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PROPAGATION, PHYSIOLOGY, AND BIOMASS OF GIANT CANE (ARUNDINARIA GIGANTEA) FOR CONSERVATION AND RESTORATION

A Master's Thesis

Presented to

The Graduate College of

Missouri State University

In Partial Fulfillment

Of the Requirements for the Degree

Master of Natural and Applied Science, Biology

By

Sanjeev Sharma

May 2023

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PROPAGATION, PHYSIOLOGY, AND BIOMASS OF GIANT CANE (ARUNDINARIA GIGANTEA) FOR CONSERVATION AND RESTORATION

Biology

Missouri State University, May 2023

Master of Natural and Applied Science

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ABSTRACT

Giant cane (*Arundinaria gigantea)* is a native species to 22 states in the U.S. The species and its ecosystem are considered critically endangered, and the species has been reduced to 2% of its original extent. The species has a long cultural and conservation history. Large canebrakes were commonly found in Missouri in bottomland forests, stream and riverbanks, and margins of lakes. My research goals were to: 1) examine methods for propagation success from field to greenhouse to field; 2) examine the physiology of cane at one of the few current canebrakes, for greenhouse propagated plants, and field planted cane; and 3) develop an allometric equation to estimate biomass of a current canebrake allowing biomass estimation from non-destructive measurements. I used the number of shoots produced (new growth) as a metric for propagation success. The number of new shoots depended on rhizome length, watering regime, and whether propagation was attempted with the rhizome alone or with an existing culm. I recorded 100% propagation success from every rhizome with culm cut at 2nd internode, 25% propagation success from non-regular watering rhizome alone and 90% propagation success with regular watering on rhizome alone. Leaf chlorophyll of *A. gigantea* values ranged from 329 *u*mol/m^2 in sun leaves to 354 *u*mol/m^2 in shade leaves in October 2022. During a mild drought summer 2022, leaves-maintained water potential of -1.8 MPa with photosynthetic rates as high as 12 *u*mol CO_2/m^2/s. Biomass models based on pole diameter and height were established, allowing an estimate of carbon storage. I estimated that 5.8 metric tons of carbon was stored by a 0.17 ha canebrake at Mincy Conservation Area. My data provide baseline data for understanding the role of *A. gigantea* and canebrakes in ecosystem functioning in existing canebrakes, and habitats where *A. gigantea* could be restored.

KEYWORDS: biomass, photosynthesis, chlorophyll, propagation, carbon sequestration, photosynthetically active radiation, model

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Approved:

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In the interest of academic freedom and the principle of free speech, approval of this thesis indicates the format is acceptable and meets the academic criteria for the discipline as determined by the faculty that constitute the thesis committee. The content and views expressed in this thesis are those of the student-scholar and are not endorsed by Missouri State University, its Graduate College, or its employees.

ACKNOWLEDGEMENTS

The success and outcome of this thesis required a lot of guidance and assistance from many people and I extremely fortunate to have got this all along the completion of my thesis. Whatever I have done is only due to the full support and the guidance given by the lovely and outstanding people. I express my sincere gratitude and respect to our respected professor Dr. D Alexander Wait who has given me his full support, cooperation, and suggestions to complete my thesis work. I would like to thank Webster Groves Nature Study Society (Menke Scholarships) for providing a $2040 and Missouri State University graduate college and Biology department for funding $1000.

Similarly, I would like to express my sincere gratitude to the committee professors Dr. Jay McEntee and Dr. Toby Dogwiler who helped me in many aspects with the completion of my thesis. I would like to thank other professors who taught me great skills in data analysis Dr. Sean P Maher and report writing Dr. Debra S Finn.

I thank my parents for inspiring me through their hard work. I am indebted for their support throughout while I am away from home, for giving me freedom to explore the world, and to catch my dreams.

I dedicate this thesis to my mother and father.

