Intentional introgression of a blight tolerance transgene to rescue the remnant population of American chestnut

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Abstract
In contrast to many current applications of biotechnology, the intended consequence of the American Chestnut Research & Restoration Project is to produce trees that are well-adapted to thrive not just in confined fields or orchards, but throughout their natural range. Our primary focus is on disease tolerance, but we believe it will also be critically important that optimal restoration trees should have robust genetic diversity and resilience, which can be supplied by a full complement of their wild-type genes. Chestnut restoration offers a unique case study because many restoration or intervention options have been attempted: doing nothing, planting non-native chestnut species, planting hybrids, mutagenesis (exposing seeds to high levels of radiation to induce random mutations), backcross breeding, and now genetic engineering. Any of these techniques may be advantageous independently or in combinations, depending on the specific goals of land managers or restoration practitioners, but genetic engineering offers a unique opportunity to enhance blight tolerance while minimizing other changes to the genome.

Keywords
biotechnology, conservation alternatives, genetic engineering, intended consequences, restoration

The loss of the American chestnut (Castanea dentata; Figure 1) due to the introduction of an exotic, invasive pathogen is the classic example of how world trade can change the environment. The introduction of the fungus that causes chestnut blight (Cryphonectria parasitica) led to the first Plant Quarantine Act in 1912 (Waterworth & White, 1982), which was intended to help prevent such catastrophes in the future. Early in the efforts to contain the blight, it was realized that quarantines, sanitation, and the use of fungicides were not going to stop the spread of the pathogen. Therefore, alternative paths were taken with the intended consequence of genetically altering the chestnut tree to be resistant to the blight. These included introducing blight resistant chestnut species from Asia and making hybrid trees between chestnut species (Diller, Clapper, & Jaynes, 1964; Miller, Woeste, Anagnostakis, & Jacobs, 2014) with the goal of replacing the susceptible American chestnut in the forests. To date, these efforts have had little success: Asian chestnuts and hybrids can have good blight resistance, but tend to be shorter and express other differing phenotypes, so in most cases they are unable to thrive in eastern U.S. forests where American chestnut once lived (Anagnostakis, 2012; Miller et al., 2014). A further refinement of these efforts is a
backcross breeding program, initiated in 1983 by The American Chestnut Foundation. The intended consequences of this program are genetically modifying chestnuts in a way that retains most of the American chestnut genome, to retain its adaptability to its forest ecosystem, and to incorporate a small percentage of the Asian genome to confer blight resistance. To date, this program has only been partially successful due to the recent discovery that many separate Asian chestnut genes contribute to blight resistance, which significantly complicates breeding (Steiner et al., 2017; Westbrook et al., 2020).

In 1990, an effort was initiated to genetically engineer blight resistance into the American chestnut using only a few genes, with the goal of introgressing this blight resistance trait into the surviving remnant population (Powell, Newhouse, & Coffey, 2019). This effort follows the same intended consequence as earlier efforts: genetically altering the chestnut tree to be resistant to the blight. But unlike previous genetic modifications, the genetically engineered (GE) trees retain all of their wild-type genes and genome structure, with only the blight resistance trait added on. Because the GE American chestnut has not lost any of its traits, it should be fully adapted to its native forest ecosystem. Breeding (outcrossing) GE chestnuts with wild relatives from other parts of the range can incorporate the blight resistance trait into even more diverse and adaptable offspring generations (Westbrook, Holliday, Newhouse, & Powell, 2020). The new blight resistance trait in GE American chestnuts is unique in that it degrades the fungal toxin

