a. Project title
Predicting growth and carbon uptake of American chestnut in current and future climates.

b. Project summary
Reintroducing American chestnut may increase forest carbon uptake and alter carbon cycling. Predicted increases in temperatures and CO₂ will lengthen growing seasons and may increase chestnut growth, but the coupled impact of these (or other stresses) in eastern deciduous forests are not fully resolved because different species respond in different ways. Physiological data needed to accurately predict and model chestnut growth (carbon uptake) under current and future climates are lacking. We address this knowledge gap by assessing how current climate and future predicted increases in temperature and CO₂ affect the physiology of American chestnut and hybrids created for restoration.

c. Principal investigators and affiliations
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d. Duration of project
The fund requested here will support 1 year of consumable supplies to collect data on an already established field experiment and some supplied are needed to run a chamber experiments. We are also requesting seeds of assorted lineages from TACF (see methods).

e. Amount requested
We are requesting $2825.60 for field sampling supplies and consumables use to measure carbon uptake, and for chemical analyses of leaf and soil samples.

f. Short and long term goals
This project has already started. In winter of 2015 I was awarded funding the Ohio University Research Council (OURC $7961.00) to procure, assemble and test the equipment necessary to control CO₂ in four environmental growth chambers at Ohio university. The short term goals of this proposal if funded are 1) to assess how natural environmental variation affects carbon assimilation on pure American chestnut trees that I have already established in permanent plots at Ohio University and 2) assess the physiological responses of BC3F3 chestnuts to simulated climate change (i.e. elevated temperature and CO₂). The long term goals are two fold. First, we continue to monitor seasonal physiology in Ohio each summer and second I will seek funding next year to collect eco-physiological data in orchards from the southern to northern edge of the range to compare to those collected at the Ohio field site.
g. Narrative

The restoration of American chestnut (*Castanea dentata*) as a canopy dominant tree is important for ecological, economic and aesthetic reasons. As an ecological foundation species it is likely to affect population, community and ecosystem processes in eastern American forests [1, 2]. Chestnuts also provide a rich food source for wildlife. In addition to providing tangible ecosystem services, it is a versatile tree for wood production and other agricultural products. Historically, chestnut bark was valued for its tannins which are a critical component of leather processing. It was also valued for its large decay resistant timbers and its nuts were an important agricultural commodity. Moreover, its popularity with the public also adds less tangible but equally important esthetic and recreational values to the forest.

Chestnut restoration efforts aim to introduce millions of blight resistant trees back into eastern deciduous forests. Over the long term the reintroduction of this previous dominant tree has the potential to alter forest carbon uptake and nutrient cycling [2]. However no quantitative physiological data exist to accurately predict seasonal carbon uptake for American chestnut or blight resistant hybrids under current or future climate. The specific aim of the proposed is to quantify the response of carbon uptake and assimilation in 5 to 6 year old saplings to natural diurnal and seasonal variation in light, water, and temperature. These data can then help us to predict precisely how natural environmental variation affects assimilation and growth in chestnut saplings. We also propose to assess the response of selected hybrid American x Chinese chestnuts [3] to simulated increases in carbon dioxide concentration ([CO2]) and temperature in controlled environment growth chambers. Data from the chamber experiment will be used to inform a mechanistic photosynthesis model [4, 5] which will allow us to more accurately predict chestnut response to and growth under climate change.

Do we need to know if chestnuts will respond to global change? Mean land-surface temperatures have increased by over 1°C over the last century [6] and are expected to increase further. Rising atmospheric [CO2] and emissions of other more potent greenhouse gases due to anthropogenic activities are likely to increase global mean air temperatures by ≥ 3°C before the end of this century [7]. Some regions are expected to increase even faster than the global mean experiencing increases of between 3 and 4 °C as early as mid-century [8]. Forest tree growth will be stimulated by increasing [CO2] in the long term because CO2 is the substrate for photosynthetic carbon assimilation [9, 10] and longer growing seasons are already stimulating carbon uptake in eastern deciduous forests [11]. Globally, photosynthesis accounts for the largest flux of CO2 from the atmosphere into ecosystems and is the driving process for terrestrial ecosystem function. Deciduous forests account for about 25% of the world total carbon uptake! Yet, the coupled impacts of increasing atmospheric CO2 and concomitant increases in temperature (or other stresses) on photosynthetic carbon assimilation and acclimation in forests are not fully resolved because different species respond in different ways [reviewed in 12, 13].