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INTRODUCTION

1.1 Historic Occurrence

Bamboo is a woody grass with 1,250 species. Scurlock et al. (2000) estimated that it is used daily by about 2.5 billion people, mostly within Asia. Uses include bioenergy and fiber crop for niche markets including construction, furniture, and animal feed. Bamboo has great potential for carbon farming and trading (Nath et al. 2015), and there are multiple markets for *A. gigantea* products, including a global market valued to reach over $98,759.9 million by 2026. Expansive monotypic clonal stands of bamboo (Bambusoidae: Poaceae) occur throughout the world (Janzen, 1976). However, bamboo in the United States is much more restricted taxonomically, where it is restricted to one genus with three species and is generically referred to as "cane". Cane (*Arundinaria* spp.), including giant cane (*A. gigantea*), hill cane (*A. appalachiana*) and switch cane (*A. tecta)*, once formed extensive stands or "canebrakes" throughout the southeastern United States (Fig. 1; Hughes 1966; Marsh 1977).

Giant cane (*A. gigantea)* is a native to 22 states in the U.S. (USDA 2021; Fig. 1). Canebrakes were usually located on bluffs, natural levees, and in mixed cane savannas located along waterways and in backwater areas and floodplains (Platt & Brantley1997); all areas which experienced moderate disturbance. Land conversion for agricultural purposes and urban development, in combination with overgrazing and fire suppression are considered the major variables reducing canebrake habitat to less than 2% of historical occurrence (Noss et al. 1995; Platt & Brantley 1997). Recovering a historic landscape feature by reestablishing canebrakes to the bottomland hardwood forest mosaic, managers can provide habitat for a variety of wildlife and restore ecosystem functions.

1.2 Wildlife Habitat

A. gigantea plays a significant role in providing habitat for aquatic and terrestrial species. At least 50 animal species including 23 mammals, 15 birds, four reptiles, and seven invertebrates are negatively affected because of lack of *A. gigantea* habitat (Brantley & Platt 2001). Historically, canebrakes were used extensively by the Louisiana black bear (*Ursus americanus*) for cover and denning sites (Simek et al. 2012). The huge destruction of *A. gigantea* stands were presumed to be a major factor contributing to the extinction of Bachman's Warbler (*Vermivora bachmanii*) and currently, IUCN least concern bird the Swainson's Warbler (*Limnothlypis swainsonii*) (Remsen 1986, U.S. Fish and Wildlife Service 2008). As an evergreen species, *A. gigantea* may provide forage and cover for wildlife throughout the year (Blattel et al. 2009) and has been associated with several species of hunting interest, such as snipe (*Gallinago delicata*.), woodcock (*Scolopax minor*), bobwhite (*Colinus virginianus*), wild turkey (*Meleagris gallopavo*), and white-tailed deer (*Odocoileus virginianus*) (Platt et al. 2013). *A. gigantea* is rich in nutrients in its aboveground structures, below-ground structures, and seeds (Janzen 1976). It was preferred as browse for livestock during settlement and is still considered the highest value native fodder for livestock in the southeast (Halvorson et al. 2011). *A. gigantea* rhizomes are high in nutrients (Griffith et al. 2009). Rice and wheat seeds are comparable to the *A. gigantea* seeds in terms of nutrients and *A. gigantea* seed may have been a food source for many birds like passenger pigeon and Carolina Parakeet (Janzen 1976). Historically, *A. gigantea* has good forage values for many wildlife species like white tailed deer, wild turkey, and bison (Steinberg 2010).

1.3 Soil Anchor and Bank Stabilization

Canebrakes provide significant positive impacts on soil erosion, stream bank stabilization, infiltration of water into soil, uptake and storage of nitrate, phosphorous and environmental toxins that would otherwise enter streams (USDA 2021). These systems were maintained by periodic disturbance, especially fire, which reduced woody competition and encouraged canebrake uniformity (Hughes 1966). Canebrakes were commonly found in alluvial floodplains and bottomlands. When *A. gigantea* is planted as a riparian buffer, the high density of *A. gigantea* culms lower flood velocity (Schoonover et al. 2005). For example, sediment deposition increases with decrease in water velocity and decreases erosion (Schoonover et al. 2005); improving overall water quality. Canebrakes increase bank structural stability due to the dense, shallow rooting structure that filter excess nutrients and sediments before they are deposited in herbaceous wetlands. Sediments and nutrients are a major stressor in restored wetlands especially in an agricultural setting (Cooper & Lipe 1992); therefore, Canebrakes help in preventing wetland degradation and aid in the establishment of target plant assemblages.

