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Save Our Species: A Blueprint for Restoring Butternut (*Juglans cinerea*) across Eastern North America

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Abstract

Butternut is a relatively uncommon hardwood tree native to eastern North America. The species' abundance has declined over the past 50 years, primarily because of an invasive pathogen (*Ophiognomonia clavigignenti-juglandacearum* [*Oc-j*]) and loss of suitable habitat for regeneration. Although genetic diversity of butternut is highest along the southern range edge, genetic diversity rangewide is fairly high, except in small and isolated populations. Although there is little evidence for even moderate resistance in native butternut, hybrids with Japanese walnut, a closely related species, display enough tolerance to infection to persist on the landscape and bear abundant nut crops year after year. Cryostorage of native embryogenic axes has yielded promising initial results as a strategy for gene conservation, but additional action is needed to conserve the remaining native gene pool. We describe a strategy for canker-resistance breeding in butternut using naturally occurring hybrids, hybrids in research orchards, and sources of native trees from as many regions as possible. Forest managers are encouraged to find surviving trees and collect seed for planting in suitable habitat to develop actionable knowledge that will enable the restoration of butternut with enough resistance to be self-sustaining on the landscape.

Study Implications: We are soliciting help from the forestry community to take these steps to help save butternut: (1) Collect butternuts to propagate and feature in botanic gardens and arboreta. (2) Upload images and locations to TreeSnap, a free mobile phone application developed by scientists at the University of Kentucky and University of Tennessee, so the trees may be used for future breeding efforts. (3) Include butternut in upland plantings in high light environments, especially on sites that can be protected from white-tailed deer. (4) Collaborate with researchers, nonprofit organizations, and breeders to develop actionable knowledge—for example, to improve the canker-resistance screening process and to understand the pathogen's biology.

Keywords: *Juglans cinerea*, butternut canker disease, tree breeding, genetic conservation

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Butternut, *Juglans cinerea* L., is a shade-intolerant, uncommon hardwood tree species that occurs across a large swath of the eastern United States and southern Canada (Rink 1990). The species is valued by many indigenous communities for a variety of medicinal uses (Moerman 1998) and sustenance (Erichsen-Brown 1989); its mast was respected for the value to wildlife. Butternut was also used to build dugout canoes (Waldman 2005) and was commonly used as a dye. The soft, light-colored wood is highly valued for veneer, wood carving (see Figure 1), furniture, and cabinet production (Sargent 1885, Snow 1903, Schroeder 1972). The steep decline in butternut abundance over the past 60 years has two primary causes: an introduced fungal pathogen and lack of suitable habitat for seedling establishment. The pathogen most closely associated with this demise, *Ophiognomonia clavigignenti-juglandacearum* (*Oc-j*), was first noticed in the late 1960s but may have been present, albeit unnoticed, decades earlier (Broders et al. 2015). Butternut requires high-light environments and soil disturbance to regenerate, conditions often excluded from modern forest management practices in hardwood regions. The combined pressures of low light in natural forests and heavy infection has led to a steep decline in census (see details below under "Species Status"), and a sharp increase in the number of stands where butternut is now locally extinct, across the United States and Canada (Morin et al. 2018).