FIGURE 1 Top: “Ghost forest” of blighted American chestnuts in Virginia (Library of Congress Prints and Photographs Division). Bottom left: Rare large surviving American chestnut tree in central New York state. Bottom right: Young, third-generation transgenic American chestnut seedling with introgressed blight tolerance in a trial shelterwood restoration planting.
oxalic acid, which is major virulence factor of the pathogen (Chen, Sun, Narayanan, Nuss, & Herzberg, 2010), rather than targeting the pathogen itself (Hu, Telenko, Phipps, & Grabau, 2016; Zhang et al., 2013). Thus, there is logically not a strong selection pressure against the pest. The same mechanism of fungal toxin degradation is expressed in many crop plants (Lane, 2002), and a similar mechanism is present in Chinese chestnuts: in both cases the pathogen resistance has been stable and durable, without increases in pathogen virulence. We expect blight tolerance in GE chestnuts to be similarly robust, but we are nevertheless testing combinations of other traits (through either GE or backcross breeding) or treatments (such as biocontrols) that should provide additional durability and resilience across temporal and ecological scales (Powell et al., 2019; Westbrook, 2018).

Intentional introgression has previously been applied as a restoration strategy for both animals (Benson et al., 2011; Smith, Peterson, & Houston, 2003) and plants (Hamilton & Miller, 2016; Suarez-Gonzalez, Lexer, & Cronk, 2018). However, most of these examples involve the introgression of broad genetic diversity into populations that were either severely restricted or locally extirpated (Hostetler et al., 2010; Kauffuß & Reisch, 2017; Weisenberger, Weller, & Sakai, 2014). Uncontrolled introgression of multiple traits may result in various mixtures of desired, neutral, and undesired outcomes. In fact, genetic introgression by translocation or hybridization with non-native species has occasionally been described as a threat to conservation (Bohling, 2016; Rhymer & Simberloff, 1996). But the intentional introgression of specific traits to benefit existing populations has been successfully applied to agriculture (Hao et al., 2020; Zamir, 2001) and experimentally studied to elucidate evolutionary principles (Bradshaw & Schemske, 2003). Similar concepts of trait introgression for conservation are being investigated for enhancing tolerance of various species to changing climatic conditions (Aitken & Whitlock, 2013; Cleves et al., 2020). The American Chestnut Foundation’s backcross breeding program incorporates elements of both population replacement and trait introgression, as would similar projects being proposed for other threatened trees (Romero-Severson & Koch, 2017; Sniezko & Koch, 2017). Thus, the intention for restoration with the GE American chestnut (i.e., to rescue a diverse population via introgression of a single trait; Westbrook, Holliday, et al., 2020) represents a novel application of biotechnology for conservation.

Humans have been modifying the genomes of plants for thousands of years, but the ability to intentionally introduce individual genes into plants started about four decades ago (Tepfer, 1984). This was a watershed moment because these new tools allowed plant breeders to achieve a much more focused result while greatly reducing unintended consequences. Although this was always the goal, it wasn’t until the recent age of genomic sequencing that this has been confirmed. Genetic engineering, and now the more precise offshoot known as genome editing, both produce far fewer changes to plant genomes than more traditional methods such as species hybridization, selective breeding, and mutagenesis (natural or induced). GE can result in genomic changes outside the insertion site of the intended trait, but it is important to understand that these changes are not limited to GE, and in fact are more common via traditional methods. To explore and illustrate this concept, researchers sequenced the genomes of soybean cultivars produced through various modification techniques, specifically to look for structural variations in the DNA. They found the number of genes affected by structural variations in GE plants was an order of magnitude less than that of fast neutron mutants (i.e., plants modified by mutagenesis), and two orders of magnitude less than the rates observed between traditionally bred cultivars (Anderson et al., 2016). Hybridization between species can cause especially extensive genomic changes (Lai et al., 2005). One study noted that “...hybrid offspring receive a genomic shock due to mixing of distant parental genomes, which triggers a myriad of genomic rearrangements, for example, transpositions, genome size changes, chromosomal rearrangements, and other effects on the chromatin (i.e., DNA structures). Recently, it has been reported that, besides genomic rearrangements, hybridization can also alter the somatic mutation rates in plants.” (Bashir, Mishra, Hasan, Mohanta, & Bae, 2018). This does not mean that these more intrusive methods should be avoided—in some cases, traditional breeding, mutagenesis, or hybridization may be quicker, easier, and more effective tools for genetic modification in plants, despite resulting genomic changes. In chestnuts, just as in soybeans, hybridization can result in unintended consequences such as intermediate traits, male-sterile offspring (Anagnostakis, 2012), a semi-lethal condition called “cracked bark” (Jaynes, 1964), or Internal Kernel Breakdown, in which approximately 40% of the nuts decay in their shell (MSU Extension, n.d.; Fulbright et al., 2014). These consequences of breeding are not generally seen as threats or risks to chestnuts as a whole, but they require management through careful breeding and selection processes.

