Morphological and physiological adaptations to shade affect drought susceptibility in American chestnut (*Castanea dentata* [Marsh] Borhk)

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a) b)

Planted chestnut seeding a) and mature chestnut tree (b)

Introduction

American chestnut (*Castanea dentata*) was a long lived, large hardwood tree (Horton, 2010), whose native range in the eastern United States once encompassed approximately 800,000 square kilometers (Russel, 1987) (Figure 1). It was one of the most dominant trees in eastern North America comprising an estimated 25% of hardwood forests (Burnham, 1988). Chestnut's light weight rot-resistant wood made it a desired timber species and it abundant nut crop was enjoyed by both wildlife and man (Smith, 2000; Youngs 2000).

In the early 20th century chestnut blight (*Cryphonectria parasitica*) was introduced from Asia. Within 50 years nearly all four billion chestnuts within the its native range were infected and killed (Hepting, 1974). Due to its high value there have been several restoration attempts. The most recent and promising is a back-crossing program undertaken by The American Chestnut Foundation (Figure 2). The newest hybrid, the BC3F3, contains roughly 94% American chestnut DNA, is phenotypically identically to pure American chestnut, (Diskin et al., 2006) and will be used for current and future restoration programs.

Figure 1

Figure 2

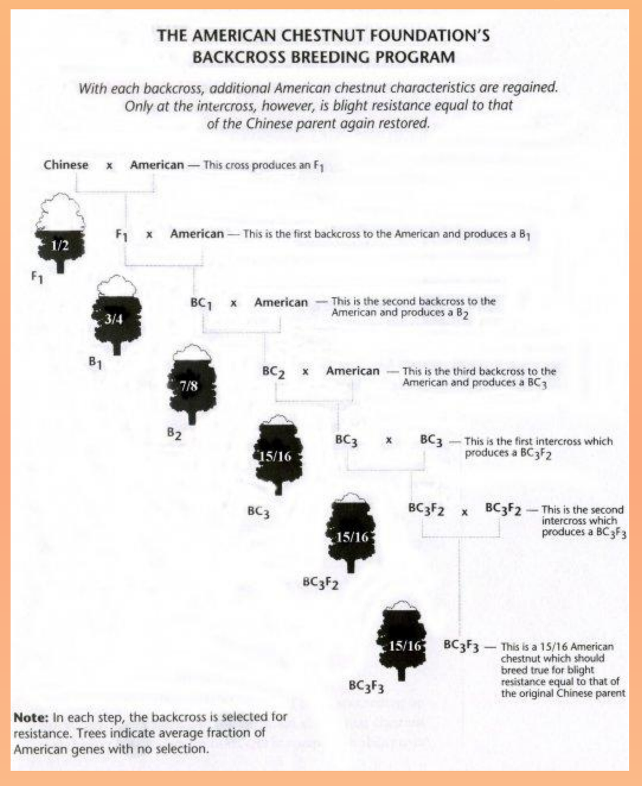

Figure 1. Natural range of the American chestnut, as adapted from Little (1977) Figure 2. Graphically explains the American Chestnut Foundation's backcross breeding program. Spring 2004 Volume of the "The Journal of the American Chestnut Foundation" on page 15 in an article by Dr. Paul Sisco.

Justification

One likely reforestation method for chestnut hybrid reintroduction is underplanting beneath an intact forest canopy. Several recent studies have found that densely shaded understory conditions make seedlings more susceptible to drought because of acclimations to low light levels such as increased leaf allocation at the expense of root growth and lower stored reserves which to endure stress with. Shaded conductions are also thought to decrease a seedlings control over stomatal aperture, reducing instantaneous water use efficiency, and lower hydraulic conductance (Rodriguez-Calcerrara et .al., 2008). The innate drought susceptibility of transplanted seedlings, the high potential for chestnut reforestation on drought prone, well-drained upland slopes where it was historically present, and the increasing amount of drought events forecasted by some climate models indicate a high likelihood that seedlings will face moisture stress during establishment.

Objectives

Examine first year seedlings responses and susceptibility to drought under varying light levels in a controlled field trail. This will allow for precise morphological and physiological measurements that will help elucidate basic chestnut ecophysiology and aid in determining appropriate silvicultural treatments during reforestation efforts.

Materials and Methods

Planting stock, growing medium, and treatment establishment

One year old bareroot (0+1) BC3 half sib American chestnut seedlings where received from Indiana DNR Vallonia nursery in early 2011 and placed into cold storage until planting occurs in mid April. Seedlings will be planted into 45.42 liter pots containing 1:1:1 ratio of sand, peat moss, and silt loam top soil. Three light levels: 90, 50, and 10% of full sunlight will be created by administering shade cloth and greenhouse film over 7.32 x 2.44 x 2.54 meter (length, width, and height respectively) cold frames. Ten randomly chosen seedlings will be placed inside each cold frame (Figure 3). Upon transplant all seedlings will be well watered as needed. Irrigation water will be acidified to a pH of approximately 5.6. Once seedlings have acclimated to their respective light levels half the seedlings inside each cold frame will randomly be selected to undergo a watered stress treatment, the remaining seedlings will continue to well watered throughout the study. In the water stressed treatment the soil water potential will be lowered to -1.5 kpa. Without intervention it is likely that the soil under different light treatments will reach -1.5 kpa at different rates due to difference rates of seedling transpiration. To insure moisture stress is even across light treatments volumetric water content of water stressed seedlings will be taken bi-weekly and pots which have significantly lower volumetric water contents will receive irrigation. Volumetric soil water content will be correlated to soil water potential through as soil moisture retention curve. This experiment will be a 3x2 split plot design consisting of three replicate blocks.

