American chestnut restoration©

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INTRODUCTION

Since 1983, the restoration of the American chestnut (*Castanea dentata*) has been pursued by The American Chestnut Foundation (TACF) in partnership with a number of other organizations, research partners, and citizen scientists. The fungus that causes chestnut blight, *Cryphonectria parasitica*, was accidentally imported to the United States in the late 1800s and identified as a new pathogen in 1904. American chestnut has little to no resistance to the fungal disease and by 1950 close to 4 billion trees on 200 million acres of eastern forest had succumbed (Figure 1). Fortunately, American chestnut successfully re-sprouts from the root collar and as a result, there are still native chestnut sprout populations on the landscape. While we generally consider the species to be "functionally extinct", occasionally trees escape blight infection long enough to flower and produce nuts, allowing for their inclusion in breeding efforts to retain the genetic diversity of the species.

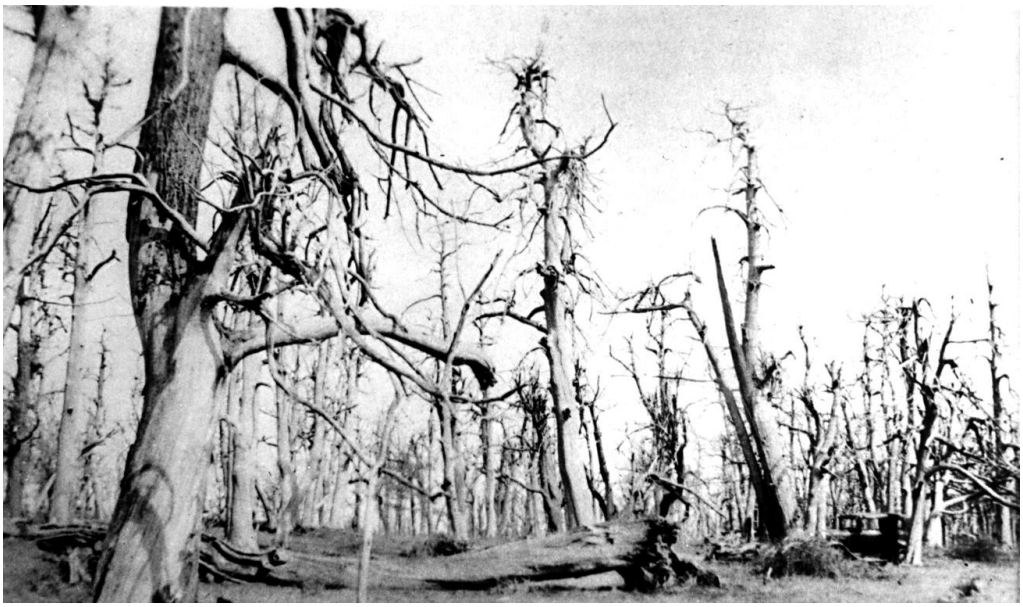

Figure 1. Dead chestnut trees in shenandoah national park (Credit Shenandoah National Park archives, copy photo by John Amberson).

The American Chestnut Foundation, along with many collaborators, has pursued three main strategies for species conservation and restoration: traditional breeding; genetic modification and biotechnology; and biocontrol through hypovirulence. Each of these efforts to restore the American chestnut has included propagating and planting hundreds of thousands of chestnuts in research plantings across the landscape. Direct-seeding chestnuts is the most common planting method, however use of bare-root and containerized stock is also standard. Grafting and micropropagation techniques have been refined within the past 20 years. Rooting cuttings is the propagation technique which has largely eluded successful application (Keys, 1978; Galic et al., 2014), and would be an indispensable tool for various

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aspects of restoration efforts should it be developed.

DISCUSSION

Breeding American chestnuts for resistance to chestnut blight has been on-going in some capacity since the early twentieth century. Early efforts focused on hybridizing American chestnut with Chinese chestnut (*Castanea mollissima*) and Japanese chestnut (*Castanea crenata*), in order to capture the pathogen resistance of these Asian species. The breeding program of The American Chestnut Foundation was developed utilizing some of the lessons learned by early breeders, and built upon earlier efforts. TACF's program utilizes an initial hybrid cross to Chinese chestnut in order to capture blight-resistance, several successive backcrosses to American chestnuts to increase the representation of the American species, as well as the genetic diversity of the breeding population, and a series of intercrosses to bolster resistance further. This program is currently at a point where we can evaluate its efficacy to-date, and determine what kinds of adjustments could be made to improve blight resistance further.

