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Poster Session Abstracts

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Chestnut Restoration Branching Out? Biotechnology, Reintroduction, & Stakeholder Engagement

Barnhill-Dilling, S. K. 2019.
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Biotechnology offers highly promising solutions to the perennial challenge of being able to protect and restore much-beloved species, including the American chestnut tree. However, public and stakeholder sentiment remains mixed about the use of genetic technologies for species protection and restoration. I will review results from two engagement activities that explored perspectives about the possible use of a genetically engineered American chestnut tree for restoration: (1) interviews with Haudenosaunee environmental and community leaders in New York State (Barnhill-Dilling & Delborne, 2019) and (2) a deliberative stakeholder workshop held in April 2018 (Delborne et al., 2018). Drawing on the results of these engagement activities, I will highlight questions, concerns, and excitements that emerged consistently across different sectors and stakeholder groups. Guided by these different perspectives, I will offer a roadmap for considering how to engage stakeholders and other public groups about the potential use of a genetically engineered organism to restore the American chestnut tree.

References:

Barnhill-Dilling, S.K., & Delborne. J.A. (2019). The genetically modified American chestnut as opportunity for reciprocal restoration in Haudenosaunee communities. Biological Conservation 232, 1-7. https://doi.org/10.1016/j.biocon.2019.01.018

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Overexpression of acid phosphatase cisgene in American chestnut enhances resistance to *Cryphonectria parasitica* in leaf assays

Bathula, Y.R.; Northern, L, and Powell, W.A. 2019.
SUNY College of Environmental Science and Forestry, Syracuse, NY

Restoration of the American chestnut (*Castanea dentata*) depends on enhancing resistance to the chestnut bight pathogen, *Cryphonectria parasitica*. One approach is to overexpress putative resistance enhancing genes from blight-resistant Chinese chestnut (*Castanea mollissima*). An acid phosphatase (APase) gene was tested because it is more highly expressed in Chinese chestnut blight canker margins than in American chestnut margins. Using a vector containing the APase gene driven by a constitutive UBQ11 promoter, Agrobacterium-mediated transformation was performed to insert the gene into the plant's genome. Leaf inoculation assay results showed 2.5-3.5 fold decrease in in necrosis caused by the blight fungus (*C. parasitica*) in the transgenic leaves compared to the wild-type leaves, indicating that the overexpression of the acid phosphatase gene may enhance blight resistance.

Silviculture of the American Chestnut in the Cumberland Uplands of Tennessee

Crawford, H. L.; Craddock, J. H. 2019.
The University of Tennessee at Chattanooga, Department of Biology, Geology, and Environmental Science, 615 McCallie Avenue, Chattanooga, TN

Castanea dentata growth and reproduction in forest ecosystems is limited, at least part, by the light made available through canopy gaps (Ashe 1911, Paillet 2002). Artificial and existing canopy/light gaps can be used for *C. dentata* seedling establishment for restoration efforts. However, the lack of blight resistant planting material has limited what is known about the silvicultural requirements of *C. dentata*, including establishment in light gaps (Clark et al. 2011, Rhodes et al. 2009). During the 2019 season, we began studying the survival and growth of 715 TACF seedlings in 35 various sized light gaps in the Cumberland Uplands. We compared canopy gap size to the growth rate and survival of the saplings. The Cumberland Upland locations include two sites previously established by Tennessee Chapter of TACF volunteers at the private conservation easement of Eagle Point Railroad (Cumberland Plateau), and at the private property of Rogers Starr (Eastern Highland Rim). We established a new location near the Barker Pounds trailhead of the Cumberland Trail in the North Chickamauga Creek Gorge State Natural Area (Cumberland Plateau) with nine light gaps: 3 small, 3 medium, and 3 large (open field) plantings. We measured height, root collar diameter, and survivorship in April/May 2019 and will measure again in fall 2019. Canopy openness will be measured by hemispherical photography in September. Preliminary results at Barker Pounds show higher first-season survival in small gaps (71% survival) than in open field (57% survival), and in medium gaps (47% survival). Season two measurements will begin in early April 2020. We hope that our results will contribute to understanding silvicultural requirements of *C. dentata* for reintroduction into the forests of the Cumberland Uplands.