1.4 Reestablishment Difficulty

It appears that restoration and management for *A. gigantea* has waned because of issues related to propagation and establishment (Schoonover et al. 2011). For example, the unusual sporadic flowering patterns lead to difficulty in its propagation. It also faces difficulty due to limited pollen release, low seed yield, low seedling survival, increases in crossing and seed predation, and increased strain on plant resources, with annual flowering being selected in the species (Janzen 1976; Gagnon & Platt 2008). Between the flowering events, *A. gigantea* propagates through cloning, vegetative reproduction, and sprouting from rhizomes. *A. gigantea* seedlings culms initially grow slowly, approximately 10 cm per year (Platt & Brantley 1997), with substantial rhizome elongation required before culms grow substantially. These characteristics lend canebrakes to fragmentation and have led to the small size of existing canebrakes, which in turn may lead to decreased reproductive success of *A. gigantea*. Reproductive strategies and disjuncted bottomland habitat may lead to isolated *A. gigantea* islands, unable to reach great enough numbers and densities to establish canebrakes.

Because of agricultural conversion of Mississippi State cropland, 90% of soil carbon and 75% of soil nitrogen has been lost at the time of agricultural abandonment compared to preagricultural levels, with recovery to preagricultural levels anticipated to be 230 years for carbon and 180 years for nitrogen (Knops & Tilman 2000). In the lower Mississippi valley bottomlands, soil carbon levels in agricultural soils range from 0.25–6.0%, compared to 10–15% found in naturally occurring wetlands. Agricultural practices negatively affect soil organic carbon and soil structure, resulting in higher soil bulk densities and lower soil porosities in agricultural lands compared to wetlands in Mississippi (Ullah & Faulkner 2006). The loss of nutrients and decrease in soil porosity may negatively affect cane establishment and propagation, as *A. gigantea* grows best in loose, well drained alluvium and does best in soils of high fertility (Anderson & Oakes 2011).

1.5 Propagation Methods

Numerous studies have been published on propagation due to the interest in *A. gigantea* restoration, with the focus on techniques and methods to improve transplant success (Zaczek et al. 2004; Zaczek et al. 2009; Schoonover et al. 2011; Eade et al. 2018). In southern Illinois, bare rhizomes and containerized stock have been used to successfully propagate *A. gigantea* (Sexton et al. 2003; Zaczek et al. 2009). Sexton et al. (2003) found that the number of culms produced from transplanted rhizomes was positively influenced by exposure to sunlight and the number of internodes present.

Zaczek et al. (2009) showed propagule survival was greater when rhizomes with more buds and taller culms were used. They recommend that *A. gigantea* sources be tested beforehand for survival rather than using large-scale restorations due to differences in survival from collection sources in their study. Dattilo & Rhoades (2005) found that by hand digging clumps that are approximately 45 cm in diameter, transplanting them, and amending the soil with hardwood mulch and composted manure, 98% survival could be achieved over the first two years with the number of culms per clump doubling in the first year and quadrupling in the second year.

1.6 Physiology

Woody bamboos are an important group of plants and extensively distributed in both tropical and subtropical regions worldwide (Yang et al. 2015). Woody bamboos are increasingly being considered a possible substitute for trees as renewable forest resources and non-timber products. As clonal plants and monocots, bamboo species lack secondary growth in their culm walls and have a large opening in the center of the culm (Yang et al. 2015). Their rapid growth rates and highly developed rhizome systems connecting culms underground suggest that woody bamboos may have different hydraulic architectures and water use strategies compared with dicotyledonous woody plants. The woody bamboos exhibit high root pressure, which may be used to repair xylem cavitation during the night. The root pressure is common in bamboo species and the occurrence of root pressure is important for woody bamboo to maintain diurnal water balance (Cochard et al. 1994; Saha et al. 2009; Wang et al. 2011; Cao et al. 2012; Yang et al. 2012). Water transport derived by root pressure may also be used to recharge the culm water storage, mainly culm parenchyma surrounding all vascular bundles (Liese & Köhl 2015). Almost ~52% of the bamboo culm constitute parenchyma cells (Liese 1998), which could potentially serve as a large storage for water.

