathology. Restoration of BBD-resistant American beech is important to retain as diverse a pool of genotypes possible, to allow for retention of genes which may be neutral in the fight against BBD, but potentially important in mitigating BLD. At this time, so little fundamental information is known about BLD, that we have little understanding of the mechanisms that may confer resistance or resilience to the disease. The greater genetic diversity that can be maintained in selection of BBD-resistant beech, the better chance we may have to enhance against future pressures.

Many gaps exist in our knowledge of propagating American beech. Continued research in the methods described in this review is necessary to improve management techniques for the species. Direct control of beech bark disease is not currently feasible, but possible biological controls have been identified. Reliable transplanting measures should be developed to increase success of restoration activities, including the transfer of plantlets created through organogenesis to soil. While grafting rates have been improved with the application of hot-callus grafting, continued research would allow for a greater volume of plant material production. Better understanding of the propagation of American beech would benefit not only mitigation of beech bark disease, but improve the outlook for the species in the face of other emerging challenges.

Table 1. Definitions of Terminology. Terms are marked with an asterisk (*) on their first appearance in the manuscript. [1]Helms 1998; [2]O'Hara, 2018; [3]Diner, 1999; [4]Collins, 2020.

Term	**Definition**
Advance regeneration[1]	Seedlings or saplings that develop or are present in the understory
Artificial regeneration[1]	A group or stand of young trees created by direct seeding or by planting seedlings or cuttings
Biological control[1]	The artificial application of a natural control agent to regulate pest species
Breeding arboretum[1]	A collection of selected trees or species established for breeding
Chemical control[1]	The application of an insecticide as the primary means of controlling a pest
Cluster planting	Planting young trees in a clustered or aggregated arrangement. Species may be clustered within a planting arrangement, or a planting may be clustered within an existing stand
Cultural control	The manipulation of vegetation to control a pest or disease
Conversion	A method of restoration where all of the existing overstory is removed and replaced by new species
Disturbance severity	The level of magnitude of a disturbance event. Disturbance severity is assessed based on the amount of biological material removed by the disturbance event. Severity can range from minor (where partial biological

	communities are removed) to major (where all biological communities may be removed) to complete (where all biological communities and legacies are removed)
Disturbance regime[2]	Trends in the type of disturbance, its duration, severity, frequency, size, seasonality and timing, and spatial pattern disturbances and their effects on the forest ecosystem over time
Enrichment planting[1]	The improvement of the percentage of desirable species or genotypes or increasing biodiversity in a forest by interplanting
Embryogenesis[3]	Using plant tissue to initiate many entire embryos, which have pre-formed root and shoot embryonic structures, then maturing the embryos to fully formed trees
Foundational species	Species which provide inputs through their structure or function which form the basis for structuring communities
Improvement planting[1]	Any planting done to improve the value of a stand, but not to establish a plantation
Infection court[1]	The site of infection by a pathogen
Interplanting[1]	Planting young trees among existing forest growth
Micropropagation[1]	The *in vitro* vegetative propagation of plants producing plantlets, micropropagules, or somatic embryos
Natural regeneration[1]	The establishment of a plant or plant age class from natural seeding, sprouting, suckering or layering

Nucleation	A restoration technique where clusters of plants, or "nuclei" are placed in a degraded environment with the goal of either creating refuge to draw seed dispersers and desired species into an area, or to supply seed of desirable species to be dispersed out from the nucleus
Organogenesis[3]	Initiating many shoots from a single piece of plant tissue, which then form roots from the shoot
Physical control	Removal of the pest organism from a host plant by means of physical intervention, such as scrubbing or suffocation by oils
Plantlet[1]	A plant produced in vitro
Seed tree[1]	A tree left standing (after cutting) for the sole or primary purpose of providing seed; a method of natural regeneration
Seed orchard[1]	A plantation consisting of clones or seedlings from selected trees for early and abundant production of seed and to promote balanced, random mating
Transformation	Partial removal of species is used to make space for some new species
Underplanting[1]	The setting out of young trees or sowing of seed under an existing stand

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2.10 Literature Cited

Arthur, M.A., Weathers, K.C., Lovett, G.M., Weand, M.P., Eddy, W.C. 2017. A beech bark disease induced change in tree species composition influences forest floor acid–base chemistry. *Canadian J. of For. Res.* 47, 875-882. https://doi.org/10.1139/cjfr-2016-0341

Barker, M.J., Pijut, P.M., Ostry, M.E., Houston, D.R. 1997. Micropropagation of juvenile and mature American beech. *Plant Cell, Tissue and Organ Culture.* 51, 209-213. https://doi:10.1023/A:1005953212568

Bayraktarov, E., Saunders, M.I., Abdullah, S., Mills, M., Beher, J., Possingham, H.P., Mumby, P.J., Lovelock, C.E. 2016. The cost and feasibility of marine coastal restoration. *Ecological Applications.* 26, 1055-1074. https://doi.org/10.1890/15-1077

Bernard, A., Gélinas, N., Duchateau, E., Durocher, C., Achim, A. 2018. American beech in value-added hardwood products: assessing consumer preferences. *BioResources*. 13, 6893-6910.

Boanares, D., Azevedo, C.S.d. 2014. The use of nucleation techniques to restore the environment: a bibliometric analysis. *Natureza & conservação*. 12, 93-98. https://doi:10.1016/j.ncon.2014.09.002.

Bohn, K. K., Nyland, R.D. 2003. Forecasting development of understory American beech after partial cutting in uneven-aged northern hardwood stands. *For. Ecology and Management.* 180, 453-461. https://doi:10.1016/S0378-1127(02)00614-X.

Bonner, F.T., Leak, W.B. 2008. *Fagus* L. The woody plant seed manual. Eds: Bonner, F.T., & Karrfalt, R.P. Agric. Handbook No. 727. Washington, DC. US Department of Agriculture, Forest Service. 1223 p., 727.

Burger, T.L., Kotar, J. 2003. A guide to forest communities and habitat types of Michigan. University of Wisconsin--Madison, Department of Forestry Ecology and Management.

Burke, D.J., Hoke, A.J., Koch, J. 2020. The emergence of beech leaf disease in Ohio: Probing the plant microbiome in search of the cause. *For. Pathology.* 50, e12579.

Burns, B. S., Houston, D.R. 1987. Managing beech bark disease: Evaluating defects and reducing losses. *North. J. of Appl. Forestry*. 4, 28-33. https://doi:10.1093/njaf/4.1.28.

Cale, J. A., Garrison-Johnston, M.T., Teale, S.A., Castello, J.D. 2017. Beech bark disease in North America: Over a century of research revisited. *For. Ecology and Management.* 394, 86-103. https://doi:10.1016/j.foreco.2017.03.031.

Cale, J. A., Letkowski, S.K., Teale, S.A., Castello, J.D. 2012. Beech bark disease: an evaluation of the predisposition hypothesis in an aftermath forest: Evaluating the

beech bark disease predisposition hypothesis. *For. Pathology (J. de pathologie forestière).* 42, 52-56. https://doi:10.1111/j.1439-0329.2011.00722.x.

Cale, J. A., McNulty, S.A., Teale, S.A., Castello, J.D. 2012. The impact of beech thickets on biodiversity. *Biological Invasions.* 15, 699-706. https://doi:10.1007/s10530-012-0319-5.

Cale, J. A., Teale, S.A., Johnston, M.T., Boyer, G.L., Perri, K.A., Castello, J.D. 2015. New ecological and physiological dimensions of beech bark disease development in aftermath forests. *For. Ecology and Management.* 336, 99-108. https://doi:10.1016/j.foreco.2014.10.019.

Ćalić, I., Koch, J., Carey, D., Addo-Quaye, C., Carlson, J.E., Neale, D.B. 2017. Genome-wide association study identifies a major gene for beech bark disease resistance in American beech *(Fagus grandifolia* Ehrh.). *BMC Genom.* 18, 547-514. https://doi:10.1186/s12864-017-3931-z.

Carey, D.W., Mason, M.E., Bloese, P., Koch, J.L. 2013. Hot callusing for propagation of American beech by grafting. *HortScience.* 48, 620-624.

Carpenter, R.D. 1974. American beech (*Fagus grandifolia*). FS-220

Cogbill, C.V. 2005. Historical biogeography of American beech. In: Evans, Celia A., Lucas, Jennifer A. and Twery, Mark J., eds. Beech Bark Disease: Proceedings of the Beech Bark Disease Symposium; 2004 June 16-18; Saranak Lake, NY. Gen. Tech. Rep. NE-331. Newtown Square, PA: US. Department of Agriculture, Forest Service, Northeastern Research Station: 16-24.

Collins, 2020. Collins English Dictionary. Accessed online 12/7/2020: https://www.collinsdictionary.com/dictionary/english.

Conrad, C.A., Reitsma, L. 2015. Demographic responses if myomorph rodents to mast production in a beech- and birch-dominated northern hardwood forest. *Northeast. Naturalist*. 22, 746-761.

Corbin, J. D. and K. D. Holl 2012. Applied nucleation as a forest restoration strategy. *For. Ecology and Management.* 265, 37-46. https://doi:10.1016/j.foreco.2011.10.013.

Cuenca, B., Ballester, A., Vieitez, A.M. 2000. In vitro adventitious bud regeneration from internode segments of beech. *Plant Cell, Tissue and Organ Culture.* 60, 213-220.

Cuenca, B., Vieitez, A.M. 1999. Histological Study of In vitro Development of Adventitious Buds on Leaf Explants of Oriental Beech (*Fagus orientalis* Lipsky). *In Vitro Cellular & Developmental Biology.* Plant, 35, 326-332. https://doi:10.1007/s11627-999-0043-8.

Del Tredici, P. 2001. Sprouting in temperate trees: A morphological and ecological review. *The Botanical Rev*. 67, 121-140. https://doi:10.1007/bf02858075.

Diner, A. 1999. Somatic embryogenesis in forestry: A practical approach to cloning the best trees. In: Wheeler, Robert, ed. Under the canopy; forestry and forest products newsletter of the Alaska Cooperative Extension. Fairbanks, AK: University of Alaska Fairbanks Cooperative Extension Service: 7-8.

Ehrlich, J. 1934. The beech bark disease: a *Nectria* disease of *Fagus*, following *Cryptococcus fagi* (Baer.). *Canadian J. of Res.* 10, 593-692.

Eyre, F.H. 1980. Forest Cover Types. Washington, DC: Society of American Foresters.

Ewing, C.J., Hausman, C.E., Pogacnik, J., Slot, J., Bonello, P. 2018. Beech leaf disease: An emerging forest epidemic. *For. Pathology*. 49, e12488.

Farrar, A., Ostrofsky, W.D. 2006. Dynamics of American beech regeneration 10 years following harvesting in a beech bark disease-affected stand in Maine. *Northern J. of App. For.* 23, 192-196.

Faison, E.K., Houston, D.R. 2004. Black bear foraging in response to beech bark disease in northern Vermont. *Northeast. Naturalist.* 11, 387-394. https://doi:10.1656/1092-6194(2004)011[0387:BBFIRT]2.0.CO;2

Fajvan, M. A., Hille, A., Turcotte, R.M. 2019. Managing understory *Fagus grandifolia* for promoting beech bark disease resistance in northern hardwood stands. *For. Science.* 65, 644-651.

Flachowsky, H., Hanke, M.V., Peil, A., Strauss, S.H., Fladung, M. 2009. A review on transgenic approaches to accelerate breeding of woody plants. *Plant breeding.* 128, 217-226. https://doi:10.1093/forsci/fxz023.

Garnas, J. R., Houston, D.R., Twery, M.J., Ayres, M.P., Evans, C. 2013. Inferring controls on the epidemiology of beech bark disease from spatial patterning of disease organisms. *Agricultural and For. Entomology.* 15, 146-156. https://doi:10.1111/j.1461-9563.2012.00595.x.

Garnas, J. R., Ayres, M.P., Liebhold, A.M., Evans, C. 2011. Subcontinental impacts of an invasive tree disease on forest structure and dynamics. *The J. of Ecology* 99, 532-541. https://doi:10.1111/j.1365-2745.2010.01791.x.

Giencke, L. M., Dovciak, M., Mountrakis, G., Cale, J.A., Mitchell, M.J. 2014. Beech bark disease: spatial patterns of thicket formation and disease spread in an aftermath forest in the northeastern United States. *Canadian J. of For. Res.* 44, 1042-1050. https://doi:10.1139/cjfr-2014-0038.

Gough, R.E. 2010. Juneberries for Montana Gardens. Montana Extension Service. e-resource MT198806AG.

Holloway, G.L., Malcom, J.M. 2007. Nest-tree use by northern and southern flying squirrels in central Ontario. *J. of Mammalogy* 88, 226-233. https://doi:10.1644/05-MAMM-A-368R2.1.

Hazubska-Przybył, T., Chmielarz, P., Bojarczuk, K. 2015. In vitro responses of various explants of *Fagus sylvatica*. *Dendrobiology*. 73, 135-144. https://doi:10.12657/denbio.073.014.

Held, M. 1983. Pattern of beech regeneration in the East-central United States. *Bulletin of the Torrey Botanical Club.* 110, 55-62.

Helms, J. A. 1998. Dictionary of Forestry. Society of American Foresters.

Heyd, R. L. 2005. Managing beech bark disease in Michigan. In: Evans, Celia A., Lucas, Jennifer A. and Twery, Mark J., eds. Beech Bark Disease: Proceedings of the Beech Bark Disease Symposium; 2004 June 16-18; Saranak Lake, NY. Gen. Tech. Rep. NE-331. Newtown Square, PA: US. Department of Agriculture, Forest Service, Northeastern Research Station: 128-132.

Höhl, M., Ahimbisibwe, V., Stanturf, J.A., Elasser, P., Kleine, M., Bolte, A. 2020. Forest landscape restoration- What generates failure and success? *Forests.* 11, 938.

Houston, D.R. 2005. Beech bark disease: 1934 to 2004: What’s new since Ehrlich? In: Evans, Celia A., Lucas, Jennifer A. and Twery, Mark J., eds. Beech Bark Disease: Proceedings of the Beech Bark Disease Symposium; 2004 June 16-18; Saranak Lake, NY. Gen. Tech. Rep. NE-331. Newtown Square, PA: US. Department of Agriculture, Forest Service, Northeastern Research Station: 2-13.

Houston, D. B., Houston, D.R. 1994a. Variation in American beech (*Fagus grandifolia* Ehrh.): Isozyme analysis of genetic structure in selected stands. *Silvae Genetica.* 43, 277-284.

Houston, D.R. 1994b. Temporal and spatial shift within the Nectria pathogen complex associated with beech bark disease of Fagus grandifolia. Canadian Journal of Forest Research, 24(5), 960-968.

Houston, D. B., Houston, D.R. 2000. Allozyme genetic diversity among *Fagus grandifolia* trees resistant or susceptible to beech bark disease in natural populations. *Canadian J. of For. Res.* 30, 778-789. https://doi:10.1139/cjfr-30-5-778.

Houston, D. R. 1983. Effects of parasitism by *Nematogonum ferrugineum* (*Gonstorrhodiella highlei*) on pathogenicity of *Nectria coccinea* var. faginata and *Nectria galligena*. In: Proceedings, I.U.F.R.O. Beech Bark Disease Working Party Conference; 1982 September 26-October 8; Hamden, CT. Sponsored by the USDA Forest Service, Northeastern Forest Experiment Station. Gen. Tech. Rep. WO-37. [Washington, DC]: U.S. Department of Agriculture, Forest Service: 109-114.

Houston, D.R. 1982. A technique to artificially infest beech bark with beech scale, *Cryptococcus fagisuga* (Lindinger). Res. Pap. NE-507. Broomall, PA: U.S. Department of Agriculture, Forest Service, Northeastern Forest Experiment Station. 8p.

Houston, D. R. 1994. Major new tree disease epidemics: Beech bark disease. *Annual Rev. of Phytopathology*. 32, 75-87. https://doi:10.1146/annurev.py.32.090194.000451.

Houston, D.R., O'Brien, J.T. 1983. Beech Bark Disease. Forest Insect and Disease Leaflet 75. United States Department of Agriculture. Forest Service, Northern Area State and Private Forestry.

Iverson, L.R., Prasad, A.M., Matthews, S.N., Peters, M. 2008. Estimating potential habitat for 134 eastern US tree species under six climate scenarios. *For. Ecology and Management.* 254, 390-406.

Jacobs, D. F., Dalgleish, H.J., Nelson, C.D. 2013. A conceptual framework for restoration of threatened plants: the effective model of American chestnut (*Castanea dentata*) reintroduction. *The New Phytologist.* 197, 378-393. https://doi:10.1111/nph.12020.

Jensen, P.G., Demers, C.L., Mcnulty, S.A., Jakubas, W.J., Humphries, M.M. 2012. Marten and fisher responses to fluctuations in prey populations and mast crops in the northern hardwood forest. *The J. of Wildl. Management.* 76, 489-502.

Johnson, R., Lipow, S. 2000. Compatibility of breeding for increased wood production and longterm sustainability: the genetic variation of seed orchard seed and associated risks. In: Proceedings Wood Compatibility Initiative Workshop. 18, 169-179.

Kahler, H. A., Anderson, J.T. 2006. Tree cavity resources for dependent cavity-using wildlife in West Virginia forests. *North. J. of Appl. Forestry.* 23, 114-121. https://doi:10.1093/njaf/23.2.114.

Kasson, M. T., Livingston, W.H. 2009. Spatial distribution of *Neonectria* species associated with beech bark disease in northern Maine. *Mycologia.* 101, 190-195. https://doi:10.3852/08-165.

Koch, J. L., Carey, D.W. 2014. A technique to screen American beech for resistance to the beech scale insect (*Cryptococcus fagisuga* Lind.). *J. of Vis. Exp.: JoVE.* e51515. https://doi:10.3791/51515.

Koch, J. L., Carey, D.W., Mason, M.E., Nelson, C.D. 2010. Assessment of beech scale resistance in full- and half-sibling American beech families. *Canadian J. of For. Res.* 40, 265-272. https://doi:10.1139/x09-189.

Leak, W.B. 2006. Fifty-year impacts of the beech bark disease in the Bartlett Experimental Forest, New Hampshire. *Northern J. of App. For.* 23, 141-143.

Lemaître, J., Villard, M.A. 2005. Foraging patterns of pileated woodpeckers in a managed Acadian forest: a resource selection function. *Canadian J. of For. Res.* 3510, 2387-2393. https://doi:10.1139/x05-148.

Löf, M., Dey, D.C., Navarro, R.M., Jacobs, D. F. 2012. Mechanical site preparation for forest restoration. *New Forests.* 43, 825-848.

Loo, J., Ramirez, M., Krasowski, M. 2005. American beech vegetative propagation and genetic diversity. In: Evans, Celia A., Lucas, Jennifer A. and Twery, Mark J., eds. Beech Bark Disease: Proceedings of the Beech Bark Disease Symposium; 2004 June 16-18; Saranak Lake, NY. Gen. Tech. Rep. NE-331. Newtown Square, PA: US. Department of Agriculture, Forest Service, Northeastern Research Station: 106-112.

Maiti, S.K. 2013. Raising of Saplings for Forest Trees. P 121-149 in *Ecorestoration of the Coalmine Degraded Lands*. New Delhi, Springer India.

Mason, M. E., Koch, J., Krasowski, M., Loo, J. 2013. Comparisons of protein profiles of beech bark disease resistant and susceptible American beech (*Fagus grandifolia*). *Proteome Science*. 11, 2. https://doi:10.1186/1477-5956-11-2.

Maurer, B.A., Whitmore, R.C. 1981. Foraging of five bird species in two forests with different vegetation structure. *The Wilson Bulletin*. 93, 478-490.

Marra, R. E., & LaMondia, J. A. (2020). First Report of beech leaf disease, caused by the foliar nematode, Litylenchus crenatae mccannii, on American Beech (Fagus grandifolia) in Connecticut. Plant Disease, 104(9), 2527.

Mayer, M., Allen, D.C. 1983. *Chilocorus stigma* (Coleoptera: Coccinellidae) and other predators of beech scale in central New York. In: Proceedings, I.U.F.R.O. Beech Bark Disease Working Party Conference; 1982 September 26-October 8; Hamden, CT. Sponsored by the USDA Forest Service, Northeastern Forest

Experiment Station. Gen. Tech. Rep. WO-37. [Washington, DC]: U.S. Department of Agriculture, Forest Service: 89-98.

McCullough, D. G., Heyd, R.L., O-Brien, J.G. 2001. Biology and management of beech bark disease. Michigan State University Extension Bulletin (E-2746). East Lansing, MI.

McGee, G. G. 2000. The contribution of beech bark disease-induced mortality to coarse woody debris loads in northern hardwood stands of Adirondack Park, New York, U.S.A. *Canadian J. of For. Res.* 30, 1453-1462. https://doi:10.1139/cjfr-30-9-1453.

Missouri Botanical Garden. 2020. *Fagus grandifolia*. Online Publication. http://www.missouribotanicalgarden.org/PlantFinder/PlantFinderDetails.aspx?kempercode=a865 Accessed 12/7/2020.

Morton Arboretum. 2020. American beech. Webpage. https://ag.tennessee.edu/news/Pages/POM-2015-12.aspx Accessed 12/7/2020.

Nyland, R.D. 2008. Origin of small understory beech in New York northern hardwood stands. *Northern J. of Appl. Forestry*. 25, 161-163.

Nyland, R.D., Bashant, A.L., Bohn, K.K., & Verostek, J.M. 2006. Interference to hardwood regeneration in northeastern North America: Controlling effects of American beech, striped maple, and hobblebush. *Northern J. of Appl. Forestry*. 23, 122-132.

O'Brien, J.G., Ostry, M.E., Mielke, M.E., Mech, R., Heyd, R.L., McCullough, D.G. 2001. First report of beech bark disease in Michigan. *Plant Disease.* 85, 921-921.

O'Hara, K. 2014. Multiaged silviculture: managing for complex forest stand structures. Vol. First edition, Oxford University Press.

Ostrofsky, W. D., McCormack, M.L. 1986. Silvicultural management of beech and the beech bark disease. *North. J. of Appl. Forestry.* 3, 89-91.

Papaik, M. J., Canham, C.D., Latty, E.F., Woods, K.D. 2005. Effects of an introduced pathogen on resistance to natural disturbance: beech bark disease and windthrow. *Canadian J. of For. Res.* 35, 1832-1843.

Pijut, P. M., Woeste, K.E., Michler, C.H., Janick, J. 2010. Promotion of adventitious root formation of difficult-to-root hardwood tree species. *Horticultural Rev.*, Hoboken, NJ, USA. P 213-251. https://doi:10.1002/9780470872376.ch6.

Poulsen, K.M. 1993. Predicting the storage life of beech nuts. *Seed Science and Technology.* 21, 327-337.

Ramirez, M., Krasowski, M., Loo, J. 2007. Vegetative propagation of American beech resistant to beech bark disease. *HortScience.* 42, 320-324. https://doi:10.21273/HORTSCI.42.2.320.

Reed, S.E., Qing, Y., Hoke, A., Burke, D.J., Carta, L.K., Handoo, Z.A., Kantor, M.R., Koch, J. 2020. Foliar nematode, *Litylenchus crenatae* ssp. Mccannii, population dynamics in leaves and buds of beech leaf disease-affected trees in Canada and the US. *For. Pathology.* 50, e12599–n/a. Web.

Robb., J.R., Brookhout, T.A. 1995. Factors influencing wood duck use of natural cavities. *The J. of Wildl. Management.* 59, 372-383. https://doi:10.2307/3808951

Rosemier, J.N., Storer, A.J. 2011. Assessing native small mammals' responses to an incipient invasion of beech bark disease through changes in seed production of American beech. In: Fei, Songlin; Lhotka, John M.; Stringer, Jeffrey W.; Gottschalk, Kurt W.; Miller, Gary W., eds. Proceedings, 17th central hardwood forest conference; 2010 April 5-7; Lexington, KY; Gen. Tech. Rep. NRS-P-78. Newtown Square, PA: US Department of Agriculture, Forest Service, Northern Research Station: 675-676. Vol. 78. 2011.

Roy, M.-È., Nolet, P. 2018. Early-stage of invasion by beech bark disease does not necessarily trigger American beech root sucker establishment in hardwood stands. *Biological Invasions.* 20, 3245-3254. https://doi:10.1007/s10530-018-1771-7.

Rushmore, F.M. 1961. Silvical characteristics of beech (*Fagus grandifolia*). Station Paper NE-161. Upper Darby, PA: U.S. Department of Agriculture, Forest Service, Northeastern Forest Experiment Station. 26 p.

Sapkota, S., Sapkota, S., Wang, S., Liu, Z. 2019. Height and Diameter Affect Survival Rate of Jujube Suckers Transplanted in a Semi-Arid Farmland of New Mexico. *J. of Appl. Horticulture*. 21, 249–251.

Seger, R. L., Servello, F.A., Cross, R.A., Keisler, D.H. 2013. Body mass and mast abundance influence foraging ecology of the American black bear (*Ursus americanus*) in Maine. *Canadian J. of Zoology*. 91, 512-522. https://doi:10.1139/cjz-2012-0326.

Shigo, A.L. 1972. The beech bark disease today in the northeastern US. *J. of Forestry.* 70,286-289.

Shigo, A.L. 1962. Another scale insect on beech. Station Paper NE-168. Upper Darby, PA: U.S. Department of Agriculture, Forest Service, Northeastern Forest Experiment Station. 13 p.

Sniezko, R. A., Koch, J. 2017. Breeding trees resistant to insects and diseases: putting theory into application. *Biological Invasions.* 19, 3377-3400. https://doi.org/10.1007/s10530-017-1482-5

Stephanson, C.A., Ribarik Coe, N. 2017. Impacts of beech bark disease and climate change on American beech. *Forests.* 8, 155.

Stephens, R.B., Hobbie, E.A., Lee, T.D., Rowe, R.J. 2019. Pulsed resource availability changes dietary niche breadth and partitioning between generalist rodent consumers. *Ecology and Evolution.* 9,10681-10693.

Sweeney, J., Hughes, C., Zhang, H., Hillier, N. K., Morrison, A., Johns, R. 2020. Impact of the invasive beech leaf-mining weevil, *Orchestes fagi*, on American beech in Nova Scotia, Canada. *Front. For Global Change*. 3, 1-11.

Taylor, A. R., McPhee, D. A., & Loo, J. A. (2013). Incidence of beech bark disease resistance in the eastern Acadian forest of North America. The Forestry Chronicle, 89(5), 690-695.

Theodoropoulos, C., Stamou, A., Vardakas, L., Papadaki, C., Dimitriou, E., Skoulikidis, N., Kalogianni, E. 2020. River restoration is prone to failure unless pre-optimized within a mechanistic ecological framework: Insights from a model-based case study. *Water Res.* 173, 465-487.

Tozer, D.C., Burke, D.M., Nol, E., Elliott, K.A. 2012. Managing ecological traps: logging and sapsucker nest predation by bears. *The J. of Wildl. Management*. 76, 887-898. https://doi:10.1002/jwmg.336.

Traux, B., Gagnon, D., Fortier, J., Lambert, F., Pétrin, M.A. 2018. Ecological factors affecting white pine, red oak, bitternut hickory and black walnut underplanting success in a northern temperate post-agricultural forest. *Forests.* 9, 499.

Tubbs, C. H., Houston, D.R. 1990. *Fagus grandifolia* Ehrh. American beech. In: Burns, R. M., and Honkala, B. H. tech. coords. Silvics of North America. Agriculture Handbook 654.U.S. Department of Agriculture, Forest Service, Washington, DC. vol.2, p. 325-332.

University of Tennessee. 2015. American beech. Webpage. https://ag.tennessee.edu/news/Pages/POM-2015-12.aspx Accessed 12/7/2020.

Wagner, S., Collet, C., Madsen, P., Tohru, N., Nyland, R.D., Sagheb-Talebi, K. 2010. Beech regeneration research: From ecological to silvicultural aspects. *For. Ecology and Management.* 259, 2172-2182. https://doi:10.1016/j.foreco.2010.02.029.

Wainhouse, D. 1980. Dispersal of first instar larvae of the felted beech scale, *Cryptococcus fagisuga*. *The J. of Appl. Ecology*. 17,523-532. https://doi:10.2307/2402634.

Wainhouse, D., & Gate, I. M. (1988). The beech scale. In Dynamics of forest insect populations (pp. 67-85). Springer, Boston, MA.

Walter, M., Stevenson, O.D., Amponsah, N.T., Scheper, R.W.A., Rainham, D.G., Hornblow, C.G., Kerer, U., Dryden, G.H., Latter, I., and Butler, R.C. 2015. Control of *Neonectria ditissima* with copper based products in New Zealand. *New Zealand Plant Protection.* 68, 241-249. https://doi:10.30843/nzpp.2015.68.5798.

Walter, M., Campbell, R.E., Amponsah, N.T., Scheper, R.W.A., and Butler, R.C. 2017. Evaluation of biological and agrichemical products for control of *Neonectria ditissima* conidia production. *New Zealand Plant Protection.* 1, 87-96.

Weber, R.W.S. 2014. Biology and control of the apple canker fungus *Neonectria ditissima* (syn. *N. galligena*) from a Northwestern European perspective. *Erwerbsobstbau.* 56, 95-107. https://doi:10.1007/s10341-014-0210-x

Wiggins, G.W., Grant, J.F., Eelbourne, W.C. 2001. *Allothrombium mitchelli* (Acari: Trombidiidae) in the Great Smoky Mountains National Park: Incidence, seasonality, and predation on beech scale (Homoptera: Eriococcidae). *Annals of the Entomological Society of America.* 94, 896-901. https://doi:10.1603/0013-8746(2001)094[0896:AMATIT]2.0.CO;2.

Wisconsin Department of Natural Resources (DNR). 2014. Management of beech bark disease in Wisconsin. Website.

https://dnr.wisconsin.gov/sites/default/files/topic/ForestHealth/beechBarkManagement.pdf Accessed 12/7/2020.

Woods, K.D., B. Davis, B.D. 1989. Paleoecology of range limits: Beech in the Upper Peninsula of Michigan. Ecology (Durham). 70, 681-696. https://doi:10.2307/1940219.

Wright, S. 1952. The genetics of quantitative variability. Quantitative Inheritance. Her Majesty's Stationery Office, London.

3 Beech Bark Disease in Northern Michigan after 20 years and Site Selection for a Resistant Tree Restoration Plan

3.1 Abstract

Beech bark disease (BBD) is threatening American beech in much of the eastern United States, and has been present in the western edge of American beech's range since at least 2001. Restoration activities focused around the enhancement of beech-scale resistant American beech can retain this ecologically important species in areas affected by BBD. The implementation of nucleated orchards in protected properties offers a minimally intensive solution to the problem of increasing resistant propagules to affected areas. Detailed ecological context is necessary to select planting sites for nucleated seed orchards with the greatest likelihood of success. We describe the disease and ground layer composition for two target National Park Lakeshore properties, and outline the methods for selection of appropriate planting sites. The target properties are estimated to be in differing phases of BBD, which complicates the planning process for the unified restoration plan. These selection methods are generalizable for selection of planting sites for restoration of tree species threatened by emergent diseases.

3.2 Key words

American beech, Nucleated orchards, Restoration planting

3.3 Implications

- Ecological context for two national park properties are presented, including disease description and composition
- Generalized methods and rationale are described for selecting appropriate planting sites for nucleation-based restoration in a temperate forest

3.4 Introduction

American beech (*Fagus grandifolia* Ehrh) is an ecologically important species in the eastern United States which is threatened by a number of health issues. Beechnuts drive food webs at multiple trophic levels, from rodents to bears (Faison & Houston 2004; Rosemier and Storer 2010; Jensen et al. 2012; Seger et al. 2013; Conrad & Reitsma 2015; Stephens et al. 2019). Beech snags serve an important role as nesting cavities and foraging sites for birds (Maurer & Whitmore 1981; Robb & Brookhout 1995; Lemaître & Villard 2005; Kahler & Anderson 2006; Holloway & Malcolm 2007; Tozer et al. 2012). Emerging pressures from Beech Bark Disease (BBD), climate change, and the recently described beech leaf disease (BLD), threaten to reduce American beech even further in the western edge of its range (O'Brien et al. 2001; Iverson et al. 2008; Burke et al. 2020).

3.4.1 Disease Phase Evaluation

Beech Bark Disease has been present in northern Michigan since at least 2001 and continues to cause significant mortality to American beech (O'Brien et al. 2001; Sanders & Kirschbaum 2019). This tree disease, made up of an insect and fungal component, occurs in three phases: the advance front, the killing front, and the aftermath front (Shigo 1972). Each phase of the disease is marked by relative amounts of the pathogenic organisms, and changes in new American beech mortality. In the advance front, much beech scale (*Cryptococcus fagisuga* L.) is present, but *Neonectria* (*Neonectria ditissima* Samuels & Rossman, *N. faginata* Castlebury) and beech mortality are at levels close to pre-arrival forests. In the killing front, both pathogenic organisms are present in high amounts, and beech mortality increases above regular levels. In the aftermath front, the pathogenic organisms are present in lower amounts, and beech mortality lessens, but remains at higher than background levels.

Detailed ecological context is important in planning restoration activities. The National Park Service has an established forest health monitoring plot network in northern Michigan at which they monitor general forest health issues, such as deer

browse (herbivory), earthworm impacts to the forest floor, and beech scale presence (Sanders & Kirschbaum 2019).While presence of BBD is reported on a broad scale, fine-scale information of beech bark disease impacts in stands or individual trees is not available, however it is known that the disease is present at both Pictured Rocks National Lakeshore (PIRO) and Sleeping Bear Dunes National Lakeshore (SLBE) in northern Michigan. In order to better understand the severity of BBD in these target properties, detailed surveys were performed in a number of forest types, and condition of all beech encountered was assessed. We compared the amounts of beech scale and beech mortality between the two properties to determine the disease phase which each are in, which may vary based on the amount of time BBD is present in an area, to allow for better understanding of the ecological context in which restoration activities can occur.

3.4.2 Site Selection for Restoration

A restoration plan is being developed for the two target properties, using local provenance, disease resistant, tree scions to establish nucleated seed orchards. Nucleated seed orchards have drawbacks compared to traditional seed orchards, but require little management and human input beyond their initial establishment (Corbin et al. 2016). The establishment of nucleated orchards, rather than traditional orchards, is more cost and labor-intensive up front, but potentially cheaper over extended periods, ensuring that restoration efforts will continue regardless of additional human input beyond the scope of the initial planting in the mitigation efforts (Zawahi et al. 2013; Holl & Zawahi 2018).

Nucleated seed orchards are the introduction of desirable species through cluster planting in the area targeted for restoration. This allows for dispersal of seed along existing pathways, especially in a case where the desirable species is reintroduced into an area where the target species is already challenged, such as underplanting for BBD mitigation, due to the presence of a predefined niche including wildlife dispersal vectors (Corbin and Holl 2012; Corbin et al. 2016). Nucleated seed orchards are often used in tropical forest restoration, but have not yet been frequently used in temperate forests (Boanares and Azevedo 2014).

The planting sites for nucleated seed orchards must be carefully selected. These plantings may occur under existing canopy, so it is possible to establish them without removal of overstory competition, however understory vegetation will compete with the planted trees, so trees should be planted with minimal competing vegetation, and removal of some understory vegetation will be necessary (Löf et al. 2012). This may be accomplished through physical methods such as weed pulling or tilling of soil, as chemical methods are inappropriate in wild systems due to the risk of lateral leaching, or mortality of non-target vegetation (Smith et al. 1997).

A description of the severity of BBD within the target properties of Pictured Rocks National Lakeshore and Sleeping Bear Dunes National Lakeshore is necessary to address the ultimate objective to create a plan for restoration and disease mitigation. Planting sites are selected based on desired structural characteristics and selected environmental data, with the goal of establishing nucleated seed orchards for young, resistant American beech.

3.5 Methods

3.5.1 Composition Surveys and Disease Description

Community composition data was recorded from mid-June to early August in 2017, 2018, 2019. Data was collected from Pictured Rocks National Lakeshore (PIRO) in 2017 and 2018, and from Sleeping Bear Dunes National Lakeshore (SLBE) in 2018 and 2019. A total of 88 plots in PIRO and 85 plots in SLBE were measured.

Forest composition and regeneration surveys were completed in 2017, 2018 and 2019 in forests types identified as containing American beech (Supplemental Table 4). Plots were established along a transect leading a cardinal direction away from a road edge or trail into the forest, at random length intervals between 40 and 99 meters ($40 \text{ m} \leq L \leq 99 \text{ m}$) selected from a random number table. At each plot location, coordinates were

taken and two fixed-area circular plots were established using the coordinate location as a plot center.

A 1.3 m radius circle was established on a center point and all woody regeneration above 10 cm and below 2 m in height and less than 2.3 cm diameter at breast height (DBH) were identified to species when possible. A variable radius plot was established at plot center and all trees counted as "in" with a 2m basal area factor (BAF) calibrated wedge prism were identified to species and DBH recorded. Any American beech encountered above 12.5 m DBH within 12.3 m of the plot center, or counted as "in" in the variable radius plot were assessed for vigor, a numerical scale representing overall tree health (Table 2). All species encountered are presented in Supplemental Table 5.

Every American beech with a DBH greater than or equal to 12.5 cm was assessed for additional health attributes. Two technicians independently assessed vigor score (Table 2), crown position, live crown ratio, and branch dieback from opposing sides of the tree, and scores were compared (Schomaker et al. 2007). Scale was measured at DBH as the percent of bole coverage visible in a 30 cm by 27 cm clear frame. Presence or absence of cankers or tarry spots were recorded on a whole tree basis, where one visible canker or tarry spot resulted in a score of "yes" for the tree.

Table 2. American beech vigor rating codes adapted and truncated from Petrillo et al., 2005.

Code	Criteria
1	Crown with relatively few dead twigs; foliage density and color normal; occasional small dead branches in upper crown; occasional large branch stubs on upper bole
2	Crown with occasional large dead branch in upper portion; foliage density below normal; some small dead twigs at top of crown; occasional large branch stubs on upper bole
3	Crown with moderate dieback; several large dead branches in upper crown; bare twigs beginning to show; several branch stubs on upper and mid bole
4	Approximately half of crown dead
5	Over half of crown dead
6	Tree dead; not cut, standing with fine twigs (less than 2.54 cm (1 in) in diameter) attached to branches
7	Tree dead (natural death); not cut; standing without fine twigs but still has some branches attached to bole of tree
8	Tree dead; standing but bole only, no branches attached to bole

All statistical analyses performed in R were completed with R build i386 3.5.1, using RStudio GUI (Venables and Ripley 2002; Kembel et al. 2010; R Core Team 2013; Wickham et al. 2019; Hebbali 2020). Packages included picante, vegan tidyverse, OLSRR, and MASS. To determine which phase of Beech Bark Disease each property was in, the amount of pathogenic organisms was compared using a one-sided t-test. A one-sided t-test was used to determine if overstory mortality influenced American beech regeneration in our target properties.

Stepwise regression (*stepAIC* in R 4.0.3) was used to find factors influencing the amount of woody regeneration (TPA) and mean American beech vigor. Regeneration

counts were square root transformed to normalize the data, to correct a right-tailed skew in data.

3.5.2 Planting Site Selection

Environmental and beech vigor data were used to create stepwise regression models. Environmental data (elevation, slope, aspect, average precipitation, average minimum winter temperature, and soil surface texture) were collected from publicly available spatial data for the state of Michigan. Richness and diversity of overstory composition were calculated based on composition data collected in 2017, 2018, and 2019. The direction and magnitude of factors returned as significant in the stepwise regression were used to select areas for planting from interpolated maps of the target properties. Desirable conditions were defined in accordance with silvicultural principles to be areas with low beech vigor (to create gaps for interplanting) and low regeneration (to reduce competition for the young resistant trees planted).

Regeneration was adjusted to trees per acre (TPA) counts using an area-based conversion factor. Regeneration TPA was interpolated using the Inverse Distance Weighting (IDW) tool in ARCMap 10.5.1. The IDW tool was chosen to account for spatial aggregation in the data collection points. Suggested planting sites were selected near roadside edges and parking areas, in areas with low slope, to allow access with equipment. At least one planting selection site at each property was chosen near a main parking area to facilitate public interaction, in accordance with the stakeholder's (National Park Service) identified goals.

3.6 Results

3.6.1 Composition Surveys and Disease Description

In sites which contained American beech, regeneration was greater in areas with beech mortality than those without ($t=3.66$, $df=87.9$, p-value < 0.01). The mortality of

American beech may simulate regeneration by root suckering. No data was recorded on the origin of regeneration, so while it is increased in areas heavily affected by BBD, we cannot determine whether root suckering or seed origin are the cause of the increase.

Both sites have an established BBD presence but an unknown disease phase (Sanders & Kirschbaum 2019; O'Brien et al. 2001), so we compared the disease organism prevalence and American beech mortality between the two properties with a Welch two-sample t-test, as the data were non-normally distributed. The lower scale intensity was observed at PIRO than SLBE, which was not significantly lower ($t = -0.08$, $df = 70.4$, p-value = 0.8). The proportion of dead trees was observed to be lower than in SLBE, which was significantly lower ($t = -6.17$, $df = 70.4$, p-value < 0.01).

The amount of scale observed on beech was not significantly different, but the proportion of present beech which had died was higher in PIRO than SLBE. This signifies that PIRO has entered the aftermath phase, which is marked by high levels of beech mortality, but moderate levels of scale and *Neonectria* fungus. While we observed higher levels of scale in SLBE, it was not significantly higher, therefore we describe SLBE to be in the late advance phase, marked by moderate, but increasing levels of scale and *Neonectria*, but low levels of beech mortality. We expect a rapid increase in the amount of beech mortality in coming years for SLBE, as it enters the killing phase.

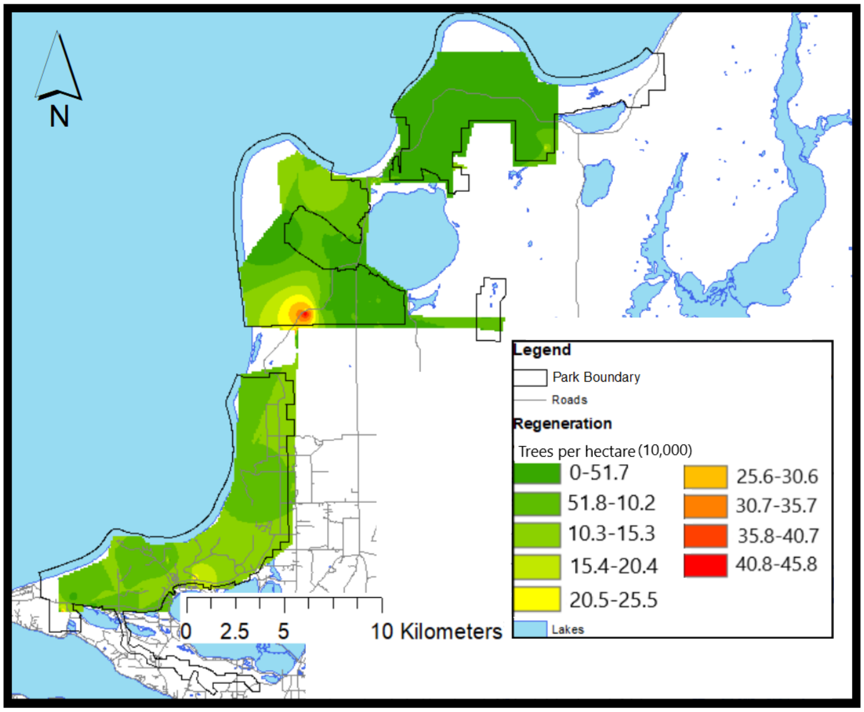
3.6.2 Planting Site Selection

Nonmetric multidimensional scaling (NMDS) ordination analysis was used to select average minimum temperature for analyses, due to the longer vector length than average maximum annual temperature (Supplemental Figure 3). The overall model had a low R^2 value (0.28) indicating a weak relationship (sqrt_Reg ~ DEM_MI + Slope_MI + PrecipInch + TempMin + Richness, Multiple $R^2 = 0.2785$; F: 11.74 on 5 and 152 df; p-value < 0.01.)

Increasing minimum temperature negatively influenced American beech vigor (p-value <0.01). This model also had a low R^2 value (0.31) indicating a weak relationship

(VigorMean ~ TempMin, Multiple $R^2 = 0.314$; F: 34.33 on 1 and 75 df, p-value < 0.01). IDW interpolation returned prediction surfaces of expected regeneration abundance in TPA (Figure 1). Planting sites were selected within areas with low expected regeneration, which fall within areas of higher elevation, precipitation, and minimum annual temperature (Figure 2).

Soil surface texture was not selected as an influential environmental factor in regeneration counts nor vigor. Planting sites were selected in areas with the most appropriate soil surface texture and flooding regime, based on life history traits of American beech.