The American Chestnut Foundation (TACF) has created trees that are both blight resistant like Chinese chestnut and morphologically similar to the American chestnut [14]. In addition to improved blight resistance, successful restoration will depend on the physiological response of chestnuts. Throughout the eastern deciduous forests of North America, high temperature and extreme events such as heatwaves, drought and flooding events are all predicted to increase with climate change [15]. Furthermore, it is predicted that nighttime temperatures will warm more than those during the day, potentially raising temperature minima in the northern part of the forest’s range closer to those experienced throughout the middle and southern edges.
of eastern temperate forests. These reports are also consistent with the prediction that, in the northeastern United States, predominantly maple-beech-birch forests may be replaced by oak-hickory forests (Spencer 2001), a forest type that historically included American chestnut. However, aside from several published studies on frost tolerance and responses to light (reviewed below), little else is known about the potential effects of current climate or future climate change on the growth and physiology of American chestnuts or the enhanced blight resistant B3F3 hybrids.

A principle restoration strategy of The American Chestnut Foundation (TACF) is field testing putative blight-resistant backcross strains in progeny arrays [3]. Ultimately seedlings success is determined over several years through growth and survival assessments [16]. Each “test” frequently requires a thousand or more seeds or seedlings because large replication is needed and plants are expected to perish under these natural field conditions. The power of this approach is that it can potentially identify if (and importantly which) progeny can survive under field conditions. The drawback is that this approach cannot specifically identify if factors other than greater blight resistance of the myriad environmental factors contributed to a seedlings success or demise. A detailed understanding of chestnut physiological responses to the environment, in addition to light responses, will allow us to more accurately predict chestnut survival in response to global environmental change and inform restoration. Thus, as a first step towards this goal we propose to gather information on a few representative individuals that will encompass the range of environmental tolerance of American chestnut.

Why use photosynthesis models in restoration? The biochemical model of leaf photosynthesis ($A$) [17] provides the basis for scaling carbon uptake from leaves to trees to canopies to ecosystems [4] and landscapes [18]. Indeed this leaf-level photosynthesis model is also a key component of earth system models [19]. Because of the mechanistic nature of the leaf photosynthesis model, it is an ideal tool to predict changes in photosynthesis over a wide range of environmental conditions. Accurate modeling and future prediction requires a physiological understanding and must consider the impact of longer-term changes, most notably increasing atmospheric [CO$_2$] and temperature change that is predicted to continue well into the future [8]. A century ago, this large canopy tree was reportedly common in eastern deciduous forests and was likely a co-dominant with oaks for several thousand years [20]. Chestnuts may have comprised up the 50% of the total tree basal area in some stands [21]. It is estimated that some 4 billion American chestnut trees were decimated by the blight [22]. Today, it is well known that chestnuts subsist mostly as short shrubby “root re-sprouts” because blighted plant die back after 10-15 years but the blight does not affect roots. If we are to reintroduce a tree that may become a dominant species in that ecosystem, and given the important role trees play in carbon storage, then it stands to reason that we should know how much it will affect the entry of carbon into eastern deciduous forest ecosystems.