Previous research and literature have indicated both stomatal and non-stomatal limitations resulting in a decline in net photosynthetic rate of common woody species in bottomland habitats (Anderson & Pezeshki 2001; Pankovic et al. 1999), which would not be the case for cane. In addition, when upland plant species are flooded, their roots lack adequate oxygen; respiration is compromised and the plant's ability to transport water decreases, resulting in a wilted appearance of the plant (Cronk & Fennessy 2001). Therefore, stomata close to decrease water loss and, consequently, photosynthetic activity decreases. However, emergent wetland plants and riparian plant species have adaptations that have allowed them to sequester oxygen and tolerate low oxygen levels found in flooded soils. *A. tecta* possess aerenchyma tissue (McClure 1963; Triplett et al. 2006; Triplett & Clark 2009), which transports gases throughout the plant, allowing oxygen to reach the buried portions of the plant (Vartapetian & Jackson 1997). The presence of aerenchyma in *A. tecta* has been thought to allow greater survival in wetter habitats than those habitats of *A. gigantea*.

The photosynthetic processes are limited by the reduction of the radiant energy on which the fitness success of a plant depends (Fitter & Hay 2002). Canopy gaps allow light to reach understory plants, thereby affecting plant growth (Battaglia et al. 2000; Saitoh et al. 2002; Wang et al. 2006). The regeneration patterns fluctuate with plant response to light in canopy gaps, as has been shown with rhododendron in Appalachian oak forests (Clinton 2003) and bottomland hardwood forests (Sharitz et al. 1992). The evergreen species may potentially possess a phenological gap advantage (Lei & Koike 1998), continuing photosynthetic activity during the temperate forest dormant season during diminished leaf area. Rivercane populations may be responding to canopy gap and disturbance related openings in the canopy (Gagnon et al. 2007; Gagnon & Platt 2008) and, as an evergreen, continue to photosynthesize during winter months.

No research has been done to study the chlorophyll content or effect of light on *A. gigantea* photosynthetic responses in the field where collected, greenhouse where propagated, and field where out planted. Leaf chlorophyll provides both a measure of nutrient status and potential ability to use light to drive photosynthesis. Therefore, leaf chlorophyll content in *A. gigantea* as a function of growth environment, propagation, leaf age or canopy position all together is not known.

1.7 Biomass Estimation (Allometry)

Woody plants serve a major function in carbon storage. However, most studies of woody species have focused on assessing the capability of trees to sequester carbon (Rotzer et al. 2010; Tian et al. 2011; Fayolle et al. 2013); few studies have examined woody grass such as bamboos. The accurate assessment of biomass is helpful for tracking changes in the carbon stocks (Yen & Wang 2013; Yen & Lee 2011; Goswami et al. 2014). Biomass estimation helps in quantifying the amount of carbon dioxide which can be sequestered from the atmosphere (IPCC 2006). The few studies with bamboo have observed significant variations in the estimation of carbon storage across moso bamboo (Chen et al. 2009; Wang et al. 2013). Both direct methods (destructive techniques) and indirect methods (biomass equations) are generally used for biomass estimation in forestry. Indirect methods of using biomass equations are cost effective and less time consuming as compared to destructive methods and are therefore the preferred approach for biomass estimation (Montes et al. 2000; Nogueira et al. 2008; Nath et al. 2009; Daryaei &

Sohrabi 2016), as rapidly growing bamboo has high carbon stock production and potential in carbon sequestration. There is a need for a method of accurately estimating the biomass and growth of bamboo where it is being restored and would replace much of the current vegetation.

The objectives of this study were (1) to determine an appropriate and best method for propagation of cane rhizome for better success. (2) to examine the physiology of *A. gigantea* in a current canebrake, greenhouse, and field for understanding functional propagation success and management (3) to develop a model (biomass equation) to estimate biomass and carbon based on culm diameter and height.

METHODS

2.1 Study Location

Data collection was done in Missouri, USA, at Mincy Conservation Area (MCA) Taney County (36°32′ N latitude, 93°5′W longitude), Rockspan Farm (privately owned), Greene County (37°14′N latitude, 93°23′W longitude) and the Missouri State University Biology Department greenhouse in Springfield MO. MCA has a 1,720m^2 plot of *A. gigantea* which is effectively a canebrake. The climate at MCA is continental. The average annual temperature ranges from 89.6° F to – 24.8° F and the average annual rainfall is 1092.2 mm. MCA has a karst topography, with elevation ranging from 180 to 340 meters. Rockspan farm is in Greene County, Missouri along the Sac river watershed. A large freshwater spring flows into the Sac river and then north to Stockton Lake where it provides water for the region, including Springfield, Missouri. Over the course of the year, the temperature typically varies from 37°F to 91°F and is rarely below 25°F or above 98°F, Elevation of 383.74 meters (Fig. 2 & 3).