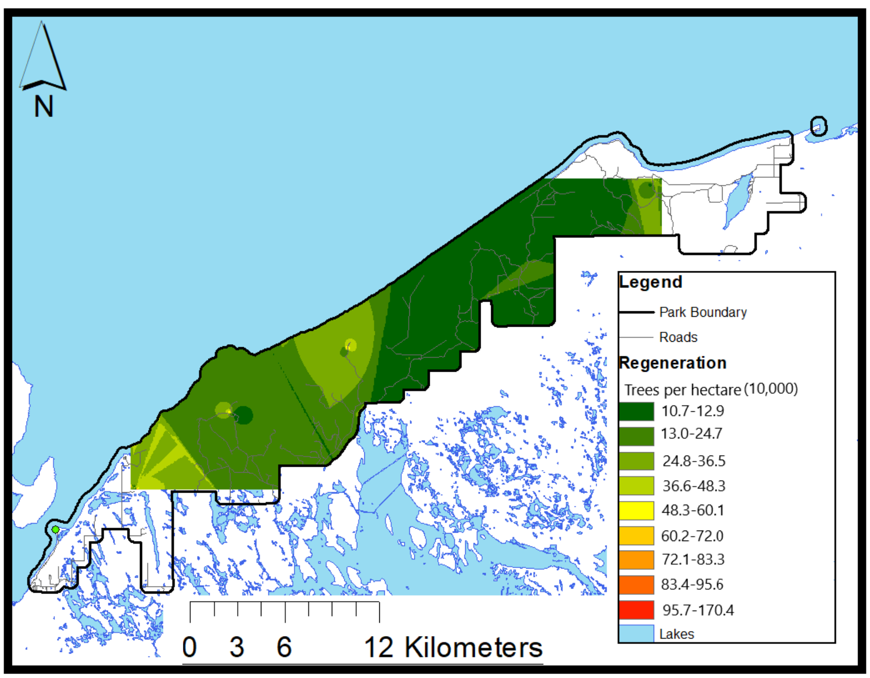


Figure 3. Regeneration interpolation surfaces in trees per hectare for Sleeping Bear Dunes National Lakeshore (above) and Pictured Rocks National Lakeshore (below). Inverse-distance weighting was used to interpolate expected regeneration counts in trees per hectare, based on surveys conducted in 2017, 2018 and 2019.

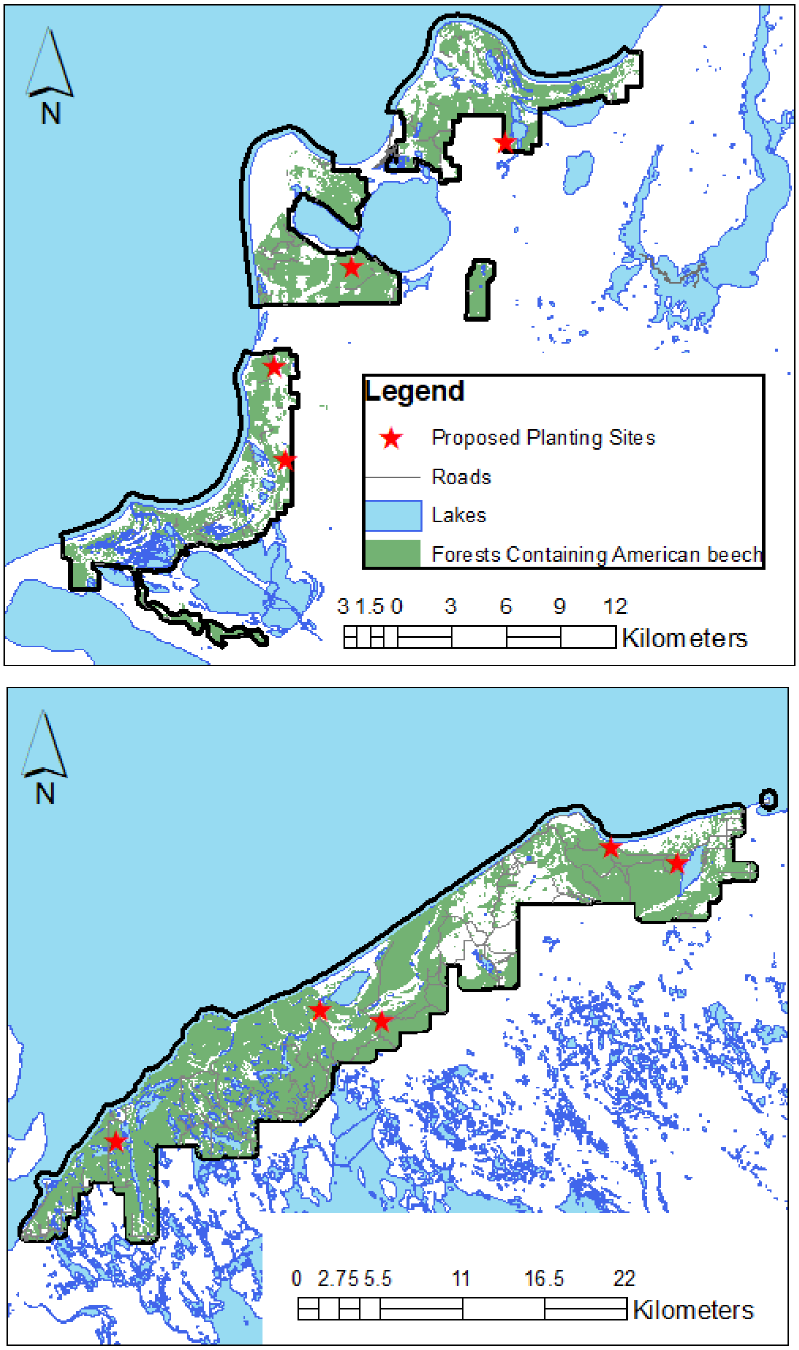


Figure 4. Proposed planting sites selected in Sleeping Bear Dunes National Lakeshore (above) and Pictured Rocks National Lakeshore (below). Forests containing beech were selected based on National Park Service internal composition surveys. Sites were selected based on projected environmental counts and proximity to roadsides. Lakes imported from national hydrography datasets via NRCS geospatial data gateway.

3.7 Discussion

3.7.1 Composition Surveys and Disease Description

We found support for our hypothesis that the two properties are in different phases of the disease, with PIRO in the aftermath front and SLBE in the late advance front. Both PIRO and SLBE had relatively similar levels of scale present, but PIRO had a higher proportion of overstory beech that had died. This agrees with Shigo's hypothesis of the three phase progression of BBD (Shigo 1972.)

These findings will affect the choice of restoration activities. In the future, as later stages of the work agreement occur, including final selection of orchard locations within the proposed sites, overstory gaps can be selected in PIRO based on current gap openings, as the killing front has passed and many canopy gaps currently exist. In SLBE, forecasting of future gap openings may be necessary, as overstory beech is declining, but has not yet occurred. Identifying beech that is declining rapidly may increase the availability of suitable of planting sites in SLBE. When ground-truthing the planting sites, beech of poor vigor should be identified in SLBE for underplanting, while current canopy gaps should be selected for PIRO.

Lower minimum temperature was associated with poor American beech vigor. While lower minimum temperatures can reduce scale populations, about 4 years of temperatures below -25°C (-13°F) to reduce populations, or -30°C (-22°F) are needed to eliminate populations in winter (Kasson and Livingston 2012). Average minimum temperatures in PIRO and SLBE are 0°C (31°F) and 2°C (36°F), respectively. Alger and Benzie counties, where the properties are located, reached minimum temperatures of only -1.7°C (-3.1°F) and -1.1°C (-1.9°F) from 2017 to 2019 (NOAA 2021), thus minimum temperatures in these properties are not low enough to limit scale populations. Additionally, minimum temperatures were higher closer to the Great Lakes shorelines, suggesting there may be unexplored environmental factors driving this change in vigor, but additional research is necessary to illuminate the relationships.

3.7.2 Planting Site Selection

The composition and disease severity surveys performed were used to select appropriate planting sites (Figure 2; Appendix C) for the nucleated seed orchards based on forest dynamic theory. Sites were sought that have overstory gaps of American beech due to BBD, to provide above-ground light resources for the nucleated seed orchards. Sites were also selected with minimal woody regeneration, to reduce competition for below-ground resources and minimize site preparation and disturbance to forest communities. The freed resources described combine to provide growing space for the planted trees to occupy (Oliver & Larson 1996).

Sites were also selected for nearby confirmed or putatively resistant American beech, in order to increase the likelihood of resistant tree pollen to reach the trees. Under complex canopies, European beech (*Fagus sylvatica*) pollen can travel about 60 to 160 meters (Millerón et al. 2012). Where ecological information is missing for American beech, information for European beech is often substituted (Bonner & Leak 2008), so we assume the average dispersal distance of American beech under forest canopy is about 100 meters.

While influential environmental factors were identified, the strength of their influence was low based on the low R^2 value of the analysis. This is perhaps due to the low variability of factors within the target properties. As both properties cover a large amount of suitable forested land (22,864.8 ha in PIRO; 14,443.13 ha in SLBE) by their nature as National Lakeshore properties, the shape of the property boundaries follows a long contour of the natural boundaries of Lake Michigan and Lake Superior. The inland, forested portions of the parks are relatively narrow, naturally excluding a large amount of the park from suitable planting area (forested, with existing American beech-containing forest types). This long, narrow geographic range results in a narrow range of independent variable values. We have recommended the best planting sites considering ecological context and stakeholder's objectives for American beech within this range (Table 3).

Table 3. Summary of conditions at proposed site locations for planting young beech bark disease resistant trees in nucleated seed orchards. Symbol + indicates desirable conditions, - indicates undesirable conditions, / indicates neither desirable nor undesirable conditions.

Proposed Planting Site Location	Slope	Soil	Regeneration	Forest Type	Parking Area
Sleeping Bear Dunes National Lakeshore					
Wilco Road	/	+	+	+	+
Burnham Road	+	/	+	+	-
School Lake Road	+	-	+	+	-
Fowler Road	+	-	/	+	-
Pictured Rocks National Lakeshore					
Beaver Basin Overlook	+	+	/	+	-
Little Beaver Lake Campground	+	+	-	+	-
Grand Sable Lake	+	+		+	-
Miners Falls Trailhead	+	+	-	+	/
Log Slide Overlook	+	+	/	+	+

Additionally, the properties are located near the western edge of American beech's current distribution (Tubbs & Houston 1990). The soil surface texture where beech exists within the properties is not indicated as ideal for American beech. While optimal conditions would ensure the strongest success for the restoration planting, based on life history traits the 'ideal conditions' for beech do not occur simultaneously within the target properties. This may explain the tendency of beech to occur as a single

dominant or co-dominant species within the parks, rather than a monotypic dominant, pure-stand tree as occurs in the eastern parts of the range.

We have focused on the selection of sites with low beech regeneration (avoiding beech thickets) to minimize site preparation and potential impacts to surrounding vegetation at proposed planting sites. Proposed treatments for the sites are limited to mechanical measures for the same reasons. Acceptable methods of site preparation for the stakeholder include scarification to reduce grass and sedge competition, and uprooting and removal of woody vegetation. Small-scale scarification will facilitate planting by loosening any compacted soils and removal of rocks from the planting site. Underplanting should eliminate the need for wind protection of young trees, and sites are selected to eliminate the need for hydrologic interference. Planted trees will need intervention from rodents, which can be accomplished simply through the application of tree collars at the time of planting, and the removal of surrounding grasses and sedges. Tree collars should remain in place until trees are large enough to resist rodent girdling and tall enough to escape deer browse.

While interpolation was used to estimate regeneration at the selected sites, additional ground surveys will be required to confirm suitability of the sites for planting. Wandering surveys to locate as many resistant trees as possible were conducted in SLBD by NPS personnel, and a similar effort at PIRO will enhance recommendations for optimal planting selection sites.

Additional work continues on this restoration project to enhance the likelihood of success. Specific timing and techniques for planting will be identified which are appropriate to meet stakeholder needs and the ecological demands of American beech. While general activities are proposed in this article, little is published about the specific handling, propagation management, and transplanting needs of American beech for underplanting, thus planting trials and monitoring of the initial nucleated seed orchards are recommended.

Creating nucleated seed orchards will meet stakeholder objectives for restoration activities to mitigate beech bark disease impacts on NPS national lakeshores. Nucleated orchards may be implemented with a minimum of disruption to existing forests, and have high short term investment to establish, but relatively low long term investment to ensure success. Careful consideration of ecological context must be made for selection of planting sites to ensure success. Future work is necessary to confirm that selected sites meet the projections made with interpolation and continue with the next stages of planting disease resistant trees.

3.8 Acknowledgements

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3.9 Literature Cited

Boanares D, Azevedo CSd (2014) The use of nucleation techniques to restore the environment: a bibliometric analysis. *Natureza & conservação* 12: 93-98

Bonner FT, Leak WB (2008) *Fagus* L. Pages 520-524 In: The Woody Plant Seed Manual. Bonner FT, Karrfalt RP (eds) Agric. Handbook No. 727. Washington, DC. US Department of Agriculture, Forest Service. 1223 p., 727.

Burke DJ, Hoke AJ, Koch J (2020) The emergence of beech leaf disease in Ohio: Probing the plant microbiome in search of the cause. *Forest Pathology* 50: e12579.

Conrad CA, Reitsma L (2015) Demographic responses if myomorph rodents to mast production in a beech- and birch-dominated northern hardwood forest. *Northeastern Naturalist* 22: 746-761

Corbin JD, Holl KD (2012) Applied nucleation as a forest restoration strategy. *Forest Ecology and Management* 265: 37-46

Corbin JD, Robinson GR, Hafkemeyer LM, Handel SN (2016) A long-term evaluation of applied nucleation as a strategy to facilitate forest restoration. *Ecological Applications* 26: 104-114

Faison EK, Houston DR (2004) Black bear foraging in response to beech bark disease in northern Vermont. *Northeastern Naturalist* 11: 387-394

Hebbali A (2020) olsrr: Tools for Building OLS Regression Models. R package version 0.5.3. https://CRAN.R-project.org/package=olsrr

Holl KD, Zahawi RA (2018) Applied nucleation is a straightforward, cost-effective forest restoration approach: reply to Ramírez-Soto et al. *Restoration Ecology* 26: 618-619

Holloway GL, Malcom JM (2007) Nest-tree use by northern and southern flying squirrels in central Ontario. *Journal of Mammalogy* 88: 226-233

Hop K, Lubinski S, Dieck J, Menard S, Drake J (2011) National Park Service Vegetation Inventory Program: Sleeping Bear Dunes National Lakeshore, Michigan. Natural Resource Report NPS/GLKN/NRR—2010/201. National Park Service, Fort Collins, CO, 1-15

Hop K, Menard S, Drake J, Lubinski S, Dieck J (2010) National Park Service Vegetation Inventory Program: Pictured Rocks National Lakeshore, Michigan. Natural Resource Report NPS/GLKN/NRR—2010/201. National Park Service, Fort Collins, CO, 1-15

Iverson LR, Prasad AM, Matthews SN, Peters M (2008) Estimating potential habitat for 134 eastern US tree species under six climate scenarios. *Forest Ecology and Management* 254: 390-406

Jensen PG, Demers CL, Mcnulty SA, Jakubas WJ, Humphries MM (2012) Marten and fisher responses to fluctuations in prey populations and mast crops in the northern hardwood forest. *The Journal of Wildlife Management* 76: 489-502

Kahler HA, Anderson JT (2006) Tree cavity resources for dependent cavity-using wildlife in West Virginia forests. *Northern Journal of Applied Forestry* 23: 114-121

Kasson M, Livingston W (2012) Relationships among beech bark disease, climate, radial growth response and mortality of American beech in northern Maine, USA: Relationships among beech bark disease, climate, growth, & beech mortality. *Forest Pathology* 42: 199–212

Kembel SW, Cowan PD, Helmus MR, Cornwell WK, Morlon H, Ackerly DD, Blomberg SP, Webb CO. (2010) Picante: R tools for integrating phylogenies and ecology. *Bioinformatics* 26:1463-1464

Lemaître J, Villard MA (2005) Foraging patterns of pileated woodpeckers in a managed Acadian forest: a resource selection function. *Canadian Journal of Forest Research* 3510: 2387-2393

Löf M, Dey DC, Navarro RM, Jacobs DF (2012) Mechanical site preparation for forest restoration. *New Forests* 43: 825-848

Millerón M, López de Heredia U, Lorenzo Z, Perea R, Dounavi A, Alonso J, Gil L, Nanos N (2012) Effect of canopy closure on pollen dispersal in a wind-pollinated species (*Fagus sylvatica* L.). *Plant Ecology* 213: 1715–1728

Maurer BA, Whitmore RC (1981) Foraging of five bird species in two forests with different vegetation structure. *The Wilson Bulletin* 93: 478-490

NOAA (2021) National Centers for Environmental information, Climate at a Glance: County Time Series http://www.ncdc.noaa.gov/cag/ (accessed 3 March 2021)

O'Brien J, Ostry M, Mielke M, Mech R, Heyd R, McCullough D (2001) First Report of Beech Bark Disease in Michigan. *Plant Disease* 85: 921–921

Oliver CD, Larson BC (1996) Plant Interactions and Limitations of Growth. Pages 9-40 In: Forest Stand Dynamics. Wiley, New York, N.Y.

Petrillo H, Witter JA, Thompson EM (2005) Michigan beech bark disease monitoring and impact analysis system. Beech bark disease: Proceedings of the beech bark disease symposium. US For. Serv. Gen. Tech. Rep. NE-331. US For. Serv., Northeast. Res. Stn., Newtown Square, PA, 2005.

R Core Team (2017) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Robb JR, Brookhout TA (1995) Factors influencing wood duck use of natural cavities. *The Journal of Wildlife Management* 59: 372-383

Rosemier JN, Storer AJ (2011) Assessing native small mammals' responses to an incipient invasion of beech bark disease through changes in seed production of American beech. In: Fei, Songlin; Lhotka, John M.; Stringer, Jeffrey W.; Gottschalk, Kurt W.; Miller, Gary W., eds. Proceedings, 17th central hardwood forest conference; 2010 April 5-7; Lexington, KY; Gen. Tech. Rep. NRS-P-78. Newtown Square, PA: US Department of Agriculture, Forest Service, Northern Research Station: 675-676. Vol. 78. 2011.

Schomaker ME, Zarnoch SJ, Bechtold WA, Latelle DJ, Burkman WG, Cox SM (2007) Crown-Condition Classification: A Guide to Data Collection and Analysis. USDA Forest Service Southern Research Station General Technical Report: SRS-102

Sanders S, Kirschbaum J (2019) Forest Health Monitoring at Sleeping Bear Dunes National Lakeshore. Natural Resource Data Series NPS/GLKN/NRDS – 2019/1241

Shigo AL (1972) The beech bark disease today in the northeastern US. *Journal of Forestry* 70: 286-289

Seger RL, Servello FA, Cross, RA, Keisler DH (2013) Body mass and mast abundance influence foraging ecology of the American black bear (*Ursus americanus*) in Maine. *Canadian Journal of Zoology* 91: 512-522

Smith DM, Larson BC, Kelty MJ, Ashton MS (1997) Preparation and Treatment of the Site. Smith DM, Larson BC, Kelty MJ, Ashton MS (eds) Pages 195-233 In: The Practice of Silviculture. Wiley, New York, N.Y.

Stephens RB, Hobbie EA, Lee TD, Rowe RJ (2019) Pulsed resource availability changes dietary niche breadth and partitioning between generalist rodent consumers. *Ecology and Evolution* 9: 10681-10693

Tozer DC, Burke DM, Nol E, Elliott KA (2012) Managing ecological traps: logging and sapsucker nest predation by bears. *The Journal of Wildlife Management* 76: 887-898

Tubbs CH, Houston DR (1990) *Fagus grandifolia* Ehrh. American beech. Pages 325-332 In: Burns RM, Honkala BH (tech. coords.) Silvics of North America. Agriculture Handbook 654.U.S. Department of Agriculture, Forest Service, Washington, D.C.

Venables WN, Ripley BD (2002) Modern Applied Statistics with S. Fourth Edition. Springer, New York. ISBN 0-387-95457-0

Wickham H, Averick M, Bryan J, Winston C, McGowan LD, François R, Grolemund G, Hayes A, Henry L, Hester J, Kuhn M, Pedersen TL, Miller E, Bache SM, Müller K, Ooms J, Robinson D, Seidel DP, Spinu V, Takahashi K, Vaughan D, Wilke C, Woo K, Yutani H (2019) Welcome to the tidyverse. *Journal of Open Source Software* 4: 1686, https://doi.org/10.21105/joss.01686

Zahawi RA, Holl KD, Cole RJ, Reid JL (2013) Testing applied nucleation as a strategy to facilitate tropical forest recovery. *Journal of Applied Ecology* 50: 88-96

Table 4. All forest types within two National Park Service properties in northern Michigan containing American beech as a component. (Hop et al., 2011, Hop et al., 2010.)

Forest Type	**Map Code**
Pictured Rocks National Lakeshore	
Eastern hemlock - American beech - (sugar maple) Great Lakes forest	FHB
Eastern hemlock - (yellow birch) forest	FHC
Eastern hemlock - sugar maple - yellow birch forest	FHM
Jack Pine / blueberry species / feathermoss forest	FJB
Jack pine / balsam fir forest	FJF
Jack Pine - quaking aspen / northern bush-honeysuckle Forest	FJM
Jack pine - red pine - eastern white pine dune forest	FPD
Eastern white pine - eastern hemlock great lakes forest	FWH
Eastern white pine - (red pine) - northern red oak forest	FWO
Maple - yellow birch northern hardwoods forest (classic phase)	FMB
Maple - yellow birch northern hardwoods forest (yew phase)	FMY
Maple - yellow birch northern hardwoods forest (balsam fir shrub phase)	FMF
Maple - yellow birch northern hardwoods forest (sugar maple phase)	FMM
Sleeping Bear Dunes National Lakeshore	
Sugar maple - American beech - birch species / Canada Mayflower Forest	FBM
Quaking aspen - paper birch - (red maple, bigtooth aspen) forest	FBR
Eastern hemlock - American beech - (sugar maple) Great Lakes forest	FHB
Eastern hemlock - (yellow birch) forest	FHC
Sugar maple - white ash - American basswood / mountain maple / blue cohosh forest	FMA
Northern red oak - sugar maple - (yellow birch) forest	FOM
Eastern white pine - (red pine) - northern red oak forest	FWO

Table 5. Tree species encountered in forest composition surveys within two National Park Service properties, (Pictured Rocks National Lakeshore and Sleeping Bear Dunes National Lakeshore) in forest types containing American beech (*Fagus grandifolia*) as a component.

Species name	Common Name	Authority	PIRO	SBD
Abies balsamea	Balsam fir	L.	X	
Acer pensylvanicum	Striped maple	L.	X	X
Acer rubrum	Red maple	L.	X	X
Acer saccharinum	Silver maple	L.	X	
Acer saccharum	Sugar maple	Marshall	X	X
Betula alleghaniensis	Yellow birch	Britton	X	
Betula papyrifera	Paper birch	Marshall	X	X
Cornus florida	Flowering dogwood	L.		X
Fraxinus americana	White ash	L.		X
Fraxinus nigra	Black ash	Marshall		X
Fraxinus spp.	Fraxinus spp.[1]			X
Hamamelis virginiana	Common witch hazel	L.		X
Lonicera tatarica	Tatarian honeysuckle	L.		X
Ostrya virginiana	Eastern hophornbeam	Mill.	X	X
Picea glauca	White spruce	Voss	X	
Picea mariana	Black spruce	Mill.	X	
Pinus banksiana	Jack pine	Lamb.	X	
Pinus resinosa	Red pine	Aiton	X	X
Pinus strobus	Eastern white pine	L.	X	X
Populus grandidentata	Bigtooth aspen	Michx.		X
Prunus americana	American plum	Marshall		X
Prunus serotina	Black cherry	Ehrh.	X	X
Prunus virginiana	Choke cherry	L.		X
Quercus alba	White oak	L.		X
Quercus rubra	Northern red oak	L.		X

Robinia pseudoacacia	Black locust	L.		X
Sambucus racemosa	Red-berried elder	L.	X	
Thuja occidentalis	Northern white cedar[2]	L	X	
Tilia americana	American basswood	L.		X
Tsuga canadensis	Eastern hemlock	L.	X	X
Ulmus americana	American elm	L.		X

[1] Fraxinus spp. label was given to overstory ash which had living leaves high off the ground with no ability to inspect leaf scar/vascular bundle count; or trees which were dead with no identifiable characteristics

[2] Alternative common name: arborvitae

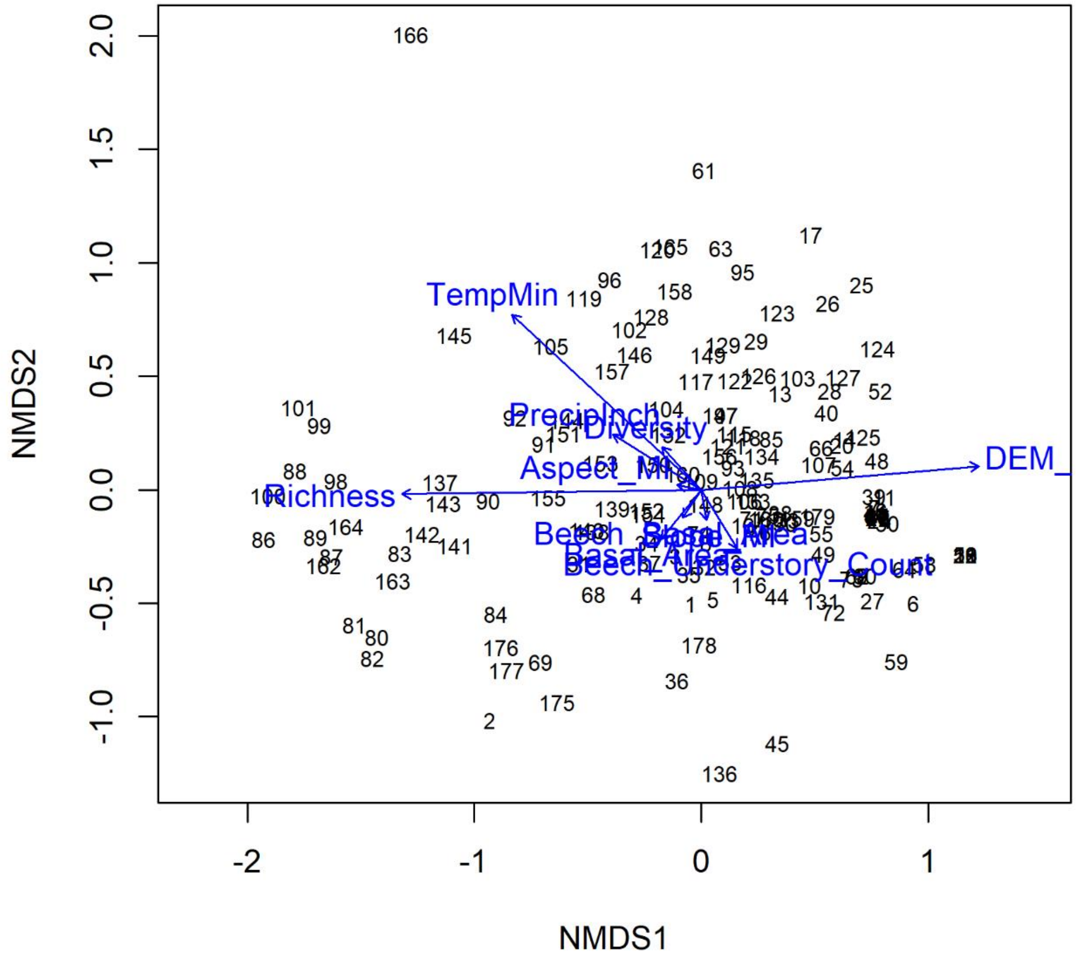

Figure 5. In the nonmetric multidimensional scaling (NMDS) ordination analysis, no convergence of factors related to beech regeneration was reached. Increasing elevation, precipitation, minimum annual temperature, and overstory richness negatively influence regeneration TPA (p-values <0.01, <0.01, <0.01, and <0.01). Increasing slope had a weak positive influence on regeneration (p-value = 0.13).

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

4.1 Abstract

A grafting program for creation of beech bark disease (BBD) resistant American beech (*Fagus grandifolia*) has been established in a cooperative agreement between Michigan Technological University and the National Park Service. The resistant trees will be used in a restoration project at Pictured Rocks National Lakeshore and Sleeping Bear Dunes National Lakeshore. This report details the methods for successful grafting of American beech to preserve knowledge, including a plain language, illustrated manual for grafting of American beech and scion collection from forest trees. Suggestions for establishing and refining a grafting program are presented. Rates of resistance are quantified for the target properties. Resistant trees were located and confirmed through field challenge tests to serve as scion donors for the grafting program. This report will inform future work as the project enters a Phase II continuation.

4.2 Introduction

Beech bark disease (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. 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 and the Michigan State University Extension Office (McCullough et al., 2001; Heyd, 2005). The US Forest Service has created resistant seed orchards through grafting of resistant trees (Koch et al., 2015). 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 (Houston, 1982; Heyd, 2005; Ramirez et al., 2007; Koch et al., 2015).

In order to enhance the proportion of American beech which are resistant to scale infestation, some type of propagation must occur, which creates 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% to 30% (Carey et al., 2013; Ramirez et al., 2007, respectively). 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 been reported to increase success rates to 57% (Carey et al., 2013).

Grafting success rates can vary widely based on a number of factors. Genotype of scion donor tree or rootstock family can affect rates, with some trees being particularly good or particularly bad donors (Carey et al., 2013). The type of graft used, and the caliper 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 is very thin; European beech, *Fagus sylvatica* displays only about 3-5 cell thickness of cambium layers in the dormant season (Oladi et al., 2011), necessitating a very precise layering of wood for successful unions. Grafting is a technically challenging process and requires skilled technicians, even in applications which use tools to simplify the process.

Beech bark disease- or BBD-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 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 the studied properties, however due to relatively lower dominance of beech, we expect to find fewer resistant trees in total.

In collaboration with the National Park Service, a grafting program to create BBD-resistant American beech was developed and propagation techniques refined, with the intention of restoration in BBD impacted areas, using local provenance genotypes from Sleeping Bear Dunes National Lakeshore and Pictured Rocks National Lakeshore, Michigan. Methods were identified using peer-reviewed literature, and adjusted in order to approach reported success rates established at other facilities. Alterations to methods were identified through trial-and-error and a working partnership with the US 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.

4.3 Methods

4.3.1 Identification and Testing of Resistant Trees

Straight transects were walked from random starting points throughout the parks, beginning at roadsides and trail edges. Lengths of transects were variable, and putatively resistant American beech were identified within 12.5 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 the trees identified during transect surveys at both properties in 2017-2019. Straight transects of varying length were performed in PIRO, and SLBE. Additional wandering surveys of variable length were performed by NPS personnel in Sleeping Bear Dunes

National Lakeshore. In wandering surveys, technicians entered from roadsides and walked at random with a GPS tracklog of their location recorded. If a beech was visible while walking these wandering surveys, it was approached and visibly assessed for presence of scale using binoculars to examine the entire bole. In order 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 per area present in the parks.

Putatively resistant trees were subjected to field resistance testing to confirm resistance to scale infestation (Houston, 1983; Koch and Carey, 2014). Scale eggs were collected from beech trees in August 2019. To collect eggs, the white mass was collected from the bole of infested beech trees. A 2-inch paintbrush was used to gently brush the bole of a tree with a visible heavy infestation of scale. The wax, insects, and eggs were collected in a plastic bag and stored in cool conditions for no more than 2 weeks. During these two weeks, the mass was gently pressed through 250 micron fine mesh sieves. This process allows separation of the eggs into a fresh container. A volumetric estimate standard was used to count out 500 eggs. This quantity (about 500 eggs) were 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 (Figure 4). 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 (Figure 5). A "Do Not Remove" message, the scientific permit number, and contact information was clearly written on each housewrap, clearly visible to park patrons. 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. An additional 5 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.

Figure 6. Field challenge testing pad applied to bole of a putatively resistant American beech. Approximately 500 beech scale eggs are applied to the underside of a damp sponge. The sponge is attached with coated wire so that the flat side makes contact with the bole of the tree.

Figure 7. Entire field challenge testing apparatus applied to bole of a putatively resistant American beech. A square of vapor-permeable housewrap is applied over the damp sponge layer, using waterproof silicone caulk, on three sides. The bottom is left open to allow drainage. The research permit number and contact information for researchers with a “Do Not Remove” message was written with sharpie on the apparatus at all locations.

After 52-57 weeks, foam pads were removed and the pad and bark surfaces were carefully inspected for presence of scale. The number of egg clusters and adults were recorded, and trees which had low scale infestations were retained in the database, but not considered suitable as scion donors. Trees were considered resistant if no more than one adult and no egg clusters were present on the bole of the tree or the foam pad. Area controls were considered successful if scale egg clusters and multiple adult scales were present.

4.3.2 Propagation

4.3.2.1 Sourcing Materials

Grafting BBD-resistant scions was initially limited by availability of commercial bare root American beech seedlings, so rootstocks were sourced from a variety of supplies. Bare root seedlings (45-60 cm tall) were purchased commercially when available. Although seedlings were purchased from an in-state grower, supply issues at that nursery necessitated shipments from unknown provenance to be mixed with the local rootstock. In addition, scale arrived on a mixed shipment of rootstock, necessitating treatment. Trees which had 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, and then treated with diatomaceous earth to prevent reinfestation by other pests.

Rootstock was also excavated from the Hiawatha National Forest under a non-commercial use permit. Survival and vigor of excavated rootstock equaled or surpassed commercially available rootstock (Myers et al., in preparation). Additional rootstock of mixed Wisconsin and Michigan provenance was donated to the project by the Oconto River Seed Orchard (United States Forest Service) under an ongoing work agreement.

Scions were collected from tested resistant trees in March 2020, November 2020, and March 2021. Scion branches were removed from the outer canopy of resistant

American beech trees using an arborist slingshot and manual chain saw in November after full senescence, or in March before trees began bud flush. An illustrated manual describing scion collection procedures is presented in Appendix A.

4.3.2.2 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 (Figure 6. The modified side graft was taught at an in-person grafting blitz hosted by the US 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. An illustrated manual describing grafting step by step procedures is presented in Appendix B.

In all grafting, a scion was selected that was no larger in diameter than the rootstock, but as close in size as possible. The scion, rootstock and all grafting tools were sanitized using an 80% ethanol solution applied with a sterile wipe. The scion should display healthy, full buds. After sanitizing the straight internodal section, the scion was trimmed so that it had between 3 and 5 healthy buds, with a strong preference for new growth. The trimmed scion was dipped from the distal end in a warm wax bath, not exceeding 55° C, to drive excess moisture from the cut end, which was dabbed away with a sterile absorbent wipe, such as a kimwipe. The scion was trimmed and dipped immediately before grafting.

In a veneer graft, the scion is cut into a wedge with two long, smooth strokes. Then a thin, long piece of bark, or veneer, is cut from the side of the rootstock to match the diameter of the scion wedge. The scion is securely laid against the rootstock, with the cambium layers aligned. The veneer flap is laid along the outer edge of the wedge and secured in place with a grafting rubber. A sap drawer should be retained on the rootstock, and the wax nicked to allow water pressure to escape and prevent lifting of the graft (Figure 7A).

In a modified side graft, the first cut is a single vertical cut of at least one inch, near the center of the scion. The wood is cut off with a shoulder from the scion. A short wedge is created with an additional cut. The scion should be sized up to the root stock. A single vertical cut is made in the rootstock to match the length and width of the scion cut. A flap is retained in the rootstock to match and slightly exceed the length of the wedge cut. The scion should be tightly fitted against the rootstock so that the cambium on at least one side matches up exactly. A grafting rubber should be firmly wrapped around the graft union, with enough space to allow for callus tissue to expand through. A sap drawer should be retained on the rootstock, and the wax nicked to allow water pressure to escape and prevent lifting of the graft (Figure 7B).

In top cleft grafting using a tool, a v-shaped blade is used to completely remove the top of the rootstock, and a scion which matches the diameter exactly is cut and wedged into the rootstock using the same v-shaped blade. While the use of a grafting tool removes the necessity of making manual cuts, the grafting tool is difficult to maintain, and the rootstock and scion must be relatively large (over 1 cm diameter) to allow clean cuts to occur. In manual top cleft grafting, a rootstock and scion of closely matching diameter are selected. The apical portion of the rootstock is removed with shears, and a single vertical cut is made in the rootstock. The scion is cut to a wedge with two long, oblique cuts. The scion is inserted into the rootstock so that the cambium layers touch, and a grafting rubber is applied to the graft (Figure 7C). Grafting rubbers should be wrapped to allow expansion of callus tissue to occur. In this graft, no sap drawer is possible.

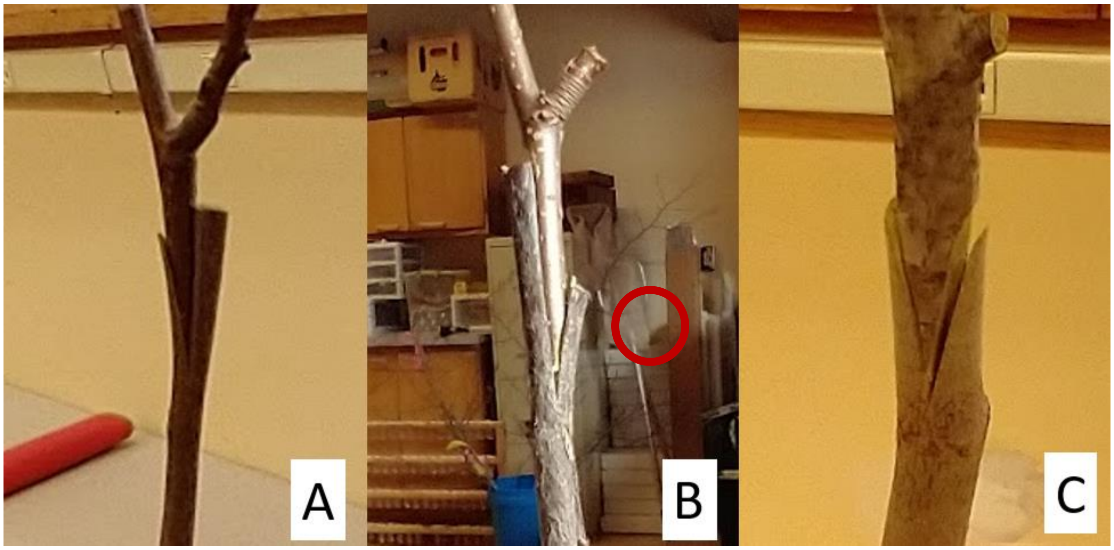

Figure 8. Grafts suitable for American beech. A. Veneer graft. B. Modified side graft. C. Top cleft machine graft. The sap drawer is indicated with a red circle. Some paraffin wax should be removed from the cut surface of the sap drawer before placement in the hot callus chamber.

Immediately after grafting, trees were placed in a hot callus apparatus. The ambient temperature of a dark room was kept at 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, which will appear as a yellow to green mass, often below the wax surface, notable for its swollen appearance (Figure 8, 9).

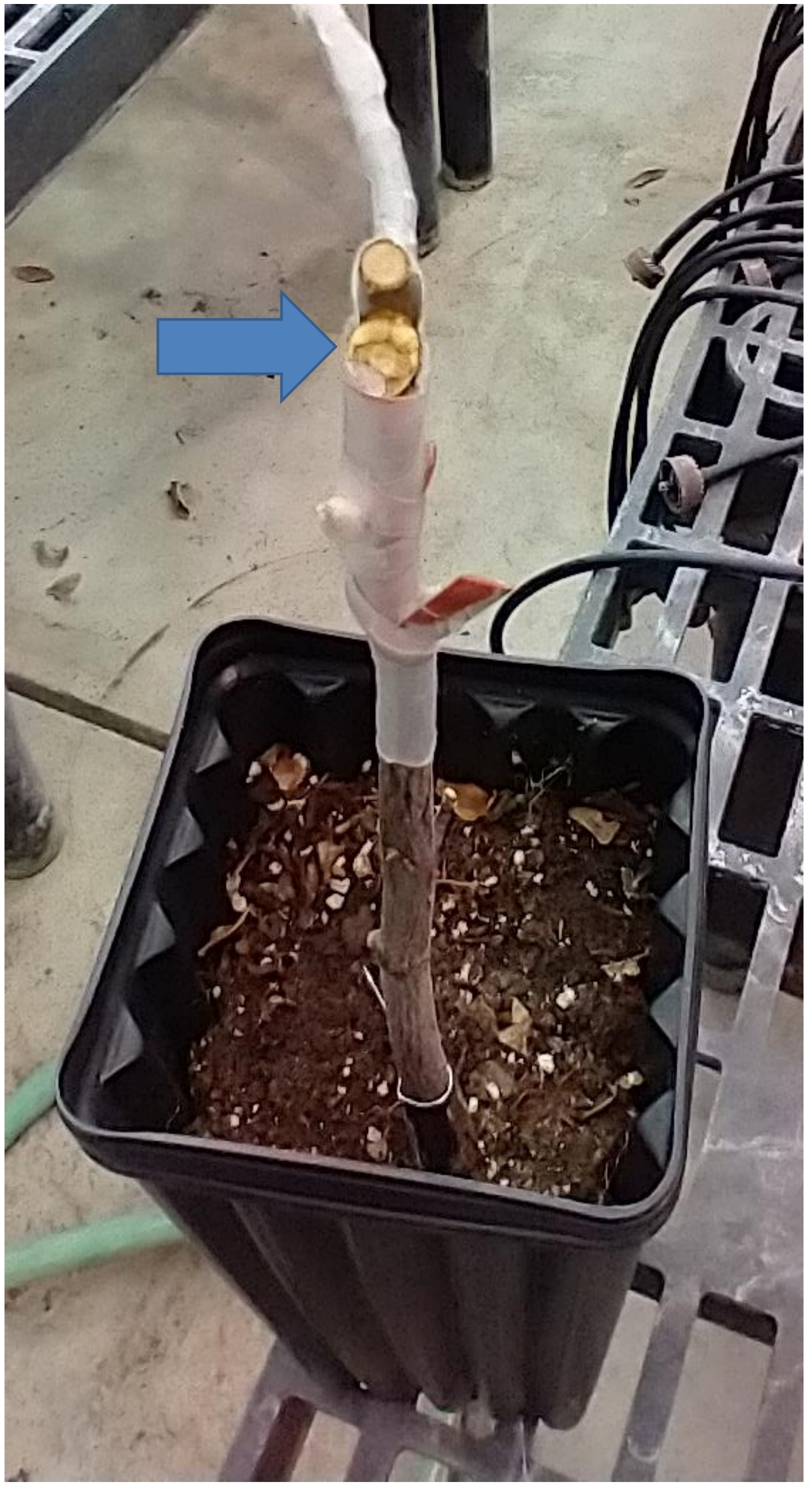

Figure 9. A grafted tree partially healed from the hot-callus apparatus. The arrow indicates growth of callus tissue, a yellow to greenish welling forming at the site of the graft union.

Figure 10. Callus tissue forming under the wax coating of a grafted tree. Although the wax is obscuring the graft union, the yellowish callus tissue is just visible, indicated by the arrow.

When callus tissue appeared, the trees were moved to an indoor, mixed-use greenhouse. Trees were lightly watered at this time and allowed to continue to heal. Overwatering the grafted trees before complete healing of the graft union may result in water pressure causing lifting and failure of the graft union. Rubbers were removed after callus tissue expansion, after the callus is visible around the majority of the union. A number of failed early grafts were attributed to twisting of a heavy scion, so plant stakes are recommended to be used to provide support scaffolding to prevent twisting, which results in lifting and failure of the graft union to heal.

4.3.2.3 Beech Maintenance

Prior to use as rootstock, beech were maintained in a mixed-use greenhouse during the late winter to mid-summer. In mid-summer, trees were moved to an outdoor space to prevent scorching by high greenhouse temperatures. In the mixed-use greenhouse, trees were watered twice a day for 3 minutes. Outdoors, trees were watered to saturation two to three times a week to maintain moderately moist soils.

The rootstock beech were treated with Neem oil in summer 2018 to remove a spider mite infestation. In spring 2019, rootstock trees were treated with diatomaceous earth to prevent further spider mite and armored scale infestations (common greenhouse pests). In the winter, rootstocks outdoors were heeled in under mulch to prevent roots from freezing. After grafting, beech are maintained in a mixed-use greenhouse in the winter and spring, and moved outdoors when temperatures rise in early June, to prevent scorching in high greenhouse temperatures. Beech are placed in a sheltered outdoor area with moderate shade, and watered deeply and allowed to drain between rain events to maintain damp soils, but prevent waterlogging. Grafted trees are moved indoor before the first projected frost event, and allowed to go dormant in the greenhouse.

4.3.2.4 Refinement of Methods

Success of grafts was determined as leaf out and survival of the scion tissue and growth of the portion above the graft. Failure codes were assigned to grafts completed and housed in Michigan Tech facilities (Table 6). No codes were assigned to failures of plants maintained in collaborator facilities at the United States Forest Service Oconto River Seed Orchard, however survival rates are presented. Trends in failure codes were analyzed to provide guidance towards refinement of methods.

Table 6. Codes of graft failure attribution.

Code	Failure
0	No failure; survival
1	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

4.4 Results

4.4.1 Resistant Trees

Trees were considered resistant if they harbored no scale egg clusters 52-57 weeks after application of the artificial infestation. Some trees had low numbers of egg clusters present, thus these trees were not classified as resistant. 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. GPS coordinates of resistant trees have been recorded and shared for internal use, but are not to be 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.

Wandering surveys performed in 2017 covered 242 ha of National Park Service properties where collaborators identified 68 putatively resistant trees after an initial survey, but only 24 trees had escaped scale infestation upon a second survey, or 0.9 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 are unknown for these surveys.

Transect surveys performed by Michigan Tech personnel in 2017, 2018 and 2019, covered an additional 13.8 ha. This effort identified 4 additional putatively resistant trees, or 0.28 disease-free trees per ha in PIRO. Of the 29 trees tested that had multi-year absence of scale reported in PIRO, 27 were confirmed resistant. In Sleeping Bear Dunes, 91 beech were encountered during transect surveys, and 5 were putatively resistant (5.49%). None of the trees identified had escaped scale infestation upon revisiting (0%). In Pictured Rocks National Lakeshore, 186 American beech were encountered during transect surveys, and 4 were disease-free (2.1%). After revisiting the sites, 4 trees were tested, and 3 were confirmed resistant (1.61%). A summary of resistance findings were compiled in Table 7 and compared to published rates in the Northeast United States.