Keywords: *Castanea dentata*, light gap, canopy gap.

Accelerated, graft-based, germplasm conservation of American chestnut (*Castanea dentata*) in the South

Deason, T.A.; Craddock, J.H. 2019.
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The American Chestnut Foundation's (TACF) breeding program can be expanded by incorporating an accelerated, graft-based, germplasm conservation method targeting under-sampled and genetically diverse American chestnut populations. Graft propagation will allow for the rapid introduction of genes into the TACF breeding program. This study targeted geographic areas of known high genetic diversity and populations thought to harbor alleles with high adaptive value. Results of preliminary trials include 12 individuals from Alabama successfully grafted to a variety of *Castanea* rootstocks. Grated plants maintained in pots can be subjected to light manipulations to test the effects of high light environments on reduction in flowering time. The twelve genotypes from two species, 7 American chestnut (28 ramets) and 5 Alabama chinquapin (*Castanea pumila var alabamensis*; 10 ramets) were used to test reduction in flowering time by light manipulation over 102 days (10 Dec 2018 to 22 Mar 2019). Individuals from each genotype were randomly assigned to two treatment groups: supplemental light (16hr photoperiod; n=23) and no supplemental light (n=16). The supplemental light treatment reduced the time for each recorded phenological event: bud break (BB) reduced by 25.61 days, catkin emergence (CE) reduced by 24.89 days, catkin maturation (CM) reduced by 26.50 days. A two-factor ANOVA found significant differences between BB (n= 37, P= <0.0001) and CE (n= 16, P= 0.0311) but not in CM. Pollen was collected from 9 genotypes, including three not previously conserved. The pollen was processed and cold-stored Feb-June 2019 until it could be used in control pollinations on 7 American chestnut/hybrid trees in two orchards. The production of seeds would represent conserved genotypes to the TACF breeding program.

Keywords: graft propagation, germplasm conservation, light chamber.

Comparing Four Nursery Production Methods on Chestnut Hybrid Seedling Quality

Evans, T.B.; Griscom, H.P. 2019.
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Successful restoration of chestnut hybrids is reliant on both genetic resistance to chestnut blight (*Cryphonectria parasitica*) and the long-term survival and reproduction of resistant individuals. Producing field-ready seedlings able to survive abiotic stresses and compete effectively with existing vegetation will require a renewed focus on seedling quality assessment. Seedling quality is important for successful restoration projects and nursery propagation techniques can affect seedling morphology to increase the likelihood of survival. Four production methods, bare-root seedlings, seedlings grown in containers, an air-pruning raised bed, and the Root Production Method® (RPM®) will be compared across several measures of seedling health in order to determine which technique produces seedlings most likely to survive out-planting. Propagation techniques that utilize air-pruning, such as the raised-bed and RPM®, have been shown to increase the number of first order lateral roots and overall root volume when compared with bare-root seedlings. Additionally, RPM® has been shown to produce seedlings with greater basal diameter, height, and a lower age at first fruiting than bare-root stock of the same age, though at many times the cost of bare-root seedlings. After one year, seedlings (n ~ 500) will be removed from their growing treatment and measured for height, root collar diameter, number of first-order lateral roots, and root volume to determine overall seedling quality of each treatment. Seedling quality, planting density, and planting costs are all considered when determining the least-cost approach to achieving a desired stocking density with seedling quality often compromised. Total cost per seedling will be determined by tracking labor hours and overhead costs to provide additional information for nurseries and practitioners who would like to produce their own chestnut hybrid seedlings for restoration plantings while making an informed decision on producing the highest quality seedlings possible.