American chestnut reintroduction efforts to date have been intermittent in scope and time, taking place on small geographic scales relative to the range of the tree. Thus far, these efforts have had limited success in terms of enhancing blight resistance while retaining American traits. Therefore, the most widespread response to chestnut blight has been to take no action toward chestnut restoration. The outcomes of this (lack of) action are
difficult to summarize on ecological or landscape scales, but the loss of American chestnuts dramatically affected food availability to many birds and mammals, along with their related food webs, and likely resulted in the extinction of at least a few species of chestnut moths (Davis, 2005; Hill, 1994). Other impacts likely included changes to nutrient cycling (Ellison et al., 2005), woody debris decomposition (de Bruijn et al., 2014), and leaf litter chemistry in freshwater pond communities (Stoler, Burke, & Relyea, 2016). Apart from broader ecological impacts, the effects on wild American chestnut populations are quite obvious: these formerly dominant trees have either disappeared entirely or been relegated to understory sprouts which rarely reach maturity (Anagnostakis, 1987; Paillet, 2002). In many forested areas, prevalence of other deciduous tree species like oaks, hickories, and maples increased as chestnut declined (Braun, 1950; Stephenson, 1986), but no other tree matches the American chestnut’s combination of size, longevity, rot resistance, economic value, and stable production of nutritious mast.

Therefore, when one examines the various methods that have been attempted to develop blight resistant American chestnut trees (i.e., doing nothing, species hybridization, mutagenesis, and genetic engineering), it is genetic engineering that best retains the integrity of the original tree’s genome while giving what appears to be suitable resistance to blight (Figure 2). This type of integrity may not be as important when growing chestnuts for purposes such as producing nut crops, attracting game for hunting, or mine land reclamation. But retaining genomic integrity is very important when developing a tree intended to reintegrate into the forest canopy and adapt into a natural forest ecosystem. Many tools are being used to restore the American chestnut tree, and genetic engineering has its place in this effort.

Unfortunately, genetic engineering tools are often seen by the public as something new and unproven, with no precedent in nature. Again, in this age of genomics, it has become clear that genetic engineering mimics natural phenomena that regularly occur in the environment. Several genomic studies have been published over the past few years on this topic: one of particular relevance (Matveeva & Otten, 2019) revealed that approximately 7% of the common flowering plant species examined had likely experienced horizontal gene transfer (the movement of DNA fragments or even whole genes from one species to another). This genetic modification occurred, with no human involvement, due to *Agrobacterium*, the same organism we used in the lab to develop the blight-tolerant GE American chestnut trees. These plants, which can now be thought of as “natural GMOs” (or naturally GE), include three species of walnut trees. In that sense, a blight-tolerant American chestnut will not be the first “GMO” to enter the natural forest environment, just the first developed intentionally for restoration.

**FIGURE 2** Blight damage after intentional inoculation on young wild-type (left) and GE (right) American chestnut stems. These trees are full siblings, so they are very similar except for expression of oxalate oxidase in the GE tree. Note the large orange area and “sunken” appearance on the wild-type stem, indicating dead tissue under the canker: the stem above this canker did not survive the season. The canker on the transgenic stem is swollen, confirming that there is live tissue responding to the blight infection: this stem survived the inoculation. Black marks indicate boundaries of 5 mm vertical wounds applied during inoculation.
Public acceptance of genetic engineering as a restoration tool may depend a great deal on the goals of a particular project and associated contextual alternatives. For example, surveys of public attitudes have suggested that many people are initially skeptical about the general idea of using biotechnology in the wild, but when presented with a concrete threat and realistic alternatives (i.e., planting or hybridizing with non-native species), acceptance is similar for these types of interventions as for biotechnological approaches (Hajjar, McGuigan, Moshofsky, & Kozak, 2014; Needham, Howe, & Petit, 2016). See the concurrent article by E. Brister in this issue for a more thorough exploration of public acceptance of these technologies for use in restoration.