Table 7. Rates of resistance in publication compared with rates found in transect surveys in PIRO and SLBE National Park properties.

	Putative Resistant	Resistant	% confirmed	Beech stems/Ha	Disease Free Trees/Ha	Resistance Rates
Tested Trees, Houston 1982	17	17	100	--	--	--
Riverdale N.S. Houston, 1982	75	--	--	2129	15	3.5
Brookside, N.S. Houston 1982	32	--	--	1872	12	1.7
Sleeping Bear Dunes Transect Surveys	5	0	66	86.88	0.58	--
Pictured Rocks Transect Surveys	4	3	75	82.08	0.28	1.1
PIRO Multi-year Surveys	25	24	96	--	--	--
SLBE Wandering Surveys	24	16	66	--	0.6	--

4.4.2 Propagation

Currently (as of Spring 2021), 18 genotypes have been propagated in the grafting program (Table 8). Two additional genotypes have been grafted and are healing. Additional genotypes are anticipated to be collected in a phase II continuation of the project.

Table 8. Results of grafting of resistant trees (2019, 2020). Number of trees are successful individuals after grafting, considered as leafing out and growing from grafted portion of tree. Success rates are percentage of trees surviving out of total graft attempts. Provenance is the target property from which scions were collected. PIRO= Pictured Rocks National Lakeshore, SLBE= Sleeping Bear Dunes National Lakeshore.

Genotype	**Trees**	**Success Rate**	**Provenance**
Faggra075	1	5%	SLBE
Faggra030	1	5%	SLBE
Faggra133	1	6.25%	SLBE
FAGR31	2	6.67%	PIRO
FAGR085	5	13.33%	PIRO
Faggra039	3	14.28%	SLBE
Faggra102	7	23.33%	SLBE
Faggra129	2	25%	SLBE
Faggra041	14	46.67%	SLBE
FAGR034	16	52.0%	PIRO
Faggra047	16	53.3%	SLBE
FAGR119	18	60%	PIRO
FAGR116	20	66.66%	PIRO
FAGR128	22	73.33%	PIRO
Faggra043	24	80%	SLBE
Faggra104	26	86.67%	SLBE
FAGR118	27	90%	PIRO
Total	233	43.96%	

Grafting accomplished under supervision by experienced grafters at Oconto River Seed Orchard had greater success than unsupervised grafting success in 2020 (66% vs 12.89%). Grafting success rates increased every year as methods were refined (Figure 10). The latest round of grafting in November 2020 resulted in success rates of 45.45%.

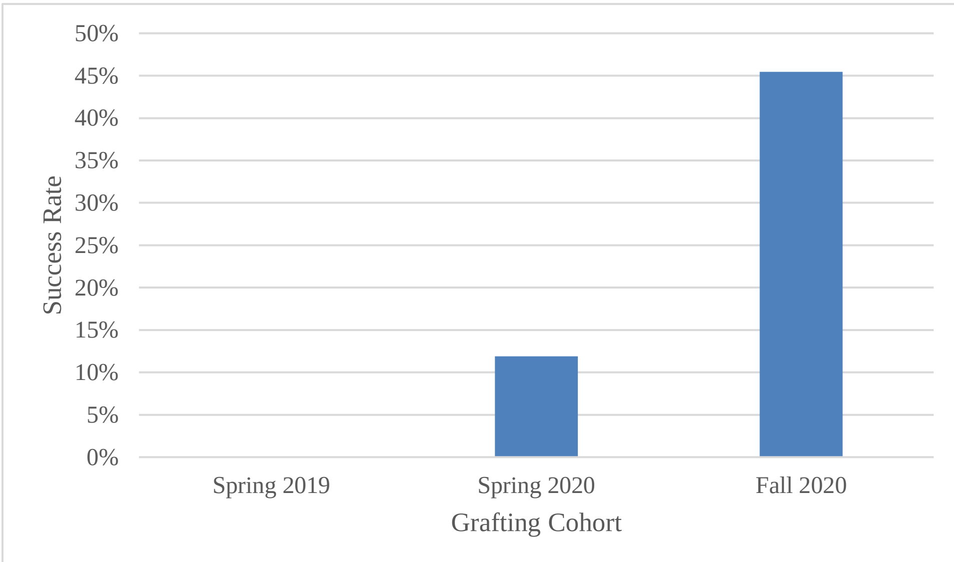

Figure 11. Grafting success rates from spring 2019, spring 2020, and fall 2020. Grafting in spring 2020 occurred before and after training at US Forest Service Oconto River Seed Orchard, and before any correction for mechanical failure. Fall 2020 grafting occurred after training and additional practice sessions in grafting technique, resulting in a dramatic increase in success rates.

4.4.3 Refinement of Methods

The failures for spring 2019 grafting were attributed to graft union failure, due to the lack of callus tissue formation. Some trees did display callus tissue formation in 2019, but suffered mechanical failure after, so were not included in survival rates. In response to the failure of graft unions, a training workshop was organized at the US Forest Service Oconto River Seed Orchard. Technicians at this facility have successfully established a tree orchard program of grafted American beech. The training workshop decreased the failures as a result of graft failure, through careful supervision and quality control of individual grafts, and the ability to troubleshoot with experienced grafters.

In spring 2020, failures were attributed to a combination of mechanical failure and a black mold infection that appeared under the wax. 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.

4.5 Discussion

4.5.1 Resistant Trees

Additional wandering surveys should be completed in Pictured Rocks National Lakeshore to match the wandering survey effort that occurred in Sleeping Bear Dunes National Lakeshore, because the identification of additional resistant trees will enhance the genetic diversity of grafted stock for use in restoration. Multi-year confirmation of disease-free trees is the most reliable predictor of true resistance, which stands to reduce the effort needed to field challenge resistant trees. Identification of trees which 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 combat BBD.

Our practice of considering trees truly resistant only if no egg clusters present agrees with methods presented elsewhere (Koch and Carey, 2014). Because infestation levels can vary over years or across environmental gradients, and the assumption of low heritability of resistance, only trees which seemingly offer no suitable feeding sites for scale are desirable for propagation.

More trees were identified at putatively resistant by NPS personnel, but a higher percentage of the trees identified by MTU personnel were confirmed resistant. This may

be a result of the length of time of infestation in the property studied. The disease is at an earlier phase in the disease in the property where NPS identified resistant trees (Myers et al., submitted b). The NPS identified trees 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 are planned to be revisited to monitor for infestation in subsequent years.

Multi-year scale absence is the most reliable indicator of resistance prior to testing. While trees may escape scale infestation in a single year through 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 3 ha is lower than reported rates, however, overstory American beech stems per ha is lower in the target properties than in the reported rates, resulting in lower trees per ha, with the same rates (Houston, 1983). This is consistent with the established hypothesis of genetic heritability of resistance, with about 1% of trees genetically resistant to scale insects (Houston, 1983; Mason et al., 2013; Ćalić et al., 2017).

4.5.2 Propagation

The success rates of our program increased over years, likely in response to gaining experience in grafting American beech. This is supported by the dramatic increase in success after receiving personalized training and successive rounds of practice grafting. It should be expected that new technicians will have low success rates when beginning grafting.

4.5.3 Refinement of Methods

The alteration of methods in response to failures also increased successful union take rates. In our process, the increased sanitizing concentration in response to pathogen infection, and the addition of supporting scaffolding to reduce mechanical failure were direct responses to observed failure points in the process. The ability to troubleshoot issues will increase success rates of grafting.

Assigning graft failure has been instrumental in the refinement of grafting methods, and evaluation of the efficacy of changes made in the grafting process. Many of the changes instrumented were the result of personal communication with experienced grafters. In order to preserve this knowledge formally, and prevent loss of knowledge in personnel turnover, an illustrated, plain-language manual is presented in appendices A and B.

4.6 Conclusions

Grafting of trees is a costly and labor-intensive process, but worth pursuing because of the high success rates and known resistance of donor trees, leading to fully resistant stock in all successes. Grafted trees take upwards of 3 years to heal, but a program which has sturdy, thicker stock at the end of this time that will more likely be able to produce resistant seeds sooner than if resistant plantlets were being produced from seeds. When establishing a grafting program, however, 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. Extra care should be exercised when determining appropriate numbers of stock for grafting, taking the difficulty of propagating beech into account.

The timeline for production of resistant trees can potentially be shortened by recording multi-year resistance data of trees before implementation, if such records exist.

An experienced grafter may require less time to acclimate to the grafting of beech. Implementing a quality check which allows timely responses to failures in the grafting system should also be included as part of the project planning. Implementing these processes will facilitate the quickest turnaround on restoration materials in the form of grafted trees ready for planting in as short a timeline as possible.

4.7 Resources

Cale, J.A., McNulty, S.A. 2018. Not dead yet: Beech trees can survive nearly three decades in the aftermath phase of a deadly forest disease complex. *Forest Ecology and Management*. 409, 372-377.

Ćalić, I., Koch, J., Carey, D., Addo-Quaye, C., Carlson, J.E., Neale, D.B. 2017. Genome-wide association study identifies a major gene for beech bark disease resistance in American beech (*Fagus grandifolia* Ehrh.). *BMC Genomics*. 18, 547-514.

Carey, D.W., Mason, M.E., Bloese, P., Koch, J.L. 2013. Hot callusing for propagation of American beech by grafting. *HortScience*. 48, 620-624.

Doran, W. 1957. Propagation of woody plants by cuttings. University of Massachusetts, College of Agriculture.

Heyd, R. L. 2005. Managing beech bark disease in Michigan. In: Evans, Celia A., Lucas, Jennifer A. and Twery, Mark J., eds. Beech Bark Disease: Proceedings of the Beech Bark Disease Symposium; 2004 June 16-18; Saranak Lake, NY. Gen. Tech. Rep. NE-331. Newtown Square, PA: US. Department of Agriculture, Forest Service, Northeastern Research Station: 128-132.

Houston, D.R. 1982. A technique to artificially infest beech bark with beech scale, *Cryptococcus fagisuga* (Lindinger). Res. Pap. NE-507. Broomall, PA: U.S. Department of Agriculture, Forest Service, Northeastern Forest Experiment Station. 8p.

Houston, D.R. 1983. American beech resistance to *Cryptococcus fagisuga*. In: Proceedings, I.U.F.R.O. Beech Bark Disease Working Party Conference; 1982 September 26-October 8; Hamden, CT. Sponsored by the USDA Forest Service, Northeastern Forest Experiment Station. Gen. Tech. Rep. WO-37. [Washington, DC]: U.S. Department of Agriculture, Forest Service: 38-42.

Koch, J.L., Carey, D.W. 2014. A technique to screen American beech for resistance to the beech scale insect (*Cryptococcus fagisuga* Lind.). *Journal of Visualized Experiments: JoVE*. e51515.

Koch, J., Allmaras M., Barnes, S., Berrang, P., Hall, T., Iskra, A., Kochenderfer, J., MacDonald, W., Rogers, S., and Rose, J. 2015. Beech seed orchard development: Identification and propagation of beech bark resistant American beech trees. Chapter 8 in K.M. Potter and B.L. Conkling, eds., Forest Health Monitoring: National Status, Trends and Analysis, 2014. General Technical Report SRS-209. Asheville, North Carolina: U.S. Department of Agriculture, Forest Service, Southern Research Station. p. 103-108.

Mason, M.E., Koch, J., Krasowski, M., Loo, J. 2013. Comparisons of protein profiles of beech bark disease resistant and susceptible American beech (*Fagus grandifolia*). *Proteome Science*. 11, 2.

McCullough, D.G., Heyd, R.L., O-Brien, J.G. 2001. Biology and management of beech bark disease. Michigan State University Extension Bulletin (E-2746). East Lansing, MI.

Myers, A.L., Dickinson, Y.L., Storer, A.J., Bal, T.L. In Preparation. A Pilot Study in Transplanting Methods for Wildling American beech (*Fagus grandifolia*). *The Journal of Forestry*.

Myers, A.L., Dickinson, Y.L., Storer, A.J., Bal, T.L. In Preparation b. Beech Bark Disease in Northern Michigan and Site Selection for a Resistant Tree Restoration Plan. *Restoration Ecology*.

Oladi, R., Pourtahmasi, K., Eckstein, D., Bräuning, A. 2011. Seasonal dynamics of wood formation in Oriental beech (*Fagus orientalis* Lipsky) along an altitudinal gradient in the Hyrcanian forest, Iran. *Trees*. 25(3), 425-433.

Ramirez, M., Loo, J., Krasowski, M.J. 2007. Evaluation of resistance to the beech scale insect (*Cryptococcus fagisuga*) and propagation of American beech (*Fagus grandifolia*) by grafting. *Silvae Genetica*. 56, 163-169.

5 A Pilot Study in Transplanting Methods for Wildling American beech (*Fagus grandifolia*)

5.1 Abstract

American beech (*Fagus grandifolia*) is currently challenged by a number of pressures, including beech bark disease, the emergent beech leaf disease, and a decreasing range as a result of climate change. Interest in propagation methods beyond traditional planting has increased in recent years in response to these new pressures and restoration efforts. This communication reports results of a pilot study for methods to transplant American beech wildling seedlings, not of root sucker origin. Transplanted wildling seedlings outperformed commercially purchased bare root seedlings. Performance was assessed based on survival after overwintering and one summer of growth of potted seedlings. This increase in survival may be due to increased individual handling time, or age of the excavated seedlings. This study will inform further work on transplanting success of American beech root suckers.

5.2 Study Implications

Many propagation methods are technically difficult and expensive. Future restoration efforts may focus efforts on transplanting root suckers, which are clones of parent trees, as a cost-effective avenue for sourcing disease-resistant young trees. Currently, transplanting methods for American beech are not published in detail, leading to the potential for losses of transplanted trees as best practices are unknown. We have completed a pilot study comparing survival rates of wildling seedlings to commercially available bare root seedlings, to explore the feasibility of transplanting as a method of propagation. In our study, survival of wildling seedlings exceeded survival of bare root seedlings.

5.3 Introduction

American beech (*Fagus grandifolia* Ehrh.) is at risk of beech bark disease (BBD) in the majority of its range in the Northeast. The invasive disease complex BBD was reported in Michigan in 2001, and has spread to the majority of American beech in the Upper Peninsula and northern Lower Peninsula in Michigan (O'Brien et al. 2001). In this disease complex, invasive beech scale (*Cryptococcus fagisuga* L.) infests the bole of American beech trees, creating infection courts for a fungus of the genus *Neonectria* to infect and weaken the tree, eventually causing mortality (Ehrhlich 1934). American beech is important as a wildlife mast species in Michigan, and conservation efforts for the species have focused on introduction of a scale-resistant genotype tree, propagated through hot-callus grafting (Koch & Rose 2015).

This method has been successfully demonstrated in a number of facilities, and by various working groups. Resistant seed has been produced through the creation of seed orchards using resistant grafted stock, however, hot callus grafting is a lengthy, costly process, needing to be conducted by individuals with high levels of technical expertise and the availability of bulky hot-callus apparatuses. Private landowners in Michigan collectively own over 9 million acres of forest land, or 45% of the forests in Michigan (MI DNR 2015). The creation of more reliable, quick and cost-effective processes to propagate resistant American beech would benefit BBD-mitigation and forest restoration goals by allowing propagation of resistant trees by entities without the facilities to propagate through other methods.

An additional challenge in grafting for production of resistant tree through grafting is rootstock sourcing. Currently, the recommended process is to purchase commercially available bare root seedlings, or grow the necessary rootstock from seed. Multiple agencies operating in a region may rapidly deplete the commercial rootstock in an area, and American beech seedlings of a suitable size for grafting take upwards of 2 years to grow in a nursery setting (personal communication, Porcupine Hollow Farm, Central Lake, MI). This may create a bottleneck in sourcing appropriate rootstocks.

Successful transplantation methods allow for supplementation of rootstock from wildling stock.

When grafting resistant trees, the apical growth of a non-resistant rootstock is replaced with donor scion material from a resistant tree, resulting in a tree that retains the characteristics of the root portion in the rootstock, and the characteristics of the scion tree in the grafted portion. Transplanting root suckers of a resistant parent tree would result in the entire tree displaying resistance to the disease.

American beech displays a vigorous root-sprouting response to stress such as the type inflicted by BBD on individual beech trees (Del Tredici 2001). Although excavation of American beech seedlings and suckers (through examination of root morphology) has been utilized to determine origin (Nyland 2008), we are unaware of attempts to propagate the excavated trees. In fact, much literature about beech propagation specifies methods only to the genus level, with most of the references originating from early propagation of European beech (*Fagus sylvatica*) and the majority of propagation focuses on the production and sowing of beechnuts (Bonner & Leak 2008).

The ability to propagate resistant American beech by transplanting suckers may save land managers time and money by eliminating the need for costly grafting procedures or purchasing and sowing disease-resistant seed (not available at the time of writing). Origin of the individual (seed vs root sucker) may be determined by presence or absence of a taproot at the time of excavation, allowing field identification of clonal offspring (Nyland 2008).

The relative amount of reproduction occurring as seedlings or root sprouts is variable, and affected by many factors. Root suckers appear more readily in situations with lateral roots close to the soil surface, such as in downslope portions of beech on hillsides (Ward 1961). Warmer conditions may lead to an increase in suckering (Held 1983). Survival and growth rates are higher in root suckers than seedlings, leading to increased dominance in the larger sapling size classes (Beaudet & Messier 2008; Nyland

2008). Beech root sprouts can disperse to a maximum of about 6 meters from the parent tree, but with mean distance closer to 3 meters (Ribbens et al. 1994; Beaudet and Messier 2008). Beechnuts may be more widely dispersed by wildlife (Johnson & Adkisson 1985; Tubbs and Houston 1990).

Root suckers of other species have been successfully transplanted for restoration, primarily for reforestation of arid landscapes. Jujube trees (*Ziziphus jujube*; Rhamnaceae) have been transplanted in New Mexico with success rates varying by height class (Sapkota et al. 2019). Small serviceberries (*Amelanchier* spp.; Roseaceae) can be transplanted as root suckers, combined with aggressive pruning (Gough 2010). Pongame oiltree (*Pongamia pinnata*; Fabaceae) has also been propagated through suckers (Maiti 2012). Propagation by root suckering is poorly represented in scientific literature, but a known route of propagation for many trees.

To our knowledge, transplanting success rates for American beech are not published. In this study, transplanted wildling American beech seedlings were compared to commercially available bare root seedlings to explore the feasibility of transplanting as an avenue for propagation. The development of these methods will inform future work transplanting root suckers, with the ultimate goal of transplanting root suckers of resistant American beech to save costs and increase survivability rates.

5.4 Methods

Bare root seedlings were purchased from a commercial nursey in Northern Michigan. Due to supply issues, an unknown number of the bare root seedlings from a second nursery in northern Wisconsin were included in the shipment. Some purchased beech arrived with scale infestation. These trees were immediately discarded and potentially contaminated trees were not included in the analysis.

Wildling plant material was collected from three field sites in the Hiawatha National Forest near Munising, MI. All sites were located along road edges, to allow

access by vehicle. Patches were identified by US Forest Service employees as containing a large number of American beech 2 to 4 feet in height. Collection occurred in November 2019, when trees had fully entered winter dormancy.

American beech regeneration was identified by twigs with cigar-shaped buds, visible above the snow. The snow layer was removed with a shovel and the leaf litter layer was removed by hand. A shovel was used to dig around each small tree about 8-10 inches from the base, and a levering motion was used to free the entire dirt clod and seedling from the ground. Dirt clods and root balls were manipulated by hand to remove as much dirt as possible while retaining as much fine root mass as possible. Roots were not rinsed prior to potting.

Trees were then packaged in damp sphagnum peat moss and placed in heavy duty black contractor bags which were twisted shut to retain moisture. At the end of the day, bags were packed with extra damp peat moss sufficient to entirely enclose all fine root mass, and buried under an insulating layer of snow to prevent freezing or overheating of the samples while being transported to cold storage facilities.

Table 9. Root mass class distinctions. Root mass class definitions were created to divide seedlings into thirds, qualitatively.

Root Class	**Description**	**Feeder Root Nodules**
High	Many intact feeder roots; Little to no breakage to large roots; Taproot primarily intact	6+
Medium	Some feeder roots intact; Many broken large roots; Taproot up to partially gone.	2-5
Low	Little to no feeder roots intact; Most large roots broken; Taproot primarily missing.	0-1

Dormant individuals were then stored at 4°C until planting, to keep the entire tree dormant prior to potting, no more than 2 weeks. At the time of potting, root collar diameter, height, bud scale scar number, and presence/absence of a taproot were recorded. Damage to the above or below-ground biomass were recorded separately. Trees were photographed (Figure 11) and later categorized into low, medium, or high fine root mass categories based on those images (Table 9). Trees were sorted into root class at a later date by photograph of root systems, so that root mass class of bare root and ample water three times a week and allowed to grow at ambient temperatures in a greenhouse setting. Survival was defined as trees which displayed new, vigorous growth through the end of September, 2020. These results therefore include losses to overwintering in pots in both wildling and bare root seedlings. excavated seedlings could be classified together.

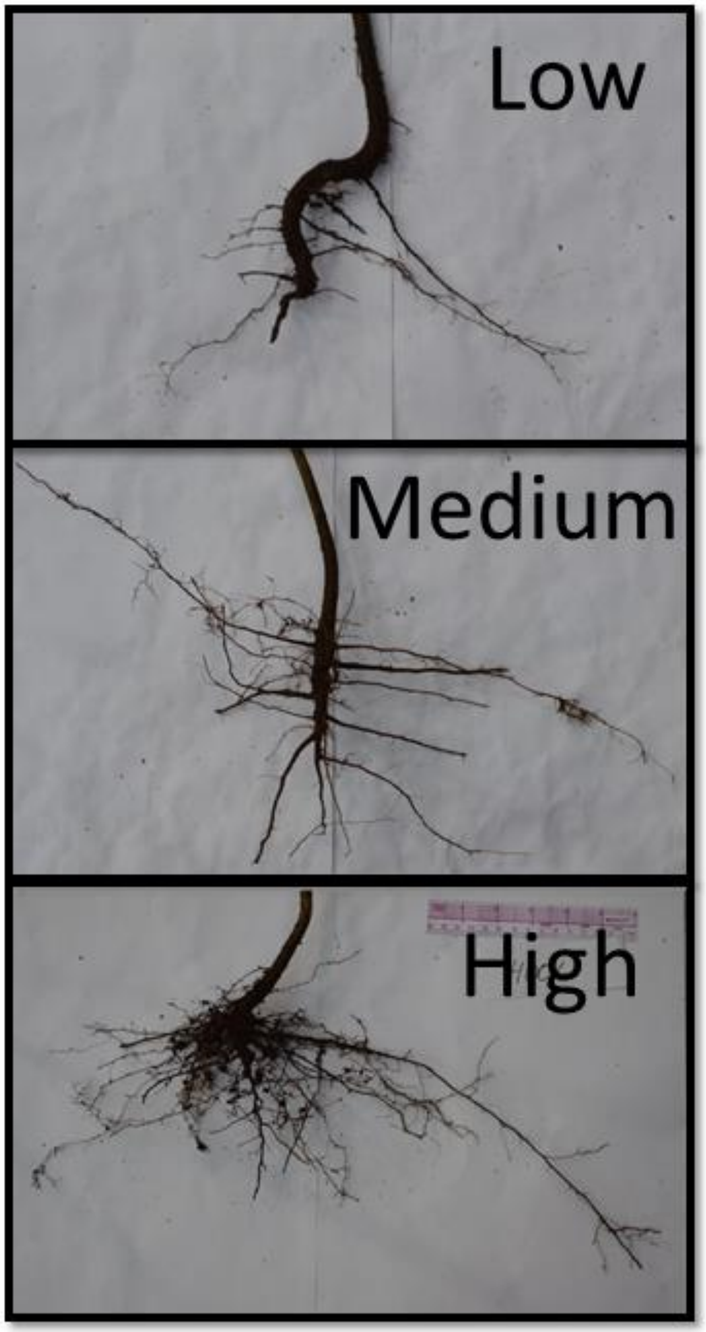

Figure 12. Example root mass classes. These root photographs are typical of American beech wildling excavated seedlings.

Chi square analyses were performed for the entire cohort and among root mass classes to confirm differences between groups. Regression analysis was performed to test simple predictor variables of aboveground height and root mass class, as these measures are most useful at the time of excavation in a field setting and making decision about which trees to select.

5.5 Results

Overall, 145 excavated seedlings and 88 bare root seedlings were compared. Though 200 bare root seedlings were ordered, many were not included in analysis due to a scale contamination issue on purchased seedlings, to avoid the issue of comparing infested purchased trees to uninfested wildling seedlings.

Excavated seedlings survived at higher rates than bare root seedlings (χ20.05, df1: 3.84, 4.098). Excavated seedlings outperformed bare root seedlings at all root mass classes, but differences were significant only in high root mass classes (High: χ2,0.05, df1: 3.84, 6.41; Medium χ2,0.05, df1: 3.84, 2.33; Low: χ2,0.05, df1: 3.84, 1.69) (Figure 12). In addition, survival was not different among root mass classes in bare root seedlings, but was significantly different among root classes for excavated seedlings. (bare root, χ2,0.05, df2: 5.99, 2.06; excavated, χ2,0.05, df2: 5.99, 6.08).

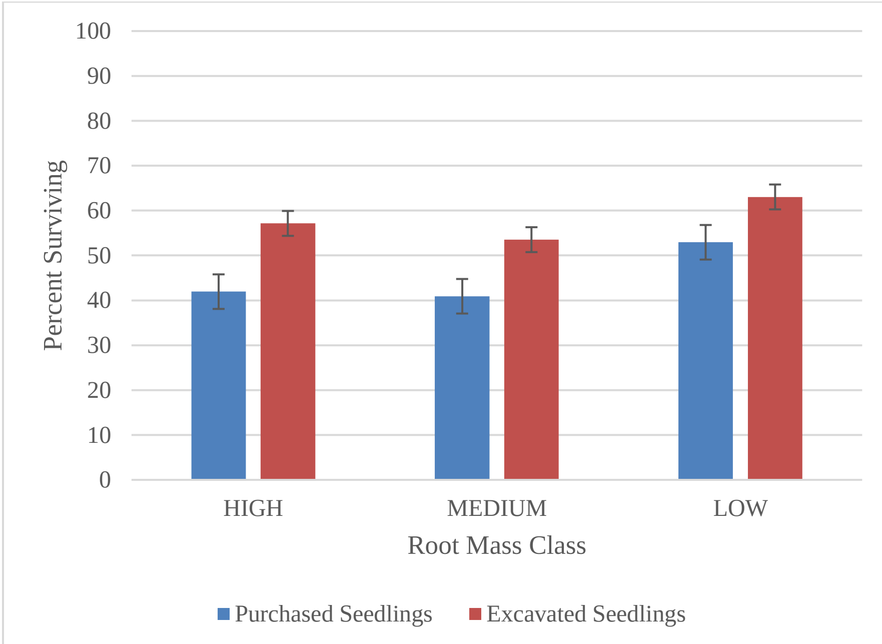

Figure 13. Comparison of survival rates of American beech seedlings of wildling and commercial bare root origin. Bare root seedlings were purchased from a commercial nursery in northern Michigan. Excavated seedlings were dug up from Hiawatha National Forest with permission. Purchased seedlings (blue) were commercially available bare root seedlings purchased from a nursery in Northern Michigan. Excavated seedlings (orange) were wildling seedlings removed from the Hiawatha National Forest in Michigan and potted in greenhouse. Survival was significantly higher for all seedlings, but not significant within root classes.

Binomial regression analysis was performed to look for significant predictors among root mass class and aboveground height influencing survival of seedling origin wildlings, and no significant predictors were found.

5.6 Discussion

The increased survival of wildling trees could be the result of increased individual handling time of wildling seedlings during excavation, resulting in more intact very fine roots. Possibly because the differences in root mass were distinct, but small between the low and high root class of wildling seedlings, and the aboveground sizes were not widely varied, the performance between root classes were not distinctly different in wildling

seedlings. This may also be the result of increased age of wildling seedlings in the same size class as bare root seedlings. Destructive sampling of wildling seedlings would be necessary to accurately age the wildlings, and is planned for future analyses, to prevent the loss of wildling seedlings from the first cohort.

Although we are pursuing alternate methods of propagation, the low viability of bare root seedlings may impact other propagation methods. The transplanted seedlings from wildling stock could be used in the pursuance of other propagation. The transplanted seedlings surviving after a second growing season will be used as grafting rootstock in a BBD-resistance tree breeding program at Michigan Technological University. The ability to successfully transplant wildling seedlings has eliminated bottlenecks in this program caused by the relatively low availability of local provenance bare root seedlings available for purchase.

5.7 Conclusion

Wildling seedlings can be successfully removed from a forest setting and maintained in a greenhouse. These trees display survival rates rivaling or exceeding bare root seedlings available for purchase. Although findings have not yet been analyzed (to be completed after 2 years growth), vigor of transplanted seedlings was similar in bare root and excavated seedlings 8 months after potting. This finding supports further research in the methods of transplanting as a propagation method, and the use of excavated seedlings as root stock in grafting.

Future work is required to assess the feasibility of transplanting wildling trees as a propagation method. The results of this pilot study are a first step towards refining methods of the process. Survival of excavated wildling root suckers compared to seedlings will be the next step to provide baseline survival rates for transplanting trees, and shorten the time to production of disease-resistant, local provenance trees for restoration efforts.

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5.9 Literature Cited

Beaudet, M., and Messier, C. 2008. Beech Regeneration of Seed and Root Sucker Origin: A Comparison of Morphology, Growth, Survival, and Response to Defoliation. *Forest Ecology and Management.* 255(10): 3659–3666.

Del Tredici, P. 2001. Sprouting in Temperate Trees: A Morphological and Ecological Review. *The Botanical Review*. 67(2): 121–140.

Gough, R.E. 2010. Juneberries for Montana Gardens. Montana Extension Service. e-resource MT198806AG.

Held, M.E. 1983. Pattern of Beech Regeneration in the East-Central United States. *Bulletin of the Torrey Botanical Club*. 110(1): 55–62.

Johnson, W.C., and Adkisson, C.S. 1985. Dispersal of beech nuts by blue jays in fragmented landscapes. *American Midland Naturalist*. 113: 319-324.

Koch, J., Allmaras M., Barnes, S., Berrang, P., Hall, T., Iskra, A., Kochenderfer, J., MacDonald, W., Rogers, S., and Rose, J. 2015. *Beech seed orchard development: Identification and propagation of beech bark resistant American beech trees*. P.

103-108 in Forest Health Monitoring: National Status, Trends and Analysis, 2014. K.M. Potter and B.L. Conkling (eds.) Asheville, North Carolina.

Nyland, R.D. 2008. Origin of Small Understory Beech in New York Northern Hardwood Stands. *Northern Journal of Applied Forestry*. 25(3): 161–163.

Maiti, S.K. 2013. Raising of Saplings for Forest Trees. P 121-149 in *Ecorestoration of the Coalmine Degraded Lands*. New Delhi, Springer India.

Michigan Department of Natural Resources (MI DNR). 2015. *Forest Stewardship in Michigan*. Electronic Bulletin. Available online at https://www.michigan.gov/documents/dnr/GeneralForestryInfo_474276_7.pdf; Last accessed Mar. 5, 2021.

O'Brien, J.G., Ostry, M.E., Mielke, M.E., Mech, R., Heyd, R.L., and McCullough, D.G. 2001. First Report of Beech Bark Disease in Michigan. *Plant Disease*. 85(8): 921–921.

Ribbens, E, Silander, J.A.Jr., and Pacala, S.W. 1994. Seedling Recruitment in Forests: Calibrating Models to Predict Patterns of Tree Seedling Dispersion. *Ecology*. 75(6): 1794–1806.

Sapkota, S., Sapkota, S., Wang, S., and Liu, Z. 2019. Height and Diameter Affect Survival Rate of Jujube Suckers Transplanted in a Semi-Arid Farmland of New Mexico. *Journal of Applied Horticulture*. 21(3): 249–251.

6 General Conclusions

Successful restoration of American beech will have a number of challenges ahead, although the progress described here regarding propagation of resistant trees is a critical step forward. A literature review was presented on advances in propagation of American beech, and the use of resistant stock in the restoration of forests affected by BBD. Some ecological information is missing for American beech (e.g., conditions for planting and maintenance, seed stratification, etc.) and the pursuit of fundamental research in the areas identified, such as the transplanting of wildling trees, and the planting conditions suitable for the species, is necessary for the next steps in successful restoration to occur. As American beech is being threatened by additional, more recently emergent issues, particularly stress from climate change and beech leaf disease, restoration efforts to preserve genetic diversity of BBD resistant trees may pave the way and save time for researchers to develop methods mitigating these issues.

While planting sites and restoration activities have been proposed for the National Park Service as a stakeholder agency in the restoration project, further work is needed to locate additional resistant trees. Ground truth surveys should occur before final selection of planting sites for resistant trees. While surveys were performed to describe the state of disease in these properties, poor correlations were found between condition of American beech and known drivers of severity of BBD. Additional work could perhaps identify better predictive factors driving severity in the properties targeted for restoration.

Grafting protocols were refined and a propagation program established at Michigan Technological University. The developments were documented in illustrated manuals to preserve institutional knowledge. This knowledge has been shared with stakeholders to ensure continuance of knowledge at both institutions and plans are in place to publish it formally as a publicly available report. The grafting program could be expanded in the future to increase the maximum output of trees. Currently, output is

limited by space and by the relative difficulty of accessing resistant trees in winter months.

Description of successful transplanting methods would allow for the use of wildling seedlings as rootstock for restoration grafting. The results of this study simply provide the first piece of the puzzle in transplanting seedlings. Rates of wildling seedlings are comparable to nursery bare-root seedlings. The next step for this research would be quantifying extended survival (3-5 years) and the success rates of transfers to a final planting site outdoors. Successful transplanting of wildling seedlings may open the door to successful transfer of root suckers, clonal reproduction of parent trees.

The conclusion of this project will be marked by the transfer of products to the stakeholder, the National Park Service. A continuation of the project has been proposed for a Phase II extension of the work, where many of these identified limitations are expected to be addressed.

7 References

Arthur, M.A., Weathers, K.C., Lovett, G.M., Weand, M.P., Eddy, W.C. 2017. A beech bark disease induced change in tree species composition influences forest floor acid–base chemistry. *Canadian Journal of Forest Research.* 47, 875-882.

Barker, M.J., Pijut, P.M., Ostry, M.E., Houston, D.R. 1997. Micropropagation of juvenile and mature American beech. *Plant Cell, Tissue and Organ Culture.* 51, 209-213.

Bayraktarov, E., Saunders, M.I., Abdullah, S., Mills, M., Beher, J., Possingham, H.P., Mumby, P.J., Lovelock, C.E. 2016. The cost and feasibility of marine coastal restoration. *Ecological Applications.* 26, 1055-1074.

Beaudet, M., and Messier, C. 2008. Beech Regeneration of Seed and Root Sucker Origin: A Comparison of Morphology, Growth, Survival, and Response to Defoliation. *Forest Ecology and Management.* 255(10), 3659–3666.

Bernard, A., Gélinas, N., Duchateau, E., Durocher, C., Achim, A. 2018. American beech in value-added hardwood products: assessing consumer preferences. *BioResources.* 13, 6893-6910.

Boanares, D., Azevedo, C.S.d. 2014. The use of nucleation techniques to restore the environment: a bibliometric analysis. *Natureza & conservação*. 12, 93-98.

Bohn, K. K., Nyland, R.D. 2003. Forecasting development of understory American beech after partial cutting in uneven-aged northern hardwood stands. *Forest Ecology and Management.* 180, 453-461.

Bonner F.T., Leak W.B. 2008. *Fagus* L. Pages 520-524 In: The Woody Plant Seed Manual. Bonner FT, Karrfalt RP (eds) Agric. Handbook No. 727. Washington, DC. US Department of Agriculture, Forest Service. 1223 p. 727.

Burger, T.L., Kotar, J. 2003. A guide to forest communities and habitat types of Michigan. University of Wisconsin--Madison, Department of Forestry Ecology and Management.

Burke, D.J., Hoke, A.J., Koch, J. 2020. The emergence of beech leaf disease in Ohio: Probing the plant microbiome in search of the cause. *Forest Pathology.* 50, e12579.

Burns, B.S., Houston, D.R. 1987. Managing beech bark disease: Evaluating defects and reducing losses. *Northern Journal of Applied Forestry*. 4, 28-33.

Cale, J.A., Garrison-Johnston, M.T., Teale, S.A., Castello, J.D. 2017. Beech bark disease in North America: Over a century of research revisited. *Forest Ecology and Management.* 394, 86-103.

Cale, J.A., Letkowski, S.K., Teale, S.A., Castello, J.D. 2012. Beech bark disease: an evaluation of the predisposition hypothesis in an aftermath forest: Evaluating the beech bark disease predisposition hypothesis. *Forest Pathology (J. de pathologie forestière).* 42, 52-56.

Cale, J.A., McNulty, S. A. 2018. Not dead yet: Beech trees can survive nearly three decades in the aftermath phase of a deadly forest disease complex. *Forest Ecology and Management*. 409, 372-377.

Cale, J.A., McNulty, S.A., Teale, S.A., Castello, J.D. 2012. The impact of beech thickets on biodiversity. *Biological Invasions.* 15, 699-706.

Cale, J.A., Teale, S.A., Johnston, M.T., Boyer, G.L., Perri, K.A., Castello, J.D. 2015. New ecological and physiological dimensions of beech bark disease development in aftermath forests. *For. Ecology and Management.* 336, 99-108.

Ćalić, I., Koch, J., Carey, D., Addo-Quaye, C., Carlson, J.E., Neale, D.B. 2017. Genome-wide association study identifies a major gene for beech bark disease resistance in American beech (*Fagus grandifolia* Ehrh.). *BMC Genomics*. 18, 547-514.

Carey, D.W., Mason, M.E., Bloese, P., Koch, J.L. 2013. Hot callusing for propagation of American beech by grafting. *HortScience*. 48, 620-624.

Carpenter, R.D. 1974. American beech (*Fagus grandifolia*). FS-220

Castlebury, L., Rossman, A., Hyten, A. 2006. Phylogenetic relationships of *Neonectria/Cylindrocarpon* on *Fagus* in North America. *Canadian Journal of Botany*. 84(9), 1417–1433.

Chaverri, P., Salgado, C., Hirooka, Y., Rossman, A. Y., Samuels, G. J. 2011. Delimitation of *Neonectria* and *Cylindrocarpon* (Nectriaceae, Hypocreales, Ascomycota) and related genera with *Cylindrocarpon*-like anamorphs. *Studies in Mycology*. 68, 57-78.

Cogbill, C.V. 2005. Historical biogeography of American beech. In: Evans, Celia A., Lucas, Jennifer A. and Twery, Mark J., eds. Beech Bark Disease: Proceedings of the Beech Bark Disease Symposium; 2004 June 16-18; Saranak Lake, NY. Gen. Tech. Rep. NE-331. Newtown Square, PA: US. Department of Agriculture, Forest Service, Northeastern Research Station: 16-24.

Collins, 2020. Collins English Dictionary. Accessed online 12/7/2020: https://www.collinsdictionary.com/dictionary/english.

Conrad C.A., Reitsma L. 2015. Demographic responses if myomorph rodents to mast production in a beech- and birch-dominated northern hardwood forest. *Northeastern Naturalist.* 22, 746-76.

Corbin J.D., Robinson G.R., Hafkemeyer L.M., Handel S.N. 2016. A long-term evaluation of applied nucleation as a strategy to facilitate forest restoration. *Ecological Applications.* 26, 104-114.

Corbin, J.D., Holl, K.D. 2012. Applied nucleation as a forest restoration strategy. *Forest Ecology and Management*. 265, 37-46.

Cuenca, B., Ballester, A., Vieitez, A.M. 2000. In vitro adventitious bud regeneration from internode segments of beech. *Plant Cell, Tissue and Organ Culture*. 60, 213-220.

Cuenca, B., Vieitez, A.M. 1999. Histological Study of In vitro Development of Adventitious Buds on Leaf Explants of Oriental Beech (*Fagus orientalis* Lipsky). *In Vitro Cellular & Developmental Biology*. Plant, 35, 326-332.

Del Tredici, P. 2001. Sprouting in temperate trees: a morphological and ecological review. *The Botanical Review.* 67(2), 121-140.

Diner, A. 1999. Somatic embryogenesis in forestry: A practical approach to cloning the best trees. In: Wheeler, Robert, ed. Under the canopy; forestry and forest products newsletter of the Alaska Cooperative Extension. Fairbanks, AK: University of Alaska Fairbanks Cooperative Extension Service: 7-8.

Doran, W. 1957. Propagation of woody plants by cuttings. University of Massachusetts, College of Agriculture.

Ehrlich, J. 1934. The beech bark disease: a *Nectria* disease of *Fagus*, following *Cryptococcus fagi* (Baer.). *Canadian Journal of Research.* 10, 593-692.

Elenitsky, L., Walters, M., Farinosi, E. 2020. Tree Regeneration Structure Following Beech Bark Disease-Motivated Harvests: Factors Associated with Patterns and Management Implications. *Forests*. 11(2), 180–206.

Ewing, C.J., Hausman, C.E., Pogacnik, J., Slot, J., Bonello, P. 2018. Beech leaf disease: An emerging forest epidemic. *Forest Pathology*. 49, e12488.

Eyre, F.H. 1980. Forest Cover Types. Washington, DC: Society of American Foresters.

Faison E.K., Houston D.R. 2004. Black bear foraging in response to beech bark disease in northern Vermont. *Northeastern Naturalist.* 11, 387-394.

Fajvan, M. A., Hille, A., Turcotte, R.M. 2019. Managing understory *Fagus grandifolia* for promoting beech bark disease resistance in northern hardwood stands. *Forest Science.* 65, 644-651.

Farrar, A., Ostrofsky, W.D. 2006. Dynamics of American beech regeneration 10 years following harvesting in a beech bark disease-affected stand in Maine. *Northern Journal of Applied Forestry.* 23, 192-196.

Flachowsky, H., Hanke, M.V., Peil, A., Strauss, S.H., Fladung, M. 2009. A review on transgenic approaches to accelerate breeding of woody plants. *Plant breeding.* 128, 217-226.

Garnas, J. R., Ayres, M.P., Liebhold, A.M., Evans, C. 2011. Subcontinental impacts of an invasive tree disease on forest structure and dynamics. *The Journal of Ecology* 99, 532-541.

Garnas, J. R., Houston, D.R., Twery, M.J., Ayres, M.P., Evans, C. 2013. Inferring controls on the epidemiology of beech bark disease from spatial patterning of disease organisms. *Agricultural and Forest Entomology.* 15, 146-156.

Gemmel, P., Nilsson, U., Welander, T. 1996. Development of oak and beech seedlings planted under varying shelterwood densities and with different site preparation methods in southern Sweden. *New Forests*. 12(2), 141–161.

Giencke, L.M., Dovčiak, M., Mountrakis, G., Cale, J.A., Mitchell, M.J. 2014. Beech bark disease: spatial patterns of thicket formation and disease spread in an aftermath forest in the northeastern United States. *Canadian Journal of Forest Research*. 44(9), 1042-1050.

Gómez-Cortecero, A., Saville, R., Scheper, R., Bowen, J., Agripino De Medeiros, H., Kingsnorth, J., Xu, X., Harrison, R. 2016. Variation in Host and Pathogen in the *Neonectria/Malus* Interaction; toward an Understanding of the Genetic Basis of Resistance to European Canker. *Frontiers in Plant Science*. 7, 1365–1365.

Gough, R.E. 2010. Juneberries for Montana Gardens. Montana Extension Service. e-resource MT198806AG.

Hazubska-Przybył, T., Chmielarz, P., Bojarczuk, K. 2015. In vitro responses of various explants of *Fagus sylvatica*. *Dendrobiology*. 73, 135-144.

Hebbali, A. 2020. olsrr: Tools for Building OLS Regression Models. R package version 0.5.3. https://CRAN.R-project.org/package=olsrr

Held, M. 1983. Pattern of beech regeneration in the East-central United States. *Bulletin of the Torrey Botanical Club.* 110, 55-62.

Helms, J. A. 1998. Dictionary of Forestry. Society of American Foresters.

Heyd, R. L. 2005. Managing beech bark disease in Michigan. In: Evans, Celia A., Lucas, Jennifer A. and Twery, Mark J., eds. Beech Bark Disease: Proceedings of the Beech Bark Disease Symposium; 2004 June 16-18; Saranak Lake, NY. Gen. Tech. Rep. NE-331. Newtown Square, PA: US. Department of Agriculture, Forest Service, Northeastern Research Station: 128-132.

Höhl, M., Ahimbisibwe, V., Stanturf, J.A., Elasser, P., Kleine, M., Bolte, A. 2020. Forest landscape restoration- What generates failure and success? *Forests.* 11, 938.

Holl, K.D., Zahawi, R.A. 2018. Applied nucleation is a straightforward, cost-effective forest restoration approach: reply to Ramírez-Soto et al. *Restoration Ecology*. 26: 618-619.

Holloway, G.L., Malcom, J.M. 2007. Nest-tree use by northern and southern flying squirrels in central Ontario. *Journal of Mammalogy*. 88: 226-233.

Hop, K., Lubinski, S., Dieck, J., Menard, S., Drake, J. 2011. National Park Service Vegetation Inventory Program: Sleeping Bear Dunes National Lakeshore, Michigan. Natural Resource Report NPS/GLKN/NRR—2010/201. National Park Service, Fort Collins, CO, 1-15.