Response of Restoration Chestnut Seedlings to Forest Management Strategies

Ingram, C.; Bergman, Z.; Griscom, H. 2019.
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The American chestnut tree (*Castanea dentata*) was a keystone species in the Appalachian Forest once numbering as many as one and every third tree. Since the accidental introduction of the chestnut blight (*Cryphonectria parasitica*), this iconic tree has become functionally extinct as all efforts to stop the spread of the fungus failed. Efforts to create a resistant chestnut hybrid tree for restoration purposes began in the early 1980s. Our research seeks to identify the most effective forest management strategies for introducing the hybrids into forested ecosystems. Hybrid chestnut (BC3F3) trees were planted in large and small gaps. Within these gaps we also tested the effect of landscape fabric and tree shelters on half of the seedlings within each plot. All plantings had a 2-meter deer fence erected around them. After four years, seedlings planted in the smaller gaps (40% canopy openness) had significantly greater diameter and survival rates than those planted in larger gaps (60% canopy openness). Landscape fabric and tree shelters had no significant effect on seedlings in small gaps. However, in large gaps there was a significant positive effect of landscape fabric on seedling height. We recommend planting seedlings in small gaps without landscape fabric or tree shelters given these findings. If seedlings are planted in large gaps, landscape fabric should be used to deter competition and increase height.

The Role of the CpSec66 gene in *Cryphonectria parasitica* pathogenicity in chestnut

Levine, B.J., Wu, Y., Xiao, S. 2019.
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The ability of pathogenic fungi to colonize and cause disease in plants is based on a broad range of complex molecular interactions between pathogen and host. These interactions can be specific to a particular pathosystem, but many involve highly conserved genes found in numerous pathosystems. Reverse genetics can be used to explore the role of suspected pathogenicity genes in genetically tractable fungal pathogens, but not in obligate biotrophic pathogens, such as powdery mildew fungi, which cannot be grown in culture or genetically transformed. However, by using complementary pathosystems, involving related pathogens, such as the chestnut blight fungus, *Cryphonectria parasitica* (*Cp*), and the Arabidopsis powdery mildew fungus *Golovinomyces cichoracearum* (*Gc*), one can study the role of genes conserved between the two species.

A bioinformatic search of the genomes of *Cp* and *Gc* yielded six suspected pathogenicity genes with homologues in both species. Deletion of these genes by homologous gene replacement was attempted in C*p*, with one success, *TG4*. *TG4* is a homologue of the Saccharomyces cerevisiae gene Sec66, which is involved in the selective translocation of incomplete proteins across the endoplasmic reticulum membrane, after which they are targeted to specific intra- or extracellular locations. In *Cp*, *TG4* appears to be involved in the delivery of proteins associated with several cellular processes, including light sensing and nutrient sensing or uptake. The *TG4*-knockout strain of *Cp* that we developed showed changes in phenotype and significantly reduced fungal virulence against chestnut.

Further work to silence the *Gc* homologue of *TG4* using host-induced gene silencing (HIGS) in Arabidopsis would demonstrate whether the gene plays a similar role in *Gc*. In either case, *TG4* appears to be a promising target for host-induced gene silencing (HIGS) in transgenic American chestnut. The use of homologues from genetically tractable species like *Cp* can help overcome the obstacles to performing reverse genetics on intractable, biotrophic fungi such as *Gc*. Experiments underway involving the silencing and ectopic overexpression of the *Gc* homologues of the target genes also provide a rapid method to study *Cp* genes, including to screen additional candidate genes as future targets for HIGS.

Success of American chestnut hybrid seedlings in a Walland, TN backcross seed orchard