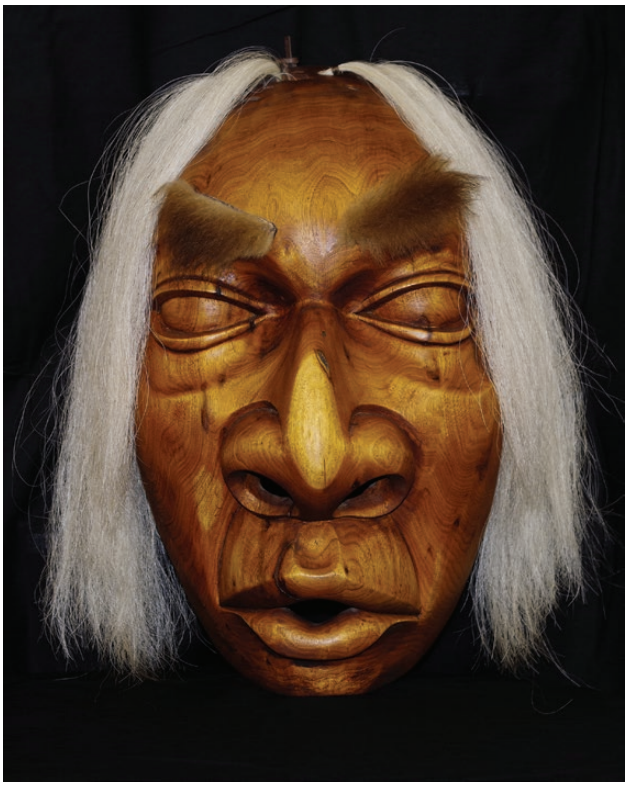

Figure 1. Napeh Kaso Yinew Kiskeyitan 16″ × 22″ by Ned Bear. The mask is made from butternut wood, horsehair, epoxy resin, varethane, and leather. The mask was created by the late Ned Bear, a master carver from the Wolistoqiyik First Nations Community in Fredericton, New Brunswick. (Photo by J. E. Letourneau, Natural Resources Canada.)

Conditions for the maintenance and restoration of butternut to some landscapes have changed considerably since the previous conservation assessment of butternut (Schultz 2003) and forest manager's guides were written (Woeste et al. 2009). The objectives of this article are to provide an update on efforts to conserve butternut across North America, propose a strategy to conserve the remaining genetic diversity, and solicit help from the forest management community as a part of this strategy.

Silvics of Butternut

Butternut is an early successional species that favors site conditions similar to the eastern black walnut, *J. nigra* L. (i.e., full sun and loamy, moist, but well-drained soils typically found in riparian zones) (Rink 1990, Cogliastro et al. 1997). Fast-growing but seldom surviving past 80 years of age, butternut bears regular nut crops at 20 years of age (USDA NRCS 2020) and younger in cultivation. The bark has a similar furrowed appearance to black walnut (Figure 2) and oval-shaped nuts (Figure 3), but unlike black walnut, it tolerates dry, rocky, limestone soils and even ruderal sites. Butternut thrives also at higher elevations than black walnut, up to 1,500 meters (Morin et al. 2018), and extends further north than the northernmost populations of black walnut (Rink 1990). Well-drained sites are essential, as the species does not tolerate wet, heavy clay soils (Cogliastro et al. 1997, 2003). Canopy tree species often found with butternut include basswood (*Tilia americana*), black cherry (*Prunus serotina* Ehrh.), American beech (*Fagus grandifolia* Ehrh.), black walnut (*J. nigra* L.), eastern hemlock (*Tsuga canadensis* [L.] Carr.), hickory (*Carya* spp.), and oaks (*Quercus* spp.) (Rink 1990, Morin et al. 2018).

The main resource limitation for butternut is sunlight: seedlings rarely regenerate in forests with few light gaps (Rink 1990, Brosi 2010). Studies conducted in the Nicolet National Forest from 1993 to 2007 tested the conditions needed for successful butternut establishment, including tree removal and deer protection strategies. The results confirmed the need for large gaps for light penetration, soil disturbance, reduction

Figure 2. Large butternut tree growing in a natural forest. The bark furrows are similar to black walnut but have less relief (photo by A. Brennan).

Figure 3. Oval-shaped nuts of the butternut tree are similar in size to black walnuts (photo by M. Williams).

of competition from other species, and protection from deer (Ostry et al. 2017). Single-tree selection is suitable for regenerating primarily shade-tolerant species (Dey 2014, Leak et al. 2014), but group/patch selection is necessary to provide enough of a canopy opening to regenerate shade-intolerant species, such as butternut. Land formally used for agriculture may be well suited to butternut regeneration (Brosi 2010), but dry, upland sites in the upper Midwest and Northeast may be best suited for restoration plantings of canker-resistant butternut (Morin et al. 2018). Ongoing work at the Morton Arboretum seeks to identify environmental conditions most suitable for butternut, including prediction of suitable sites in response to climate change, which is needed to build on prior work (Chambers et al. 2013).