Finally, a substantial consideration surrounding potential use of GE plants for restoration is government regulatory approval. While some agencies in the United States and elsewhere are beginning to relax rules for certain types of minor genome modifications (Benítez, Nidia, & Vicién, 2020; Bratlie et al., 2019; Stokstad, 2020), the process of seeking regulatory approval is still a daunting and expensive process for most GE product developers and university researchers. Our experience with the transgenic chestnut (Powell et al., 2019) has shown that regulators are responsive and helpful in navigating this process, but that they are bound by complex legal frameworks which are costly and convoluted, especially when applied to wild organisms outside agricultural boundaries. Nevertheless, we have submitted a petition to the USDA-APHIS for consideration of nonregulated status (Newhouse et al., 2020), and we are making progress toward submitting documentation to both the FDA and EPA as well. As a result, we do not believe that government regulations are necessarily an insurmountable obstacle to restoration, but they could be improved by better reflecting relative risks among plant breeding methods. It is worth noting that both the organism and the new trait determine regulatory oversight in the United States: trees that are not food products (e.g., ash or hemlock) would not be regulated by the FDA, and modifications that are not associated with pests (e.g., climactic adaptability) would likely not be regulated as pesticides by the EPA. This pesticide regulation by the EPA may be one of the most logically problematic parts of the chestnut regulatory review, especially given the non-pesticidal mechanism of the new trait as described above. Moving forward, we hope that familiarity with safe use of biotechnology in general, and with its applications to conservation, will help streamline the process and allow a focus on realistic risks in the context of non-regulated alternatives.

It is worth noting that many of the rules or expectations from regulatory agencies overlap with our own goals in creating a safe and effective restoration tree. While we understand that reintroducing an ecologically important tree would likely have numerous effects on many aspects of the ecosystem, we would not want to release a tree if the transgene product might be detrimental to neighboring native plants, animals, or beneficial soil fungi (Brown, Newhouse, Powell, & Parry, 2020; Goldspiel, Newhouse, Gibbs, & Powell, 2019; Newhouse et al., 2018). Legal mandates of the regulatory agencies address some of the same types of questions, such as protecting the environment from novel pests and ensuring safety of human food & animal feed (McHughen & Smyth, 2012). Additionally, increasingly efficient whole-genome sequencing methods should allow both developers and regulators to quickly identify any novel irregularities in transgenic lines, and also to more fully understand the scale of genomic changes in traditionally produced products.

Perhaps most importantly, many of the ecosystem impacts associated with species restoration would be similar regardless of the method used to produce the new trait, so it is critical to consider the context of non-regulated alternatives when making these decisions. Both our interests as scientists and the interests of the regulators are to conserve and protect human health and the environment, and genetic engineering is one of many tools that help us accomplish these intended consequences. The intended consequence of the American Chestnut Research & Restoration Project is to introgress blight resistance into the remnant American chestnut population in a way that best retains their genomic integrity, allowing retention of local adaptations and ecosystem services provided by the trees. We are optimistic that the tools of genetic engineering, combined with traditional breeding methods, can accomplish these goals.

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CONFLICT OF INTEREST
The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS
Andrew E. Newhouse: Wrote the bulk of the manuscript; reviewed, edited, and approved the manuscript.
William A. Powell: Conceptualized the topic; reviewed, edited, and approved the manuscript.