Maley, K.N.; Unger, D.E. 2019.
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The introduction of an ascomycete called *Cryphonectria parisitica*, or the chestnut blight fungus, to Eastern North America at the turn of the 20th century led to the functional extinction of *Castanea dentata*, the American chestnut tree. The ongoing effort to breed blight-resistant American chestnut hybrids through backcross breeding for the reintroduction of the species to its native habitat has been a goal of The American Chestnut Foundation (TACF) for over 35 years. This study examined the success of 690 American chestnut hybrid seeds in a seed orchard in Walland, TN throughout the first 6 months of life. Germination percent, timing of emergence, growth rate, final height, survival percent, and resilience to fungal infection were assessed among 3 different cross types. Results of this study show that trees of different cross types differed significantly in terms of timing of seedling emergence ($p<0.001$), number of sprouts ($p=0.017$), growth rate ($p<0.001$), height ($p<0.001$), and meristem damage ($p<0.001$). The findings of this study suggest that greater proportions of American chestnut genes tend to produce seedlings with faster establishment, but greater proportions of Chinese chestnut genes seem to produce seedlings with faster growth and more resilience to fungal pathogens.

The Asian Chestnut Gall Wasp Dryocosmus kuriphilus Yasumatsu (*Hymenoptera: Cynipidae*) in Eastern Pennsylvania and Surroundings

Mapes, C.C.; Setliff, G.P.; Bothur, K.; and Courtney, R.S. 2019.
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The Asian chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu is an invasive species of global concern that has been introduced into North America where it induces gall formation on chestnut species including *Castanea mollissima* Blume, *C. crenata* Siebold & Zucc., and *C. sativa* Mill.as well as on the American chestnut, *C. dentata* (Marsh.) Borkh. The Asian chestnut gall wasp was first introduced into the United States in the 1970's and since its initial introduction in Georgia, it has been reported to have spread to 13 other states. It has been documented as far to the northeast as Connecticut and Massachusetts and as far to the northwest as Michigan. The introduction and spread of *D. kuriphilus* is of concern given the goal to restore American chestnuts into the North American landscape. The purpose of this study was to gain a better understanding of the distribution of the Asian chestnut gall wasp in Eastern Pennsylvania and surroundings. We report the presence of Asian chestnut galls in a large number of counties in Eastern Pennsylvania as well as in some localities in neighboring states. For further confirmation of identity, we reared *D. kuriphilus* from galls collected at several sites. These findings document the expansion of the range of this gall-forming invasive pest species within the Mid-Atlantic region.

Nutrition, composition, and dietary safety of transgenic chestnuts

Newhouse, A. E.; Powell, W.A. 2019.
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Transgenic American chestnut trees have been developed using an enzyme called oxalate oxidase to tolerate chestnut blight. Previous tests have demonstrated blight tolerance and environmental safety of these trees, but since chestnuts are notably delicious and nutritious, dietary safety of the nuts is an important consideration for any new restoration material. Nut composition is also an important factor for wildlife, for whom American chestnuts were a valuable food source. Oxalate oxidase enzymes are found in a variety of foods, so novel dietary risks are unlikely, but careful safety assessments are important for both government regulatory evaluations and responsible restoration efforts.

Pollen has been collected from transgenic trees grown indoors and used to perform controlled pollinations on wild-type mother trees in permitted plots. These have resulted in two generations of transgenic nuts, samples of which have been analyzed by independent testing laboratories for proximal nutritional content, fatty acid composition, and tannin concentrations. All analyses include non-transgenic full-sibling nuts from the same crosses, as well as various other non-transgenic chestnuts for comparison. The oxalate oxidase enzyme has also been screened against multiple databases to test for potential allergenicity, toxicity, and associations with gluten. Analyses have shown that transgenic and non-transgenic chestnuts are nearly identical in terms of nutrient and tannin content. Fatty acid profiles are also generally similar for all tested American chestnuts (regardless of transgene presence), dominated by monounsaturated oleic acid (~65% of total fat) and polyunsaturated linoleic acid (~17%). While transgenic and non-transgenic nuts are nutritionally very similar, there are notable differences between nuts from similar trees in different plots, between nuts from the same trees over multiple years, and between different species of chestnuts. Database searches revealed that oxalate oxidase amino acid sequences have no matches to known allergens, toxins, or gluten proteins.