Disease Threats

Butternut canker disease, caused by the fungal pathogen *Ophiognomonia clavigignenti-juglandacearum* (*Oc-j*), was first reported in Wisconsin in the late 1960s (Renlund 1971). The fungus, previously known as *Sirococcus clavigignenti-juglandacearum*, produces sticky conidia that are dispersed primarily through rain splash (Tisserat and Kuntz 1983a, 1983b). The fungus is presumed to be exotic given the lack of resistance in its primary host and lack of genetic diversity; however, it has not been observed anywhere except the United States and Canada (Nair et al. 1979, Furnier et al. 1999, Broders and Boland 2011). The disease is characterized by the presence of numerous fusiform annual or perennial cankers (Figure 4) that can occur on branches of any size, the main stem, and at the base of the tree near the soil line. Disease progression generally follows a pattern of increased incidence of cankers in the main crown followed by cankering of the main stem and base. Mortality often occurs over several years to decades depending on site conditions. Crowns affected by numerous infection sites lead to a decline in tree vigor and subsequent infection by secondary pathogens. The biology of *Oc-j* is described in more detail elsewhere (Nair et al. 1979, Orchard et al. 1982, Tisserat and Kuntz 1982, 1983a, 1983b, Halik and Bergdahl 2002, Stewart et al. 2004, Ostry and Moore 2007, Broders and Boland 2011, Broders et al. 2015, Moore and Ostry 2015, Moore et al. 2015).

Butternut tends to survive longer on sites characterized as upland: locally high elevations with well-draining soils and good air circulation (Rink 1990). The incidence and severity of butternut canker as well as *Armillaria* root disease is higher on low-elevation sites with heavy soils and in humid sites with continuous canopies (Tisserat and Kuntz 1982, LaBonte et al. 2015, Moore and Ostry 2015, Sambaraju et al. 2018). Butternut trees that are open grown or

Figure 4. Two different canker types produced on stems of butternut caused by *Ophiognomonia clavigignenti-juglandacearum*. Annual type canker with extensive callus tissue and new smaller cankers forming (arrows) on callus ridge (left). Perennial type canker (approximately 3 years old) with prominent ridges of callus that have been overcome by subsequent fungal growth (right). (Photo by J. Jacobs.)

dominant tend to endure fewer infections or have greater vigor than trees that are intermediate or suppressed (Sambaraju et al. 2018).

Species Status

The devastating impact of canker disease on the abundance of butternut became clear through assessments by USDA Forest Service Forest Inventory and Analysis (FIA) and other survey data. Between 1966 and 1986, a 77% reduction in butternut was reported throughout the range of the species with declines of over 80% documented in some states (Anderson and LaMadeleine 1978, Ostry et al. 1994). A recent FIA assessment revealed a steep decline in the number and volume of butternut trees (58%) from the 1980s to 2015 (Morin et al. 2018), with the sharpest declines in the Midwest and lake states. In 2019, a threat assessment rated butternut as among the most severely threatened eastern tree species based on an analysis that combined multiple threat attributes and species biology (Potter et al. 2019). Butternut was federally listed in Canada in 2005 under the SARA (Species at Risk Act) and is listed as imperiled or critically imperiled in all three Canadian jurisdictions (COSEWIC 2017). Butternut is not federally listed in the United States but is listed in several states as a species of "special concern" (Kentucky), "exploitably vulnerable" (New York), "threatened" (Tennessee), and "endangered" (Minnesota) (see Brosi 2010, Farlee et al. 2010a for a complete list).