DATA AVAILABILITY STATEMENT
No new data were generated for this perspectives article.
REFERENCES

Aitken, S. N., & Whitlock, M. C. (2013). Assisted gene flow to facilitate local adaptation to climate change. *Annual Review of Ecology, Evolution, and Systematics*, 44(1), 367–388. https://doi.org/10.1146/annurev-ecolsys-110512-135747

Anagnostakis, S. L. (1987). Chestnut blight: The classical problem of an introduced pathogen. *Mycologia*, 79(1), 23–37. https://doi.org/10.2307/3807741

Anagnostakis, S. L. (2012). Chestnut breeding in the United States for disease and insect resistance. *Plant Disease*, 96(10), 1392–1403. https://doi.org/10.1094/PDIS-04-12-0350-PE

Anderson, J. E., Michno, J.-M., Kono, T. J. Y., Stec, A. O., Campbell, B. W., Curtin, S. J., & Stupar, R. M. (2016). Genomic variation and DNA repair associated with soybean Transgenesis: A comparison to cultivars and mutated plants. *BMC Biotechnology*, 16(1), 41. https://doi.org/10.1186/s12896-016-0271-z

Bashir, T., Mishra, R. C., Hasan, M. M., Mohanta, T. K., & Ba, H. (2018). Effect of hybridization on somatic mutations and genomic rearrangements in plants. *International Journal of Molecular Sciences*, 19(12), 3758–3771. https://www.mdpi.com/1422-0067/19/12/3758

Benitez, C., Nidia, D. F. R., & Vicién, C. (2020). Paraguay’s path toward the simplification of procedures in the approval of GE crops. *Frontiers in Bioengineering and Biotechnology*, 8, 1023. https://doi.org/10.3389/fbioe.2020.01023

Benson, J. F., Hostetler, J. A., Onorato, D. P., Nichols, J. D., Johnson, W. E., Rockefeller, D. W., Stadt, S., Medina-Mora, C., Mandujano, M., Bae, H., & Davis, D. E. (2005). Historical significance of American chestnut to Appalachian culture and ecology. *Proceedings of Conference on Restoration of American Chestnut to Forest lands*. Steiner, K. C., & Carlson, J. E. (eds.).

Bohling, J. H. (2016). Strategies to address the conservation threats posed by hybridization and genetic introgression. *Biological Conservation*, 203(November), 321–327. https://doi.org/10.1016/j.biocon.2016.10.011

Bradshaw, H. D., & Schemske, D. W. (2003). Allele substitution at a flower colour locus produces a pollinator shift in monkeyflowers. *Nature*, 426(6963), 176–178. https://doi.org/10.1038/nature02106

Bratlie, S., Halvorsen, K., Myskja, B. K., Mellegård, H., Bjorvatn, C., Frost, P., et al. (2019). A novel governance framework for GMO. *EMBO Reports*, 20(5), e47812. https://doi.org/10.15252/embr.201947812

Braun, E. L. (1950). CHAPTER 7 the oak-chestnut Forest region. In *Deciduous forests of eastern North America* (pp. 192–258). Philadelphia, PA: The Blakiston Company.

Brown, A. J., Newhouse, A. E., Powell, W. A., & Parry, D. (2020). Comparative efficacy of gypsy moth (Lepidoptera: Erebidae) Entomopathogens on transgenic blight-tolerant and wild-type American, Chinese, and hybrid chestnuts (Fagales: Fagaceae). *Insect Sci.*, 27, 1067–1078. https://doi.org/10.1111/1744-7917.12713

Chen, C., Sun, Q., Narayanan, B., Nuss, D. L., & Herzberg, O. (2010). Structure of Oxalacetate Acetylhydrolase, a virulence factor of the chestnut blight fungus. *Journal of Biological Chemistry*, 285, 26685–26696. https://doi.org/10.1074/jbc.M110.117804

Clevs, P. A., Tinoco, A. I., Bradford, J., Perrin, D., Bay, L. K., & Pringle, J. R. (2020). Reduced thermal tolerance in a coral carrying CRISPR-induced mutations in the gene for a heat-shock transcription factor. *Proceedings of the National Academy of Sciences*, 117(46), 28899–28905. http://dx.doi.org/10.1073/pnas.1920779117