Hop, K., Menard, S., Drake, J., Lubinski, S., Dieck, J. 2010. National Park Service Vegetation Inventory Program: Pictured Rocks National Lakeshore, Michigan. Natural Resource Report NPS/GLKN/NRR—2010/201. National Park Service, Fort Collins, CO, 1-15.

Houston, D. B., Houston, D.R. 1994. Variation in American beech (*Fagus grandifolia* Ehrh.): Isozyme analysis of genetic structure in selected stands. *Silvae Genetica.* 43, 277-284.

Houston, D. B., Houston, D.R. 2000. Allozyme genetic diversity among *Fagus grandifolia* trees resistant or susceptible to beech bark disease in natural populations. *Canadian Journal of Forest Research.* 30, 778-789.

Houston, D.R. 1982. A technique to artificially infest beech bark with beech scale, *Cryptococcus fagisuga* (Lindinger). Res. Pap. NE-507. Broomall, PA: U.S. Department of Agriculture, Forest Service, Northeastern Forest Experiment Station. 8p.

Houston, D. R. 1983. Effects of parasitism by *Nematogonum ferrugineum* (*Gonstorrhodiella highlei*) on pathogenicity of *Nectria coccinea* var. faginata and

Nectria galligena. In: Proceedings, I.U.F.R.O. Beech Bark Disease Working Party Conference; 1982 September 26-October 8; Hamden, CT. Sponsored by the USDA Forest Service, Northeastern Forest Experiment Station. Gen. Tech. Rep. WO-37. [Washington, DC]: U.S. Department of Agriculture, Forest Service: 109-114.

Houston, D.R. 1983. American beech resistance to *Cryptococcus fagisuga*. In: Proceedings, I.U.F.R.O. Beech Bark Disease Working Party Conference; 1982 September 26-October 8; Hamden, CT. Sponsored by the USDA Forest Service, Northeastern Forest Experiment Station. Gen. Tech. Rep. WO-37. [Washington, DC]: U.S. Department of Agriculture, Forest Service: 38-42.

Houston, D. R. 1994a. Major new tree disease epidemics: Beech bark disease. *Annual Rev. of Phytopathology*. 32, 75-87. https://doi:10.1146/annurev.py.32.090194.000451.

Houston, D.R. 1994b. Temporal and spatial shift within the *Nectria* pathogen complex associated with beech bark disease of *Fagus grandifolia*. *Canadian Journal of Forest Research*. 24(5), 960-968.

Houston, D.R. 2001. Effect of harvesting regime on beech root sprouts and seedlings in a north-central Maine forest long affected by beech bark disease. Res. Pap. NE-717. Newtown Square, PA: U.S. Department of Agriculture, Forest Service, Northeastern Research Station. 20 p.

Houston, D.R. 2005. Beech bark disease: 1934 to 2004: What's new since Ehrlich? In: Evans, Celia A., Lucas, Jennifer A. and Twery, Mark J., eds. Beech Bark Disease: Proceedings of the Beech Bark Disease Symposium; 2004 June 16-18; Saranak Lake, NY. Gen. Tech. Rep. NE-331. Newtown Square, PA: US. Department of Agriculture, Forest Service, Northeastern Research Station: 2-13.

Houston, D.R., O'Brien, J.T. 1983. Beech Bark Disease. Forest Insect and Disease Leaflet 75. United States Department of Agriculture. Forest Service, Northern Area State and Private Forestry.

Iverson, L.R., Prasad, A.M., Matthews, S.N., Peters, M. 2008. Estimating potential habitat for 134 eastern US tree species under six climate scenarios. *Forest Ecology and Management.* 254, 390-406.

Jacobs, D. F., Dalgleish, H.J., Nelson, C.D. 2013. A conceptual framework for restoration of threatened plants: the effective model of American chestnut (*Castanea dentata*) reintroduction. *The New Phytologist.* 197, 378-393.

Jensen P.G., Demers C.L., Mcnulty S.A., Jakubas W.J., Humphries M.M. 2012. Marten and fisher responses to fluctuations in prey populations and mast crops in the northern hardwood forest. *The Journal of Wildlife Management.* 76, 489-502.

Johnson, W.C., Adkisson, C.S. 1985. Dispersal of beech nuts by blue jays in fragmented landscapes. *American Midland Naturalist*. 113, 319-324.

Johnson, R., Lipow, S. 2000. Compatibility of breeding for increased wood production and longterm sustainability: the genetic variation of seed orchard seed and associated risks. In: Proceedings Wood Compatibility Initiative Workshop. 18, 169-179.

Kahler H.A., Anderson J.T. 2006. Tree cavity resources for dependent cavity-using wildlife in West Virginia forests. *Northern Journal of Applied Forestry*. 23, 114-121.

Kasson, M.T., Livingston, W.H. 2009. Spatial distribution of *Neonectria* species associated with beech bark disease in northern Maine. *Mycologia.* 101, 190-195.

Kasson, M., Livingston, W. 2012. Relationships among beech bark disease, climate, radial growth response and mortality of American beech in northern Maine, USA:

Relationships among beech bark disease, climate, growth, & beech mortality. *Forest Pathology*. 42: 199–212

Kembel, S.W., Cowan, P.D., Helmus, M.R., Cornwell, W.K., Morlon, H., Ackerly, D.D., Blomberg, S.P., Webb, C.O. 2010. Picante: R tools for integrating phylogenies and ecology. *Bioinformatics* 26, 1463-1464.

Koch, J. 2010. Beech Bark Disease: The Oldest "New" Threat to American Beech in the United States. *Outlooks on Pest Management*. 21(2), 64–68.

Koch, J.L., Carey, D.W. 2014. A technique to screen American beech for resistance to the beech scale insect (*Cryptococcus fagisuga* Lind.). *Journal of Visualized Experiments: JoVE*. e51515.

Koch, J.L., Carey, D.W., Mason, M.E., Nelson, C.D. 2010. Assessment of beech scale resistance in full- and half-sibling American beech families. *Canadian Journal of Forest Research.* 40, 265-272.

Koch, J., Allmaras M., Barnes, S., Berrang, P., Hall, T., Iskra, A., Kochenderfer, J., MacDonald, W., Rogers, S., and Rose, J. 2015. Beech seed orchard development: Identification and propagation of beech bark resistant American beech trees. Chapter 8 in K.M. Potter and B.L. Conkling, eds., Forest Health Monitoring: National Status, Trends and Analysis, 2014. General Technical Report SRS-209. Asheville, North Carolina: U.S. Department of Agriculture, Forest Service, Southern Research Station. p. 103-108.

Koch, J.L., Heyd, R.L. 2013. Battling beech bark disease: establishment of beech seed orchards in Michigan. *Newsletter of the Michigan Entomological Society*. 58 (1 & 2), 11-14.

Kunstler, G., Curt, T., Bouchaud, M., Lepart, J. 2005. Growth, mortality, and morphological response of European beech and downy oak along a light gradient

in sub-Mediterranean forest. *Canadian Journal of Forest Research*. 35(7), 1657–1668.

Leak, W.B. 2006. Fifty-year impacts of the beech bark disease in the Bartlett Experimental Forest, New Hampshire. *Northern Journal of Applied Forestry*. 23, 141-143.

Lemaître, J., Villard, M.A. 2005. Foraging patterns of pileated woodpeckers in a managed Acadian forest: a resource selection function. *Canadian Journal of Forest Research.* 3510, 2387-2393.

Löf, M., Dey, D.C., Navarro, R.M., Jacobs, D.F. 2012. Mechanical site preparation for forest restoration. *New Forests.* 43, 825-848

Loo, J., Ramirez, M., Krasowski, M. 2005. American beech vegetative propagation and genetic diversity. In: Evans, Celia A., Lucas, Jennifer A. and Twery, Mark J., eds. Beech Bark Disease: Proceedings of the Beech Bark Disease Symposium; 2004 June 16-18; Saranak Lake, NY. Gen. Tech. Rep. NE-331. Newtown Square, PA: US. Department of Agriculture, Forest Service, Northeastern Research Station: 106-112.

Mahoney, E., Milgroom, M., Sinclair, W., Houston, D. 1999. Origin, Genetic Diversity, and Population Structure of *Nectria coccinea* var. faginata in North America. *Mycologia*. 91(4), 583.

Maiti, S.K. 2013. Raising of Saplings for Forest Trees. In: Ecorestoration of the Coalmine Degraded Lands. New Delhi: Springer India.

Marra, R.E., & LaMondia, J.A. 2020. First Report of beech leaf disease, caused by the foliar nematode, *Litylenchus crenatae* Mccannii, on American Beech (*Fagus grandifolia*) in Connecticut. *Plant Disease*. 104(9), 2527.

Mason, M.E., Koch, J., Krasowski, M., Loo, J. 2013. Comparisons of protein profiles of beech bark disease resistant and susceptible American beech (*Fagus grandifolia*). *Proteome Science*. 11, 2.

Maurer B.A., Whitmore R.C. 1981. Foraging of five bird species in two forests with different vegetation structure. *The Wilson Bulletin* 93: 478-490.

Mayer, M., Allen, D.C. 1983. *Chilocorus stigma* (Coleoptera: Coccinellidae) and other predators of beech scale in central New York. In: Proceedings, I.U.F.R.O. Beech Bark Disease Working Party Conference; 1982 September 26-October 8; Hamden, CT. Sponsored by the USDA Forest Service, Northeastern Forest Experiment Station. Gen. Tech. Rep. WO-37. [Washington, DC]: U.S. Department of Agriculture, Forest Service: 89-98.

McCullough, D.G., Heyd, R.L., O-Brien, J.G. 2001. Biology and management of beech bark disease. Michigan State University Extension Bulletin (E-2746). East Lansing, MI.

McGee, G.G. 2000. The contribution of beech bark disease-induced mortality to coarse woody debris loads in northern hardwood stands of Adirondack Park, New York, U.S.A. *Canadian Journal of Forest Research.* 30, 1453-1462.

Michigan Department of Natural Resources (MI DNR). 2015. Forest Stewardship in Michigan. Electronic Bulletin. https://www.michigan.gov/documents/dnr/GeneralForestryInfo_474276_7.pdf

Millerón, M., López de Heredia, U., Lorenzo, Z., Perea, R., Dounavi, A., Alonso, J., Gil, L., Nanos, N. 2012. Effect of canopy closure on pollen dispersal in a wind-pollinated species (*Fagus sylvatica* L.). *Plant Ecology*. 213, 1715–1728.

Missouri Botanical Garden. 2020. *Fagus grandifolia*. Online Publication. http://www.missouribotanicalgarden.org/PlantFinder/PlantFinderDetails.aspx?kempercode=a865 Accessed 12/7/2020.

Modrý, M., Hubený, D., Rejšek, K. 2004. Differential response of naturally regenerated European shade tolerant tree species to soil type and light availability. *Forest Ecology and Management*. 188(1), 185–195.

Morton Arboretum. 2020. American beech. Webpage. https://ag.tennessee.edu/news/Pages/POM-2015-12.aspx Accessed 12/7/2020.

Myers, A.L., Dickinson, Y.L., Storer, A.J., Bal, T.L. In Preparation a. A Pilot Study in Transplanting Methods for Wildling American beech (*Fagus grandifolia*).

Myers, A.L., Dickinson, Y.L., Storer, A.J., Bal, T.L. In Preparation b. Beech Bark Disease in Northern Michigan and Site Selection for a Resistant Tree Restoration Plan.

NOAA National Centers for Environmental information, Climate at a Glance: County Time Series http://www.ncdc.noaa.gov/cag/ (accessed 3 March 2021)

Nyland, R.D. 2008. Origin of Small Understory Beech in New York Northern Hardwood Stands. *Northern Journal of Applied Forestry*. 25(3), 161–163.

Nyland, R.D., Bashant, A.L., Bohn, K.K., & Verostek, J.M. 2006. Interference to hardwood regeneration in northeastern North America: Controlling effects of American beech, striped maple, and hobblebush. *Northern Journal of Applied Forestry*. 23(2), 122-132.

O'Brien, J., Ostry, M., Mielke, M., Mech, R., Heyd, R., McCullough, D. 2001. First Report of Beech Bark Disease in Michigan. *Plant Disease.* 85, 921–921.

O'Hara, K. 2014. Multiaged silviculture: managing for complex forest stand structures. First edition, Oxford University Press.

Oladi, R., Pourtahmasi, K., Eckstein, D., Bräuning, A. 2011. Seasonal dynamics of wood formation in Oriental beech (*Fagus orientalis* Lipsky) along an altitudinal gradient in the Hyrcanian forest, Iran. *Trees*. 25(3), 425-433.

Oliver, C.D., Larson, B.C. 1996. Plant Interactions and Limitations of Growth. Pages 9-40 In: Forest Stand Dynamics. Wiley, New York, N.Y.

Ostrofsky, W.D., McCormack, M.L. 1986. Silvicultural management of beech and the beech bark disease. *Northern Journal of Applied Forestry.* 3, 89-91.

Papaik, M.J., Canham, C.D., Latty, E.F., Woods, K.D. 2005. Effects of an introduced pathogen on resistance to natural disturbance: beech bark disease and windthrow. *Canadian Journal of Forest Research.* 35, 1832-1843.

Petrillo, H., Witter, J.A., Thompson, E.M. 2005. Michigan beech bark disease monitoring and impact analysis system. In: Beech bark disease: Proceedings of the beech bark disease symposium. US For. Serv. Gen. Tech. Rep. NE-331. US For. Serv., Northeast. Res. Stn., Newtown Square, PA, 2005.

Pijut, P.M., Woeste, K.E., Michler, C.H., Janick, J. 2010. Promotion of adventitious root formation of difficult-to-root hardwood tree species. p. 213-251. In: Horticultural Review. Hoboken, NJ, USA.

Poulsen, K.M. 1993. Predicting the storage life of beech nuts. *Seed Science and Technology.* 21, 327-337.

R Core Team. 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Ramirez, M., Krasowski, M., Loo, J. 2007. Vegetative propagation of American beech resistant to beech bark disease. *HortScience*. 42, 320-324.

Ramirez, M., Loo, J., Krasowski, M.J. 2007. Evaluation of resistance to the beech scale insect (*Cryptococcus fagisuga*) and propagation of American beech (*Fagus grandifolia*) by grafting. *Silvae Genetica*. 56, 163-169.

Reed, S.E., Qing, Y., Hoke, A., Burke, D.J., Carta, L.K., Handoo, Z.A., Kantor, M.R., Koch, J. 2020. Foliar nematode, *Litylenchus crenatae* ssp. Mccannii, population dynamics in leaves and buds of beech leaf disease-affected trees in Canada and the US. *Forest Pathology.* 50, e12599.

Ribbens, E., Silander, J.A.Jr., and Pacala, S.W. 1994. Seedling Recruitment in Forests: Calibrating Models to Predict Patterns of Tree Seedling Dispersion. *Ecology*. 75(6), 1794–1806.

Robb, J.R., Brookhout, T.A. 1995. Factors influencing wood duck use of natural cavities. *The Journal of Wildlife Management*. 59, 372-383.

Rosemier, J.N., Storer, A.J. 2011. Assessing native small mammals' responses to an incipient invasion of beech bark disease through changes in seed production of American beech. In: Fei, Songlin; Lhotka, John M.; Stringer, Jeffrey W.; Gottschalk, Kurt W.; Miller, Gary W., eds. Proceedings, 17th central hardwood forest conference; 2010 April 5-7; Lexington, KY; Gen. Tech. Rep. NRS-P-78. Newtown Square, PA: US Department of Agriculture, Forest Service, Northern Research Station: 675-676.. Vol. 78. 2011.

Roy, M.È., Nolet, P. 2018. Early-stage of invasion by beech bark disease does not necessarily trigger American beech root sucker establishment in hardwood stands. *Biological Invasions.* 20, 3245-3254.

Rushmore, F.M. 1961. Silvical characteristics of beech (*Fagus grandifolia*). Station Paper NE-161. Upper Darby, PA: U.S. Department of Agriculture, Forest Service, Northeastern Forest Experiment Station. 26 p.

Sanders, S., Kirschbaum, J. 2019. Forest Health Monitoring at Sleeping Bear Dunes National Lakeshore. Natural Resource Data Series NPS/GLKN/NRDS – 2019/1241

Sapkota, S., Sapkota, S., Wang, S., and Liu, Z. 2019. Height and Diameter Affect Survival Rate of Jujube Suckers Transplanted in a Semi-Arid Farmland of New Mexico. *Journal of Applied Horticulture* 21(3), 249–251.

Schomaker, M.E., Zarnoch, S.J., Bechtold, W.A., Latelle, D.J., Burkman, W.G., Cox, S.M. 2007. Crown-Condition Classification: A Guide to Data Collection and Analysis. USDA Forest Service Southern Research Station General Technical Report: SRS-102

Seger, R.L., Servello, F.A., Cross, R.A., Keisler, D.H. 2013. Body mass and mast abundance influence foraging ecology of the American black bear (*Ursus americanus*) in Maine. *Canadian Journal of Zoology*. 91: 512-522.

Shigo, A.L. 1972. The beech bark disease today in the northeastern US. *Journal of Forestry*. 70, 286-289.

Shigo, A.L. 1962. Another scale insect on beech. Station Paper NE-168. Upper Darby, PA: U.S. Department of Agriculture, Forest Service, Northeastern Forest Experiment Station. 13 p.

Smith, D.M., Larson, B.C., Kelty, M.J., Ashton, M.S. 1997. Preparation and Treatment of the Site. Pages 195-233 In: The Practice of Silviculture. Wiley, New York, N.Y.

Sniezko, R.A., Koch, J. 2017. Breeding trees resistant to insects and diseases: putting theory into application. *Biological Invasions*. 19, 3377-3400.

Stephanson, C.A., Coe, N.R. 2017. Impacts of beech bark disease and climate change on American beech. *Forests.* 8, 155.

Stephens, R.B., Hobbie, E.A., Lee, T.D., Rowe, R.J. 2019. Pulsed resource availability changes dietary niche breadth and partitioning between generalist rodent consumers. *Ecology and Evolution*. 9, 10681-10693.

Sweeney, J., Hughes, C., Zhang, H., Hillier, N. K., Morrison, A., Johns, R. 2020. Impact of the invasive beech leaf-mining weevil, *Orchestes fagi*, on American beech in Nova Scotia, Canada. *Front. For Global Change*. 3, 1-11.

Taylor, A.R., McPhee, D.A., Loo, J.A. 2013. Incidence of beech bark disease resistance in the eastern Acadian forest of North America. *The Forestry Chronicle*. 89(5), 690-695.

Theodoropoulos, C., Stamou, A., Vardakas, L., Papadaki, C., Dimitriou, E., Skoulikidis, N., Kalogianni, E. 2020. River restoration is prone to failure unless pre-optimized within a mechanistic ecological framework: Insights from a model-based case study. *Water Restoration.* 173, 465-487.

Tozer D.C., Burke D.M., Nol E., Elliott K.A. 2012. Managing ecological traps: logging and sapsucker nest predation by bears. *The Journal of Wildlife Management*. 76, 887-898.

Traux, B., Gagnon, D., Fortier, J., Lambert, F., Pétrin, M.A. 2018. Ecological factors affecting white pine, red oak, bitternut hickory and black walnut underplanting success in a northern temperate post-agricultural forest. *Forests*. 9, 499.

Tubbs, C.H., Houston, D.R. 1990. *Fagus grandifolia* Ehrh. American beech. Pages 325-332 In: Burns RM, Honkala BH (tech. coords.) Silvics of North America. Agriculture Handbook 654.U.S. Department of Agriculture, Forest Service, Washington, D.C.

University of Tennessee. 2015. American beech. Webpage. https://ag.tennessee.edu/news/Pages/POM-2015-12.aspx Accessed 12/7/2020.

Venables, W. N. Ripley, B. D. 2002. Modern Applied Statistics with S. Fourth Edition. Springer, New York. ISBN 0-387-95457-0

Wagner, S., Collet, C., Madsen, P., Nakashizuka, T., Nyland, R., Sagheb-Talebi, K. 2010. Beech regeneration research: From ecological to silvicultural aspects. *Forest Ecology and Management*. 259(11), 2172–2182.

Wainhouse, D. 1980. Dispersal of first instar larvae of the felted beech scale, *Cryptococcus fagisuga*. *The Journal of Applied Ecology.* 17, 523-532.

Wainhouse, D., Gate, I.M. 1988. The Beech Scale. Pages 67-85 In: Dynamics of Forest Insect Populations. Springer, Boston, MA.

Walter, M., Campbell, R.E., Amponsah, N.T., Scheper, R.W.A., and Butler, R.C. 2017. Evaluation of biological and agrichemical products for control of *Neonectria ditissima* conidia production. *New Zealand Plant Protection.* 1, 87-96.

Walter, M., Stevenson, O.D., Amponsah, N.T., Scheper, R.W.A., Rainham, D.G., Hornblow, C.G., Kerer, U., Dryden, G.H., Latter, I., and Butler, R.C. 2015. Control of *Neonectria ditissima* with copper based products in New Zealand. *New Zealand Plant Protection.* 68, 241-249.

Weber, R.W.S. 2014. Biology and control of the apple canker fungus *Neonectria ditissima* (syn. *N. galligena*) from a Northwestern European perspective. *Erwerbsobstbau.* 56, 95-107.

Wickham, H., Averick, M., Bryan, J., Winston, C., McGowan, L.D., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T.L., Miller, E., Bache, S.M., Müller, K., Ooms, J., Robinson, D., Seidel, D.P., Spinu,

V., Takahashi, K., Vaughan, D., Wilke, C., Woo, K., Yutani, H. 2019. Welcome to the tidyverse. *Journal of Open Source Software*. 4, 1686.

Wiggins, G.W., Grant, J.F., Eelbourne, W.C. 2001. *Allothrombium mitchelli* (Acari: Trombidiidae) in the Great Smoky Mountains National Park: Incidence, seasonality, and predation on beech scale (Homoptera: Eriococcidae). *Annals of the Entomological Society of America.* 94, 896-901.

Wisconsin Department of Natural Resources (DNR). 2014. Management of beech bark disease in Wisconsin. Website. https://dnr.wisconsin.gov/sites/default/files/topic/ForestHealth/beechBarkManagement.pdf Accessed 12/7/2020.

Woods, K.D., Davis, B.D. 1989. Paleoecology of range limits: Beech in the Upper Peninsula of Michigan. *Ecology*. 70, 681-696.

Wright, S. 1952. The genetics of quantitative variability. Quantitative Inheritance. Her Majesty's Stationery Office, London.

Zahawi, R.A., Holl, K.D., Cole, R.J., Reid, J.L. 2013. Testing applied nucleation as a strategy to facilitate tropical forest recovery. *Journal of Applied Ecology.* 50, 88-96.

A SCION COLLECTION

A.1 Equipment:

Collection	Storage	PPE
Arborist slingshot + trigger	Heavy plastic bags	Work gloves
Double sided pull saw	Damp packing material (sphagnum moss, paper, sawdust)	Eye protection
Throwline/slick line (about 100 ft)	Tagging material (masking tape or flagging tape)	Hard hat
Throw bags	Water resistant permanent marker	GPS
2x Pull line (heavy rope) (About 100 ft each)	Duct tape (heavy duty or outdoors)	

Optional Equipment: Extra carabiners, extra throwline, throw cube, snowshoes, Extra pull line in a shorter length (about 60 feet)

A.2 Planning

Scion collection should occur in the spring, before greening out occurs. Generally, this occurs between December and March in Michigan (Ramirez et al., 2007; Carey et al.,

2013). Scions should be collected as close as possible to the beginning of grafting, as long-term storage may lead to loss of scion material to disease or desiccation.

Scions should be collected from confirmed resistant American beech. If field challenge tests are not possible, scions may be collected from putatively resistant trees, identified by multi-year absence of scale infestations. Resistance of grafted seedlings must be confirmed in the greenhouse regardless of scion origin, after the graft union has healed completely, but before outplanting occurs (3-5 years after grafting). Refrigerated cold storage space is necessary to store scions.

A.3 Collection

Scions should be collected from the outer edge of the canopy of beech. In order to collect upright growth, collect from the highest branch possible (Humphrey, 2019).

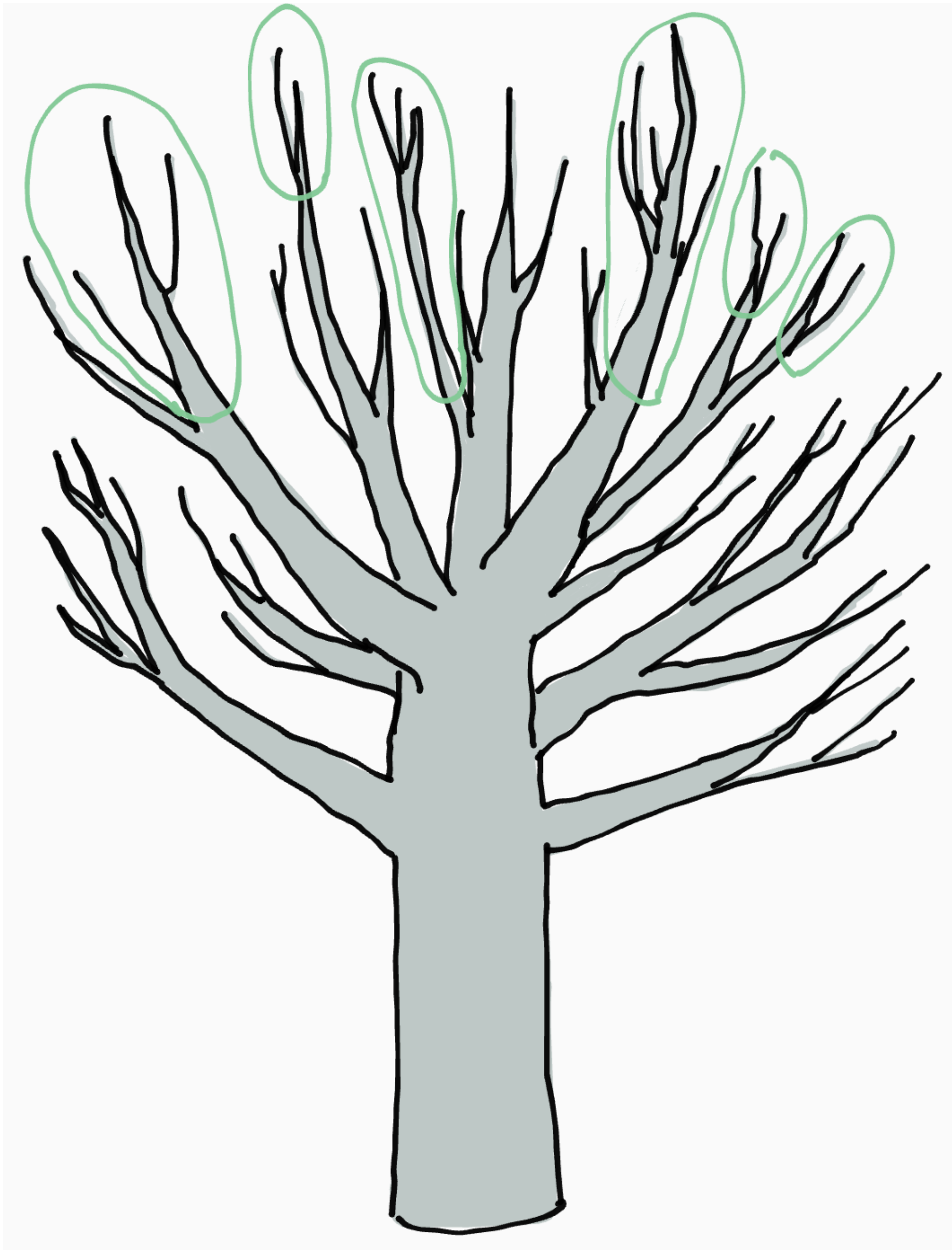

Figure A14. Branches acceptable for scion collection. Light gray circles indicate ideal branches, which demonstrate upright growth, access to sunlight, and acceptable lengths for collection.

1. Create a staging area on the ground. The staging area should be as clear from competing vegetation as possible, to prevent tangling of the lines during shooting. It will be helpful to select an area with a clear line of sight to the canopy if possible. Limbs from larger trees and midstory trees may tangle the line while shooting, or impede the trajectory of the weight bag as it is shot into the canopy.

2. In the staging area, assemble the line. Attach a throw bag to each end of the throwline using two half-hitch knots, slippery clove hitch, or a similar stable knot. Lay out the throwline in a zigzag, or if available, in a throw cube. Ensure that the throwline is free of tangles and knots.

3. Load the slingshot into the trigger according to the manufacturer's instructions. Load the lighter, or lead throw bag into the cradle of the slingshot. Select a 4-6 ft portion of limb that you would like to collect. Aim slightly higher than the branch section selected, and position the slingshot so that the throwline is beside the slingshot, and any humans are behind and well away from the shooter. Release the trigger on the slingshot, and watch the trajectory of the throw bag. You should seat the throw line in a branch crotch at the base of the selection you have chosen.

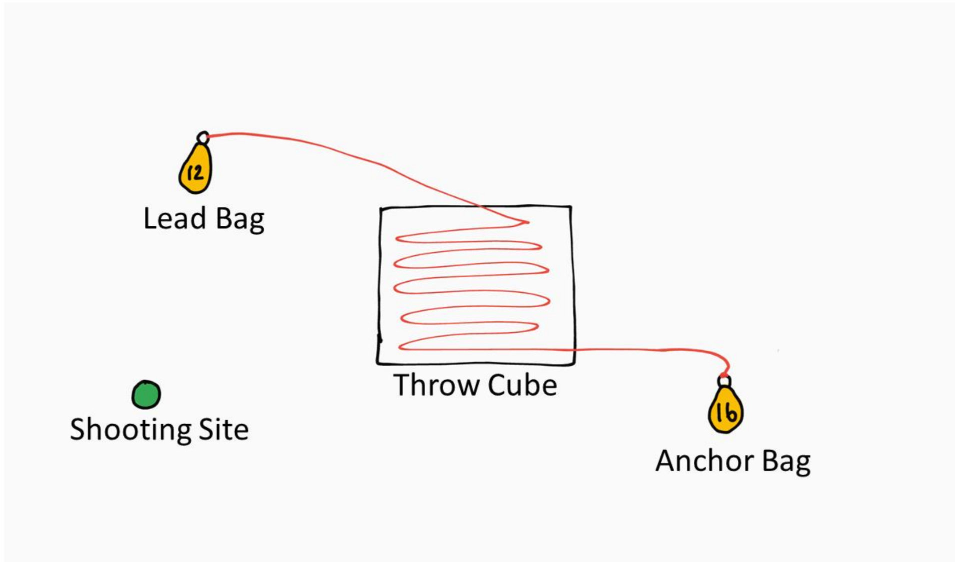

Figure A15. Throw cube line setup. Keep heavier bag in anchor position. Flake rope into throw cube to prevent knots. Select a shooting site behind and away from throw cube to prevent tangling.

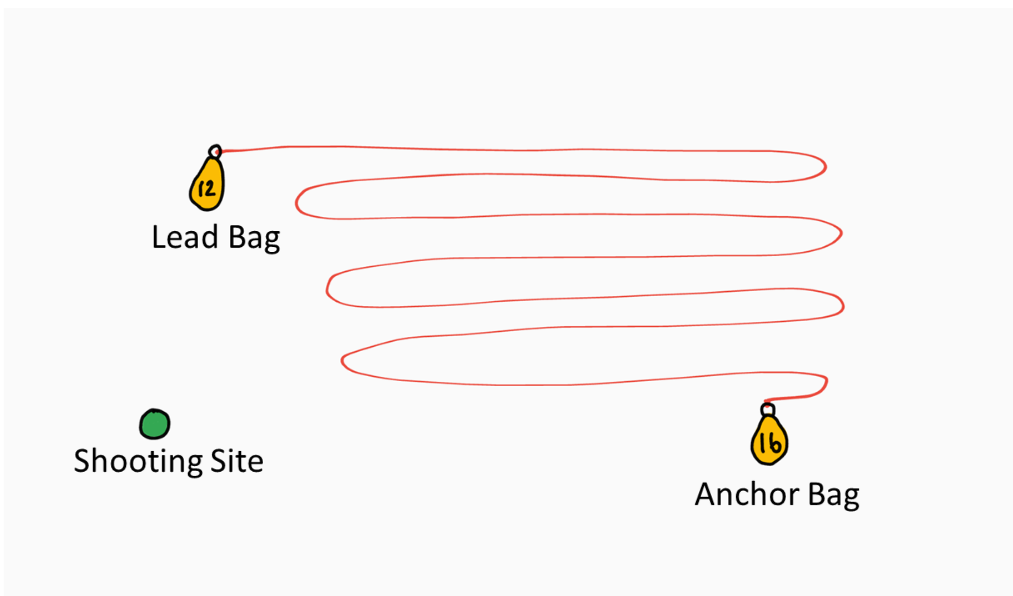

Figure A16. Bare ground line setup. Keep heavier bag in anchor position. Carefully flake line in a zigzag arrangement to prevent knots. Select a shooting site behind and away from throw cube to prevent tangling. In this arrangement it is important to prevent overlaps from occurring with careful selection of shooting site.

- It is important to watch the trajectory of the throw bag to allow corrections in any additional shots that are necessary.

- In scion collection, you should not collect entire limbs to prevent stressing the resistant donor tree, so you should not seat the rope in the branch crotch at the base of the limb.
- If you miss the selected branch portion, pull the throw bag back and re-shoot. Communicate clearly to ensure that all present are aware that the weighted bag will be returning. The weighted bag will 'jump' the branch as you pull it back, so make sure everyone present is aware of and watching the falling weight.

4. If the throw line is seated well, the lead throw bag should descend through the canopy cleanly. If the throwline catches in branches on the descent, the rope may be 'whipped' to enable the throw bag to descend.

5. After the throwline is seated, attach one end the pull rope to the end of the throwline, and the other end to the pull saw. Attach the second pull rope to the other end of the pull saw. Use the throwline to raise the pull saw to the branch.

6. After the pull saw has reached the branch, untie and store the throwline. One technician should take each pull rope in hand. Move as far from the tree as possible, so that the saw and pull ropes are perpendicular to the selected branch.
   - The farther away the technicians can stand, the more easily the saw will run across the branch, enabling a faster, cleaner cut.

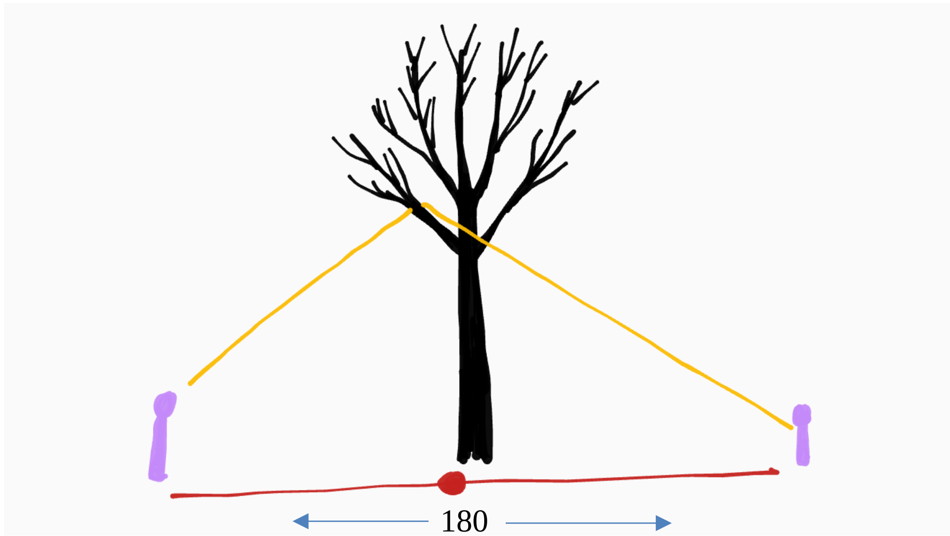

Figure A17. Cutting arrangement to remove branches. Technicians should stand as far apart as possible to create an oblique cutting angle over the selected branch to facilitate sawing. By standing in a perpendicular angle to the selected branch, the saw will operate cleanly and without kinking.

7. Use long, even strokes to saw through the branch.
   - Sawdust will fall from the tree, so be sure to wear eye protection.
   - Be aware of the falling branch. The branch may fall before you have cut cleanly through it, leading to a sharp, heavy projectile falling suddenly.
   - Limbs and other trees in the way may cause the branch to descend erratically.

A.4 Storage

After the branch has fallen, label the limb clearly with the name of the resistant tree. Pack the branch in a heavy duty plastic bag with damp packing material around the cut end to prevent desiccation of the branch during transport. Duct tape can be wrapped around the heavy plastic bag to compress lateral branches for transport, and the bag should be sealed with tape to prevent desiccation.

If being transported to a separate grafting location, scions may be stored in in a 4-6° C cold room their heavy plastic bags prior to shipment. If trees are being grafted on

site, buckets of fresh, clean water should be placed in a 4-6° C cold room, so that scions can be immediately trimmed and stored upon return. Do not allow scions to freeze, or warm beyond 4-6° C for extended periods.

A.5 Resources

Carey, D.W., Mason, M.E., Bloese, P., & Koch, J. L. 2013. Hot callusing for propagation of American beech by grafting. *HortScience*, 48(5), 620-624.

Ramirez, M., Krasowski, M.J., & Loo, J.A. 2007. Vegetative propagation of American beech resistant to beech bark disease. *HortScience*, 42(2), 320-324.

Humphrey, B. 2019. Ch 37: *Fagus* (Fagaceae) – Beech. In *The Bench Grafter's Handbook* (1st ed., Vol. 1). CRC Press. https://doi.org/10.1201/9781315171463

B GRAFTING TECHNIQUES FOR AMERICAN BEECH

B.1 Materials:

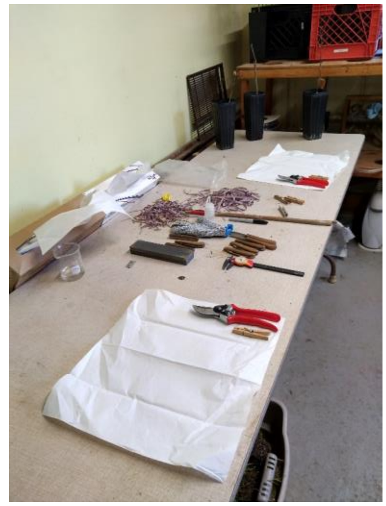

Figure A18. A typical grafting bench setup.

- ✓ Grafting knives
- ✓ Pruning shears
- ✓ 70-80% Ethanol solution
- ✓ Kimtech wipes
- ✓ Grafting Rubbers
- ✓ Hot water bath (double-boiler style)
  - o with thermometer
- ✓ Paraffin wax
- ✓ Insulating foam lid for water bath
- ✓ Pot cover for dipping grafts
- ✓ Masking tape or duct tape

<u>Personal Protective Equipment</u>

- ✓ Cut proof gloves

B.2 Preparation

1. Rootstocks should be lightly watered and in a dormant state.

2. Prepare a hot water bath. In a double-boiler setup, bring hot distilled water to (44°C ± 6°). Add a new block of paraffin wax to the distilled water and allow to melt completely. Cover the wax with an insulating foam lid when not in use. Keep the heat source at the lowest setting possible and use the insulating foam to maintain the temperature. Dipping scions in wax that is too hot can damage them, so maintain the lowest temperature that keeps the paraffin wax fully melted.

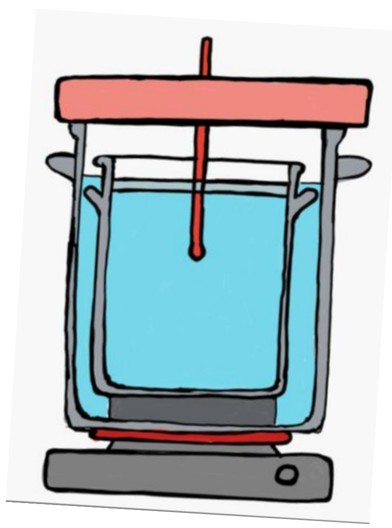

Figure A19. The hot water bath configuration. The interior pot, made of plastic or metal, should be able to fit fully inside of the exterior, metal pot. The depth of the interior pot should be sufficient to dip grafted trees with minimal bending of the scion when inverted and dipped. An insulating foam cover may be used with a thermometer inserted to prevent the cooling of wax between dipping, while still allowing a view of the temperature. Alternatively, a probe thermometer may be placed in the hot water between the inner and outer pots to monitor the temperature without lifting the lid. A spacer should be used below the inner pot to prevent direct contact between the inner pot and heating element.

Figure A20. An example hot water bath. The thermometer is placed so that the bulb reads the temperature of the wax within the inner pot, but the ideal temperature range is visible outside the insulating foam lid.

3. Use foam to create a pot cover for dipping grafts. Trees must be inverted over the wax for grafting, so a piece of foam board or heavy cardboard should be used to cover the pot and prevent debris from the pot trees are planted in from falling into the wax. Cut a square 2-3 inches larger on each side than your pot size. Cut a slit halfway through the square. When dipping grafts, place the cover tightly against the top of the pot and hold in place while inverting the plant. Using cardboard will allow you to make additional covers if yours gets dirty or wet, however foamboard is more rigid and easier to hold in place.

4. Scions should be stored upright in cool, clean water.
    - Move one genotype to the grafting area. Working with one genotype at a time will prevent mislabeling of grafts.

Figure A21. Scions ready for grafting, stored in cool water with freshly trimmed ends.

5. Bring rootstocks to the grafting area. Aim to bring 3x the amount of grafts that you intend to make. This will allow you to select a rootstock and scion of matching diameter.

6. Prepare tags for the genotype you will be working with. Masking or flagging tape can be used for short term identification, but soft aluminum tags are preferable for long-term storage and identification. The tags will need to last upwards of 5 years.

    - Use a meaningful labelling system. Our system of identification is:

Resistant Tree Code

Rootstock source #

Grafting Date, Grafting #

Example:

FAGR31

HWBR 105

12/3/2020 1

7. Prepare a witness stick for your hot callus apparatus. Using a piece of bamboo, place it flush against the insulating material of the hot callus apparatus. Use tape to mark the upper and lower edge of the insulated chamber. All of your grafts should fall within the upper and lower boundary. If necessary, you can graft just below the lower boundary, but never above. You can use shims to raise the bottom of the pot, but a graft that is too tall cannot be adjusted down to fit the chamber.

8. Sharpen and hone your knives immediately before grafting. Grafting knives should never be used for any purpose other than grafting cuts. Use a single bevel-style grafting knife for beech grafting. Right and left-handed models are available. When holding the grafting knife in your dominant hand, the beveled surface of the knife faces toward your body, and the flat surface faces away from your body. Pruning shears should be used for any additional cuts, including trimming the scions to length and trimming rootstocks.

B.3 Grafting

For small diameter rootstock (below 1 cm diameter) use a **side veneer graft [1]** or **modified veneer graft [2]**. For larger rootstocks (at least 1 cm diameter) a **top cleft graft [3]** using the top-cleft grafting machine may be used, unless you prefer the side or modified side graft. We recommend the modified side graft if sufficiently experienced grafters are available.

[1] Side Veneer Graft (Figure A20)

a. Use a witness stick to find a rootstock which is suitable for grafting at the desired height.

b. Measure the rootstock with calipers. For a side veneer graft, it is important that the rootstock and scion are the same diameter.

c. Select a scion that matches the diameter of the rootstock at a straight intermodal section.

d. Trim the scion so that it has 3 to 6 healthy buds. Use new, terminal growth whenever possible.

e. Dip the trimmed scion into a paraffin wax bath (44°C +-6°). Blot any moisture off the cut end of the scion.

In a veneer graft, the scion is cut into a long wedge shape using two single-cut strokes.

> A. To make the first cut on the scion, place the flat side of the grafting knife against the scion and pull towards your body. The knife should cut into the scion with little resistance. Use the whole length of the blade to make a single cut with a long, flat, elliptical surface. The cut should be over 1 inch long.
>
> B. Turn the scion over and line up the flat side of the knife parallel to the first cut, on the opposite side of the branch. Make a second long, flat cut, which meets the first cut at a sharp angle at the end of the scion. Both cut surfaces should be about an inch or more long.
>
> C. Trim the rootstock about ¼ inch above the required diameter. Place the flat edge of the grafting knife against the rootstock. Pull down in a gentle sweeping motion just until the knife catches in the bark. Use your non-dominant hand to

firmly cut a thin slice of bark, leaving it attached at the bottom. This flap is the “veneer”.

D. Insert the scion so that the wedge fits firmly in the base of the veneer cut. Be sure that the cambium lines up exactly on at least one side of the cut, but an exact match on both sides is ideal.

E. Use a clothes pin to hold the veneer and scion in place while wrapping the cut with a grafting rubber. Dip the tree in paraffin past the entire graft union site.

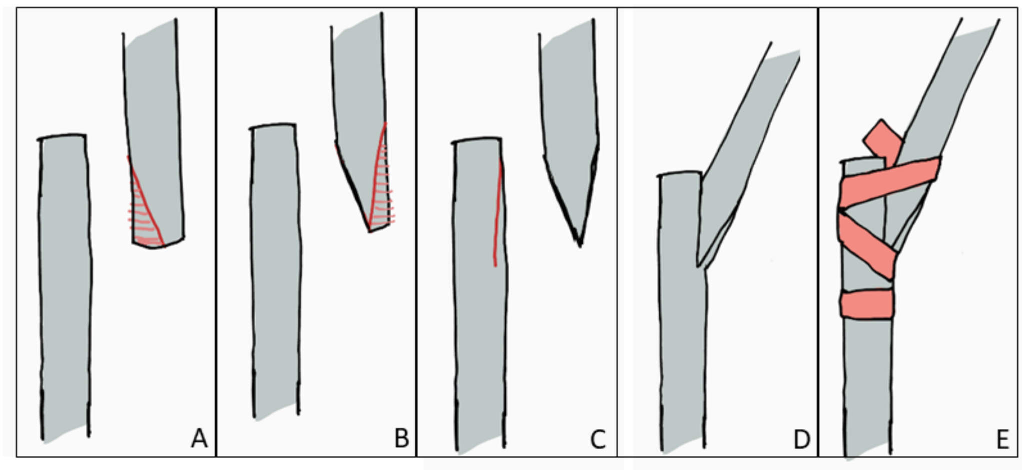

Figure A22. Side veneer graft, as described in Graft section [1].