Genetic Diversity

Butternut is an outcrossing, wind-pollinated tree with moderate to high genetic diversity and relatively low levels of population structure, except for strong differentiation between the main range and the geographically disjunct New Brunswick populations (Hoban et al. 2010). It shows some population-level genetic differentiation across an east–west gradient that may have originated in distinct Ice Age refugia. Butternut also exhibits a moderate genetic cline from south to north, with higher genetic diversity in the southern populations (Hoban et al. 2008, 2010, Laricchia et al. 2015), but appreciable diversity exists in the north as well. Anecdotally, some small populations show indicators of inbreeding such as albinism (S. Schlarbaum, pers. commun., 2019). Genetic data have revealed very low diversity and inbreeding in some small, isolated populations (Hoban et al. 2010). Butternut's heavy fruits probably limit effective seed dispersal to about

100 m in most cases, which may constrain natural dispersal and regeneration (Hoban et al. 2012), though immigrant pollen can connect stands across farther distances and waterways may disperse seeds further (Hoban and Schlarbaum 2014). Across the range, genetic diversity has not yet shown signs of a bottleneck attributable to butternut canker, but this may be due to diseased adult trees that persist long enough to be genotyped in spite of low vigor. A genetic bottleneck is on the horizon as these trees die and the few seeds they produce are consumed by granivores. The window for butternut gene conservation is closing rapidly along its southern range edge.

Butternut naturally hybridizes with Japanese walnut (*J. ailantifolia* Carr.), native to Japan. Nursery owners introduced Japanese walnut into the United States in the early 1900s for ornamental use and as a food source (Bixby 1919). Progeny that result from the hybrid cross between Japanese walnut and butternut are known as *J.* × *bixbyi* (USDA-NRCS 2020) or buartnuts. The two species and hybrids are commonly grown for small-scale nut production in orchards (for more information see nutgrowing.org). These hybrids often exhibit a higher growth rate, larger reproductive capacity, and higher tolerance to butternut canker than does *J. cinerea* (McKenna et al. 2011, Boraks and Broders 2014, Brennan et al. 2020). Complicating efforts to identify hybrids in the field, F_1, F_2 and backcross hybrids may resemble either parent or have traits of both parents, which can mask their genetic identity. Purdue University developed a guide to assist with identifying pure versus hybrid butternut and describes characteristics of Japanese walnut and butternut (Farlee et al. 2010b). The guide is useful for identifying many putative hybrids in the wild, but given the overlap in species phenotypes, DNA-based genotyping is required to accurately detect evidence of interspecific ancestry. Relatively inexpensive methods for genotyping both the chloroplast and nuclear genomes are published (Hoban et al. 2009, 2012, Zhao and Woeste 2011). Species-specific chloroplast DNA markers detect the species type of the maternally inherited chloroplast (McCleary et al. 2009), but this alone does not reveal whether the nuclear genome has hybrid ancestry. The published set of nuclear DNA markers (Hoban et al. 2008) reliably detects F_1 hybrids and hybrids more than one generation removed (backcross and F_2 hybrids).

Hybrids between butternut and Japanese walnut are not uncommon and can be locally abundant, especially near human habitation. They have spread across many sites including natural stands, especially in Connecticut, Massachusetts, and North Carolina (Hoban et al. 2009). Populations of native butternut with few or no hybrids (Hoban et al. 2012) can still be found, especially in national forests and other areas of contiguous forest such as the Menominee Reservation in Wisconsin, and we speculate that native butternuts still outnumber hybrids overall. The number and frequency of hybrids in any location depends on the local landscape/habitat; hybrids are most often found in old fields, roadsides, fencerows, abandoned farms, or very small woodlots, essentially places near to where Japanese walnut was planted. Often 50% to 100% of trees in these "near farm" locations are hybrids, whereas in large continuous forests (larger than a farm woodlot) hybrids are rare (less than 5%) (Hoban et al. 2012). Nearly all (90%) of hybrid trees identified have a Japanese walnut chloroplast (Hoban et al. 2012), indicating that the maternal parent was Japanese walnut. Backcrosses usually involve butternut pollen and a *J.* × *bixbyi* seed tree, which means most backcrosses have approximately 75% butternut ancestry and 25% *J. ailantifolia* ancestry. However, the 10% of hybrids with butternut chloroplast demonstrates that the cross can occur both ways under natural conditions.