American Chestnut (*Castanea dentata*) Reintroduction in Kentucky: A GIS Approach

Pease, J.R. 2019.
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Anthropogenic factors have been the causal agents in the decline of tree species globally. From logging and development to disease and poor management, threats to our forests have taken their toll. One species, the American chestnut (*Castanea dentata*) was once an integral basal tree species in Appalachian forests but was eliminated from the landscape by chestnut blight by the mid-1900s and is now functionally extinct. Blight-resistant chestnuts have been created to reintroduce this species back into its historical range. Efficient and effective reintroduction will require optimal restoration sites that will maximize planting success. I propose to conduct a large-scale project focusing on site suitability of Kentucky's US Forest Service lands. Specifically, I will evaluate the degree to which site suitability criteria from literature can predict chestnut locations in both Land Between the Lakes National Recreation Area (LBL) and Daniel Boone National Forest (DBNF). Resulting analyses from remotely sensed data and maps will be used to further refine our knowledge of chestnut reintroduction and determine how land managers can optimize and expedite the reintroduction process across large landscapes. This project will provide a geospatial framework for chestnut reintroduction and improve methodologies for creating future frameworks for other at-risk species. The results of this project thus have the potential to serve as a model for land managers to utilize GIS and remotely sensed data as tools for the restoration of degraded landscapes.

Genetic Transformation of the Ozark Chinquapin (*Castanea ozarkensis*)

Pilkey, H.C.; McGuigan L.D.; Powell, W.A. 2019.
SUNY College of Environmental Science and Forestry, Forestry Drive, Syracuse, NY

The arrival of the invasive fungal pathogen, *Cryphonectria parasitica* (cause of the chestnut blight), decimated the native *Castanea* tree species in the eastern U.S. and southern Ontario, Canada. Billions of American chestnuts and chinquapins were killed. The extirpation of this keystone genus severely impacted the ecology of our forests. Today, the trees persist naturally as stump sprouts, but rarely achieve reproductive maturity. Efforts to restore this genus are critical, as re-sprouting ability is diminishing with time leading to a loss of genetic diversity. Researchers at ESF developed a blight-tolerant American chestnut via genetic engineering by incorporating a common plant defense gene, Oxalate-Oxidase (OxO), into the tree's genome. OxO detoxifies the oxalic acid produced by the pathogen and inhibits its ability to produce deadly cankers. The objective of this research was to determine if the methodology used to develop a transgenic American chestnut (*C. dentata*), could be applied to the Ozark chinquapin (*C. ozarkensis*). Foundational research demonstrated that established methods could be used to initiate somatic Ozark chinquapin embryo cultures and regenerate them into whole plants in-vitro. Somatic embryos were then transformed using established Agrobacterium-mediated transformation methods using an AGL1 strain containing the p35S-OxO binary vector. The presence of the OxO transgene was confirmed in one embryo culture by PCR. It was determined that a transgenic Ozark chinquapin can be developed using the same framework for American chestnut transformation. Continuing research will focus on further optimization of the tissue culture and transformation protocols, determining transgene copy number and expression in the transgenic events, and eventually blight resistance assays. These tools of genetic engineering may help the restoration of the Ozark chinquapin.

Habitat Suitability for American Chestnuts in Eastern Hardwood Forests

Smith, H.L. 2019.
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In 1904 *Cryphonectria parasitica*, a pathogen that causes the chestnut blight was identified in New York, likely arriving from the shipping industry. The chestnut blight has wiped out 99% of American chestnuts, and in 1983 The American Chestnut Foundation (TACF) was formed. The American Chestnut Foundation's goal is to establish blight-resistant American chestnut trees back into the eastern hardwood forests. The objective of this project is to identify possible suitable habitat for American chestnuts in five Eastern U.S. states using saturated hydraulic conductivity, soil pH, and soil slope. With these maps, the TACF will have areas to search for surviving American chestnuts to increase genetic diversity in their future generations. They will also have suitable habitat mapped out for future chestnut planting research sites. Known tree location data for Pennsylvania was used to identify a preference soil type for habitat suitability, due to lack of tree locations for other states.

Notes

Notes

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