2.2 Data Collection

2.2.1 Rhizome Collection from Mincy Conservation Area and Greenhouse Propagation. Rhizomes or rhizomes with culm were collected in March ($n = 8$), June ($n = 22$), August ($n = 12$), September ($n = 22$), and November ($n = 34$) 2022 when the soil was unfrozen and unflooded by hand-digging from the Mincy conservation area using shovel and fork. Rhizomes were collected with great care without any deformation and brought to greenhouse or Rockspan Farm. Rhizomes were kept moist and cool until processing at the MSU greenhouse within 4 hours after collecting. Rhizomes were washed with water before propagation to remove the soil. Rhizomes or rhizomes with culm were cut into 20 or 35 cm sections for rhizome length treatments. Each rhizome was provided with at least 3 buds. Rhizomes with culm and only rhizomes cuttings from collection were processed and planted in either perlite (Aero-Soil, Industries, Inc.) or soil mix (Pro-Mix BX) into 8 x 15.6-inch pots (Stowe and Sons, Inc.). Pots were filled to ¾ to a constant weight of planting medium. I planted using different pots to evaluate pot depth and soil capacity; however, the success rate was high in 8 x 15.6-inch pots and therefore I eventually only used that pot size. Pots were placed in a heated greenhouse under a misting regime during daylight hours. Different experiments were done (Table 1) to determine the appropriate methods of propagation. I used the new shoot as an indicator and counted all the new shoots above 5cm from the soil-mix or perlite surface. Rhizomes or rhizomes with culm were placed randomly on pots and labelled. Experiments were done on the pots (Tables 1 & 2).

Additionally, I found different height of new shoots from the rhizome which were propagated at same time. For estimation of any relationships between propagated rhizome diameter and new shoots. 10 rhizomes were cut into 20 cm and diameter of rhizome was measured to examine relationships between rhizome diameter and new shoots height. For growth measurement, I used 10 new shoots and recorded the mean height until 5 months.

2.2.2 Rockspan Planting. I planted the greenhouse propagated cane to the Rockspan Farm which has a historic record of cane abundance. Shoemaker (2017) identified that good fertility is needed for cane propagation which includes less disturbed sites from agriculture and urbanization. I also found abundant light is needed, so I tried with an open canopy area. I found all criteria available for cane propagation at Rockspan Farm, which includes a portion of Sac River. *A. gigantea* in the riparian area will help to infiltrate the sediment as cane has high sediment infiltration capacity compared to other species which help in water quality maintenance

(Singh et al. 2019). Rhizomes with culm were transplanted from Mincy to Rockspan Farm (*n = 5*). Rhizomes that formed new shoots in the greenhouse were later transplanted Rockspan Farm to determine future field survival and growth as indicated by (Zaczek et al. 2004). I compared new shoots from rhizome with culm between Mincy to Rockspan Farm (*n = 5*) and Mincy to greenhouse and later to Rockspan Farm (*n = 4*).

2.2.3 Assimilation Rate Measurement. Photosynthetic rates were measured using a LI-6800XT portable gas exchange system (LI-COR Biosciences, Lincoln, NE, USA). Measurements were done in June, August, and November. 150, 700 and 1400 umol/m^2/s photosynthetic active radiation (PAR) were used to compare the assimilation rate as a function of light. Measurements were performed at three different levels of photosynthetic active radiation (1400, 700 and 150 PAR) across three locations (Mincy, Rockspan Farm and greenhouse).

2.2.4 Chlorophyll Measurement. Chlorophyll concentrations were measured with an MC-100 Chlorophyll Concentration Meter (Apogee Instruments, Inc., Logan, UT, USA). Measurements were done by clipping the sensor onto the leaf. Leaves were selected from different culm and measurement was done. Measurement was done on different leaves based upon age (*n = 45*) (expanded, expanding, and newly initiated), different locations (*n = 100*) (Mincy, Rockspan Farm and greenhouse), canopy positions (*n = 36*) (upper, middle, and lower crown cover), and sun and shade leaves (different times of year summer (*n = 22*) and fall (*n = 40*). Selection of leaves was done haphazardly (Table 3).