Gene Conservation

Techniques to propagate butternut include direct seeding (Young and Young 1992, Bonner 2008, Brennan and Jacobs 2020), grafting (Stefan et al. 1984, Moore and Ostry 2005), rooted cuttings (Pijut and Moore 2002), and micropropagation techniques (Pijut 1993, 1997, Williams et al. 2019a), although propagation from grafting and seed are most widespread. In Canada, a procedure to cryobank the embryonic axis (EA), the embryo and some of the cotyledonary tissue, was developed to safeguard genetic diversity. The in-vitro protocol for tree regeneration after six years of EA cryogenic storage resulted in over 80% viability (Williams et al. 2019a). So far, more than 35,000 EAs representing 329 seed lots (individual and bulk), with most mother trees genotyped, have been conserved (e.g., embryo and some cotyledonary tissue) from New Brunswick populations for long-term storage. There is interest in expanding this collection to include germplasm from Quebec and Ontario (Williams, pers. commun., 2020). The estimated cost for maintaining this collection in cryostorage is $0.06 USD/EA/year, and this includes the one-time cost of a 185-liter cryotank (30,000 EAs capacity with 6 racks/tank, 10

boxes/rack, 100 tubes/box, 5 EAs/tube), consumables (tubes and boxes), and the cost of the liquid nitrogen required for tank refill over a 10-year period. The United States has not previously used cryostorage for butternut gene conservation, but this approach merits consideration for conserving genetic diversity in areas at greatest risk of butternut extirpation.

Genetic conservation efforts include an ongoing project by the Minnesota Landscape Arboretum to collect genetic material to share among botanic gardens, large plantations of saplings, and active conservation of large adult trees in Ontario. Clonal collections are maintained at various national forests across the eastern United States, and a repository of southern sources at the Forest Service's Beech Creek Orchard. University of Tennessee has found surviving trees, genotyped a subsample of their progeny, and planted those with pure *J. cinerea* pedigrees into progeny tests with plans to convert into seed orchards (S. Schlarbaum, pers. commun., 2016). The Menominee reservation maintains a geodatabase of live butternuts that foresters report. Purdue University's Hardwood Tree Improvement and Regeneration Center maintains an archive of various hybrid and pure genotypes. Lastly, an ongoing research collaboration between the United States and Canada is further investigating the genetic diversity in New Brunswick, Quebec, and Ontario populations.

Resistance Breeding Efforts: Past, Present, and Future

The success of any butternut canker resistance breeding program hinges on whether traits that contribute to resistance are heritable. The potential for traditional breeding to improve resistance to butternut canker disease remains unknown, in part because of the lack of an effective, reproducible screening protocol. Host response on small seedlings may have low predictive power for host response on mature trees in a forest setting because, in general, forest trees grow less vigorously due to advanced age or biotic and abiotic stress and become more susceptible to fungal pathogens than healthy seedlings or saplings (Isabel et al. 2019). LaBonte et al. (2015) used molecular techniques and an in-depth site inventory to study a woodlot where others had collected many promising selections and reported little evidence for heritable differences in disease characteristics related to morbidity and mortality, although heritability estimates may have been reduced because dead trees were excluded from the analysis. This included the previously described "dark-barked" phenotype (Ostry and Woeste 2004), which was thought to be a predictor of survival, although another study found no association between bark phenotype and disease susceptibility (Sambaraju et al. 2018). Other observations by McKenna et al. (2011) and Ostry and Moore (2008) suggest genetic basis for differences among selections in response to inoculation with *Oc-j*, but confirmation is lacking. The long-term multigeneration studies required for reliable estimates of variance attributable to genotype remain unrealized.