Photosynthetic carbon assimilation ($A$) is a fundamental determinant of plant growth, so one approach to predict growth rates is to assess photosynthetic responses to environmental variation. For instance, shade-tolerant species grow more slowly but have properties that maximize carbon gain efficiency under low light which can include lower photosynthetic rates, lower respiration rates, ability to maintain carbon uptake at low light levels, and capturing photons more efficiently than shade-intolerant plants [23, 24]. American chestnut shows characteristics similar to shade-tolerant species in studies conducted in rainout shelters [25] and in the field [26]. However, [25] reported a light saturation point of American chestnut that was higher than that of red maple (Acer rubrum L.), suggesting the potential for American chestnut to
respond to high light levels with rapid growth. The latter observation is consistent with Joesting et al. [27] who found that the maximum photosynthetic rate was greater for American chestnut seedlings growing under a thinned canopy (i.e., increased light) than for seedlings growing under an intact canopy. These results, when coupled with growth measurements from other studies [28-30], indicate that American chestnut is able to respond to canopy disturbance with rapid growth in response to increased light availability. What about the blight resistant hybrid (B3F3) chestnuts’ response to light? Knapp et al. [31] compared the light response of three backcross breeding generations in the Southern Appalachians and found that the light response of the B3F3 generation hybrids were similar to American Chestnuts. Taken together, then, this body of work suggest that American chestnut and more importantly the B3F3 generation respond to light and exhibit similar growth strategies that allow for persistence in the forest understory and the potential for rapid growth following canopy disturbances that increase light availability [1, 20, 25, 26, 32].

However, while light availability is critical to photosynthesis and seedling and sapling establishment and persistence in current forests, no studies to date have examined the combined effects of increasing CO₂ and temperature on American chestnuts or B3F3 hybrids. What we do know is that American and hybrid chestnuts tend to be less cold tolerant than co-occurring sugar maples and oaks [33] which gives maples the advantage in colder climates. We also know that red maples from Minnesota showed a greater reduction in photosynthetic capacity when grown at higher temperatures [34] showing that tree species can have thermally adapted genotypes. Indeed, it is also true that American chestnuts from colder climates tend to be less susceptible to winter frost injury than those from warmer southern sites [35]. All these observations indicate some degree of genetic and local adaptation to cold tolerance exists in American and backcross chestnut. It is not known, but it is likely, that chestnut photosynthesis also exhibits local adaptation to high temperature. If true then it is also possible that American chestnuts from the southern edge of the range will have higher temperature optima, higher photosynthesis, and greater biomass when grown at elevated temperature and CO₂.

Methods

In spring of 2016, twenty four 4 year old saplings of American chestnuts with mixed ancestry were transplanted from 10 L pots into six plots at two adjacent old field sites (N=24 n=3) with differing management histories. Photosynthesis will be measured with a portable infrared gas analyzer (LICOR biosciences) over the course of the day, 5 to 7 times during the growing season to capture how natural variation affects carbon uptake (see

Figure 1. Four year old American chestnut saplings from two of six replicated plots established at Ohio University in spring of 2016. These trees will be monitored for physiological performance for the field experiment
preliminary data for example). We will also measure leaf area, functional traits other standard growth and physiological traits every two weeks during the 2017 growing season. These data will allow us to calculate whole tree carbon gain. The photosynthesis model will be parameterized by measuring leaf responses in the field to changes in light and CO2. Leaf photosynthetic responses to increasing temperature (T), and increasing light (Q) and increasing CO2 will be systematically measured using published protocols with which the PI is highly familiar [5]. Photosynthetic responses to increasing CO2 will be fitted to a biochemical model of photosynthesis. We will account for leaf (and soil) nitrogen, which are well known to affect plant growth and photosynthesis, by analyzing leaf and soil nitrogen content.

