Can Genetic Engineering Help American Chestnut (Castanea dentata) Restorative Efforts in Northern Climates?

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MOTIVATION/BACKGROUND

Introduction

- "A major biological conservation goal is to maintain or restore species to a demographic state such that populations are stable or increasing toward a recovery threshold" (Van Drunen et al., 2017).
- Due to American chestnut, *Castanea dentata*, trees importance in ecosystems, both ecologically and economically, restoration of this species provides an effective model for restoration of other keystone species threatened by disease or pests (Jacobs et al., 2012).

Background

- American Chestnut were once one of the most prevalent species in the north east climate; 96% of its historical range occurs in the United States. (Jacobs, 2007). In the early 20th century, American chestnut trees were plagued by the chestnut blight fungus, *Cryphonectria parasitica*, reducing populations to less than 1–10% of their original size (Van Drunen et al., 2017).This fungus spread viciously throughout the country and to other parts of the world including Europe.

Figure 1. Fatal canker on American chestnut sapling, caused by *Cryphonectria parasitica*, or chestnut blight. Photo Source: American Chestnut Foundation, https://acf.org/our-community/news/new-genetically-engineered-american-chestnut-will-help-restore-decimated-iconic-tree/

Motivation

- American chestnut fruit is a highly nutritious food source for wildlife, livestock and humans; and the tree itself provides shelter for many wildlife species (Gurney et al., 2011). American chestnut trees are also an economically important tree species of deciduous forests due to its high-quality lumber (Van Drunen et al., 2017).
- The most utilized method used by conservational biologists working toward restoring American chestnut is planting a naturally resistant chestnut hybrid, *Castanea dentata x mollissima*, a cross between the American and Chinese chestnut trees (Jacobs et al., 2012). However, efforts to restore American chestnut in it's historically most northern ranges, such as Vermont, have not been as successful. This is thought to be due to the hardiness of the hybrid being less hardy than its American chestnut parent (Gurney et al., 2011).

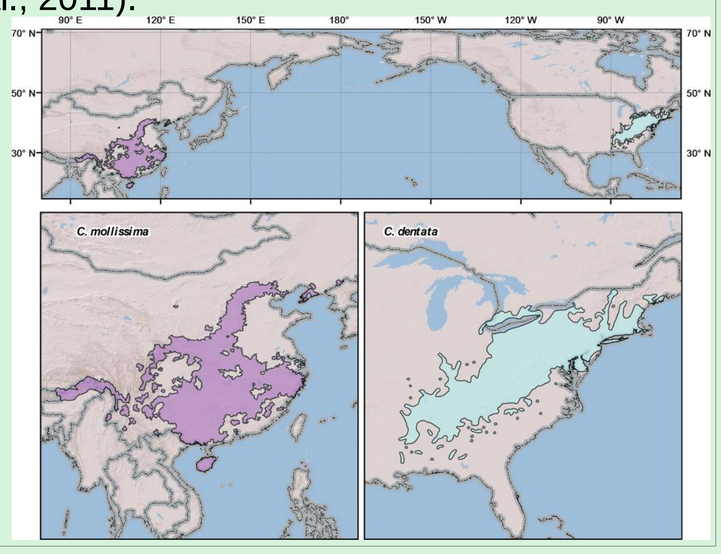

Figure 3. Native ranges of American chestnut (right) and Chinese Chestnut (left). New Phytologist, https://nph.onlinelibrary.wiley.com/doi/full/10.1111/nph.12020

Hypothesis: I hypothesize that there is a relationship between hardiness and survival of American chestnut trees in northern climates.

- I predict that genetically modified American chestnut trees, selected for resistance to chestnut blight, will have higher survival rates in northern climates than American-Chinese chestnut hybrids or hypovirulence treated American chestnut species.
- I predict that hardiness is the most limiting factor for chestnut species in northern climates; and species with similar or identical hardiness will be limited by their resistance to chestnut blight (Gurney et al., 2011).