The Forest Service's Forest Health Protection program and the Northern Research Station have made a concerted effort to locate surviving butternut trees, study the pathogen's life cycle, quantify disease progression, plant grafts of survivor trees, and plant open-pollinated seeds into germplasm banks intended to function as seed orchards. Many trees in these collections succumbed to *Oc-j* or poor site selection before seed collection or advanced breeding could occur. Resistant phenotypes, those with fewer cankers or smaller cankers, were sometimes evident (Brennan et al. 2020), so it was not possible to use traits other than survival (albeit as a highly cankered tree) to select advanced generation material. In Canada, the observed rates of canker infection is over 90% in Quebec and Ontario (COSEWIC 2017) and over 70% in the province of New Brunswick (Williams et al. 2019b), which was the last jurisdiction to be infected by the pathogen in 1997 (Harrison et al. 1998). A recovery strategy was published in 2010 (Environment Canada 2010) with the main focus involving the identification of "plus trees," or surviving trees that have either escaped infection or demonstrated tolerance. Selected surviving trees have been grafted and planted in Ontario (Forest Gene Conservation Association [FGCA]). These trees have not been artificially inoculated with the pathogen, but they are being monitored closely for canker infection. The older grafts are already producing nuts, and a small number of these nuts, all identity preserved by mother tree, were grown and planted back into orchards to monitor their health (FGCA 2020, pers. commun.).

The combined experience of multiple efforts strongly suggests that surviving trees have very low levels of genetic resistance. At Purdue University, inoculation and evaluation of disease incidence and severity on selected trees over a period of 10 years revealed high levels of mortality. Other studies working with surviving trees yielded the same disappointing results (LaBonte et al. 2015), and many survivor trees

appear to be hybrids (Boraks and Broders 2014). Japanese walnuts and F_1 interspecific hybrids with Japanese walnut, identified through a combination of genetic and phenotypic methods, develop smaller cankers than butternuts when artificially inoculated, as well as fewer and smaller cankers resulting from natural infection (Ostry and Moore 2007, Boraks and Broders 2014). Breeding programs that include hybrids may be the only cost-effective method to save the remaining standing genetic variation in butternut while providing an approximation of ecological equivalence (Michler et al. 2005, Boraks and Broders 2014). The success of naturally occurring hybrids, including backcrosses, suggests that such an approximation may already be realized on the landscape. The alternative to making use of hybrids, other than developing transgenic or gene editing approaches which take years of costly research to develop, is to let nature run its course, which will most likely lead to the replacement of pure *J. cinerea* with hybrids. A key benefit of controlled breeding is the maintenance of maternal lineages of *J. cinerea*, as assessed through chloroplasts, in otherwise hybrid populations.

Approaches to Restore Butternut

In July 2019, geneticists from the United States and Canada met at Purdue University to discuss the prospects of resistance breeding for butternut. From this effort, a modified backcross breeding plan was developed (Figure 5). This plan begins with genetically confirmed hybrids that have butternut chloroplasts, interplanted with genetically confirmed butternut to provide pollen for backcrosses to the hybrid trees. This backcross approach to breeding (or facilitated breeding in this case) has been used extensively in American chestnut (*Castanea americana*) to achieve a genome with greater than 90% *C. americana* (Diskin et al. 2006). The long-term goal for butternut is to conserve as much native germplasm as possible, introducing genes for resistance from Japanese walnut and allowing natural selection to remove susceptible