[2] Modified Side Graft (Figure A21)

a. Use a witness stick to find a rootstock which is suitable for grafting at the desired height.

b. Measure the rootstock with calipers. For a modified side graft, it is not necessary for the rootstock and scion to match in size, however the rootstock should be the same size or larger than the scion.

c. Select a scion that roughly matches the diameter of the rootstock at a straight intermodal section.

d. Trim the scion so that it has 3 to 6 healthy buds. Use new, terminal growth whenever possible.

e. Dip the trimmed scion into a paraffin wax bath (44°C ± 6°). Blot any moisture off the cut end of the scion.

A. Make a first vertical cut on the scion. Use the grafting knife to cut into the scion, and then cut straight down the scion to create a flat, vertical surface. This cut should be at least an inch.

B. Make a second cut at the base of the scion. This cut will be about 1/8 to 1/4 inch long, and meet the first cut at a sharp angle.

C. On the rootstock, make a flat, vertical cut so that the width of the cut matches the width of the scion cut. This cut should be the same length or slightly longer than the cut on the scion.

D. Make one final, horizontal cut on the rootstock so that the flap is the same length as the wedge cut on the scion.

E. Remove the piece of rootstock cut away from the flap in step D.

F. Place the flat side of the scion against the flat cut on the rootstock. Be sure that the cambium lines up exactly on at least one side of the cut, but an exact match on both sides is ideal. The scion should be wedged firmly into the rootstock, with the flap in place on the wedge cut.

G. If necessary, use a clothes pin to hold the scion in place while wrapping the graft with a grafting rubber. Dip the tree in paraffin past the entire graft union site.

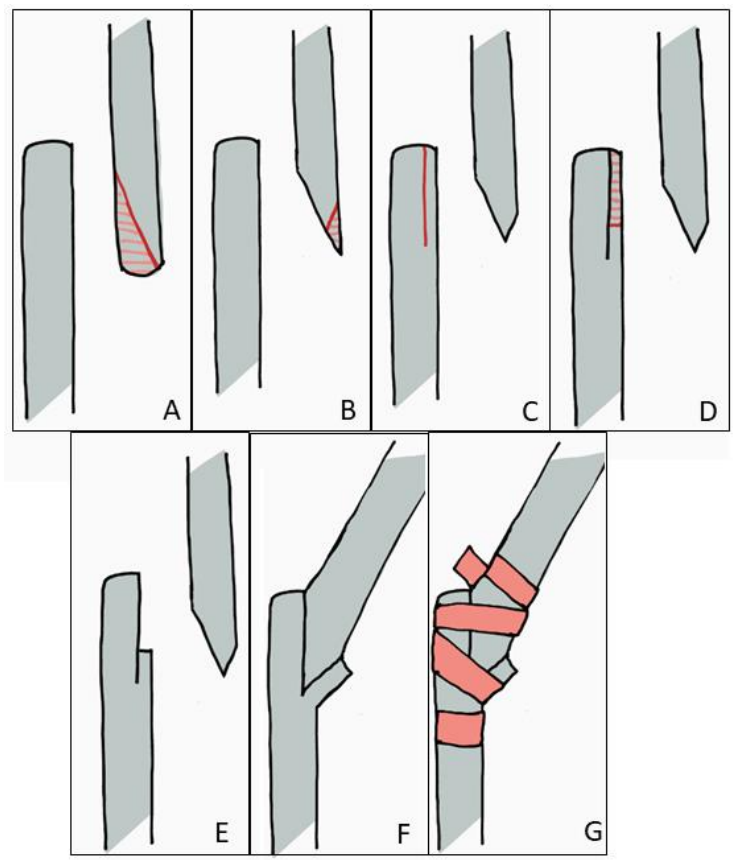

Figure A23. Figure A10. Side veneer graft, as described in Graft section [2].

[3] Top Cleft Graft (Machine Graft) (Figure A11)

a. Use a witness stick to find a rootstock which is suitable for grafting at the desired height.

b. Measure the rootstock with calipers. For a top cleft graft, it is important that the rootstock and scion are the same diameter.

c. Select a scion that matches the diameter of the rootstock at a straight intermodal section.

d. Trim the scion so that it has 3 to 6 healthy buds. Use new, terminal growth whenever possible.

e. Dip the trimmed scion into a paraffin wax bath (44°C +-6°). Blot any moisture off the cut end of the scion.

A. Place the scion in the top cleft grafting machine so that the point of the blade is centered in the scion. Place the base of the machine on the edge of a table and press down firmly in one smooth motion until the scion is completely cut through.
B. Find the matching diameter on the rootstock and line up the machine so that the point of the blade is in the center of the stem. Press down in a firm, smooth motion until the rootstock is completely cut through.
C. Place the scion into the rootstock, the fit should be snug. Be sure that the cambium lines up exactly on at least one side of the cut, but an exact match on both sides is ideal.
D. Ensure that the cut surfaces are free of debris and fit exactly.
E. Use a clothes pin to hold the scion in place while wrapping the graft with a grafting rubber. Dip the tree in paraffin past the entire graft union site.

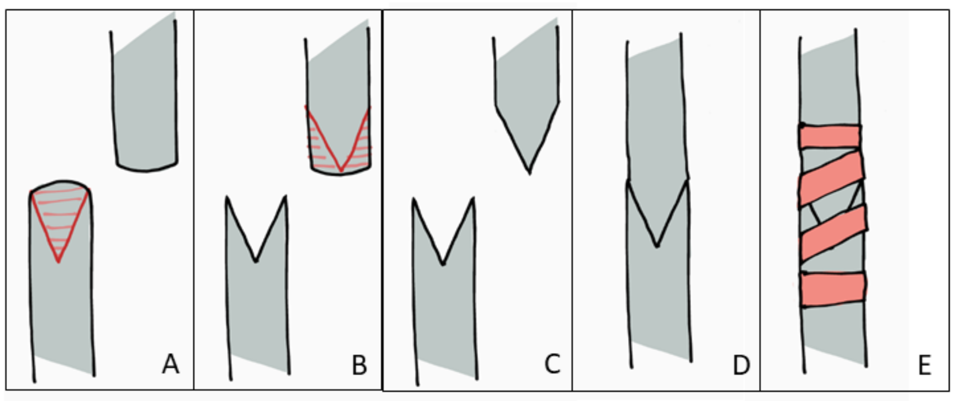

Figure A24. Top cleft (machine) graft, as described in Graft section [3].

B.4 Hot-Callus Placement

a. Place grafted trees carefully into hot-callus apparatus. Be extremely careful not to shift or move the graft union when placing the trees into the insulated foam chamber. Make sure no buds are inside the insulating foam chamber. Buds inside the insulating foam chamber will begin to grow immediately and take resources away from the healing graft union, preventing callus tissue from forming.

b. Close the insulating foam chamber around the graft using masking or duct tape.

c. Begin checking the graft union for callus tissue 4 weeks after grafting. Continue to check every 4-7 days for callus tissue formation. Be sure not to move or shift the graft when checking inside the chamber.

d. When callus tissue has begun to form, move the trees to the greenhouse. At this time, trees may be watered lightly. Do not saturate the soil fully to prevent lifting of the graft union.

e. Carefully attach scaffolding to the tree to prevent movement or twisting of the graft union as the wax softens in greenhouse temperatures.

f. Once the wax has softened sufficiently in the warm greenhouse and callus tissue has begun to expand, carefully cut away the grafting rubber to allow the callus tissue to grow.

g. Once the graft union has healed fully, you may water the trees as normal.

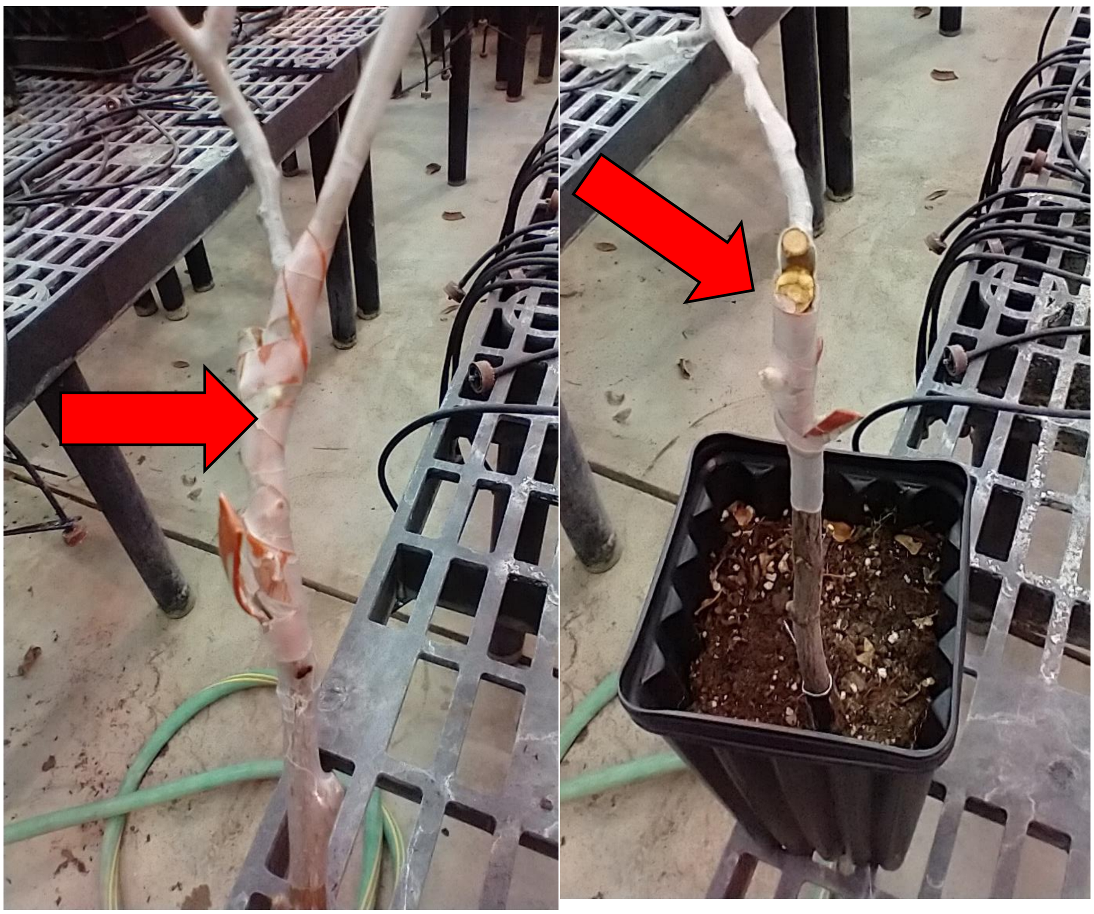

Figure A25. Callus tissue formation sufficient for removal from the hot-callus chamber. Callus can be seen through the paraffin wax layer, or may be clearly visible as the callus expands and pushes away the brittle layer of wax.

B.5 Troubleshooting:

Sharpen knives daily.

Bad cut surfaces? Be sure to hone your knife.

Twisting in the cut surface? Be sure you aren’t dropping your elbows.

Shoulder on your rootstock? Allow the knife to do the work on your downward cuts.

Uneven cut? Pull with both hands while cutting.

C Maps of Planting Site Selections Provided to National Park Service Collaborators

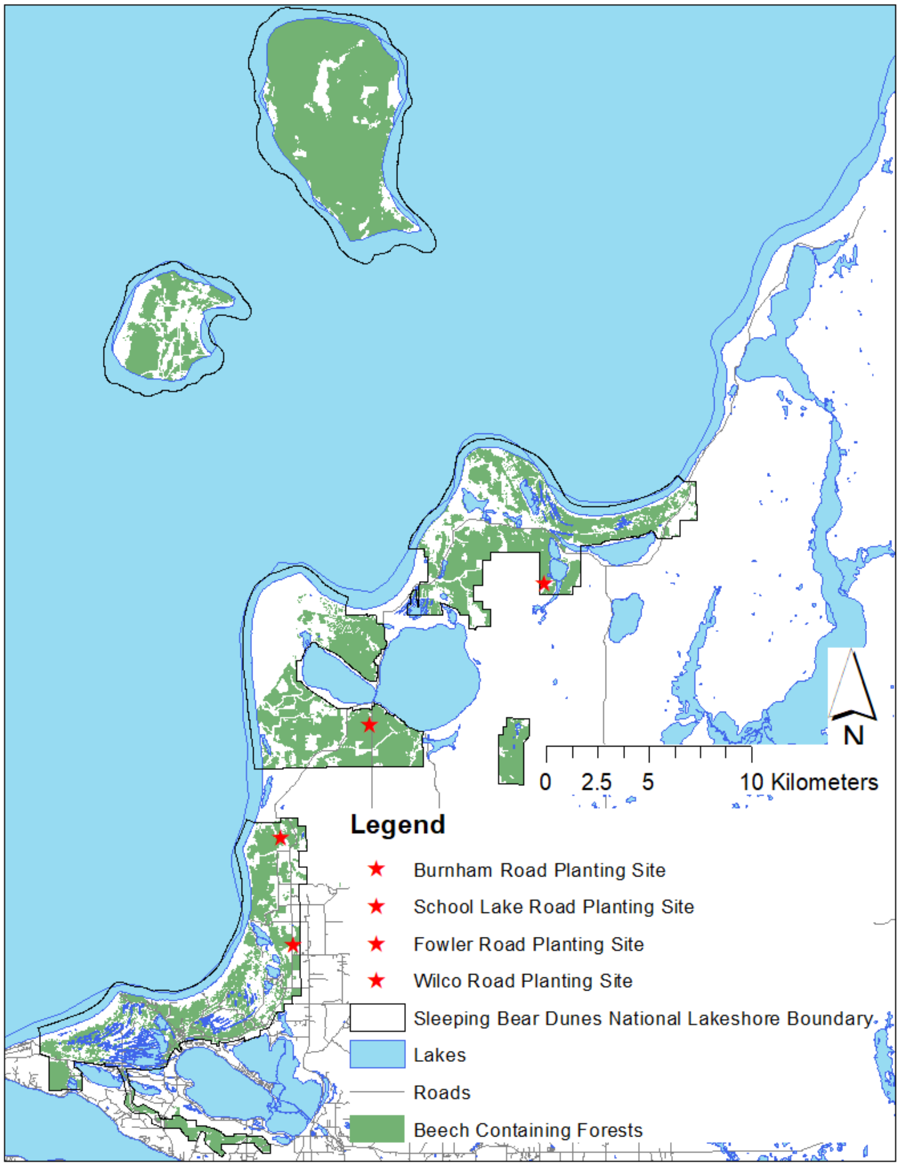

Figure A26. The overall planting site selection for Sleeping Bear Dunes National Lakeshore. Planting sites were selected using the processes described in chapter 3 of this dissertation. Forest composition data was provided by the National Park Service, Road layers are from TIGER 2015 Roads dataset, and lakes from Watershed Boundary datasets, downloaded at the NRCS Geospatial Data Gateway.

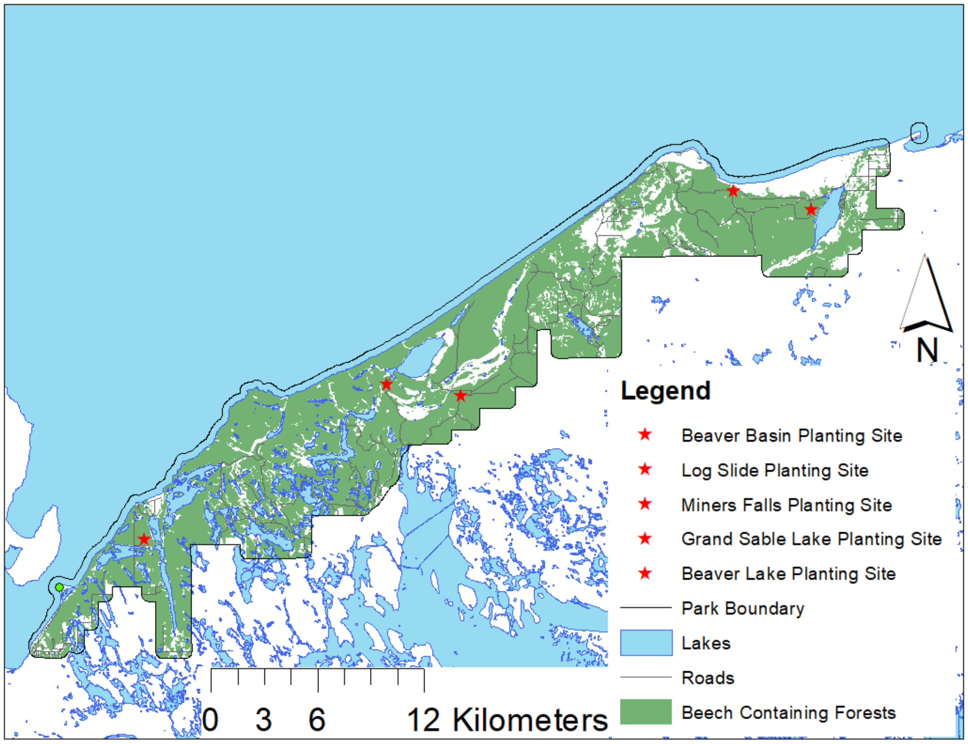

Figure A27. The overall planting site selection for Pictured Rocks National Lakeshore. Planting sites were selected using the processes described in chapter 3 of this dissertation. Forest composition data was provided by the National Park Service, Road layers are from TIGER 2015 Roads dataset, and lakes from Watershed Boundary datasets, downloaded at the NRCS Geospatial Data Gateway.

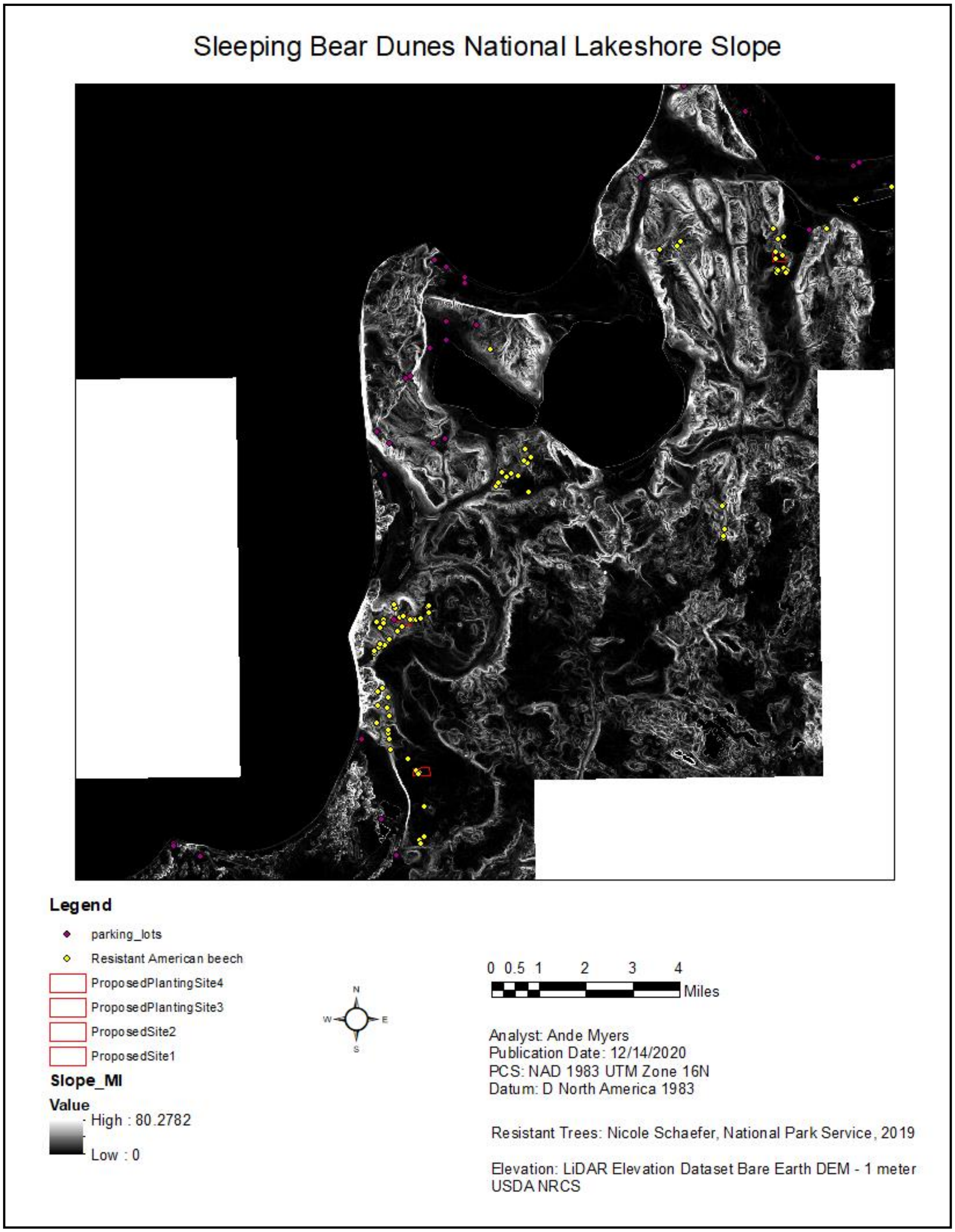

Figure A28. Slope of Sleeping Bear Dunes National Lakeshore, calculated from LiDAR Elevation Dataset via NRCS Geospatial Data Gateway.

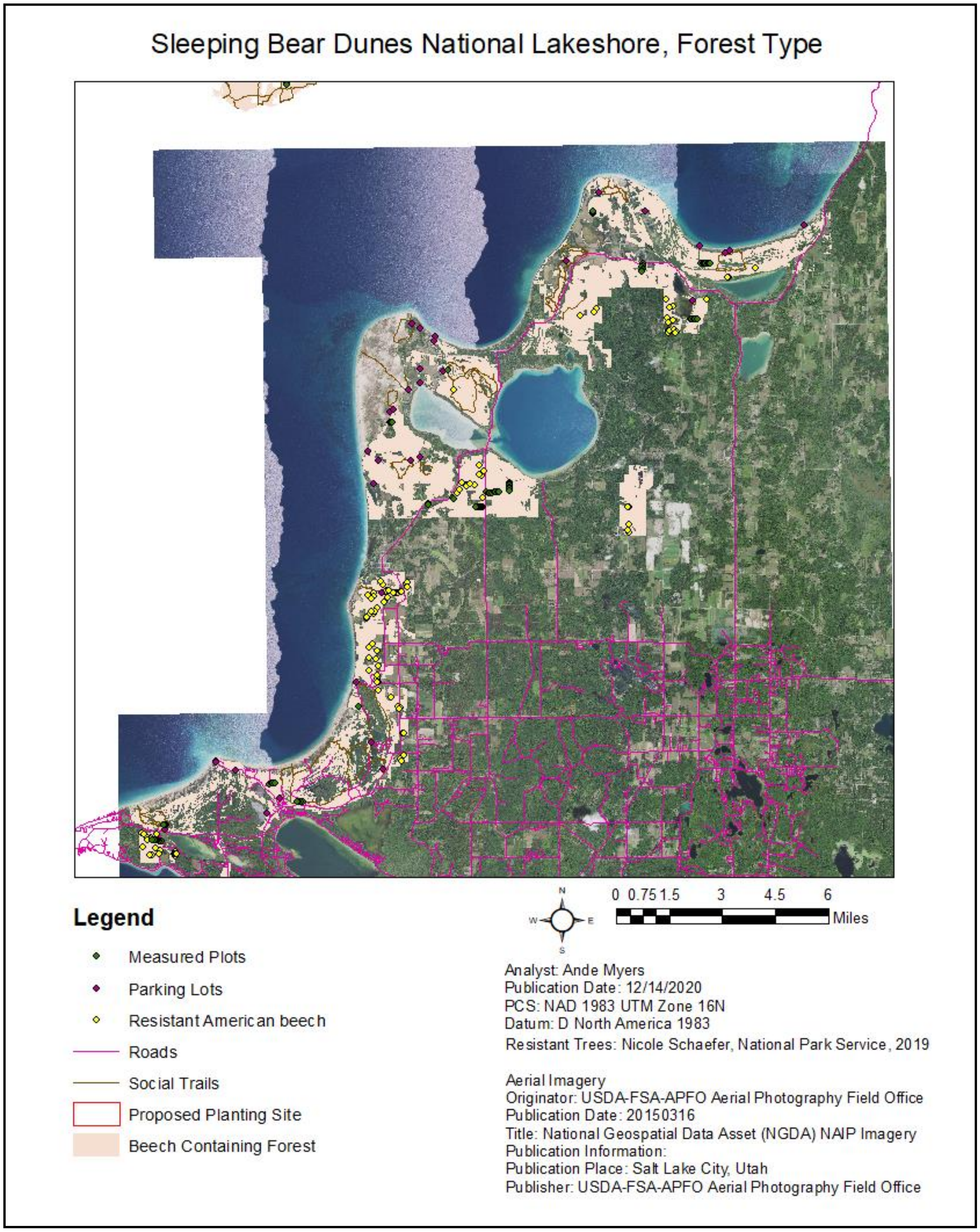

Figure A29. Points of interest for selection of planting sites in Sleeping Bear Dunes National Lakeshore. The forest types containing American beech are presented. Locations of resistant American beech and parking areas are marked, as planting sites must be located close to both.

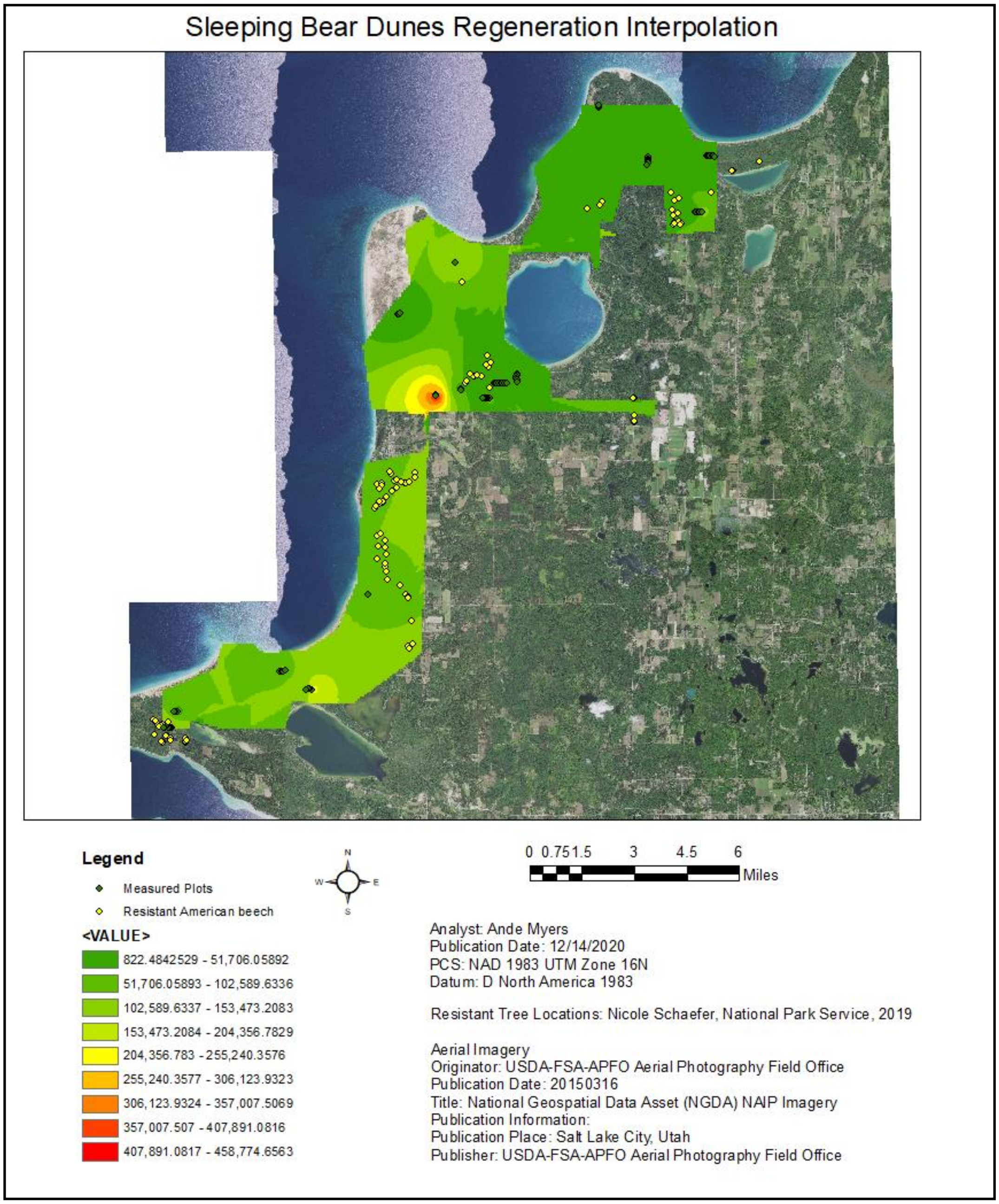

Figure A30. Prediction surface of beech regeneration created with IDW interpolation for Sleeping Bear Dunes National Lakeshore.

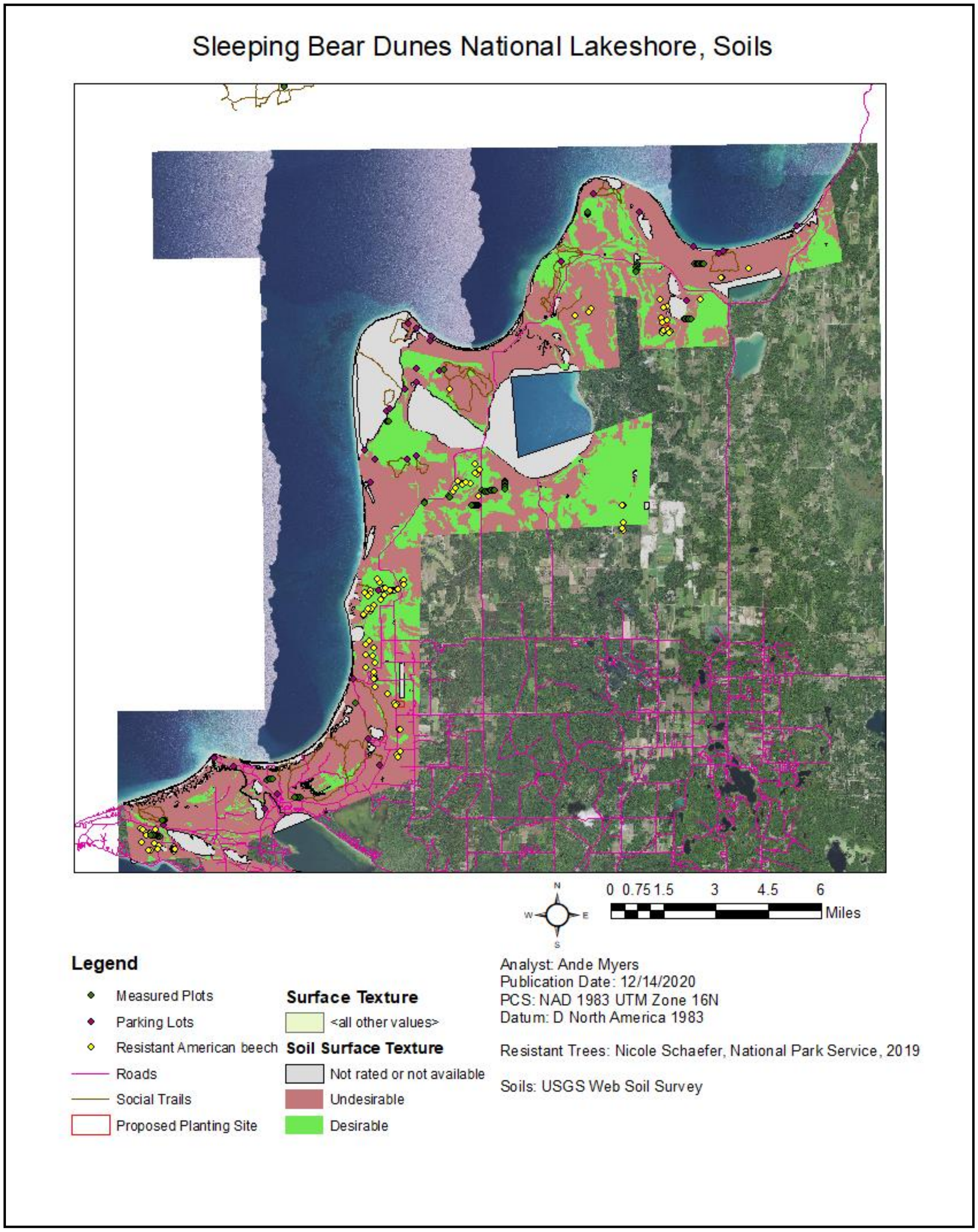

Figure A31. Soil surface texture in Sleeping Bear Dunes National Lakeshore. Soil surface texture was defined in the USGS Web Soil Survey toolset and broken into desirable and undesirable classes based on silvics of American beech.

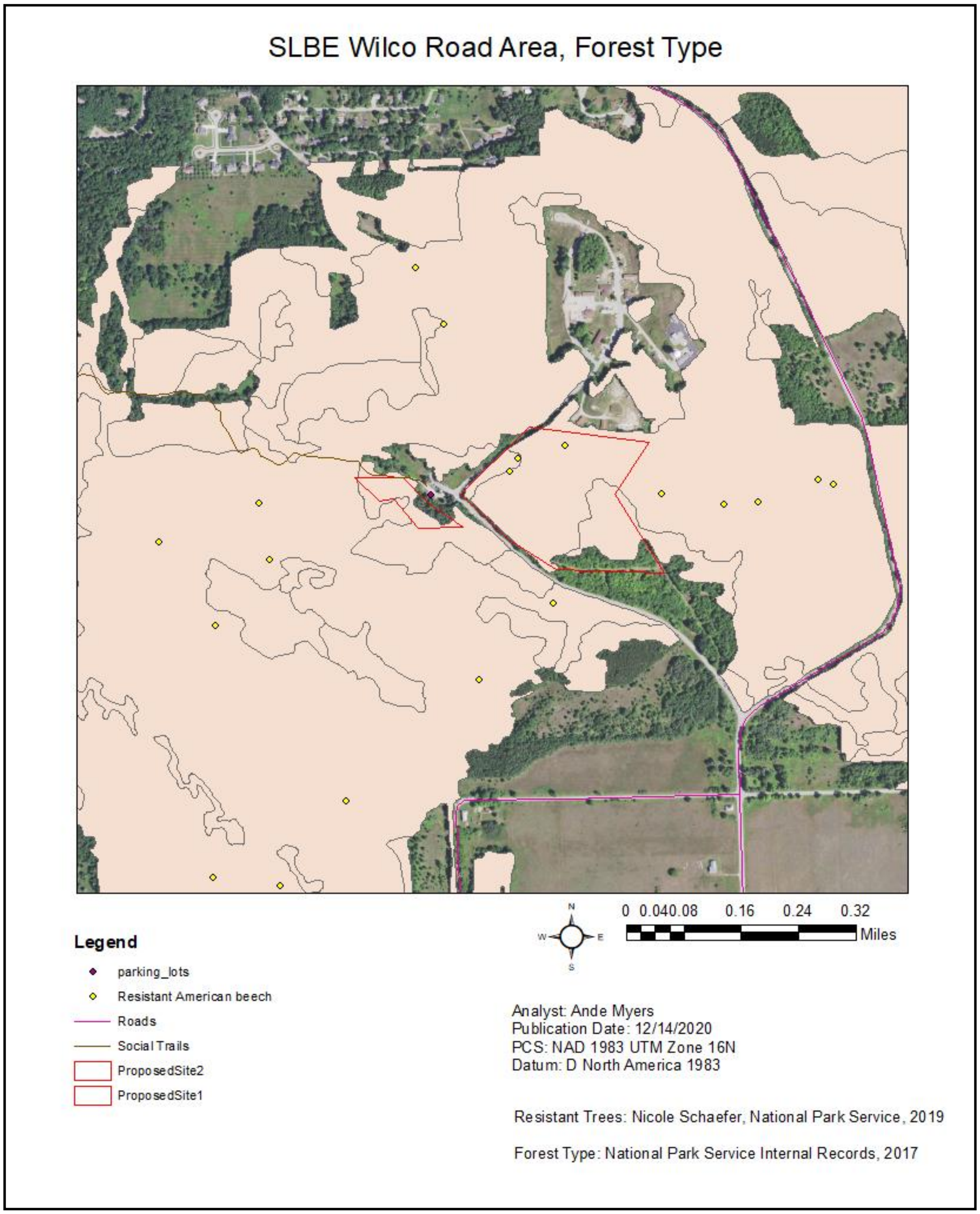

Figure A32. Points of interest for Wilco Road planting site in Sleeping Bear Dunes National Lakeshore. The forest types containing American beech are presented. Locations of resistant American beech and parking areas are marked, as planting sites must be located close to both.

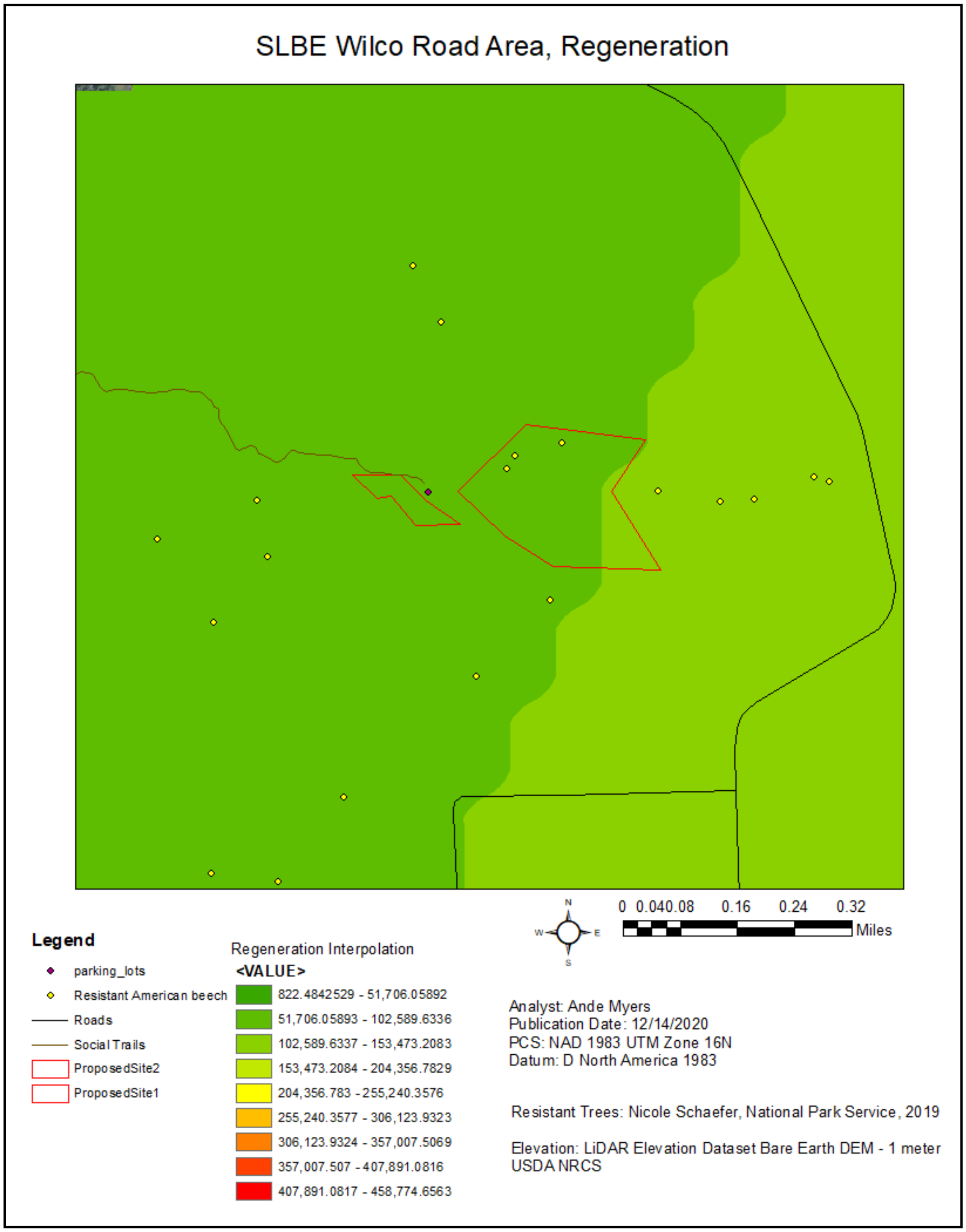

Figure A33. Prediction surface of beech regeneration created with IDW interpolation for Wilco Road planting site in Sleeping Bear Dunes National Lakeshore.

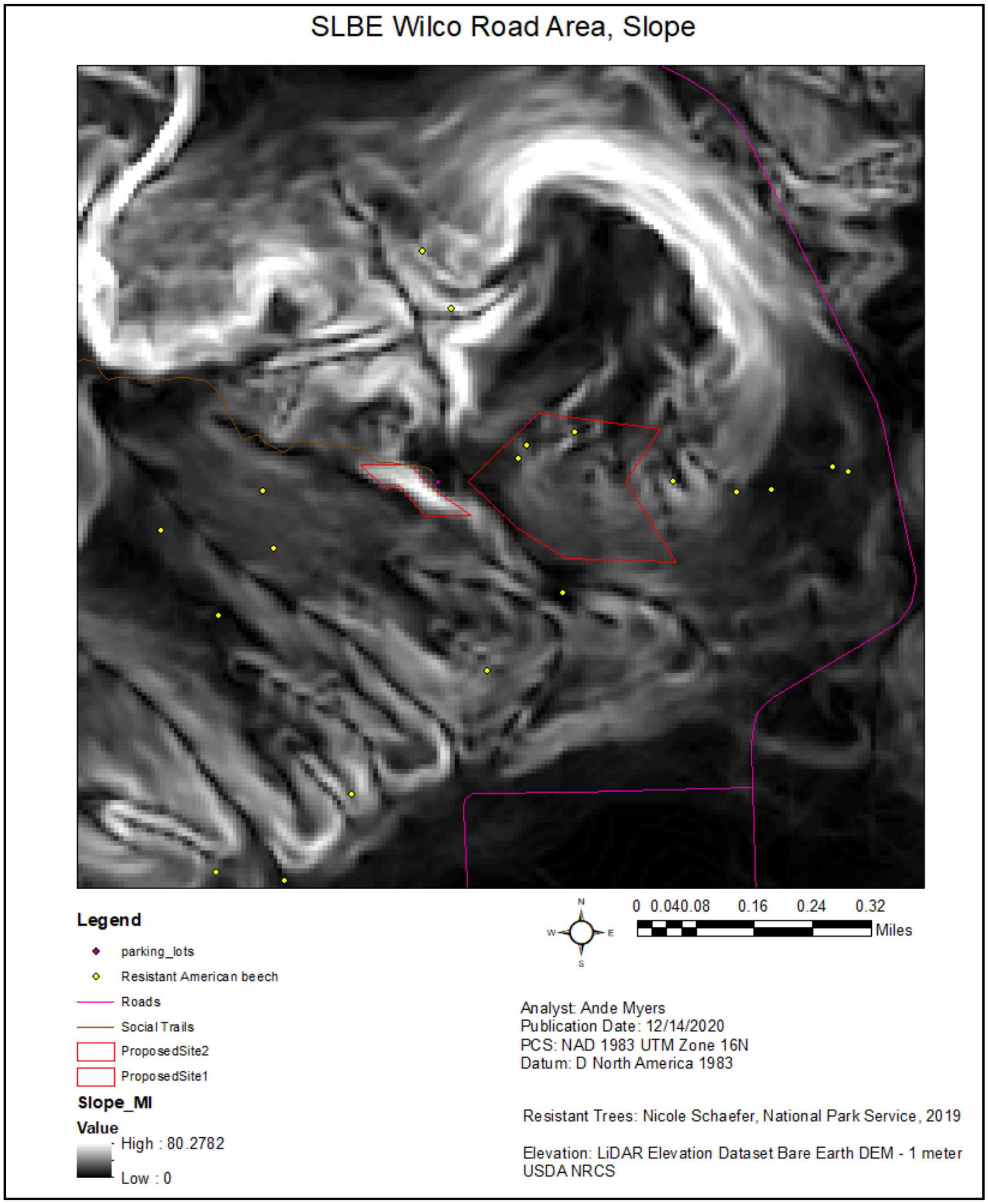

Figure A34. Slope of Wilco Road planting site in Sleeping Bear Dunes National Lakeshore, calculated from LiDAR Elevation Dataset via NRCS Geospatial Data Gateway.