**Chamber experiment.** 72 seeds selected from different families with parents vetted in the field as resistant to blight (i.e. from parents that TACF knows to be hardy) will be mixed together and then randomly assigned to one of the four treatments. Similarly 48 seeds from each of 2 populations of American chestnut trees from thermally contrasting regions (i.e NY and NC) will be selected. In early spring, seeds will be weighed and planted in Fafard 52 Mix (Sungro Horticulture), in 8” diameter x 18” high (10 Liter) large tree pots (stuwee and sons inc. Tangent OR). Large pots will be used to avoid confounding root restriction with response to treatment [9, 36] that can occur when plant are grown in elevated CO2. Pots will be kept outdoors and protected from herbivores in screen cages until all seeds have germinated and all seedlings have at least four true leaves. Selected pots will be then be placed in four environmentally controlled growth chambers (CONVIRON) housed in the department of Environmental and Plant Biology. The chambers will be maintained at current and future predicted temperature and [CO2]. Two chambers will be set to current ambient atmospheric [CO2] of 400 parts per million and ambient temperature (ACAT), two chambers at future elevated CO2 and temperature and elevated temperature (ECET). The major environmental determinants of photosynthesis include air temperature (Tair), vapor pressure deficit (VPD), and photosynthetically active radiation (PAR); these are all highly dynamic in nature but can be kept relative constant in growth chambers. Constantly controlled conditions provided by the growth chamber minimizes environmental variation and increases our power to detect genetically based differences in seedlings’ acclimation to temperature and [CO2]. To more closely mimic forest climate, chamber temperature for the ACAT treatment will be set to the average ambient spring temperature at the time of planting and the ECET will be subjected to temperature that is 3.5 °C greater. Chamber temperatures will be subsequently increased to mimic average ambient conditions every two weeks throughout spring and summer and then decreased through fall for both treatments. To mimic seasonality day length in the chambers will be also be increased every two weeks until the summer solstice and then decreased every two weeks thereafter. Chamber irradiance of approximately 300 µmol m$^{-2}$ s$^{-1}$ (a level consistent with partial shade) will be maintained at the top of the plant throughout the experiment. All plants will be watered and fertilized to maintain optimal growth conditions. Photosynthetic traits will be measured as the field experiment. Above and below ground biomass and total leaf areas will be determined in a final harvest in late fall.

The chamber experiment will analyzed as a complete randomized design. To avoid confounding position or chamber effects with treatments effects, each pot will be rotated within chambers and treatments will be reassigned to chambers every week. Data will be analyzed using a mixed model analysis of variance with chamber as a random effect and ACAT and ECET and family (i.e. genotype) as fixed effects.
**Preliminary Data.** Diurnal photosynthesis and the response of photosynthesis to intercellular CO₂ (A vs. Ci curves) were measured with a LI-COR 6400 in the field in May and August of 2016. Diurnals were measured under prevailing conditions, and A vs. Ci curves were measured at saturating light of 1500-1800 PPFD and ambient temperatures of ca. 15 to 35 °C. We found that the photosynthesis (A) in 4 year old saplings more than doubled (Compare panel C and F below) and stomatal conductance (gₛ; a measure of plant water loss) increased tenfold (B vs. E) over the two months indicating that chestnuts established well at these two sites. Note that both photosynthesis and conductance is lower in site 2 indicating site differences in soil moisture. Additional data are being collected to determine what is driving differences in soil moisture at the sites. These preliminary data are promising as we are beginning to be able to predict assimilation fairly well already (compare X and circle in panel F).

An initial chamber experiment was initiated in February of 2016. Twelve seeds of each of 4 different BC₃F₃ families and 12 from two putatively thermally contrasting genotypes of American chestnut were planted in pots and germinated in growth chambers under the treatments outlined above. Unfortunately, while germination was good for the hybrids it was much lower for Americans, making robust comparisons problematic. Second within two months of the experiments inception most BC₃F₃ seedlings showed signs of senescence potentially related to phytophthora. High humidity in the chamber in early spring may have exacerbated this problem. Interestingly while fewer of the American seeds germinated they remained generally healthier. That initial chamber experiment will be harvested but the plants are in poor health and the sample sizes are too low to draw any robust comparisons. To address these issues we have therefore modified the proposed experiment in three ways. First only plants with four leaves will be placed in growth chambers. Second germinated seedlings will not be placed in the chambers until late spring eliminating the low temperature /high humidity conditions favoring fungal and other pathogens. Third we have increased our sample size to account for low germination in American chestnuts.
h. Timeline