Davis, D. E. (2005). Historical significance of American chestnut to Appalachian culture and ecology. *Proceedings of Conference on Restoration of American Chestnut to Forest lands*. Steiner, K. C., & Carlson, J. E. (eds.).

de Bruijn, A., Gustafson, E. J., Kashian, D. M., Dalgleish, H. J., Sturtevant, B. R., & Jacobs, D. F. (2014). Decomposition rates of American chestnut (*Castanea dentata*) wood and implications for coarse Woody debris pools. *Canadian Journal of Forest Research*, 44(12), 1575–1585. https://doi.org/10.1139/cjfr-2014-0270

Diller, J. D., Clapper, R. B., & Jaynes, R. A. (1964). Cooperative test plots produce some promising Chinese and hybrid chestnut trees. (Research Paper NE-25). Northeastern Forest Experiment Station, Radnor, PA. p. 8.

Ellison, A. M., Michael, S. B., Clinton, B. D., Colburn, E. A., Elliott, K., Ford, C. R., et al. (2005). Loss of foundation species: Consequences for the structure and dynamics of forested ecosystems. *Frontiers in Ecology and the Environment*, 3(9), 479–486. https://doi.org/10.1890/1540-9295(2005)003[0479:LOFSCE]2.0.CO;2

Fulbright, D. W., Stadt, S., Medina-Mora, C., Mandujano, M., Donis-González, I. R., & Serdar, U. (2014). Kernel breakdown appears when hybrid *Castanea* cultivars are pollinized by *Castanea mollissima*. *Acta Horticulturae*, *International Chestnut Symposium*, 1019(V), 91–97. https://doi.org/10.17660/ActaHortic.2014.1019.14

Goldspiel, H., Newhouse, A. E., Gibbs, J. P., & Powell, W. A. (2019). Effects of transgenic American chestnut leaf litter on growth and survival of wood frog larvae. *Restoration Ecology*, 27(2), 371–378. https://doi.org/10.1111/rec.12879

Hajjar, R., McGuigan, E., Moshofsky, M., & Kozak, R. A. (2014). Opinions on strategies for Forest adaptation to future climate conditions in Western Canada: Surveys of the general public and leaders of Forest-dependent communities. *Canadian Journal of Forest Research*, 44(12), 1525–1533. https://doi.org/10.1139/cjfr-2014-0142

Hamilton, J. A., & Miller, J. M. (2016). Adaptive introgression as a resource for management and genetic conservation in a changing climate. *Conservation Biology*, 30(1), 33–41. https://doi.org/10.1111/cobi.12574

Hao, M., Zhang, L., Ning, S., Huang, L., Yuan, Z., Wu, B., et al. (2020). The resurgence of introgression breeding, as exemplified in wheat improvement. *Frontiers in Plant Science*, 11(252), 1–11. https://doi.org/10.3389/fpl.2020.00252

Hill, J. (1994). Wildlife value of *Castanea dentata* past and present, the historical decline of the chestnut and its future use in restoration of natural areas—Reforestation, nurseries and genetics resources. *Proceedings of the International Chestnut Conference*, Morgantown, WV. pp. 186–93.

Hosteller, J. A., Onorato, D. P., Nichols, J. D., Johnson, W. E., Roelke, M. E., O’Brien, S. J., ... Oli, M. K. (2010). Genetic
introggression and the survival of Florida Panther kittens. *Biological Conservation, 143*(11), 2789–2796. https://doi.org/10.1016/j.biocon.2010.07.028

Hu, J., Telenko, D. E. P., Phipps, P. M., & Grabau, E. A. (2016). Comparative susceptibility of Peanut genetically engineered for Sclerotinia blight resistance to non-target Peanut pathogens. *European Journal of Plant Pathology, 145*(1), 177–187. https://doi.org/10.1007/s10658-015-0831-4

Jaynes, R. (1964). Interspecific crosses in the genus *Castanea*. *Silvae Genetica, 13*(5), 146–154.