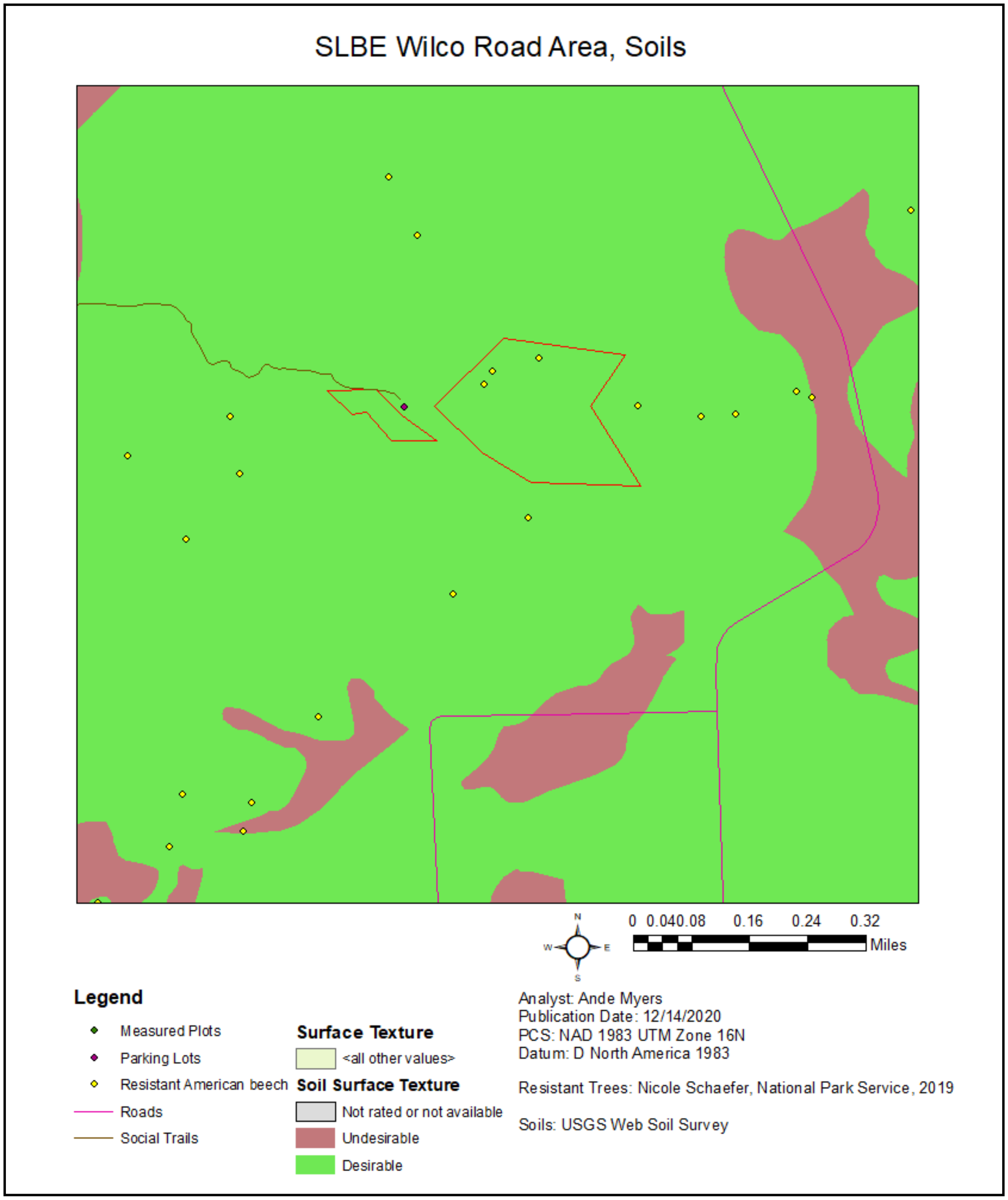

Figure A35. Soil surface texture for Wilco Road planting site in Sleeping Bear Dunes National Lakeshore. Soil surface texture was defined in the USGS Web Soil Survey toolset and broken into desirable and undesirable classes based on silvics of American beech.

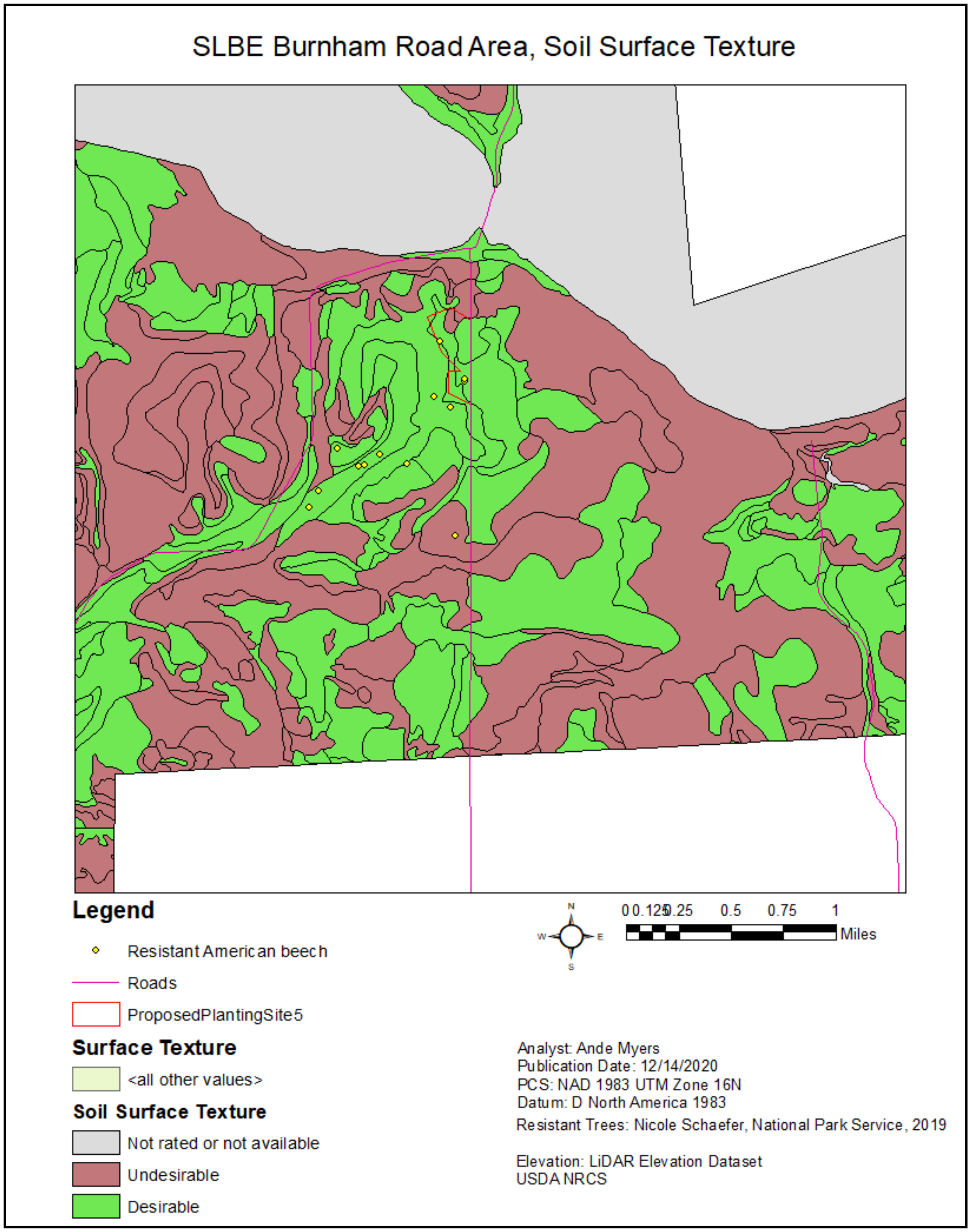

Figure A36. Soil surface texture for Burnham Road planting site in Sleeping Bear Dunes National Lakeshore. Soil surface texture was defined in the USGS Web Soil Survey toolset and broken into desirable and undesirable classes based on silvics of American beech.

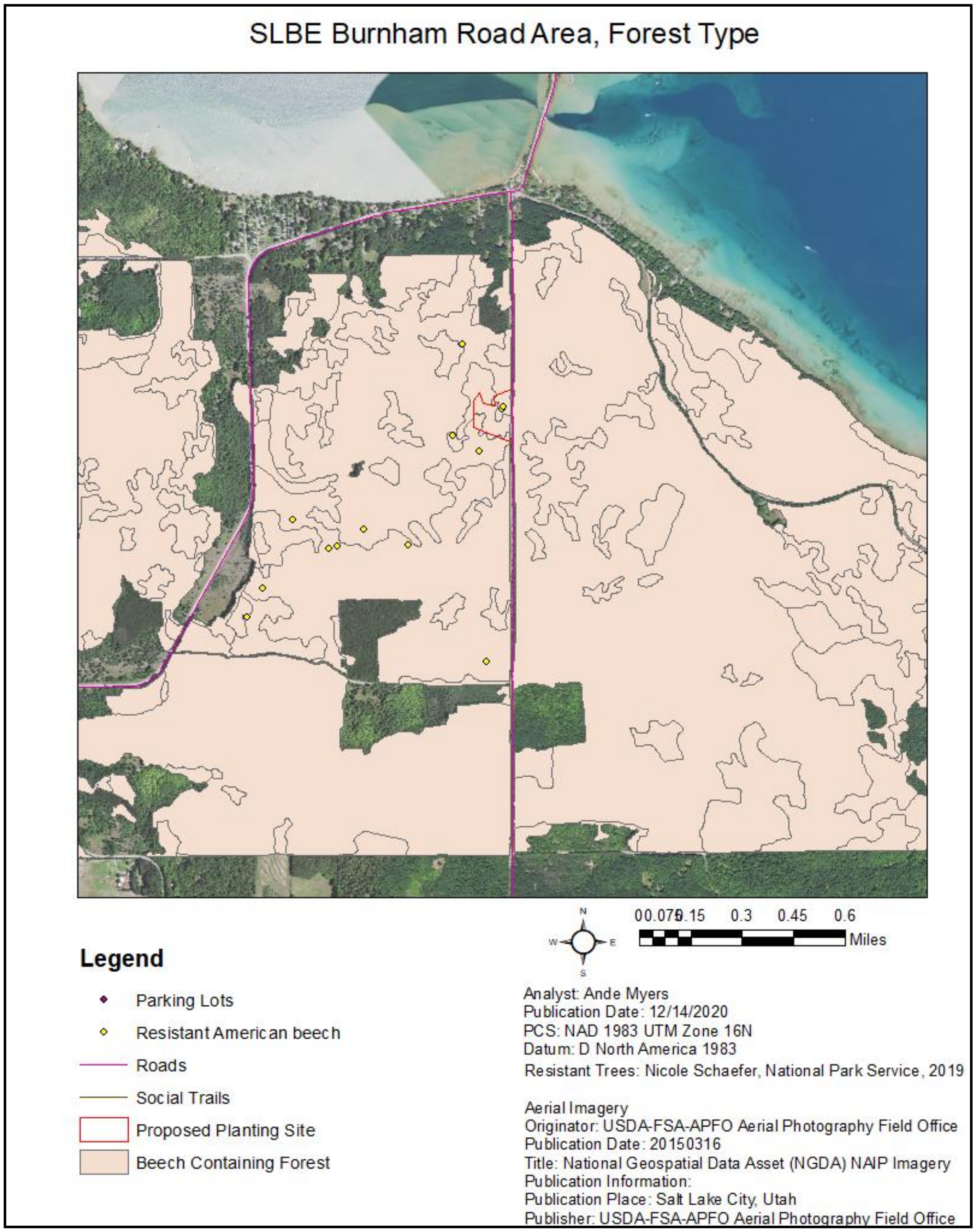

Figure A37. Points of interest for Burnham Road planting site in Sleeping Bear Dunes National Lakeshore. The forest types containing American beech are presented. Locations of resistant American beech and parking areas are marked, as planting sites must be located close to both.

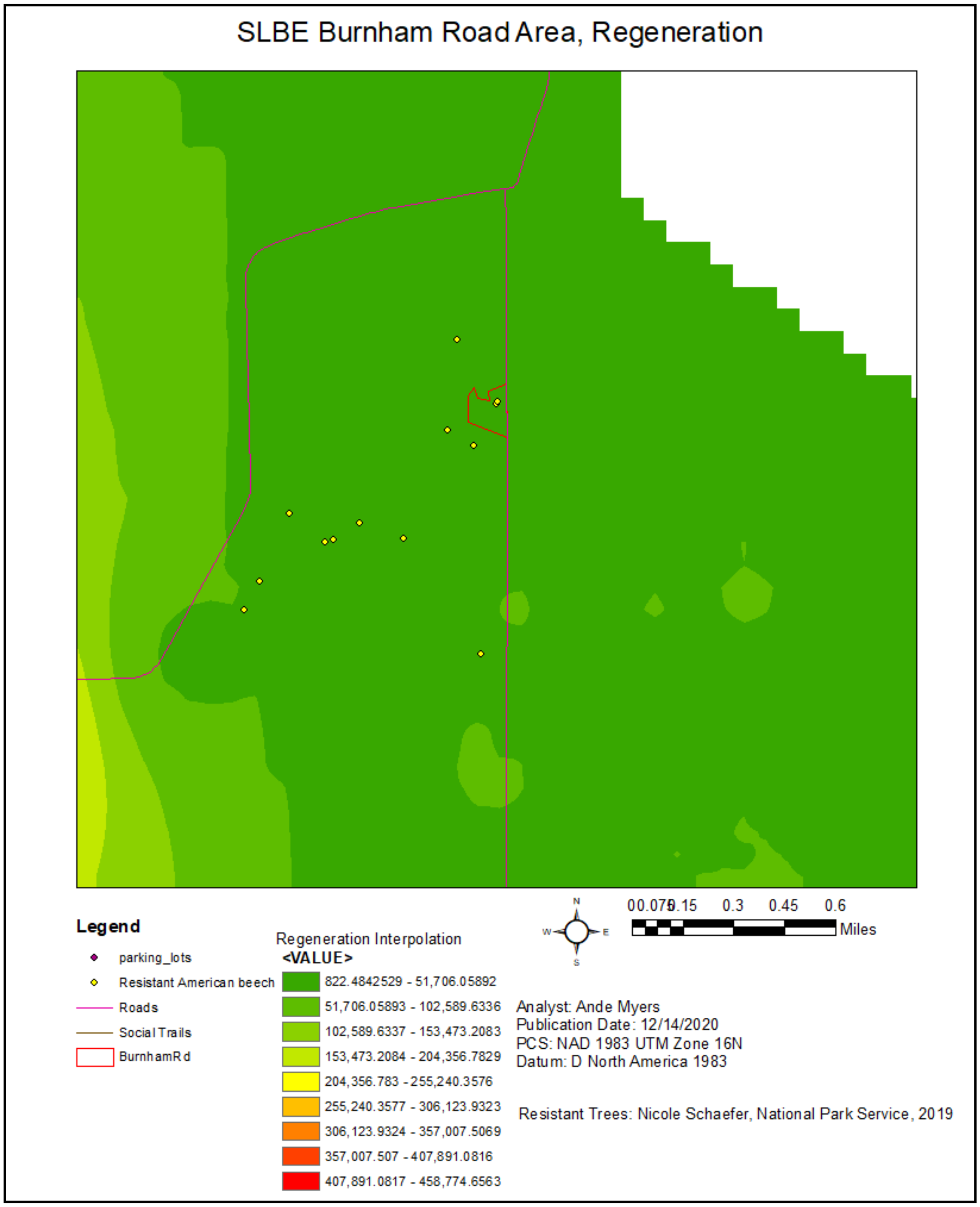

Figure A38. Prediction surface of beech regeneration created with IDW interpolation for Burnham Road planting site in Sleeping Bear Dunes National Lakeshore.

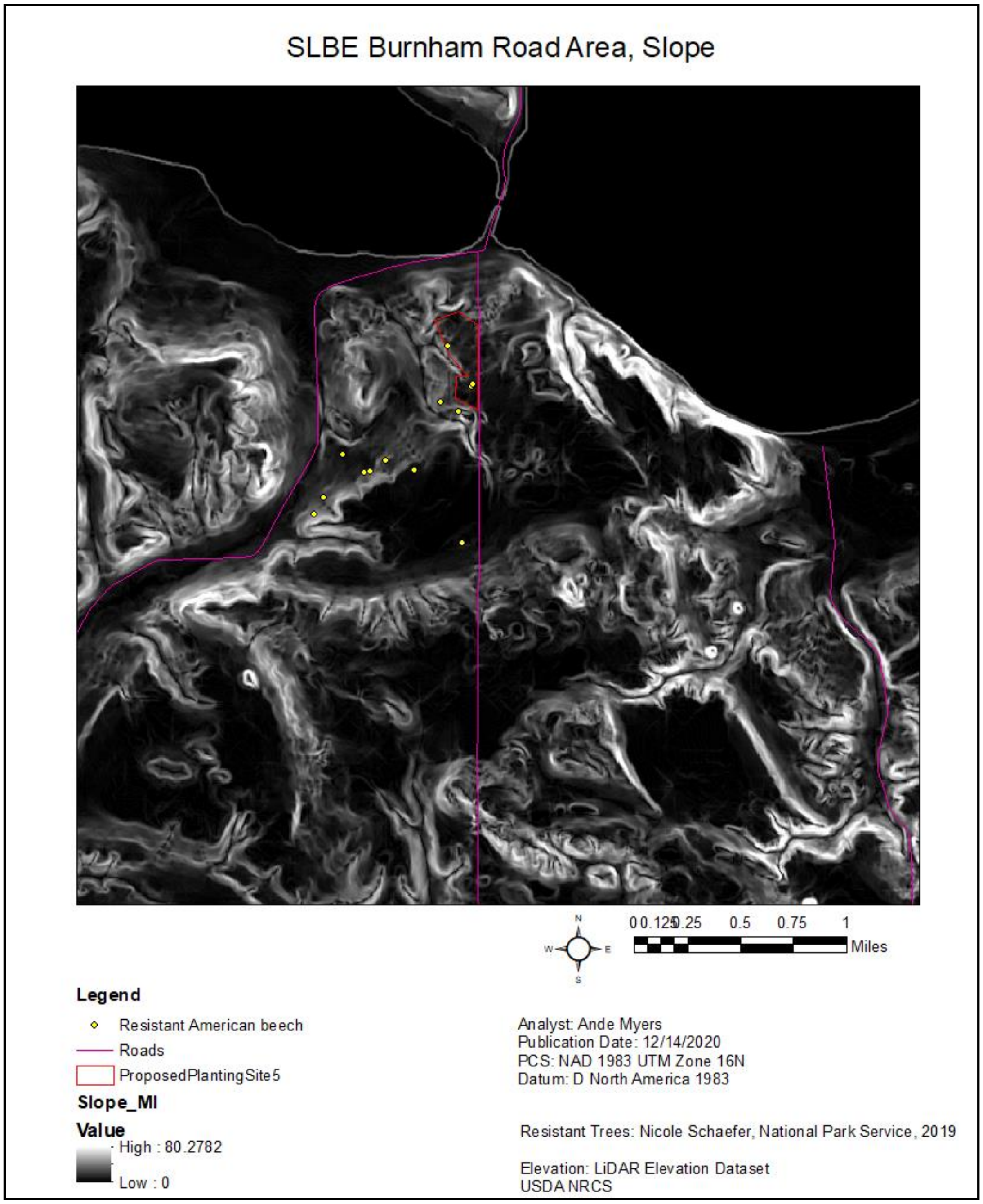

Figure A39. Slope of Burnham Road planting site in Sleeping Bear Dunes National Lakeshore, calculated from LiDAR Elevation Dataset via NRCS Geospatial Data Gateway.

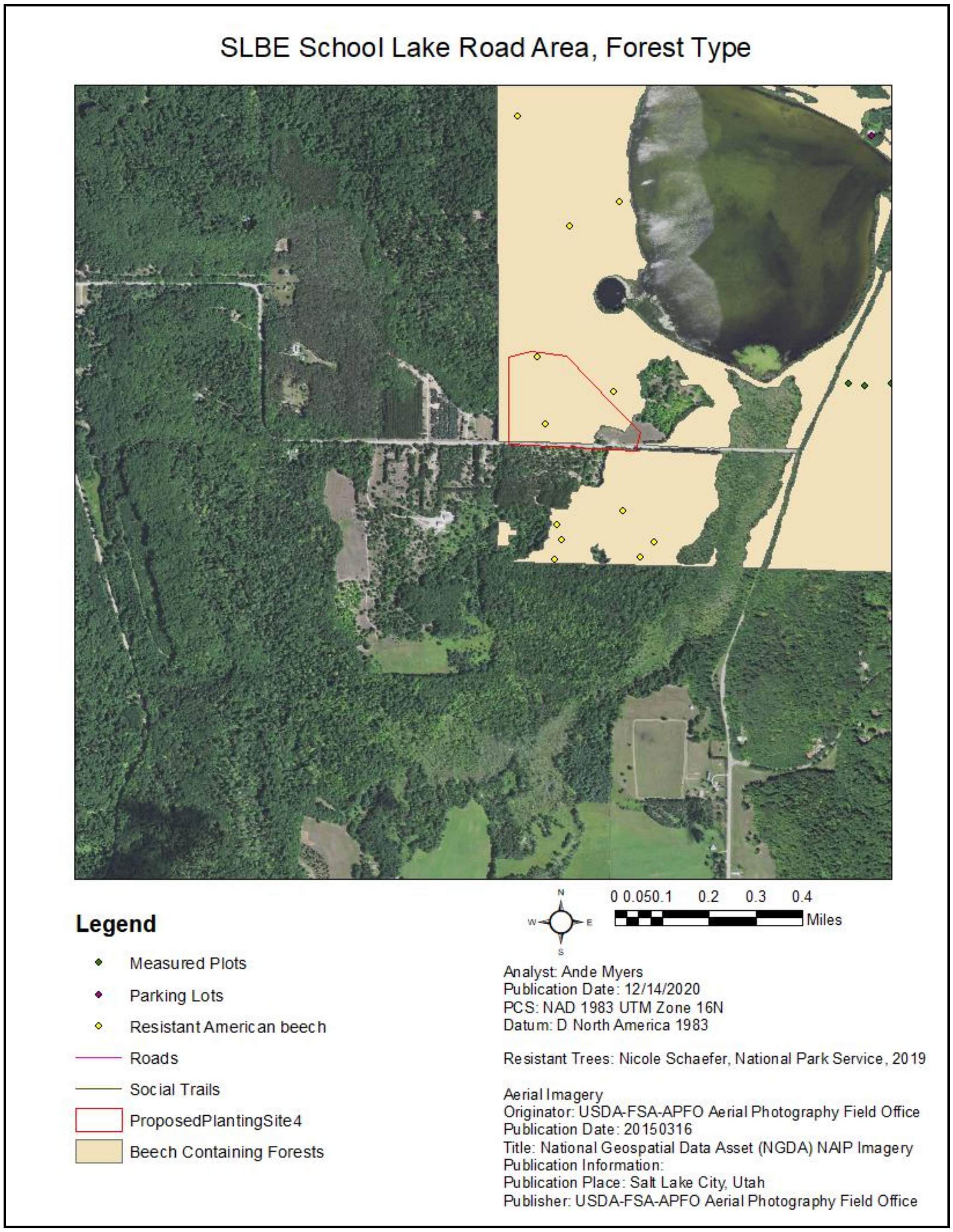

Figure A40. Points of interest for School Lake Road planting site in Sleeping Bear Dunes National Lakeshore. The forest types containing American beech are presented. Locations of resistant American beech and parking areas are marked, as planting sites must be located close to both.

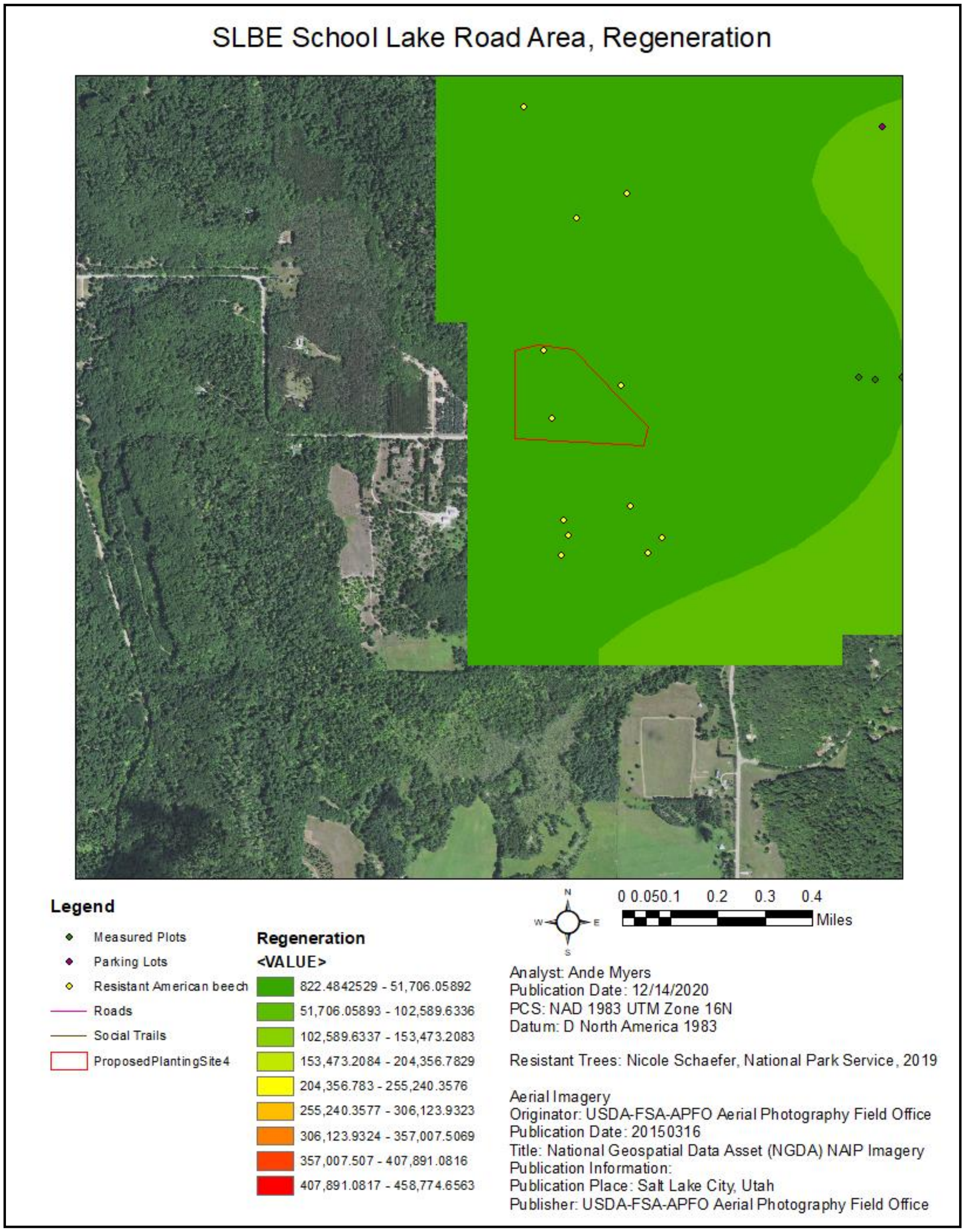

Figure A41. Prediction surface of beech regeneration created with IDW interpolation for School Lake Road planting site in Sleeping Bear Dunes National Lakeshore.

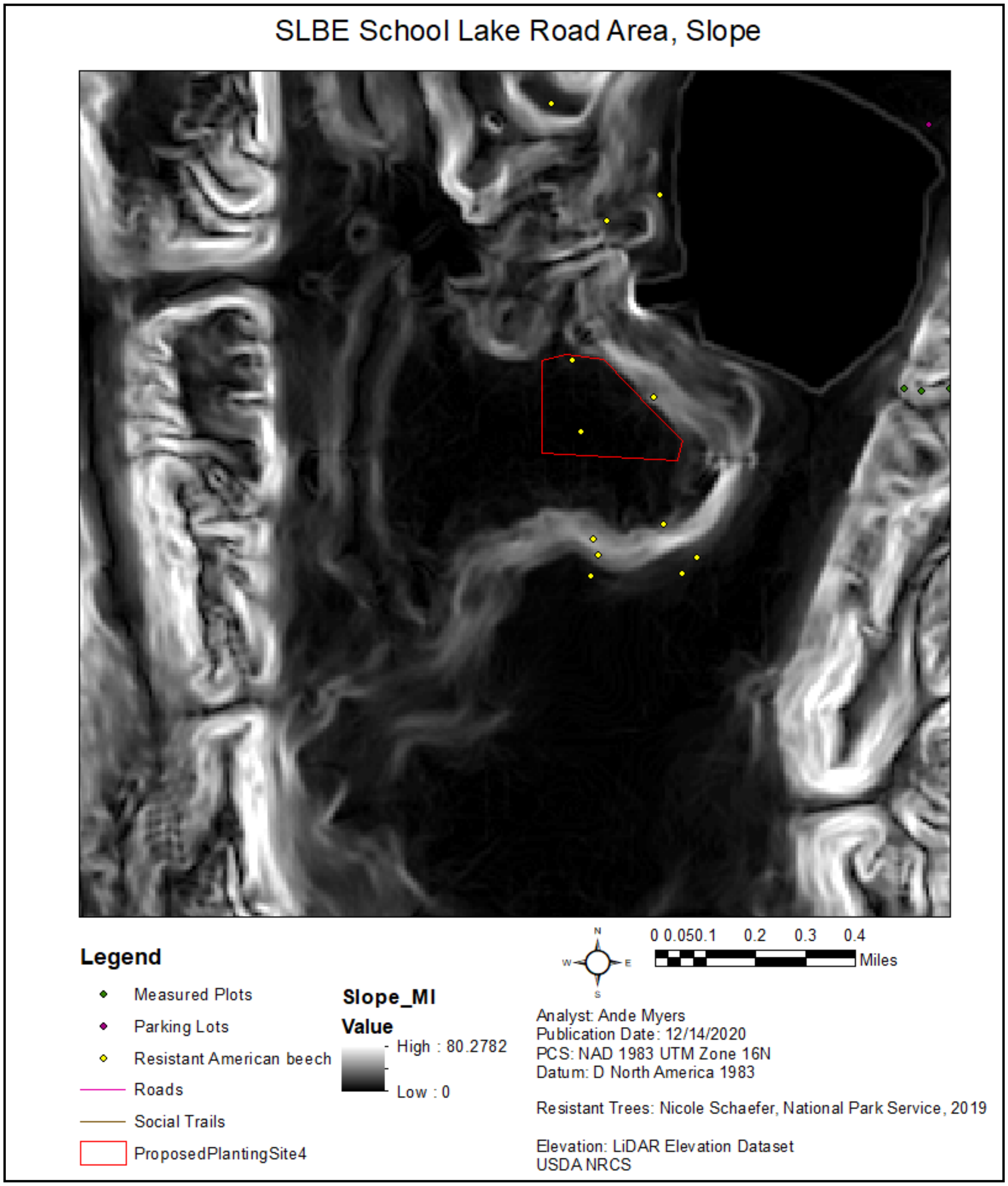

Figure A42. Slope of School Lake Road planting site in Sleeping Bear Dunes National Lakeshore, calculated from LiDAR Elevation Dataset via NRCS Geospatial Data Gateway.

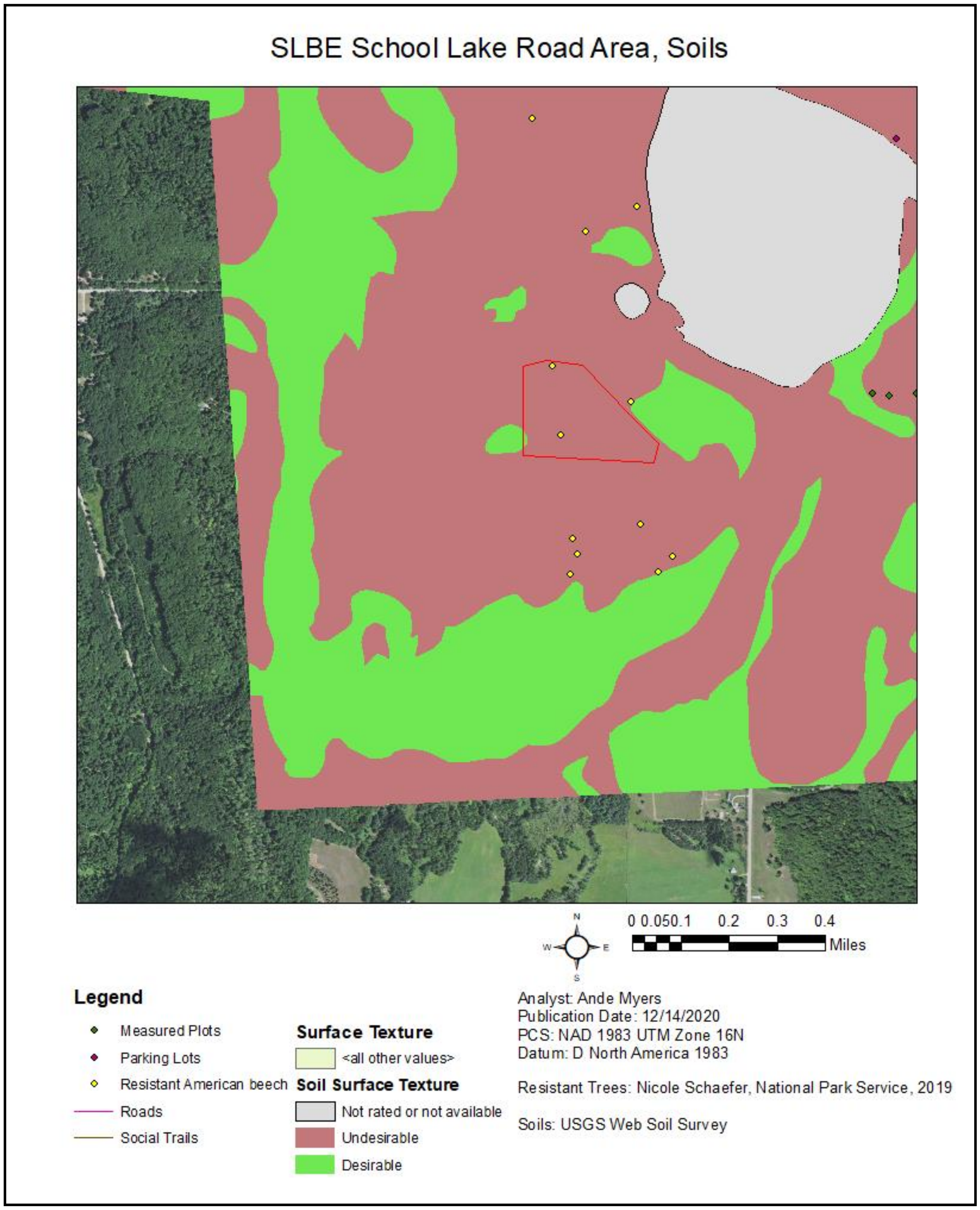

Figure A43. Soil surface texture for School Lake Road planting site in Sleeping Bear Dunes National Lakeshore. Soil surface texture was defined in the USGS Web Soil Survey toolset and broken into desirable and undesirable classes based on silvics of American beech.

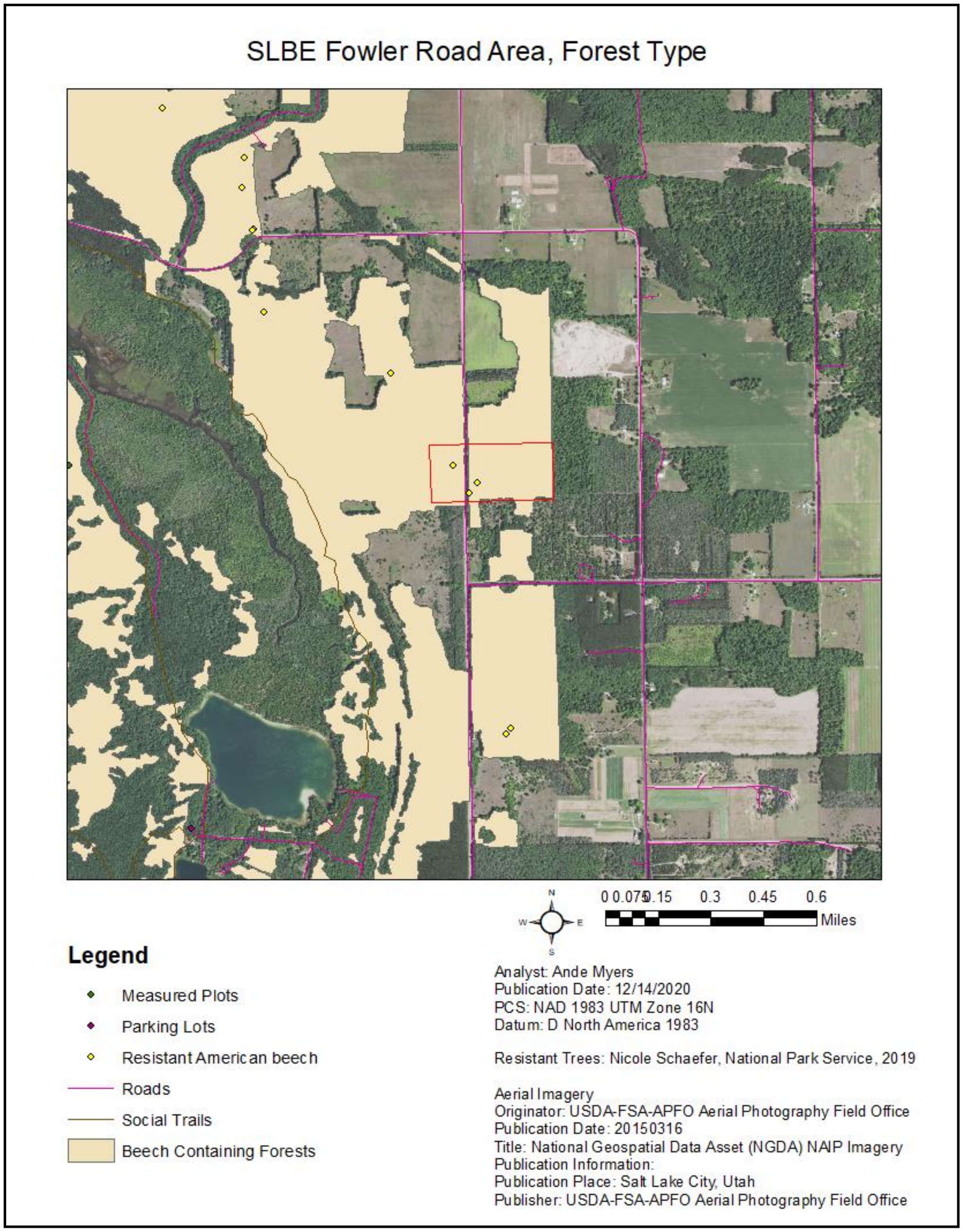

Figure A44. Points of interest for Fowler Road planting site in Sleeping Bear Dunes National Lakeshore. The forest types containing American beech are presented. Locations of resistant American beech and parking areas are marked, as planting sites must be located close to both.

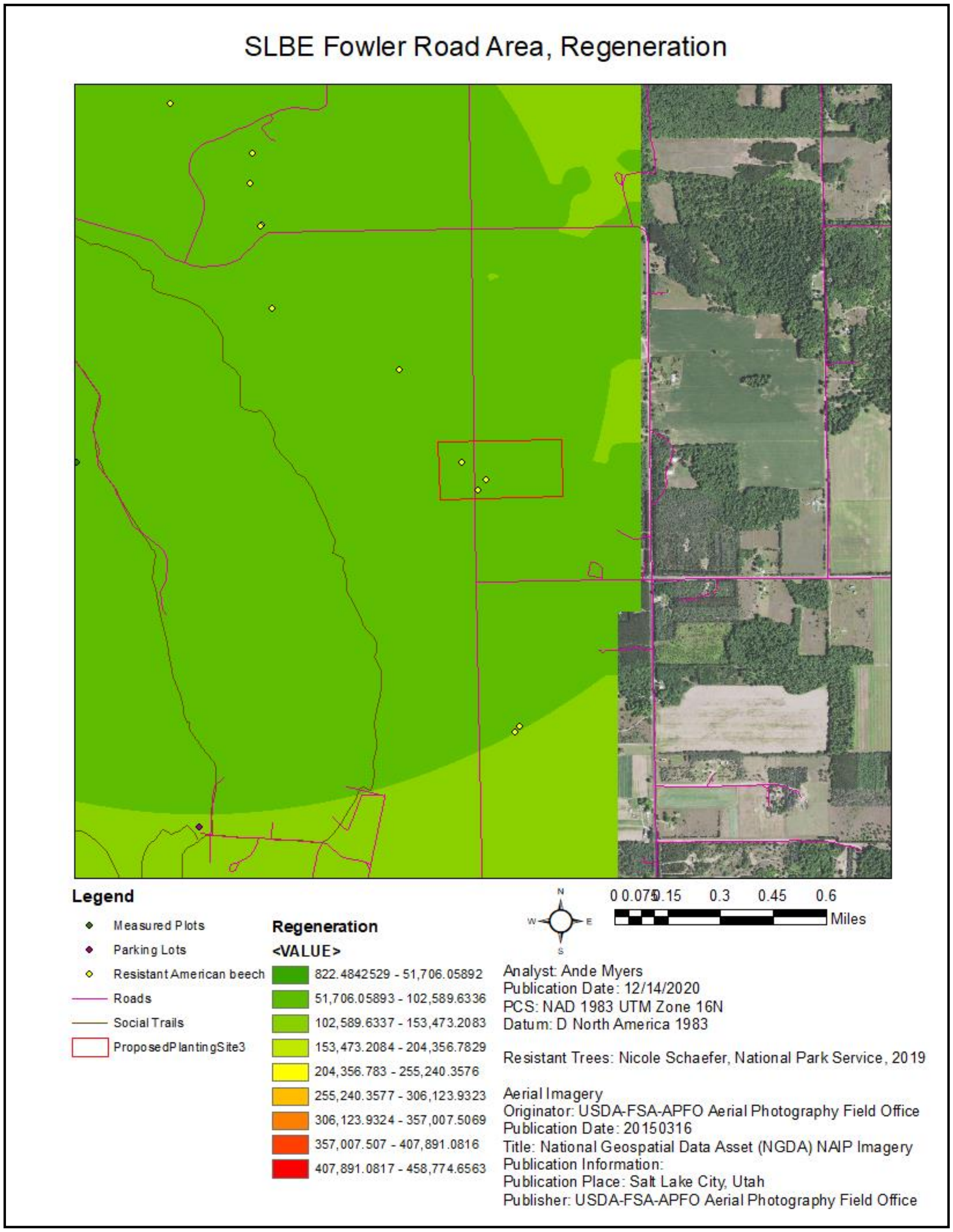

Figure A45. Prediction surface of beech regeneration created with IDW interpolation for Fowler Road planting site in Sleeping Bear Dunes National Lakeshore.

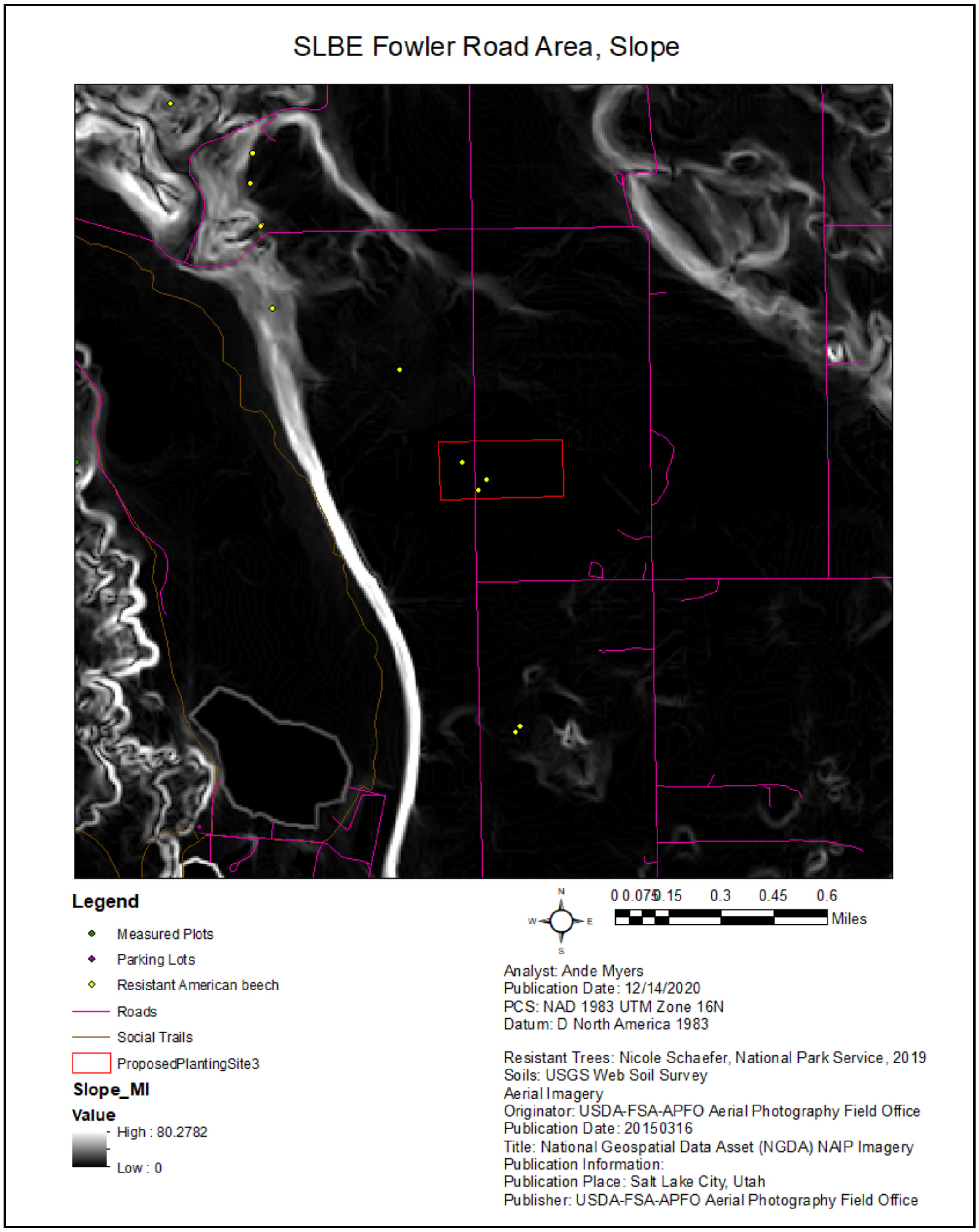

Figure A46. Slope of Fowl Road planting site in Sleeping Bear Dunes National Lakeshore, calculated from LiDAR Elevation Dataset via NRCS Geospatial Data Gateway.