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<tr>
<th></th>
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<th>Spring 2017</th>
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<th>Fall 2018</th>
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i. how results will be measured and reported

We will use the biochemical parameters of photosynthesis calculated from the light, temperature and CO₂ response curves to model and predict carbon assimilation in American chestnuts and hybrids. We show that this is possible in figure 2 panel F. The validity of the model will be tested by comparing model prediction with growth and biomass in a future field experiment under various manipulations. The results from these experiments will be presented at the TACF annual meeting, written up for publication in peer reviewed literature (i.e. Tree Physiology, New Forests). The leaf model will eventually inform a scaled up model of canopy assimilation with the potential of estimating chestnut stand (i.e. whole canopy) carbon uptake and productivity.

j. Breakdown of how and when funds will be spent

<table>
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<th>Experiment</th>
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<td>IRGA consumables</td>
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Total 2825.6
Bibliography


Biographical Sketch:

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Professional Preparation:
Ph.D. Plant Biology, 2004 – University of Georgia, Athens, GA.; M.S. Botany, 1999 – University of Wyoming, Laramie, WY; B.S. Biology, 1993 – George Mason University, Fairfax, VA.

Appointments:
2013 – Present Assistant Professor of Environmental and Plant Biology, Ohio University
2007 – 2012 Research Plant Physiologist USDA Global Change and Photosynthesis Research Unit
2005 – 2007 Research Faculty – Portland State University.
1999 – 2004 PhD Candidate / Research Assistant / Teaching Assistant – University of Georgia.

Five Relevant Products:

Five Additional Products:
Rosenthal DM and DR Ort (2012) Examining cassava’s potential to enhance food security under climate change. Tropical Plant Biology. DOI : 10.1007/s12042-011-9086-1

**Synergistic activities:**

**Funded Research:** Parameterizing photosynthesis models in American chestnuts and hybrids to inform restoration in the context of climate change—From Ohio University Research Council ($7960 2015-2016)

**Current:** Advisor (Nick Tomeo, PhD ; Kelsey Bryant, PhD; Abby Singletary, MS) **Advisory Committee Member** (Ryan Dorkowsky PhD, Kathleen Gabler, PhD; Anne Sternberger PhD; Nick Niechayev, MS Harlan Svoboda MS, Bethany Zumwalde MS; ). **Undergraduate Mentor:** 4 students in last three year in the Program to Aid in Career Exploration (PACE)


**Outreach:** 2014: Mentor/Speaker, High School Science Students, Logan High School, Logan OH; 2013 Ohio University Science Expo Judge; Ohio South-East Regional State Science Fair Judge; American Chestnut Foundation Ohio Chapter, Volunteer tree planting. 2012: Invited to Ohio Corn Growers Group, Champaign-Urbana People’s Garden community garden project to provide fresh produce to families in need 2010; University of Illinois Agronomy Day Presented work regarding climate change impacts on soybean production at an outreach conference for local agricultural practitioners 2010.

**Invited Seminars:**

2014-2015 Colloquium speaker, Voinovich School of Leadership and Public Affairs, Athens, Ohio. 2013 Guest Lecturer, Senior Capstone Seminar, Ohio University; Guest lecturer Ecology and Environmental Issues Seminar. 2012 Ohio University Department of Environmental and Plant Biology. 2011 Texas State University, San Marcos, Department of Agriculture; BASF plant Science, Research Triangle Park, North Carolina; 2010 CGIAR Climate Change Agriculture and Food Security Science Meeting; University of Illinois, Program in Plant Molecular Biology and Physiology; 2007 Oregon State University, Department of Botany and Plant Pathology; USDA Photosynthesis Research Unit. 2005 Bureau of Land Management, Eugene, Oregon; Portland State University, Biology Department; Reed College, Biology Seminar 2006 University of California, Santa Cruz; University of Fribourg, Switzerland

**Current funding**

Ohio university Research Council ($7961 awarded December 2015 expires December 2016). Awarded in part and used to establish CO2 controllers for chamber experiment proposed here.