Kauffuβ, F., & Reisch, C. (2017). Reintroduction of the endangered and endemic plant species *Cochlearia bavarica*—Implications from conservation genetics. *Ecology and Evolution, 7*(24), 11100–11112. https://doi.org/10.1002/ece3.3596

Lai, Z., Nakazato, T., Salmaso, M., Burke, J. M., Tang, S., Lane, B. G. (2002). Oxalate, Germins, and higher-plant pathogens. *Annual Review of Ecology and Systematics, 27*(1), 83–109. https://doi.org/10.1146/annurev.ecolsys.27.1.83

Romero-Severson, J., & Koch, J. L. (2017). Saving green ash. *Proceedings of Workshop on Gene Conservation of Tree Species-Banking on the Future, May 2016, Gen. Tech. Rep. PNW-GTR-963. Pacific Northwest Research Station: US Forest Service. pp. 102–110. Retrieved from https://www.fs.fed.us/pnw/pubs/pnw_gtr963.pdf.

Stokstad, E. (2020). United States relaxes rules for biotech crops. *Science, News, May 18*. https://doi.org/10.1126/science.abc8305.

Stephenson, S. (1986). Changes in a former chestnut-dominated Forest after a half century of succession. *The American Midland Naturalist, 116*(1), 173–179.

Williams, A., Burke, D. J., & Relyea, R. A. (2016). Litter chemistry and chemical diversity drive ecosystem processes in Forest ponds. *Ecology, 97*(7), 1783–1795. https://doi.org/10.1890/15-1786.1

Suarez-Gonzalez, A., Lexer, C., & Cronk, Q. C. B. (2018). Adaptive introgression: A plant perspective. *Biology Letters, 14*(3), 20170688. https://doi.org/10.1098/rsbl.2017.0688

Tepper, D. (1984). Transformation of several species of higher plants by *Agrobacterium rhizogenes*: Sexual transmission of the transformed genotype and phenotype. *Cell, 37*(3), 959–967. https://doi.org/10.1016/0092-8674(84)90430-6

Waterworth, H. E., & White, G. A. (1982). Plant introductions and quarantine: The need for both. *Plant Disease, 66*, 87–90.

Weissenberger, L. A., Wellar, S. G., & Sakai, A. K. (2014). Remnants of populations provide effective source material for reintroduction of an endangered Hawaiian plant, *Schiedea kaalae* (Caryophyllaceae). *American Journal of Botany, 101*(11), 1954–1962. https://doi.org/10.3732/ajb.1400271

Westbrook, J. W. (2018). Merging backcross breeding and transgenic blight resistance to accelerate restoration of the American chestnut: The American chestnut Foundation’s breeding and selection plan 2015–2025. Strategic Plan. Asheville, NC: The American Chestnut Foundation Retrieved from https://www.acf.org/wp-
content/uploads/2018/03/TACF_2015-2025_BreedingSciencePlan_updated2_9_18.pdf

Westbrook, J. W., Zhang, Q., Mandal, M. K., Jenkins, E. V., Barth, L. E., Jenkins, J. W., ... Holliday, J. A. (2020). Optimizing genomic selection for blight resistance in American chestnut backcross populations: A tradeoff with American chestnut ancestry implies resistance is polygenic. Evolutionary Applications, 13(1), 31–47. https://doi.org/10.1111/eva.12886

Westbrook, J. W., Holliday, J. A., Newhouse, A. E., & Powell, W. A. (2020). A plan to diversify a transgenic blight-tolerant American chestnut population using citizen science. Plants, People, Planet, 2(1), 84–95. https://doi.org/10.1002/ppp3.10061

Zamir, D. (2001). Improving plant breeding with exotic genetic libraries. Nature Reviews Genetics, 2(12), 983–989. https://doi.org/10.1038/35103590

Zhang, B., Oakes, A. D., Newhouse, A. E., Baier, K. M., Maynard, C. A., & Powell, W. A. (2013). A threshold level of oxalate oxidase transgene expression reduces Cryphonectria parasitica-induced necrosis in a transgenic American chestnut (Castanea dentata) leaf bioassay. Transgenic Research, 22(5), 973–982. https://doi.org/10.1007/s11248-013-9708-5

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