Figure 3

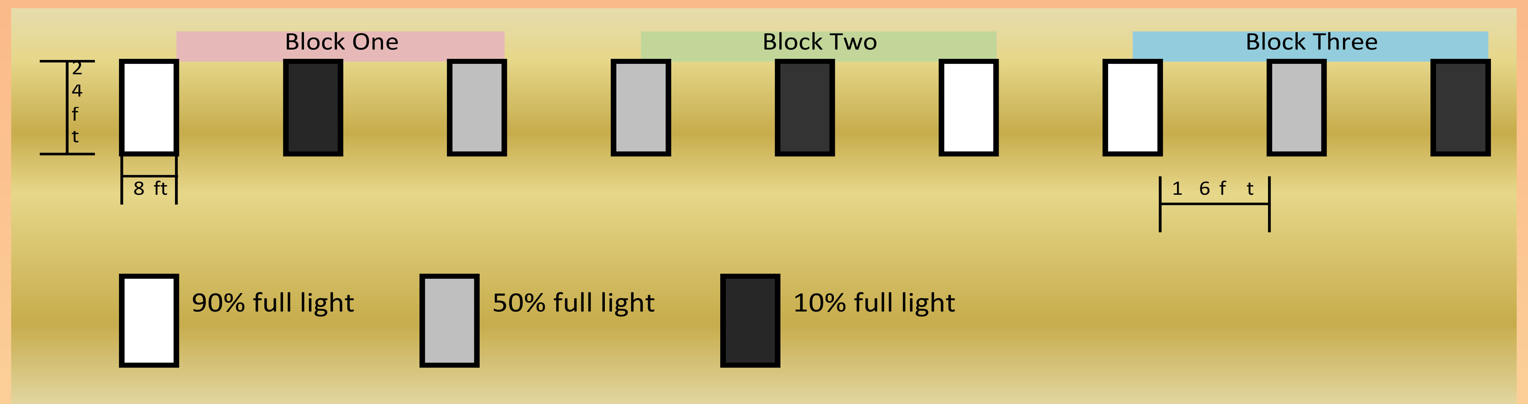

Figure 3. Diagram of light treatment layout and spacing. Each rectangle represents one 7.32 x 2.44 x 2.54 meter (length, width, and height) cold frame

Data collection and analysis

Prior to the beginning of the water stressed treatment light response curves, specific leaf area, number of fully expanded leaves, and leaf chlorophyll content will be sampled to determine if seedlings have acclimated to their respective light environments. Upon the initiation of the water stress treatment and approximately every week thereafter plant gas exchange , height , and root collar diameter will be measured. At the end of the study all seedlings will be divided into roots, stems, and leaves and weighted. Leaf area along with leaf water potential and relative water content will be measured. Data analysis will be conducted using repeated measures in SAS (Version 9.2, SAS Institute, Inc., Cary, NC, USA.)

Expected Results

Light, drought, and possible interactions.

Several studies have examined chestnuts morphological and physiological responses to varying light levels and one study has explored chestnut's ecophysiological responses to drought. Significant results from these studies are summarized below. From previous studies it can be inferred that decreasing levels of light and moisture will result in reductions in gas exchange and lower levels of overall growth for chestnut seedlings. Although other studies have examined the effects of light levels on drought response and susceptibility using other tree species no inferences will be made because of the ecological differences between chestnut and the species studied. Areas where interactive effects of light and drought would be significant if found are: instantaneous water use efficiency, specific leaf area, leaf weight root ratio, osmotic adjustment, relative changes in photosynthesis and stomatal conductance upon onset of drought, and relative changes in absolute growth rate upon on set of drought.

Table 1

Measurement	Response to increasing light	Reference
Total Biomass	↑	Wang et. al., 2006,
Root Biomass	↑	Wang et. al., 2006,
Leaf weight root ratio	↓	Wang et. al., 2006,
Height	↑	Wang et. al., 2006,
Root collar diameter	↑	Wang et. al., 2006
Number of fully expanded leaves	↑	Wang et. al., 2006, McCament and McCarthy 2005
Specific leaf area	↓	Wang et. al., 2006
Photosynthesis max	↑	Wang et. al., 2006, Joesting et al., 2007
Instantaenous water use efficeny	↑	Wang et. al., 2006
Light saturating levels of photosynthesis	↑	Joesting et. al., 2007
Dark respiration rate	↑	Joesting et. al., 2007

Table 2

Measurement	Response to decreasing soil water content	Reference
Total Biomass	↓	Bauerle et. al., 2006
Instantaenous water use efficeny	↑	Bauerle et. al., 2006
Photosynthesis max	↓	Bauerle et. al., 2006
Stomatal conductance	↓	Bauerle et. al., 2006
Leaf water potential	↓	Bauerle et. al., 2006

Table 1. Shows the results of chestnut seedlings morphological and physiological responses to increasing light levels in previous studies. Table 2. Shows the results of chestnut seedlings morphological and physiological responses to decreasing soil water content in previous studies.

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