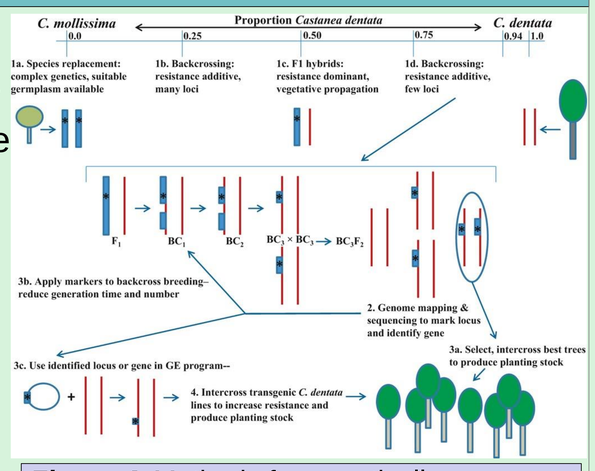

Figure 4. Methods for genetically modifying American Chestnut trees for natural resistance to chestnut fungal blight using a combination of hybridization, gene selection, and backcross breeding. Photo source: New Phytologist, https://nph.onlinelibrary.wiley.com/doi/full/10.1111/nph.12020

Study Design

• I plan to conduct a field experiment at a breeding orchard established by The American Chestnut Foundation (TACF) in Shelburne, Vermont to investigate the relationship between hardiness, resistance to chestnut blight, and overall success of the species growth. This will also be a test of the best method for American chestnut restoration in Northern climates.

- Four stands of trees will be planted with ten trees in each stand. All trees will be grown in a lab for one year under ideal conditions to maximize survival rates of saplings.
- Two randomly selected tree species from each stand will be exposed to chestnut blight after their third year of growth, since species will be established, but still extremely vulnerable to disease.
- Trees will be observed for a minimum of five years of growth to give trees time to develop and to give the chestnut blight at least two full seasons to spread to other species and cause damage.
- Treatments will be randomly assigned to each stand. Treatments and tree variety/cultivar locations will be recorded but stands will remain unmarked. Observers of indicators will be conducting blind analysis of each stand
  - **Stand #1**: pure American chestnut variety;
  - **Stand #2**: Chinese x American chestnut variety
  - **Stand #3**: genetical modified American chestnut species that are selected for chestnut blight resistance
  - **Stand #4**: American chestnut trees treated with a biological control called hypovirulence.

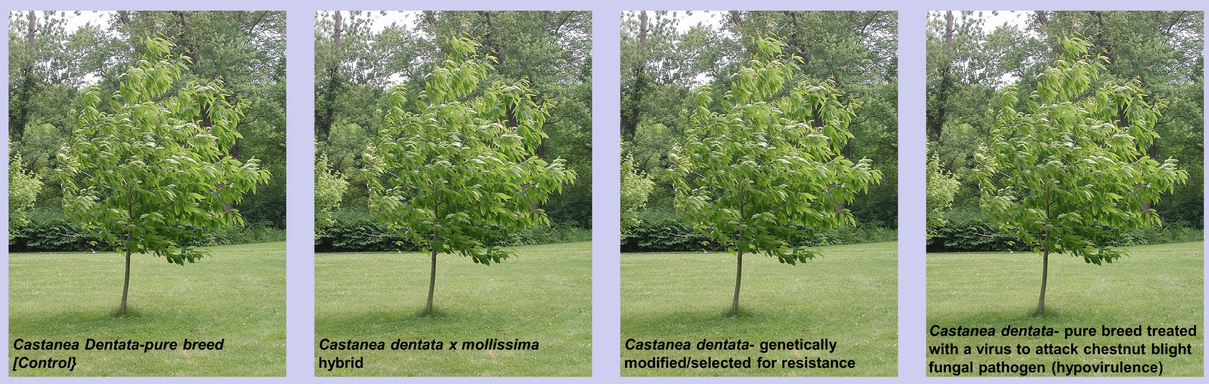

Figure 2. **American chestnut plantings showing stand #1-4 (left to right). Ten trees will be planted in each stand and be observed for up to five years. Trees shown are three years old, the age at which all trees will be exposed to chestnut fungal blight, *Cryphonectria parasitica*.** Photo source: The Pennsylvania Chapter of the American Chestnut Foundation https://www.acf.org/wp-content/uploads/2016/08/planting_manual.pdf

Intended Analysis

- I plan to measure all trees in each tree stand by the following conditions:
  - Tree Mortality*
  - Tree Damage
  - Crown Condition

  *Tree mortality will be treated as the primary indicator of a tree's success in its current growing climate (USDA Forest Service, 2002).
- Considering that our independent variable is categorical (treatment: no treatment, hybridized species with no treatment, genetically modified species with not treatment, and species with hypovirulence treatment) and our primary response/dependent variable is also categorical (tree mortality: survive or not survive), we will analyze the data using a x2 test.
- The x2 test will allow us to determine the relationship between survival and hardiness, and if genetically selected American chestnut is the best method for restoration in northern climates.
- This study is mostly a field study that is also dependent upon the site's wind protection, soil nutrients, canopy cover, herbivory interactions, and other factors. The replication of this study may vary depending on changes in these factors, which should be taken into consideration when reporting and analyzing study results.

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