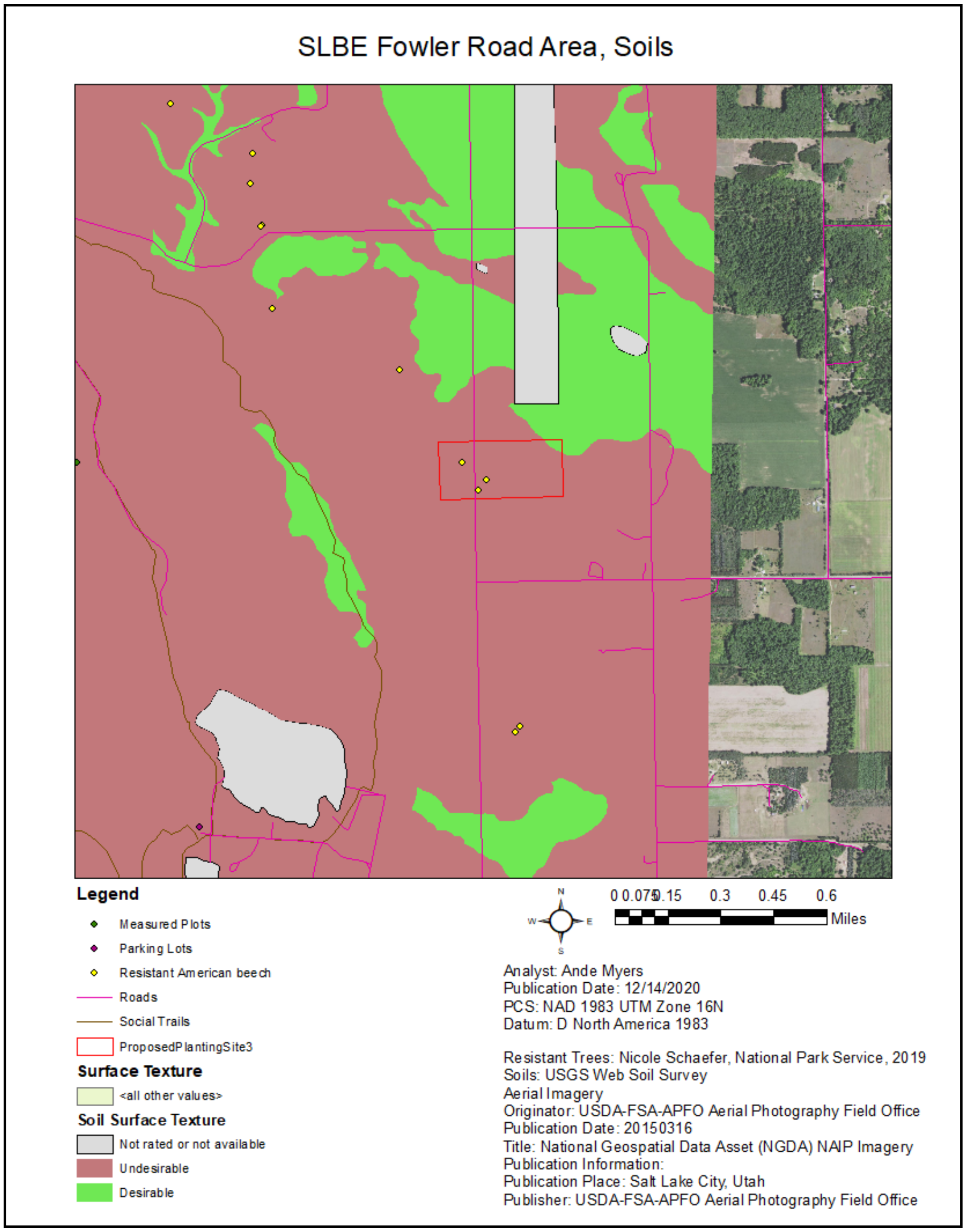

Figure A47. Soil surface texture for Fowler Road planting site in Sleeping Bear Dunes National Lakeshore. Soil surface texture was defined in the USGS Web Soil Survey toolset and broken into desirable and undesirable classes based on silvics of American beech.

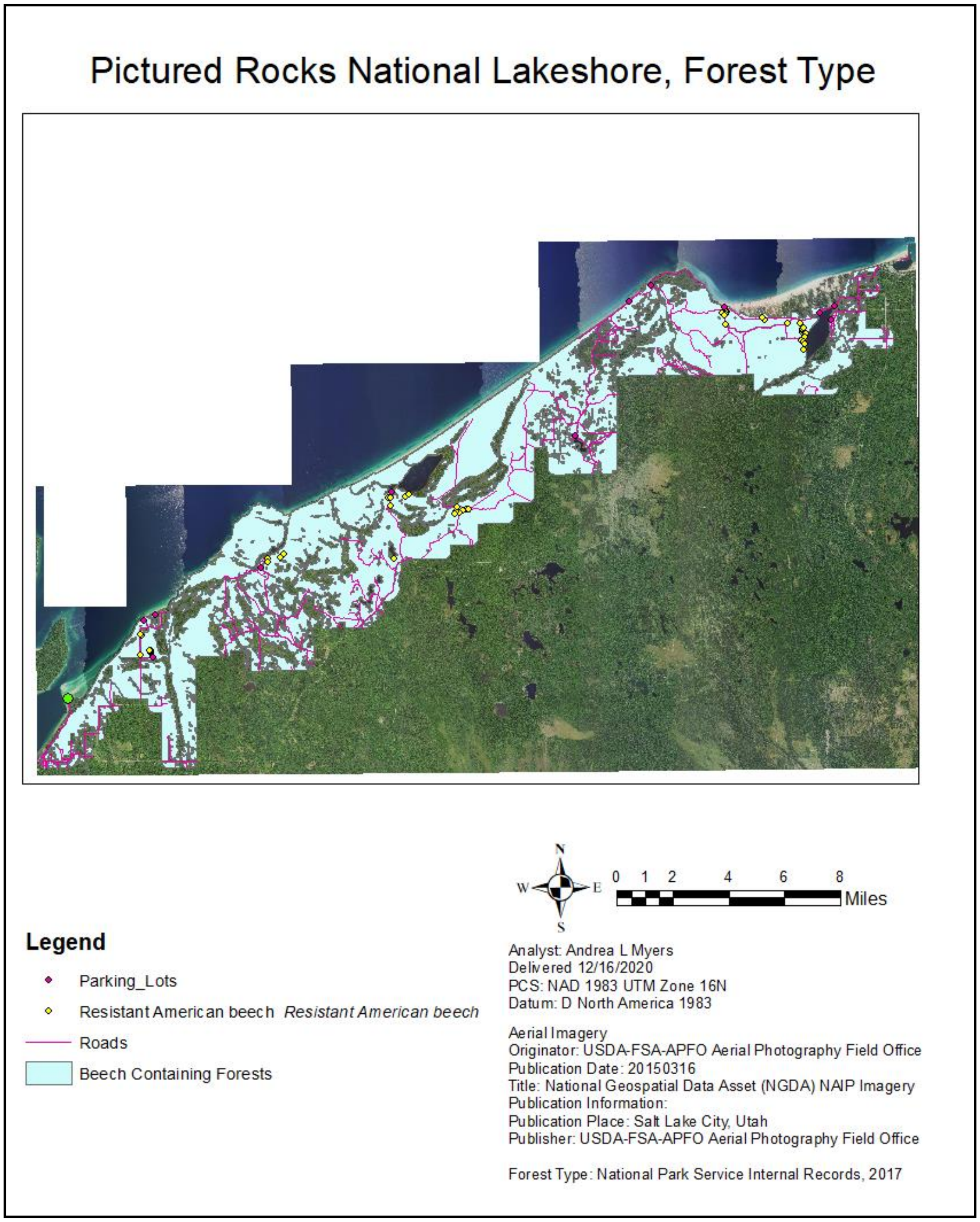

Figure A48. Points of interest for Pictured Rocks National Lakeshore. The forest types containing American beech are presented. Locations of resistant American beech and parking areas are marked, as planting sites must be located close to both.

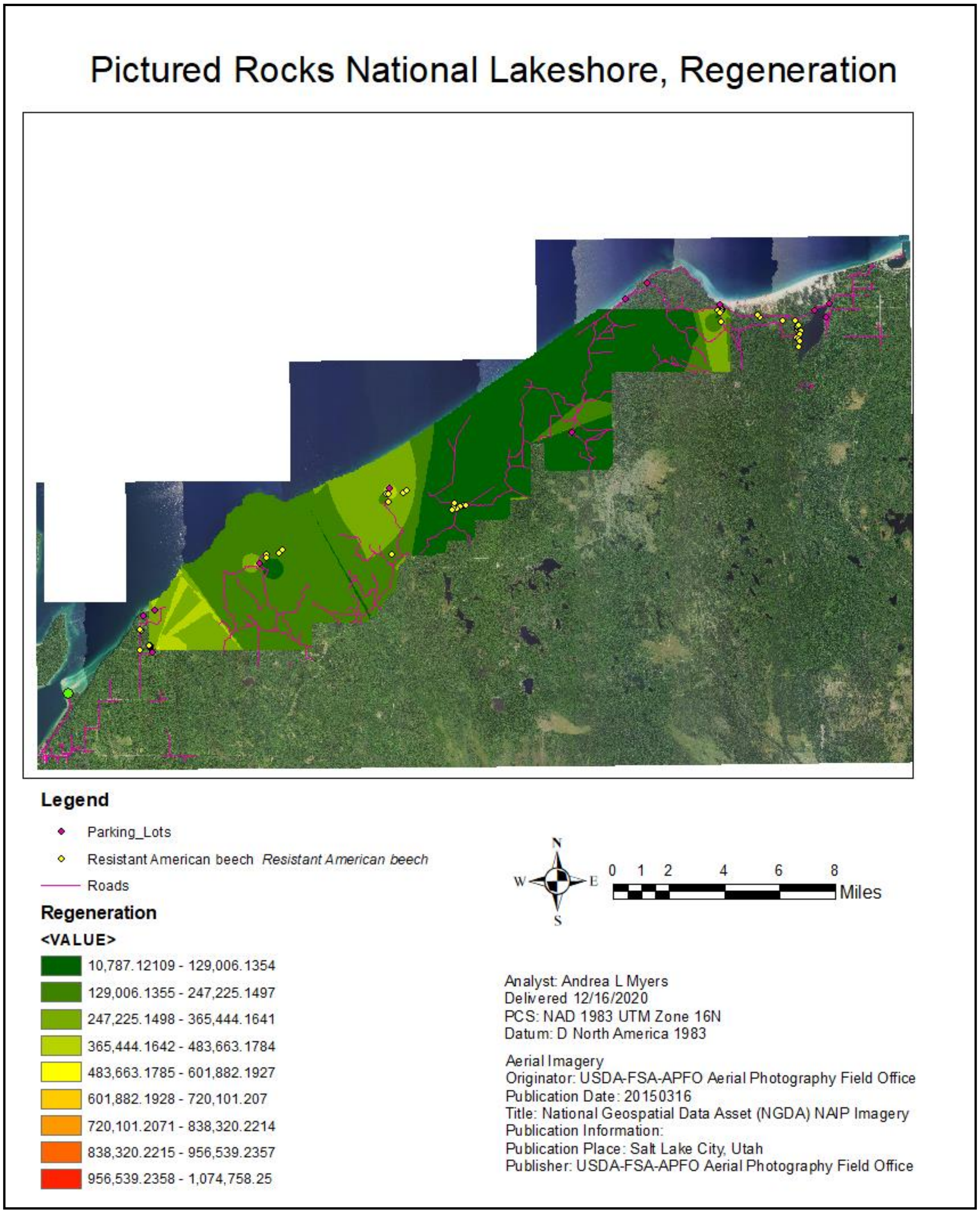

Figure A49. Prediction surface of beech regeneration created with IDW interpolation for Pictured Rocks National Lakeshore.

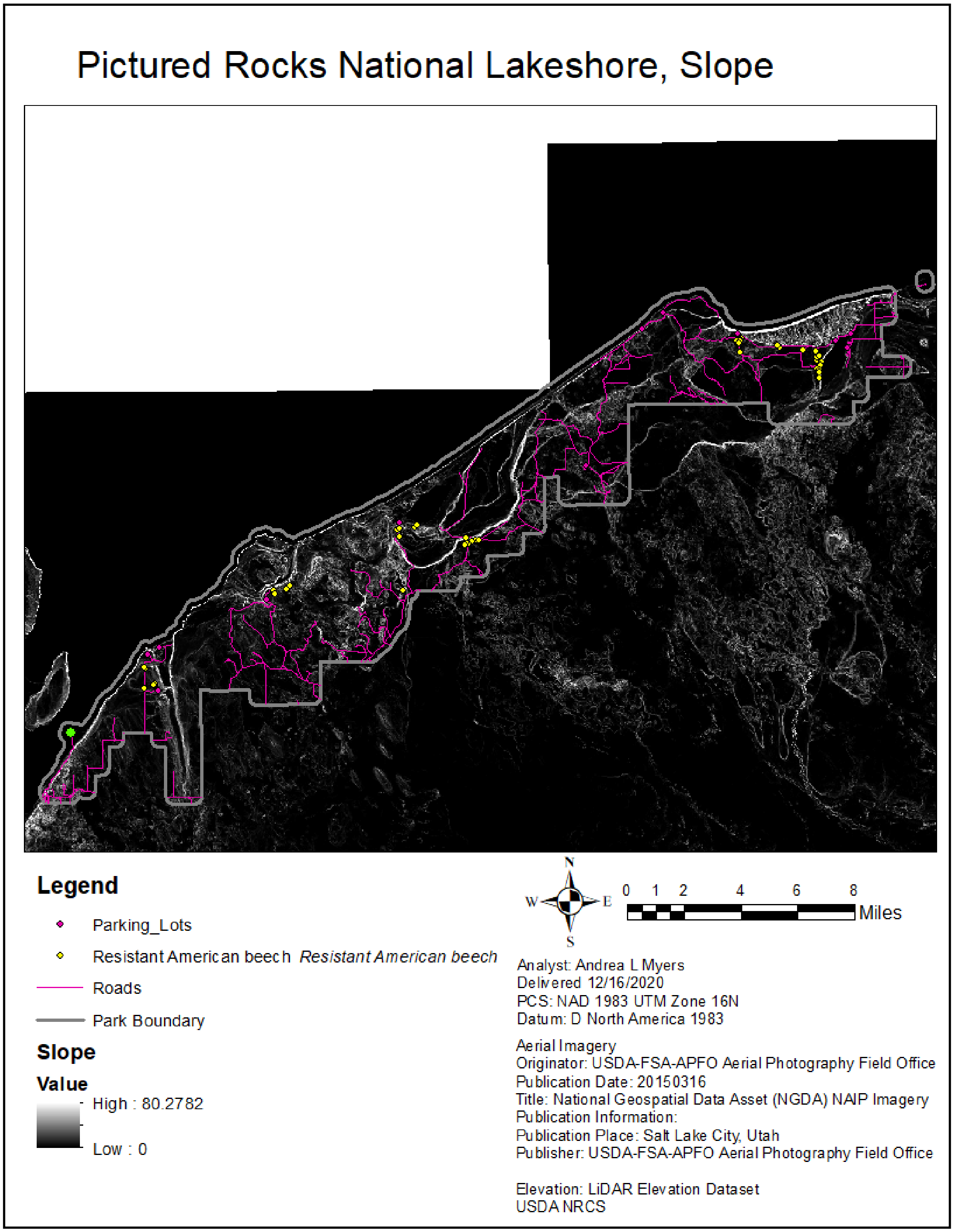

Figure A50. Slope of Pictured Rocks National Lakeshore, calculated from LiDAR Elevation Dataset via NRCS Geospatial Data Gateway.

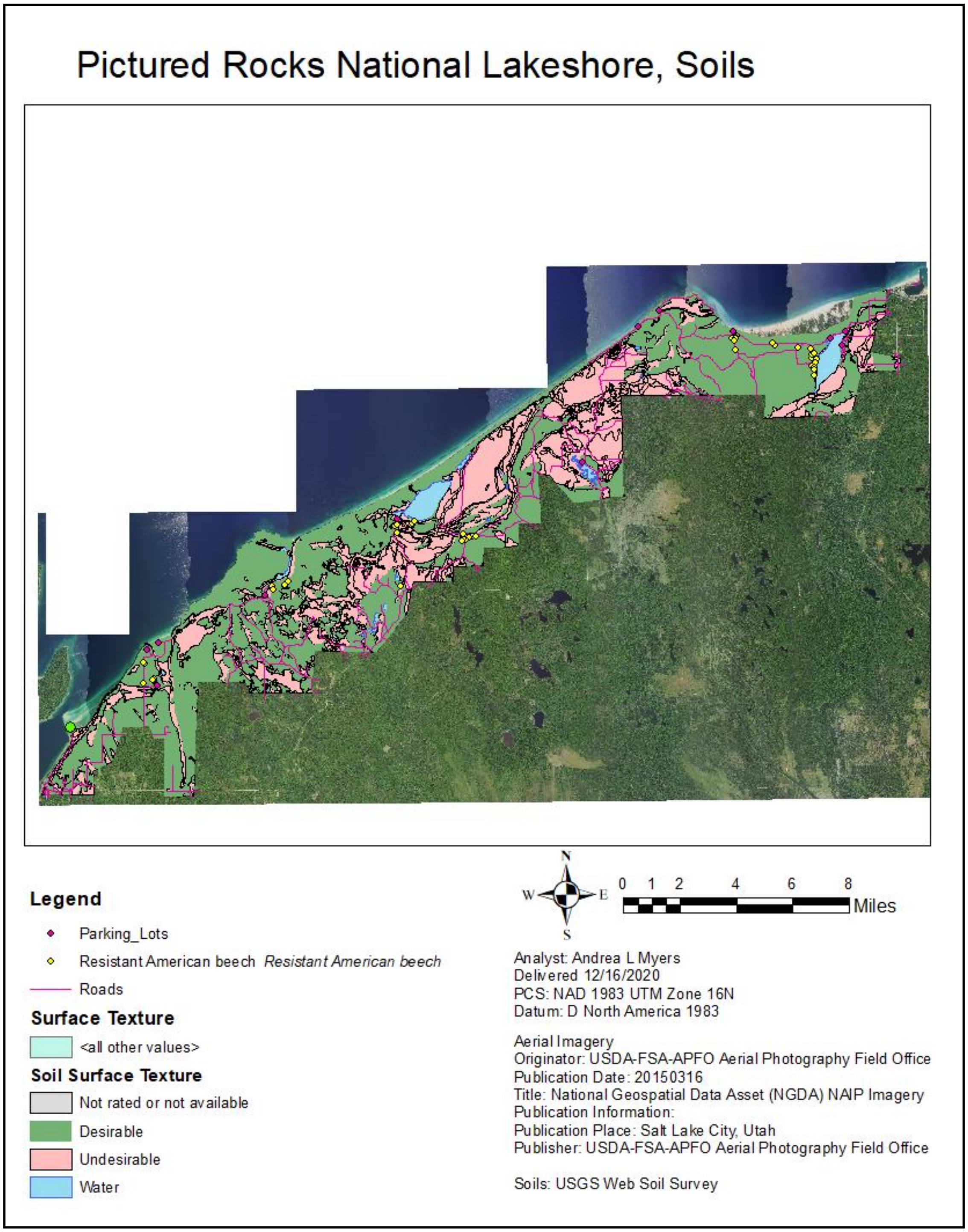

Figure A51. Soil surface texture for Pictured Rocks National Lakeshore. Soil surface texture was defined in the USGS Web Soil Survey toolset and broken into desirable and undesirable classes based on silvics of American beech.

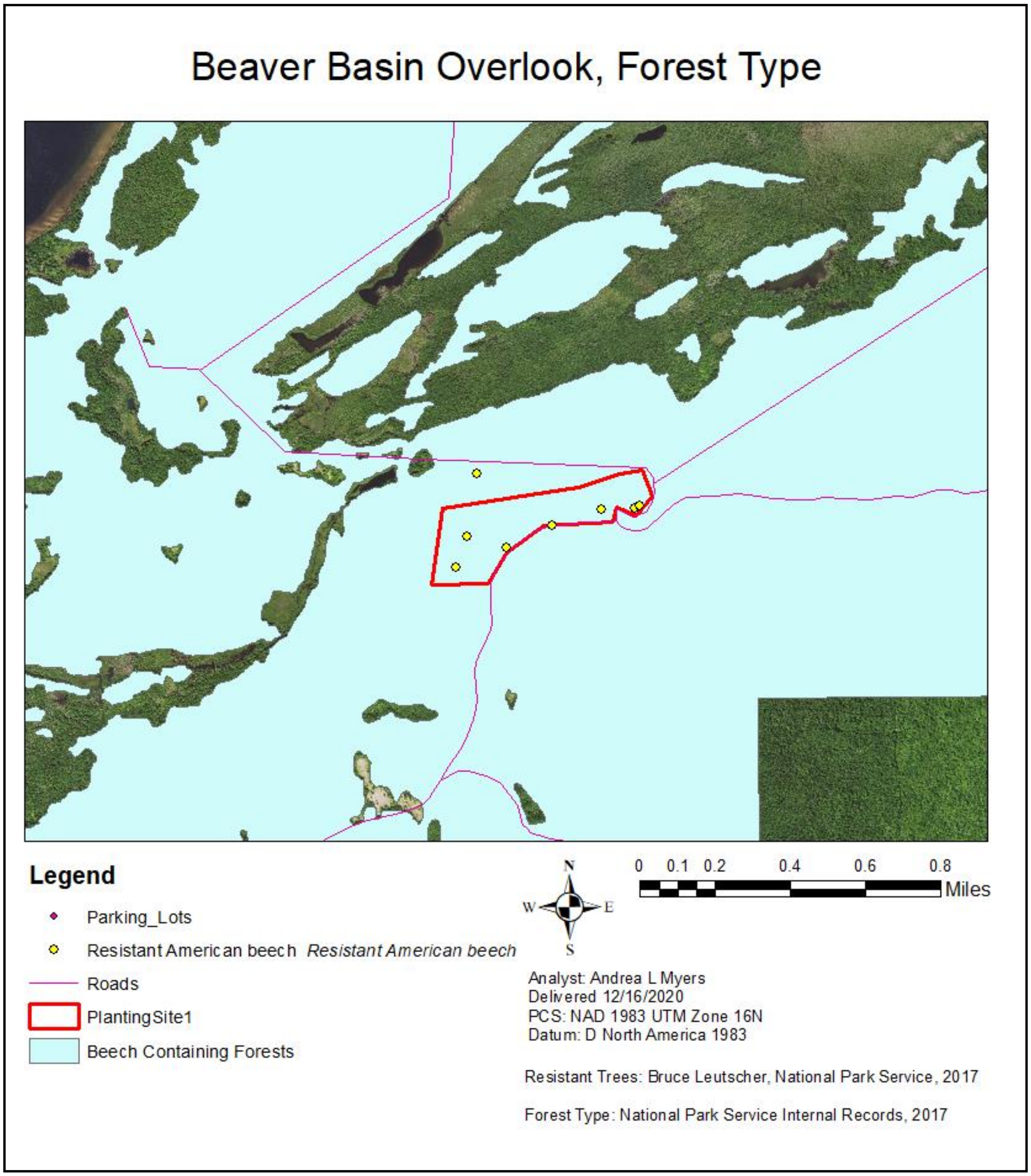

Figure A52. Points of interest for Beaver Basin Overlook planting site in Pictured Rocks National Lakeshore. The forest types containing American beech are presented. Locations of resistant American beech and parking areas are marked, as planting sites must be located close to both.

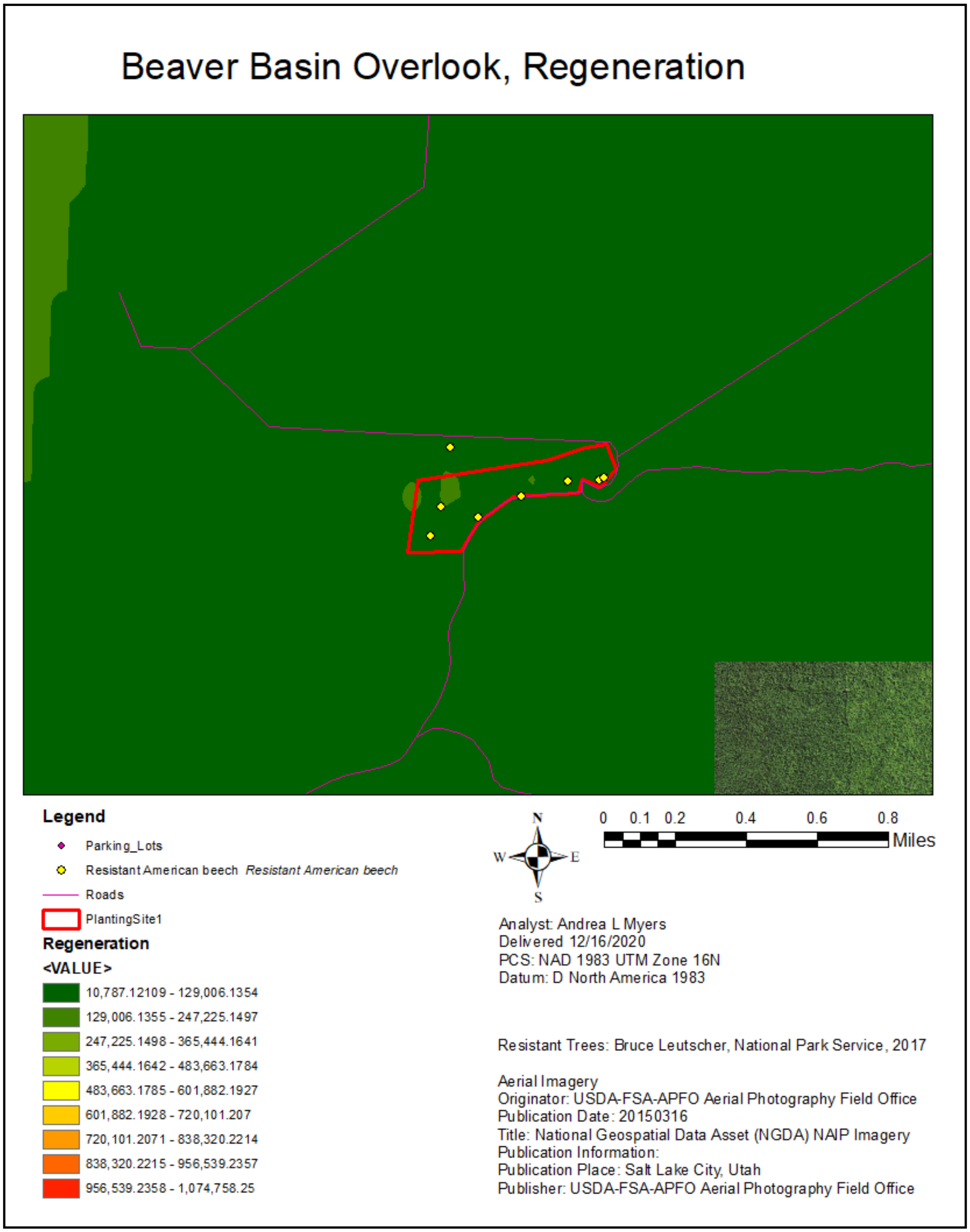

Figure A53. Prediction surface of beech regeneration created with IDW interpolation for Beaver Basin Overlook planting site in Pictured Rocks National Lakeshore.

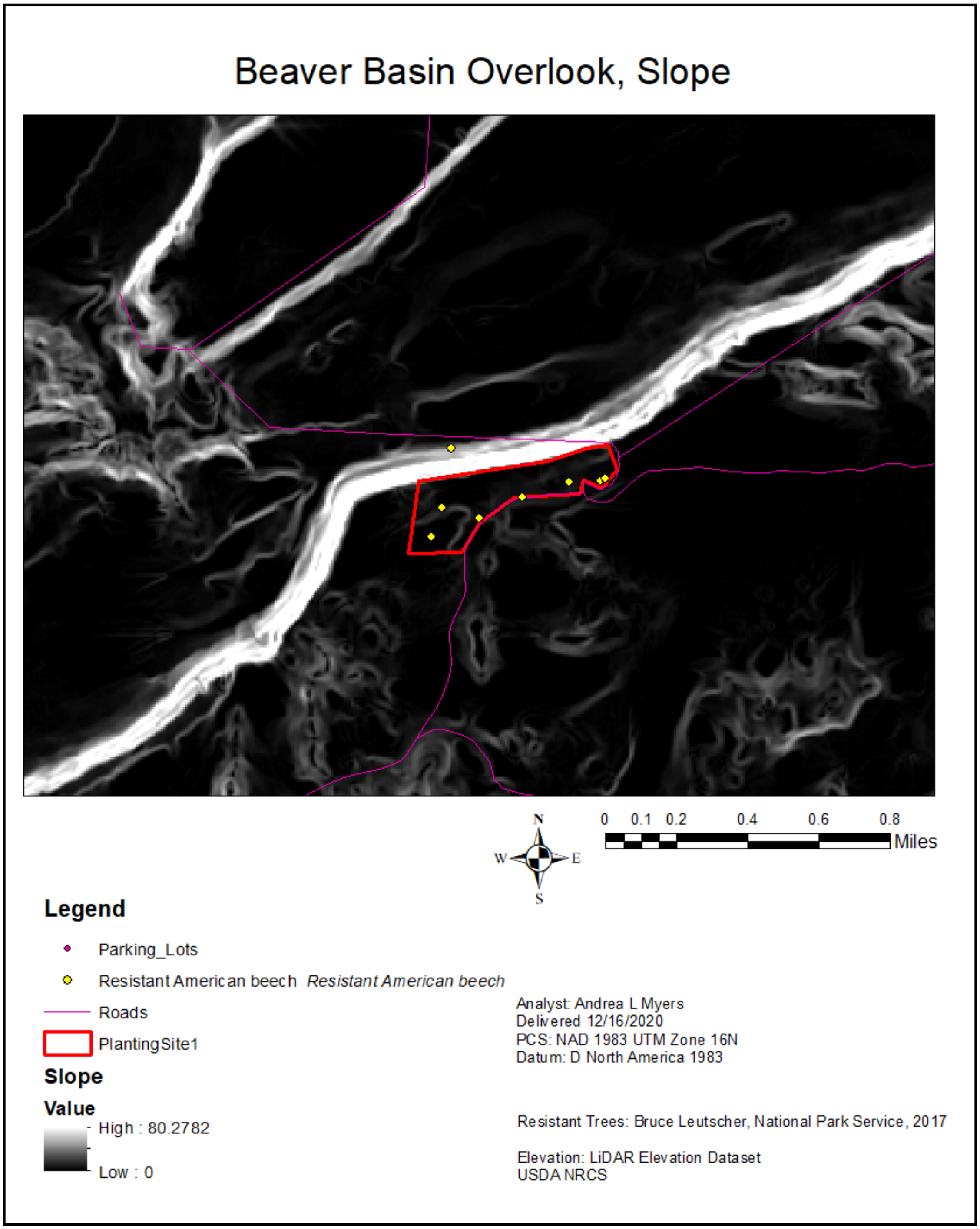

Figure A54. Slope of Beaver Basin Overlook planting site in Pictured Rocks National Lakeshore, calculated from LiDAR Elevation Dataset via NRCS Geospatial Data Gateway.

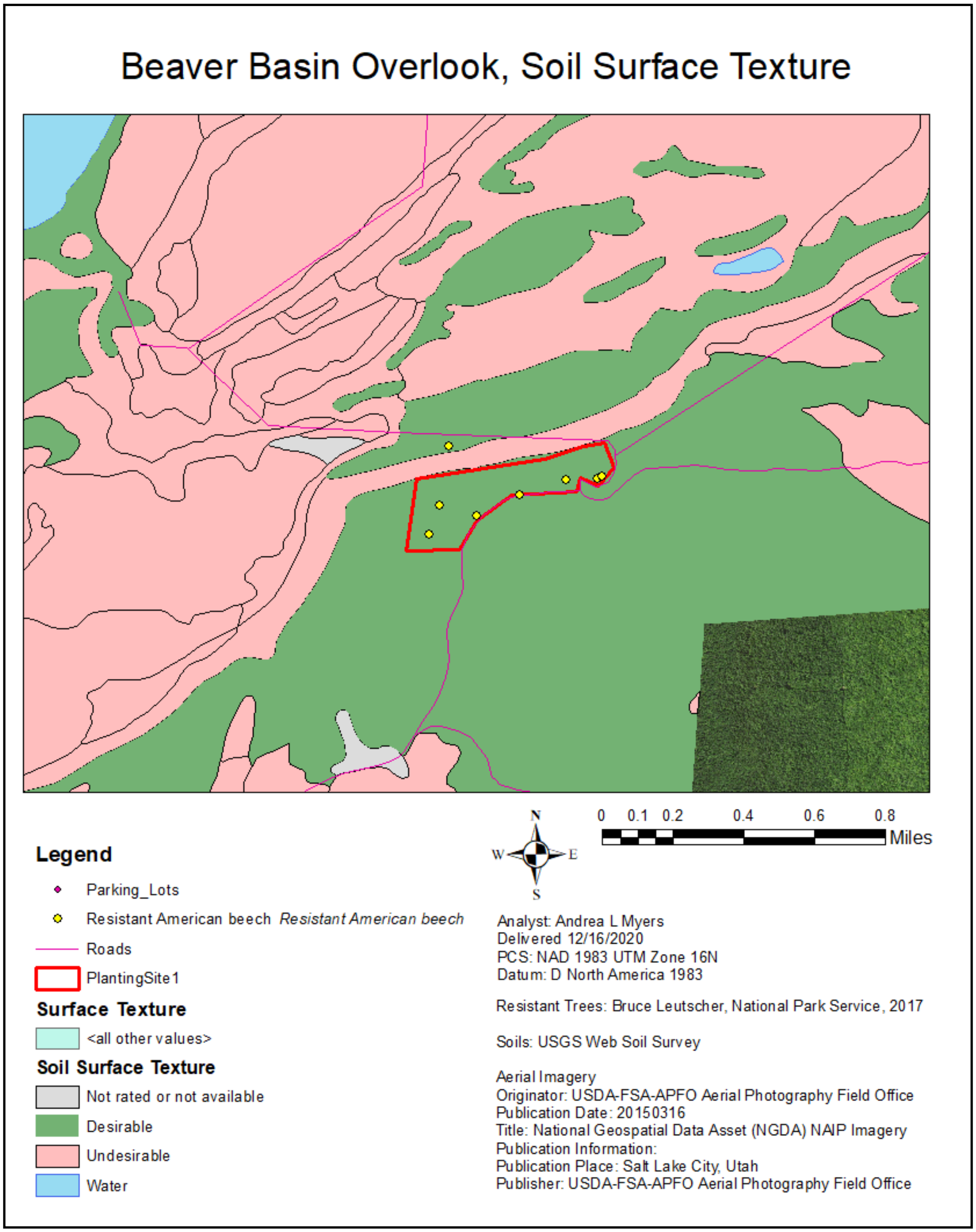

Figure A55. Soil surface texture for Beaver Basin Overlook planting site in Pictured Rocks National Lakeshore. Soil surface texture was defined in the USGS Web Soil Survey toolset and broken into desirable and undesirable classes based on silvics of American beech.

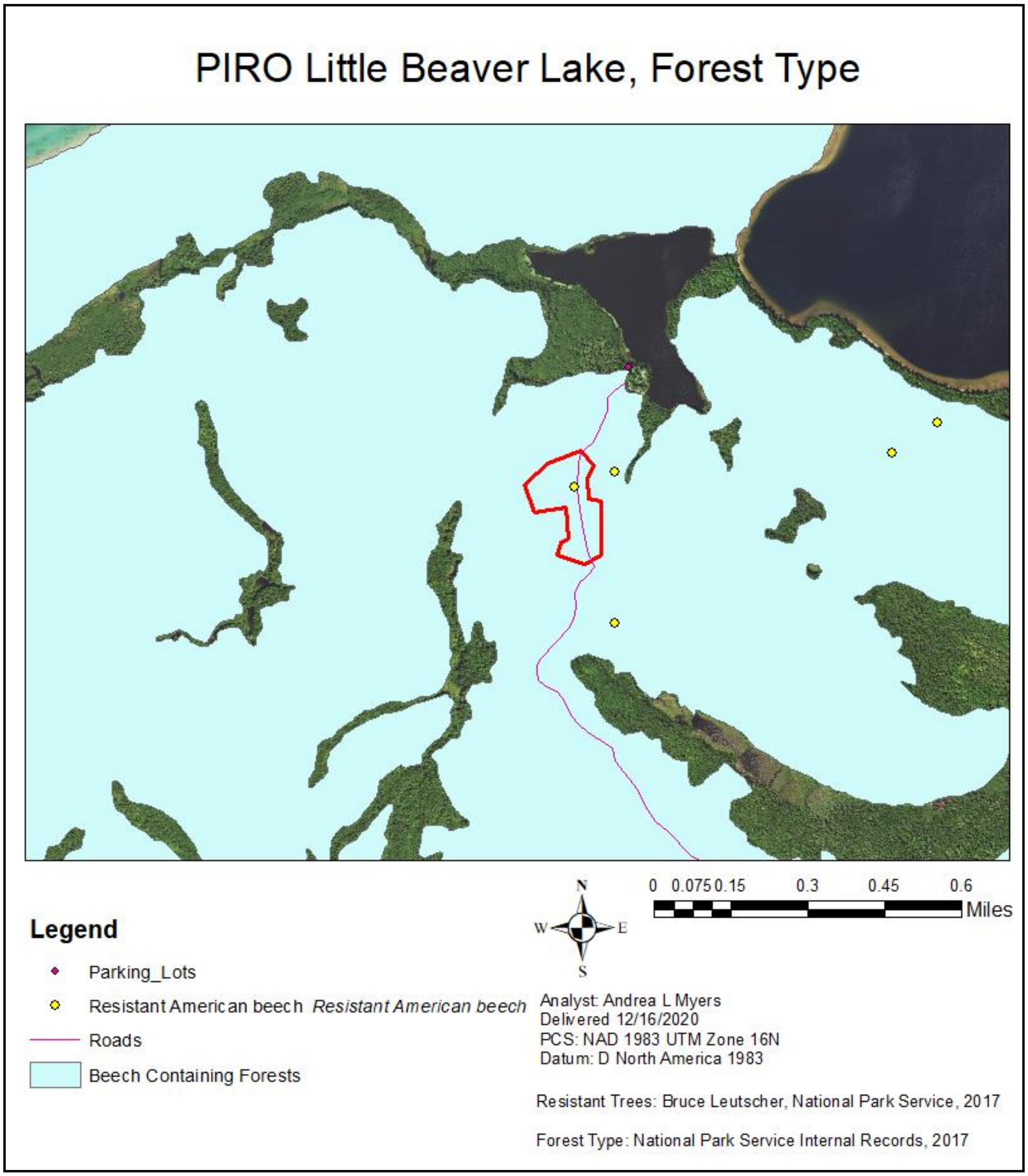

Figure A56. Points of interest for Little Beaver Lake planting site in Pictured Rocks National Lakeshore. The forest types containing American beech are presented. Locations of resistant American beech and parking areas are marked, as planting sites must be located close to both.

Figure A57. Prediction surface of beech regeneration created with IDW interpolation for Little Beaver Lake planting site in Pictured Rocks National Lakeshore.

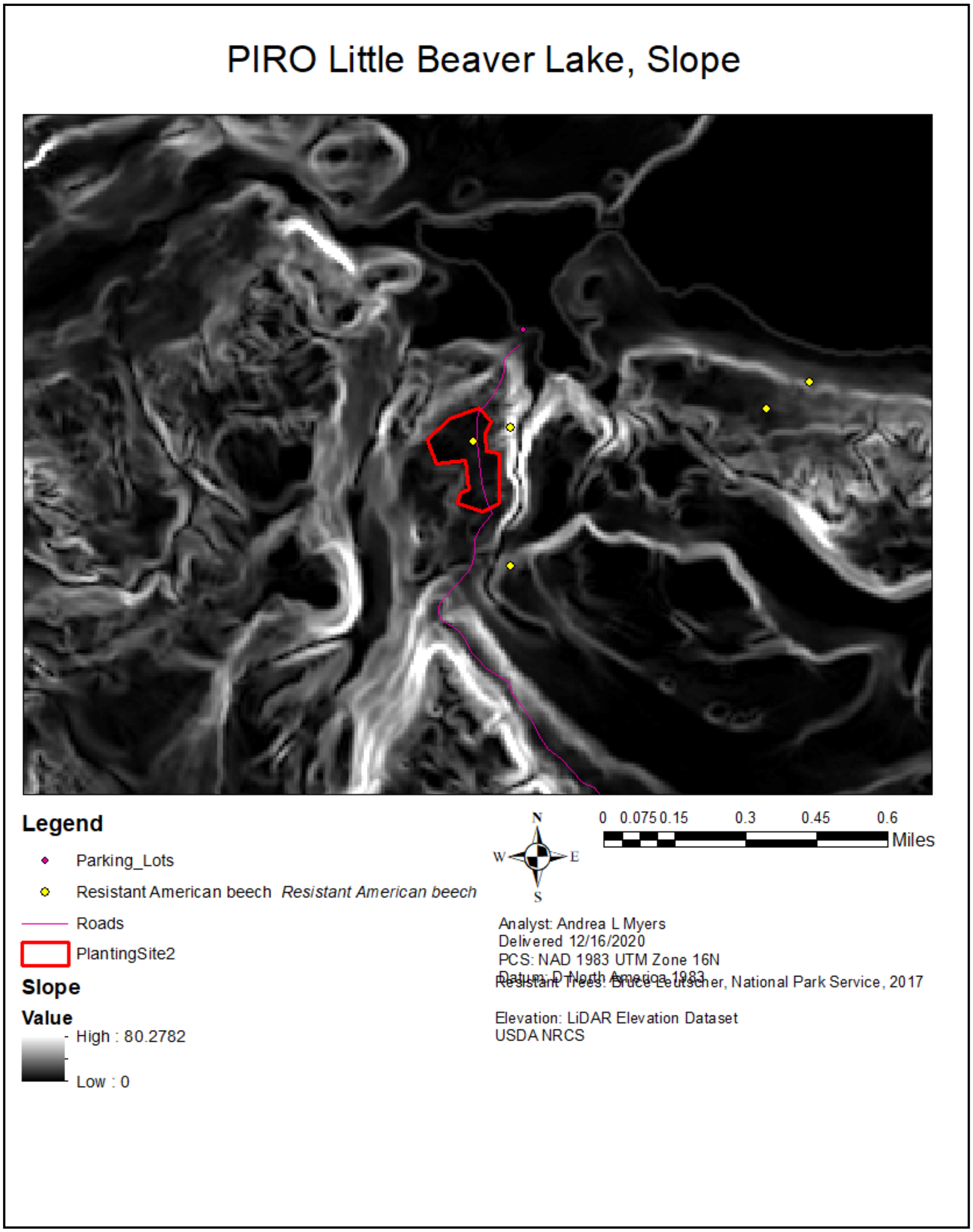

Figure A58. Slope of Little Beaver Lake planting site in Pictured Rocks National Lakeshore, calculated from LiDAR Elevation Dataset via NRCS Geospatial Data Gateway.