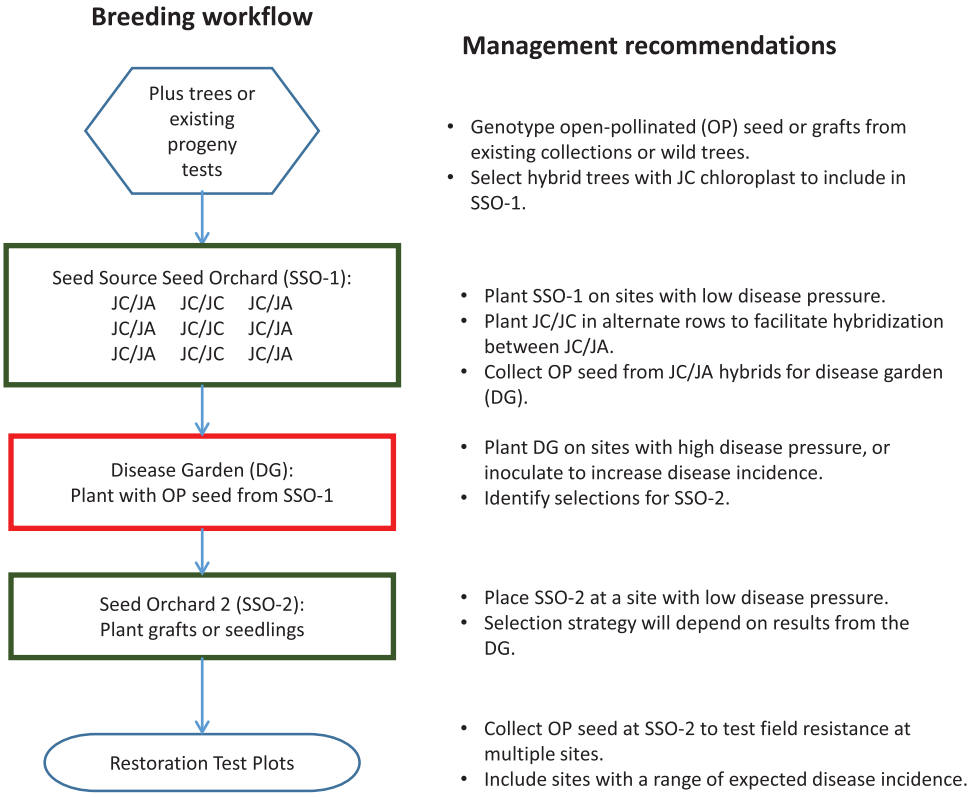

Figure 5. Framework for resistance breeding in butternut, *Juglans cinerea* (JC). The program starts with known hybrids of JC and Japanese walnut, *J. ailantifolia* (JA). Trees with a JC mother and a JA father are represented by JC/JA, as determined by the presence of a JC chloroplast. Trees with JC maternal and paternal parents are noted as JC/JC. Hybrids with JC chloroplasts will be selected for SSO-1, planted in a design to promote backcrossing of select hybrids with pure JC. Offspring from these hybrids are planted into the disease garden, at which infections are applied through a combination of artificial (stem inoculations) and natural screening. Surviving trees from the disease garden would be planted into SSO-2, from which seed would be collected and tested. Field trials, in restoration plots, would evaluate field resistance.

trees. Naturally occurring hybrid trees bear seed abundantly, and the successful survival to maturity of naturally occurring backcrosses suggests that the nuts borne on hybrid parents are viable and competitive in situations in which butternut regeneration is favorable (Hoban et al. 2010).

The duration for the proposed breeding plan is dependent on nut production to advance generations, the timing of which is variable in butternut: seven years to seed has been observed (J. McKenna, pers. commun., 2020), although 20 years are likely required for more consistent crops (USDA NRCS 2020). Timing to nut production may be earlier on grafted stock (as opposed to open-pollinatedseedlings) or accelerated by employing techniques to promote flowering (Meilan 1997). The initial seed collection garden (SSO-1 in Figure 5) should be established on sites with low disease incidence and severity (preferably outside the range of butternut to avoid disease entirely) so the trees are vigorous enough to bear fruit. Offspring from these hybrids (that were likely naturally backcrossed to *J. cinerea*) would then be planted in an area with a high incidence and severity of disease ("Disease Garden," Figure 5) to evaluate responses to natural infection progression. This could be enhanced with artificial inoculations if time and funding permit. Survivors would be selected and grafted into a new seed orchard (SSO-2 in Figure 5), employing forward or backward selection depending on heritability of traits. If improved cultivars are the main goal, then multiple progeny from the best performing maternal sources may be grafted into the seed orchard. If the primary goal is preservation of native genetic diversity, however, then a balance must be struck between maintenance of genetic diversity and incorporation of enough genetic resistance to ensure self-sustaining populations on suitable regeneration sites. The achievement of this balance is discussed in detail elsewhere (Vidal et al. 2017). If funding permits, a relatively cheap and rapid method to ascertain relatedness, such as genomic simple sequence repeats (gSSR) or Expressed Sequence Tag (EST)-SSR genotyping, could ensure the maintenance of genetic diversity and provide insurance against identity mix-ups (Hoban et al. 2012). Preservation of genetic diversity from states in the southern region of the United States is of critical importance, as this region has suffered the highest losses because of the combined impact of butternut canker and habitat loss, but genotypes from existing orchards and surviving wild trees would be candidates for the initial stages. A breeding program is not likely to advance beyond a second-stage seed orchard (SSO-2 in Figure 5) because of scarce regeneration resources for this uncommon species, so susceptible genotypes would be removed by natural selection in the future. All seed trees in the modified backcross breeding plan will have the butternut chloroplast, and all their descendants will also, assuming there were no errors in labelling during the first phase. This breeding plan would conserve maternal lineages, in the short term, at least until resistance may be fortified with the outputs from scientific or technological advances.