The use of somatic embryogenesis and genetic transformation are newer developments in efforts to restore American chestnut. The development of tissue culture techniques for American chestnut has been more difficult than initially anticipated, however current methodologies have become reliably successful (Maynard et al., 2014). Researchers at the University of Georgia have developed and refined methods for propagating, storing and multiplying somatic embryos from American chestnut (Holliday and Merkle, 2000). This technique allows for long-term preservation and storage of sources of special interest, and would also provide for the quick development of large, clonal populations. Work by researchers at the State University of New York College of Environmental Science and Forestry to genetically modify American chestnut by inserting genes that may confer enhanced blight-resistance have made the refinement of tissue culture techniques a necessity. Genes of interest are inserted into somatic embryos using *Agrobacterium*-mediated transformation. The embryos are multiplied, regenerated into shoots, rooted, and acclimatized (American Chestnut Tissue Culture and Transformation, 2016). The methodology for cloning of American chestnut through somatic embryogenesis has required more than a decade of refinement.

The on-going and varied interest in American chestnut restoration has necessitated the planting, or preservation, of hundreds of thousands of chestnut trees. While many methods of chestnut propagation are well-defined, there is certainly room for improvement or innovation by those in the propagation industry. The majority of chestnut planting is accomplished with direct-seeding of nuts. Containerized seedling production has increased in recent years to support early screening for blight resistance, reducing the number of trees planted in the field for breeding and progeny testing purposes. Bare-root production is used to support reintroduction and silvicultural trials. Methods for production of containerized and bare-root seedlings have been well-established, though not yet optimized.

Like most species in the genus *Fagaceae*, chestnut nuts are recalcitrant. Best practice is currently to stratify nuts in damp peat moss at approximately 3°C, however we have experimented recently with storage temperatures between -2-0°C. Nuts are typically planted the spring following harvest. Longer-term storage methods, as well as storage of large quantities of nuts for planting, are newer techniques that have not been well-refined.

Asexual propagation of American chestnut has been more difficult to complete successfully. Stem grafting by standard techniques is generally successful; however the species seems prone to delayed graft incompatibility, causing failures in the vicinity of the graft union several years after the graft has healed (Javier Viéitez et al., 2005). Tissue culture techniques have been developed and refined for dependable success, though chestnut has very specific requirements for media, light, and temperature. Rooting of chestnut cuttings is a technique that has long-eluded chestnut growers. For conservation of existing wild trees, or propagation of elite crosses or cultivars, this would be an ideal technique. Unfortunately, to date the only initial successes with rooting chestnuts have ultimately failed to produce viable trees.

SUMMARY

Interest in American chestnut restoration has maintained momentum for over a century, prompting the exploration and development of several conservation and propagation methodologies. Direct-seeding and growing containerized and bare-root seedlings are common practices, though optimization could still be explored. Long-term storage of nuts is a preservation technique that has received little attention. Grafting and micropropagation techniques are available for asexual reproduction, however rooting of cuttings is a technique that has proven exceedingly difficult for chestnut propagators but would be an invaluable tool for restoration efforts.

CONCLUSION

Restoration of the American chestnut is an on-going effort that continues to evolve. The American Chestnut Foundation, in collaboration with researchers and citizen scientists, has long-pursued a traditional breeding program to incorporate blight-resistance into native American chestnut populations. More recent work utilizing biotechnology tools show promise for advancing breeding efforts more efficiently. Enhancing blight resistance with a transgenic approach is an additional means of developing blight-resistant trees. The work to restore the species has necessitated the growing of hundreds of thousands of trees, with many more to be propagated it there future. Whether utilizing established best-management practices, optimizing existing protocols, or developing new methodologies, there are several ways in which the plant propagation industry could get involved with this important project.

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