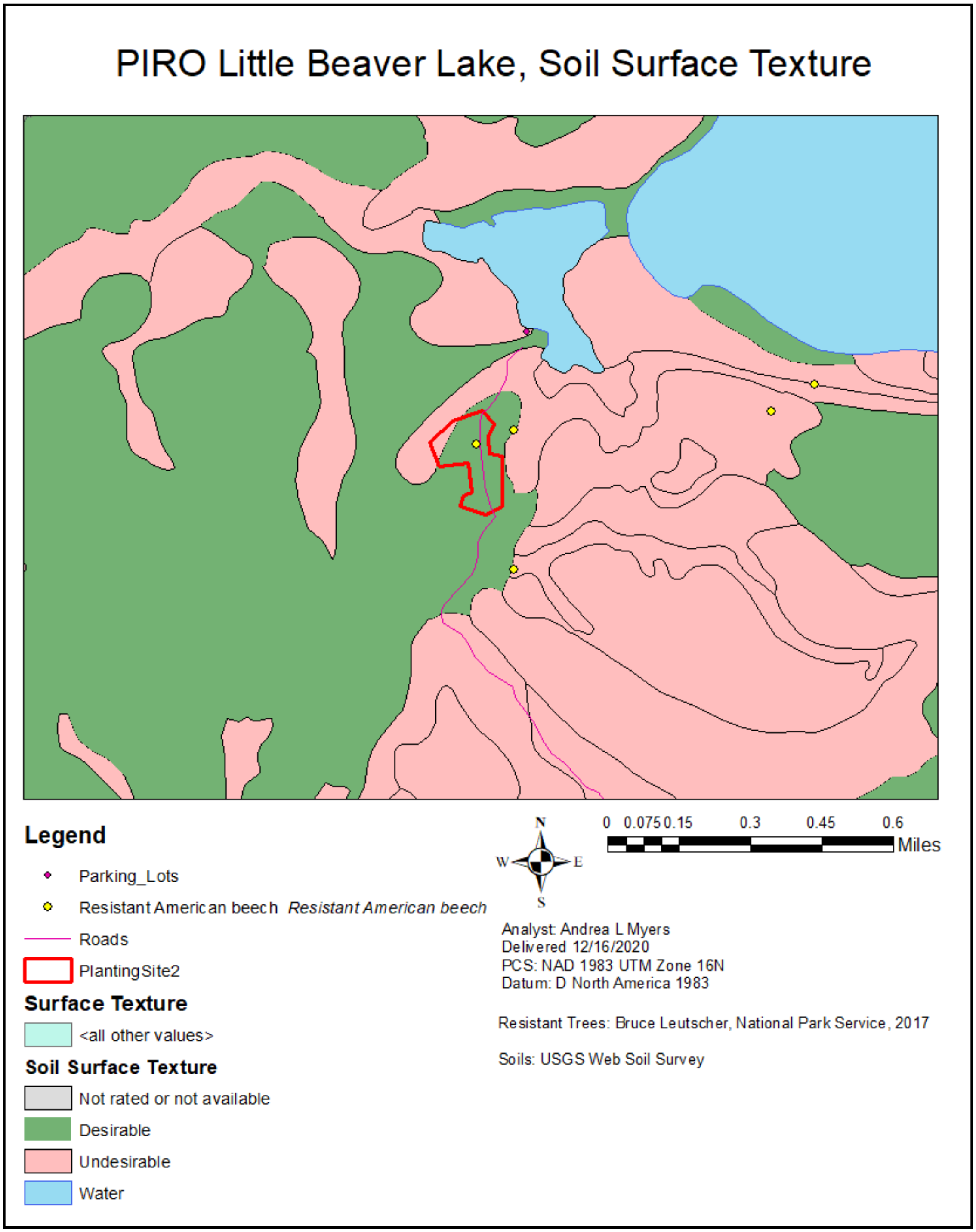

Figure A59. Soil surface texture for Little Beaver Lake planting site in Pictured Rocks National Lakeshore. Soil surface texture was defined in the USGS Web Soil Survey toolset and broken into desirable and undesirable classes based on silvics of American beech.

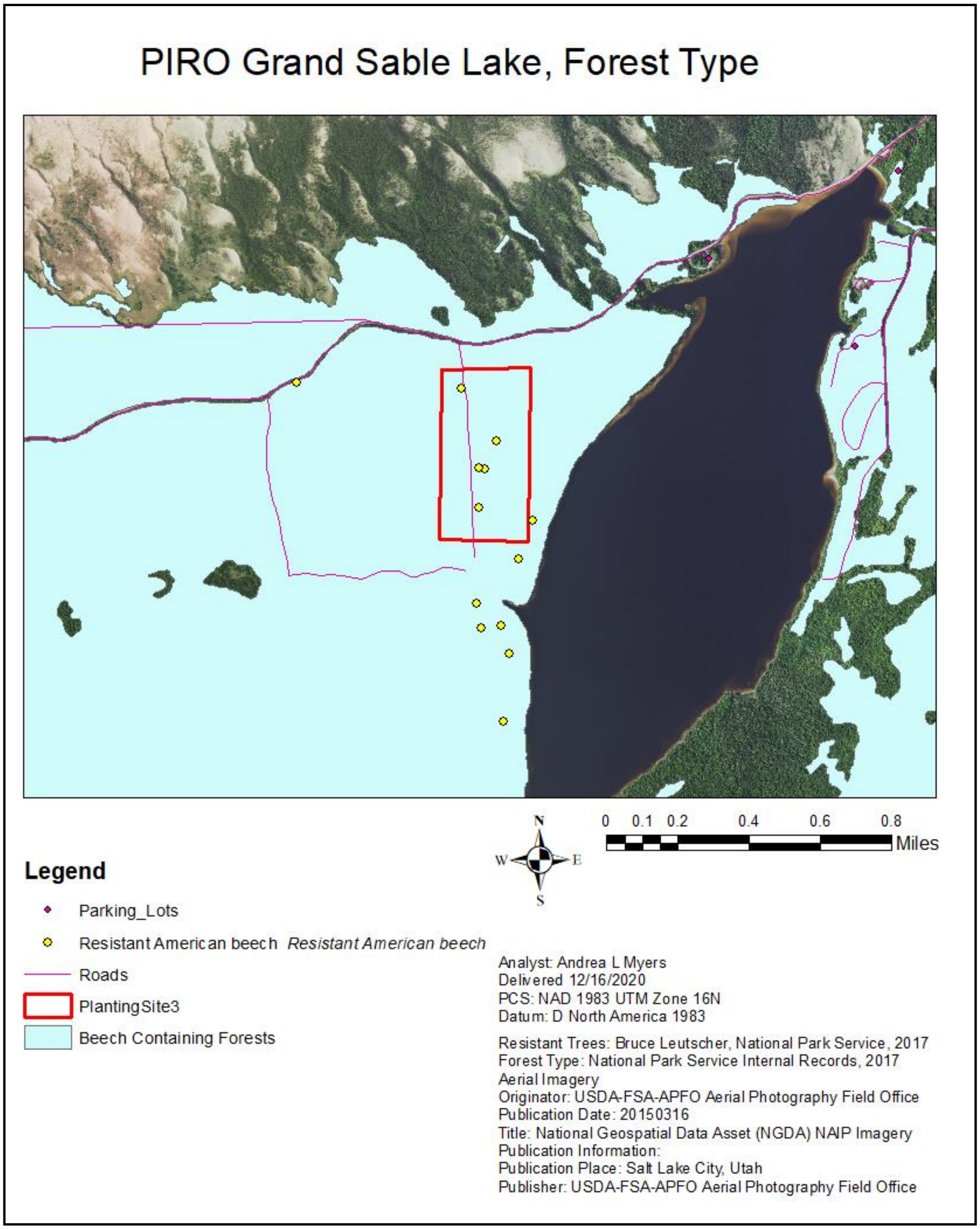

Figure A60. Points of interest for Little Beaver Lake planting site in Pictured Rocks National Lakeshore. The forest types containing American beech are presented. Locations of resistant American beech and parking areas are marked, as planting sites must be located close to both.

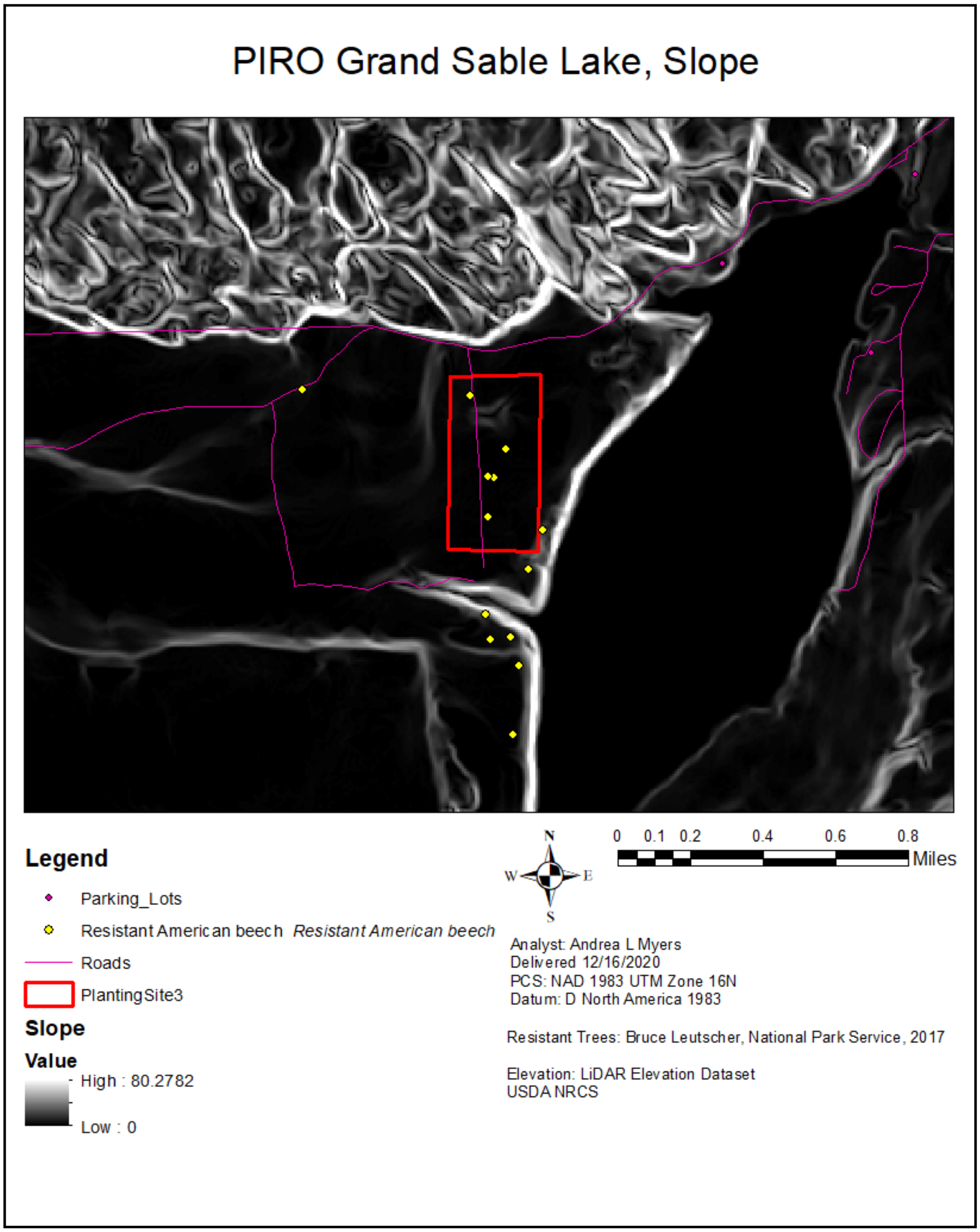

Figure A61. Slope of Grand Sable Lake planting site in Pictured Rocks National Lakeshore, calculated from LiDAR Elevation Dataset via NRCS Geospatial Data Gateway.

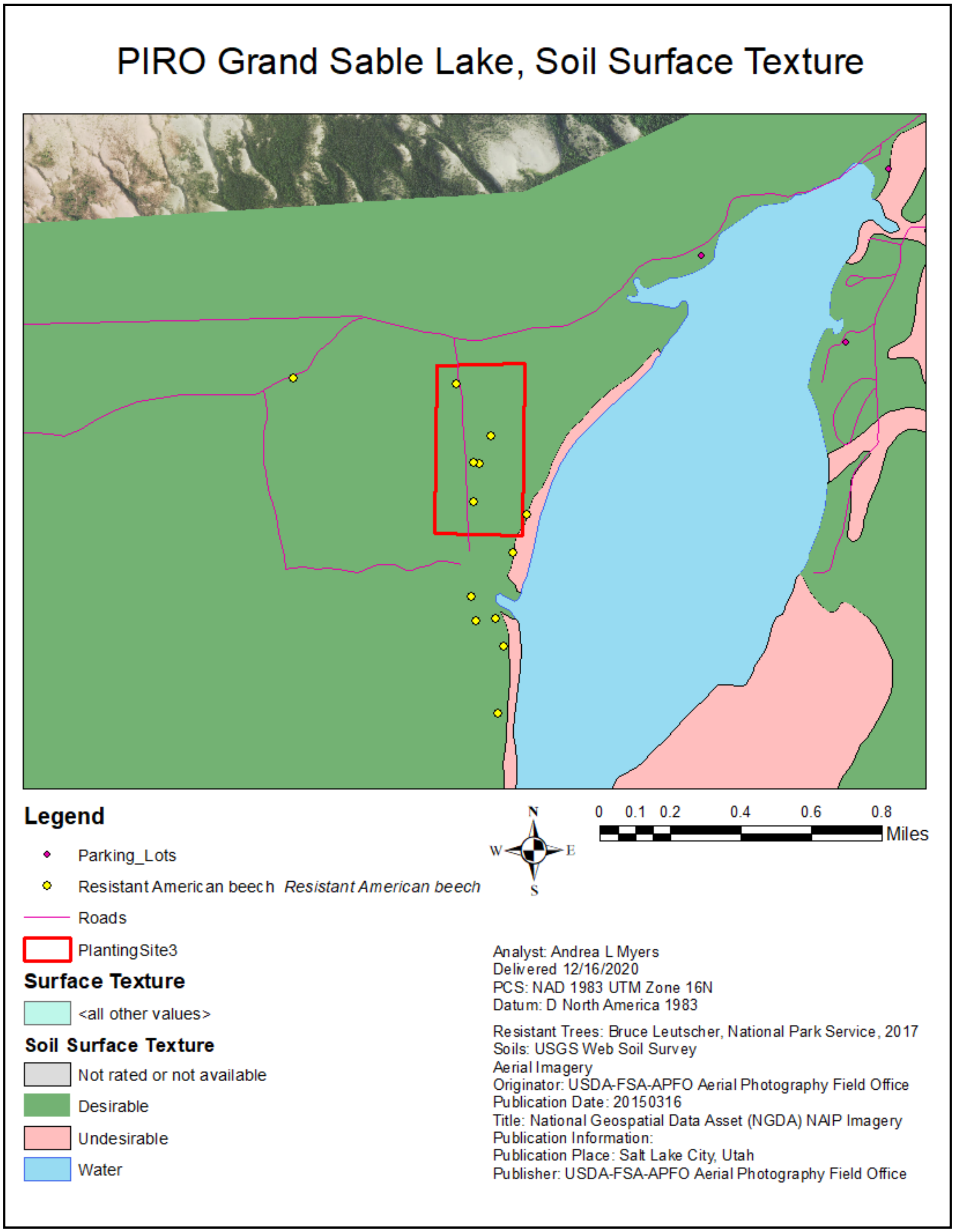

Figure A62. Soil surface texture for Grand Sable Lake planting site in Pictured Rocks National Lakeshore. Soil surface texture was defined in the USGS Web Soil Survey toolset and broken into desirable and undesirable classes based on silvics of American beech.

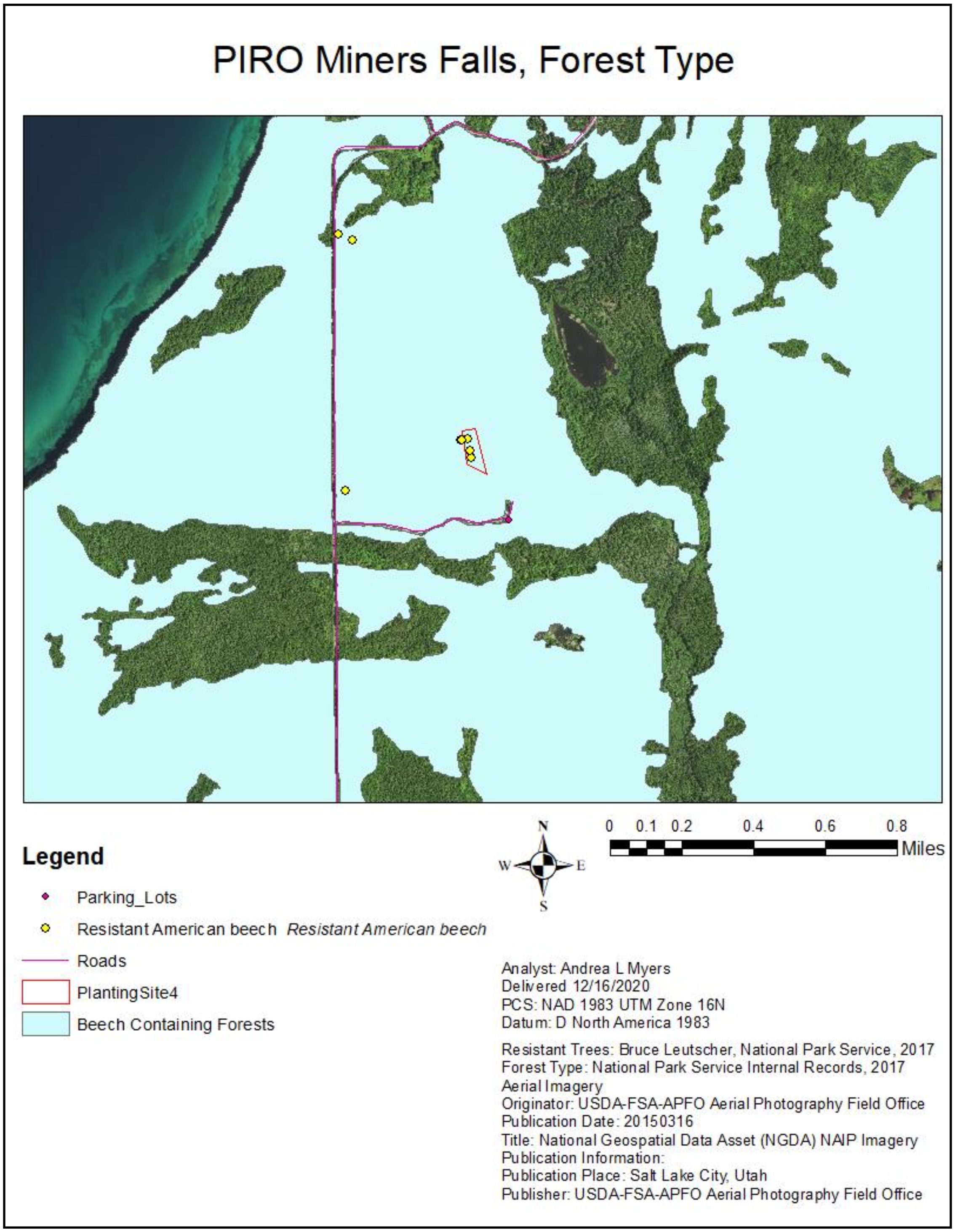

Figure A63. Points of interest for Miners Falls planting site in Pictured Rocks National Lakeshore. The forest types containing American beech are presented. Locations of resistant American beech and parking areas are marked, as planting sites must be located close to both.

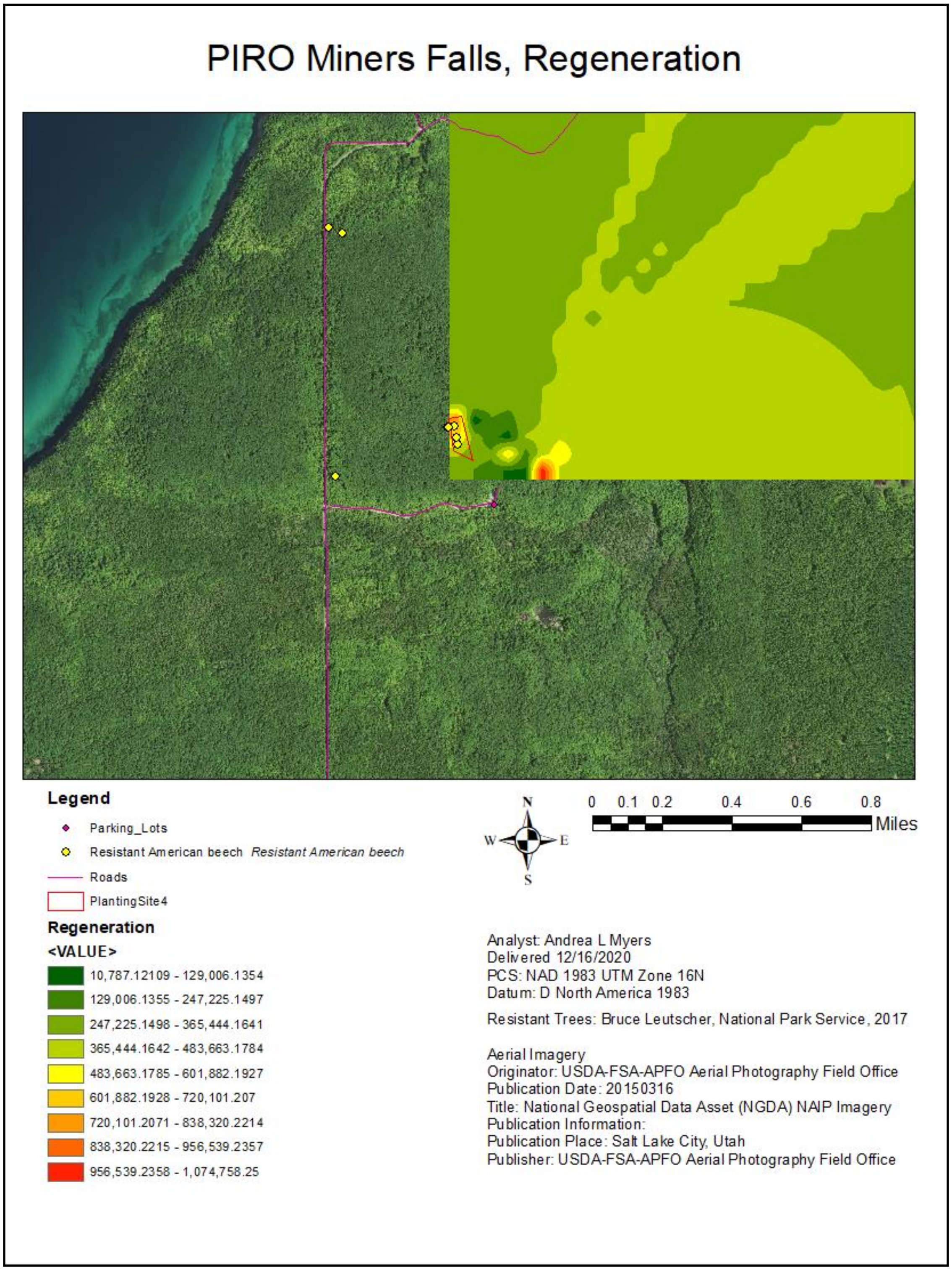

Figure A64. Prediction surface of beech regeneration created with IDW interpolation for Miners Falls planting site in Pictured Rocks National Lakeshore.

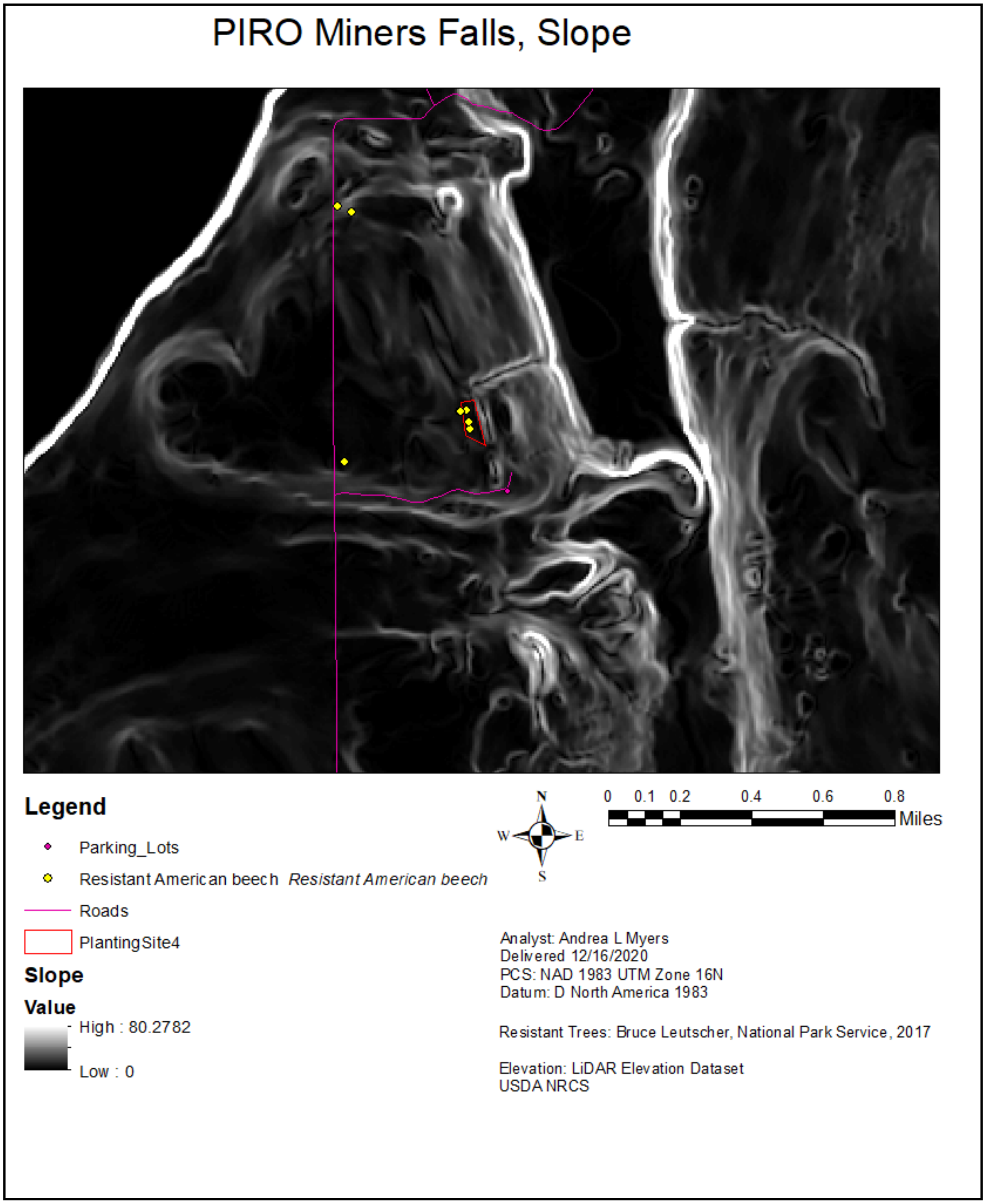

Figure A65. Slope of Miners Falls planting site in Pictured Rocks National Lakeshore, calculated from LiDAR Elevation Dataset via NRCS Geospatial Data Gateway.

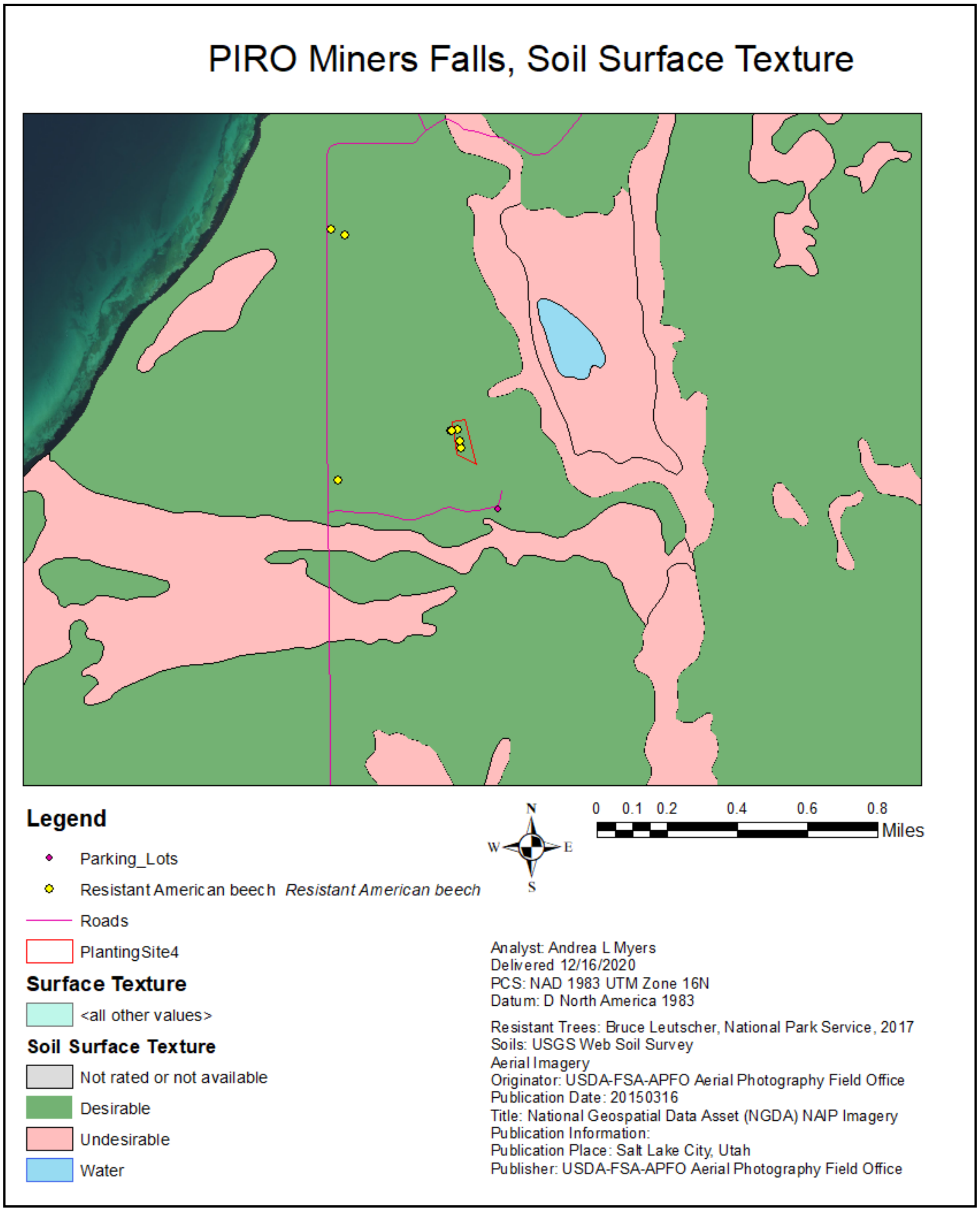

Figure A66. Soil surface texture for Miners Falls planting site in Pictured Rocks National Lakeshore. Soil surface texture was defined in the USGS Web Soil Survey toolset and broken into desirable and undesirable classes based on silvics of American beech.

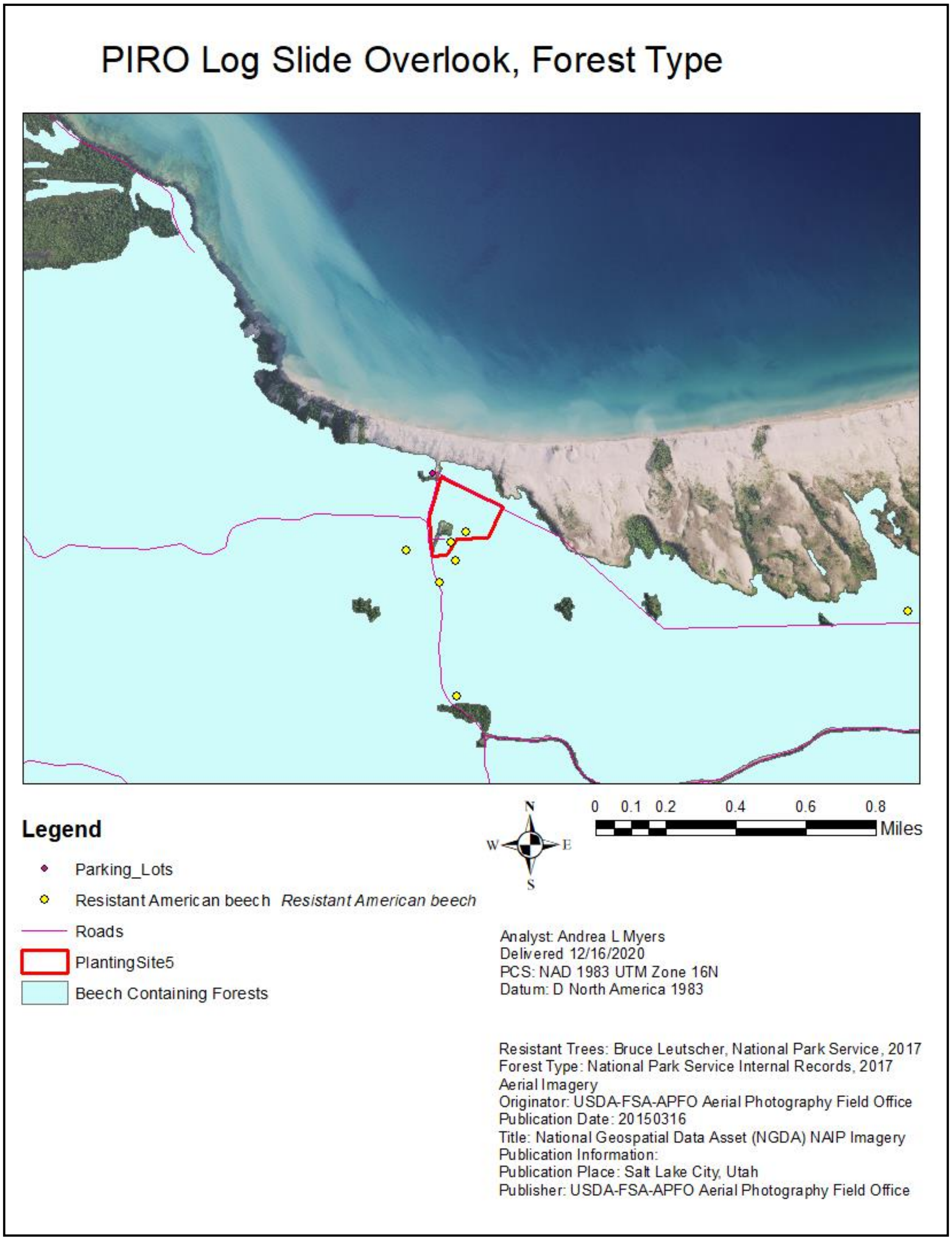

Figure A67. Points of interest for Log Slide Overlook planting site in Pictured Rocks National Lakeshore. The forest types containing American beech are presented. Locations of resistant American beech and parking areas are marked, as planting sites must be located close to both.

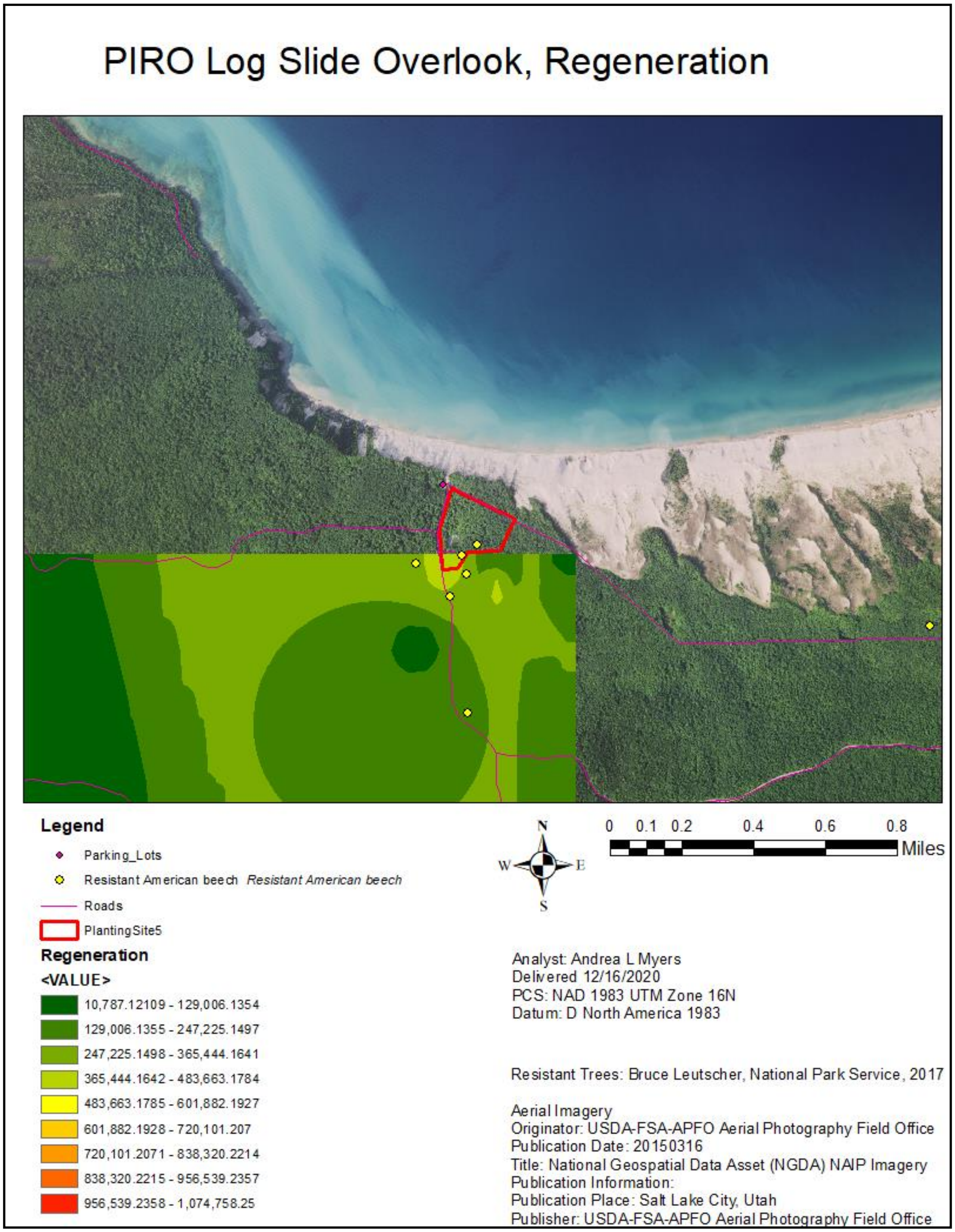

Figure A68. Prediction surface of beech regeneration created with IDW interpolation for Log Slide Overlook planting site in Pictured Rocks National Lakeshore.

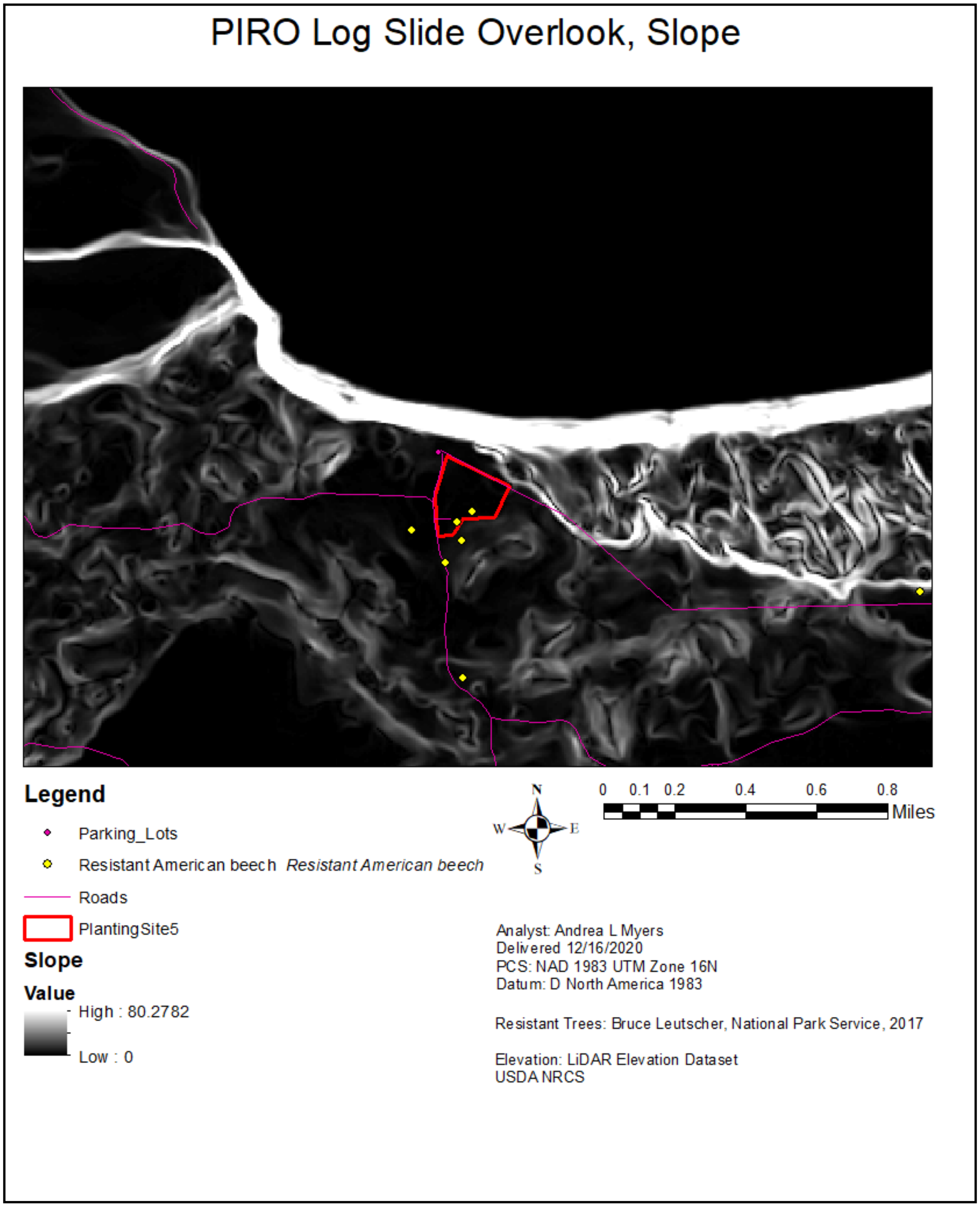

Figure A69. Slope of Log Slide Overlook planting site in Pictured Rocks National Lakeshore, calculated from LiDAR Elevation Dataset via NRCS Geospatial Data Gateway.

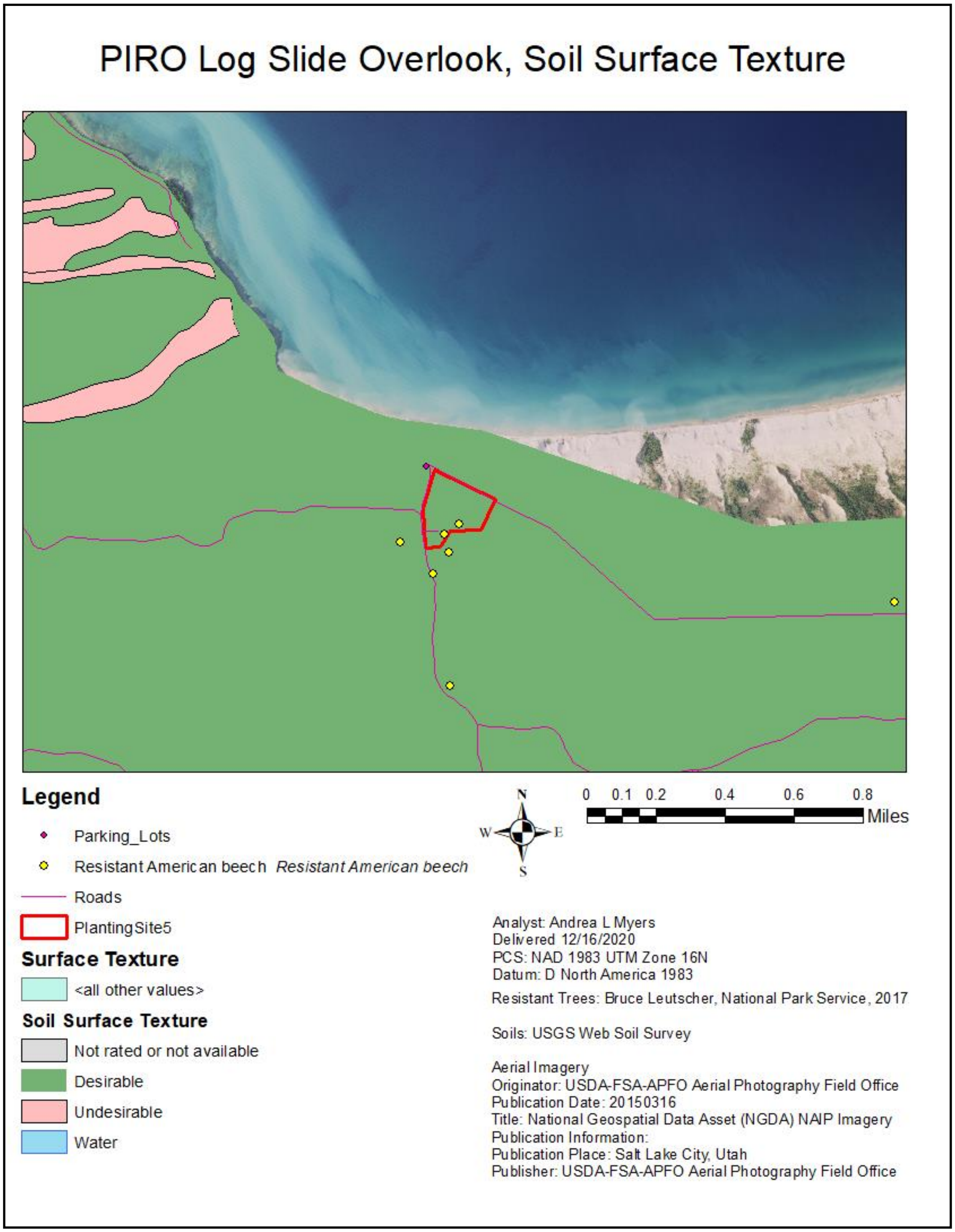

Figure A70. Soil surface texture for Log Slide Overlook planting site in Pictured Rocks National Lakeshore. Soil surface texture was defined in the USGS Web Soil Survey toolset and broken into desirable and undesirable classes based on silvics of American beech.