Good site selection is essential for this strategy to succeed. Butternut's preference for open, sunny sites make it a suitable species for planting in well-drained, abandoned pastureland, along the banks of rivers and woodlots following disturbance, provided that competition and deer browse can be controlled. Silvicultural strategies that mimic disturbance (thinning, crop tree release, or regeneration harvest) are recommended to promote butternut regeneration (Ostry et al. 1994, Farlee et al. 2010a). Scientists are encouraged to incorporate butternut into silvicultural research to further advance our knowledge of practices that favor its regeneration. Trees will require protection in all cases where white-tailed deer (*Odocoileus virginianus* Zimm.) are present. Native butternuts are expected to succumb to canker eventually, but many of the descendants of hybrids will fare better.

Forest managers with access to state and local nurseries that will buy butternuts for nursery stock are encouraged to collect butternut seed in the fall, usually in September or October (Bonner 2008, Farlee et al. 2010b) in locations where competition is intensively controlled. The nuts are usually mature when the fruit starts to fall; good nuts have a pronounced egg shape. Nuts that are lighter in weight or slightly curved inward may be empty. A detailed protocol for harvesting and germination was recently published (Brennan and Jacobs 2020). Several state nurseries in the Midwest United States grow butternuts, but highly variable seed production and a dearth of mature trees from which to collect limits the seedling supply. Naturally occurring hybrid trees often bear seed abundantly every year, and seed abundance may foretell its hybridity. State and private nurseries will likely respond to new markets if seed is available: Communicate your interests with your local nursery to make them aware of your interest in planting butternut seedlings.

The success of this strategy also depends on the incorporation of as much local genetic diversity as possible, such as wild trees that are currently not part of a breeding program. The University of Kentucky and

University of Tennessee have developed a mobile application called TreeSnap (https://treesnap.org/),[1] to enable citizen scientists to report and georeference the locations of a select group of tree species threatened by pests or disease. The app is available for free in the App Store and on Google Play. TreeSnap is unique because the information collected is directly available to researchers who work with gene conservation or resistance breeding. Pictures of surviving butternut trees can be uploaded using the app for future scion or seed collection. More information on this app is available at www.treesnap.org.

Summary

Butternut is disappearing across the landscape of North America, primarily because of a pathogen but also loss of suitable habitat. We have outlined a strategy to conserve the remaining genetic diversity and introduce enough resistance to improve restoration success by enabling the species to be self-sustaining on the landscape. Additional research is needed on the pathogen's biology, host-pathogen interactions, resistance mechanisms, and development of an effective, reproducible screening protocol. The assistance of professional foresters is essential to identify surviving trees, including suspected hybrid trees, collect nuts for the nursery trade if a market exists in their region, and plant butternut on sites where the tree can survive and thrive. The backcross breeding plan we described may be adjusted based on available resources but offers a blueprint for advancing resistance breeding for this imperiled species. The native gene pool of butternut can still be saved through the efforts of foresters, land managers, scientists, nonindustrial private landowners, and other organizations with an interest in hardwood nuts, or their wood.

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Endnote

1. Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the US Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that also may be suitable.

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