2.2.5 Water Potential. Water potential of *A. gigantea* was measured using a Scholander pressure bomb. Leaves were collected haphazardly, and leaf petiole were used for the estimation of water potential. Leaves without any deformation or visible stress were used. Measurements were done during summer in day light hour on 06/14/2022 at 1:00PM. This gave the water stress

A. gigantea was facing during summer 2022. The potential is always recorded as a negative value. Higher negative value indicates greater water stress.

2.2.6 Allometry and Biomass from Mincy Conservation Area. For allometric relationships, 32 culms were selected randomly from canebrake at Mincy conservation area. 32 culms diameter were measured at 15cm from the ground by caliper, and height of culms were measured by measuring tape. Leaf and branch were removed from each culm. Fresh weights were recorded on site, and leaves and branches were put in a bag and labeled. Likewise, the pole was cut into two to four sections. Fresh weight of poles was measured on site, and poles were put in a bag and labeled. Poles, branches, and leaves were brought to the lab. Out of 32 culms, 10 culms were oven dried at 120° F for 3-4 days until a constant weight was achieved and dry weight was recorded to estimate the dry matter content (DMC). Seventeen culms were kept for model development and 5 culms were left for validation purposes.

Dry matter content (DMC) = (Dry Weight/Fresh Weight) *100.

Dry matter content was calculated to find the relationship between the fresh weight and dry weight, which helped in building the model. Model development was done by multiplying the remaining 17 culms with DMC to get dry weight and performing a multiple regression among dry weight, diameter, and height. Validation was done by mean comparison between the culm weight applying model and culm weight after oven dried. Prediction error was also generated for the verification of the model. (Prediction Error= 100 * (sum of actual dry weight after oven dried - sum of predicted weight from model)/ sum of actual dry weight after oven dried).

Additional culms were collected from Mincy. An air-dry model was developed from additional culms collected from Mincy for valuation of culm. *A. gigantea* grower can identify the value of the *A. gigantea* with the air-dry model. The dry models above were to know the carbon; however, the air-dry models were for the sell and valuation. As *A. gigantea* leaf and branch is used for mulch and pole for the different equipment. This model can provide the biomass value applying nondestructive approach. Culms were air dried in a greenhouse and then model was built. 20 culms were again collected and processed from Mincy. Fresh weight was recorded at Mincy and brought to lab. Out of 20 culms, 5 culms were air dried until constant weight was recorded, and 10 culms were left for the model development. DMC were calculated for air dry, and 5 culms were used for a validation check of the model. This model will be helpful for *A. gigantea* growers estimate carbon uptake and storage over time without destructive methods.

2.2.6.1 Biomass Estimation. An *A. gigantea* stand was identified at Mincy conservation area. I wanted to know the carbon sequestration potential of the stand as biomass is related to carbon (IPCC 2006). An allometric model was developed to measure the biomass of the culm based upon its height and diameter. However, it was impossible to measure the diameter and height of every culm of the stand, so I needed sample plots to estimate the biomass of whole stand. To generate sample plots, I needed drone image of an area, so image processing can be done using ArcGIS Pro. A DJI Matrice 300 RTK (Lidar Drone) was used for capturing an image of the *A. gigantea* stand. Georeferencing of an image was done based on Google Earth for the exact location of an image. The raster to polygon tool in ArcGIS Pro was used to analyze the image and area was drawn by the edit tool and the total area (approx. 1720m^2) was calculated. The extract by mask tool was used to extract the exact an area of the *A. gigantea* stand. The simple random points tool on ArcGIS Pro was used to generate 25 sample plots. Point latitude and longitude values were generated and were added to a Garmin GPS. Plot finding was done through Garmin GPS and map.

Sample plot boundaries were delineated using a $0.25m^2$ square constructed of PVC tubing. Culm density (live and dead) was measured by counting individual culms within each plot. In each sample plot, and diameter and height were recorded using caliper and meter tape. From 25 samples, 182 culms were recorded. Dead and immature culms were directly cut, collected and oven dried to measure the biomass, to minimize the error. Sing et al. (2018) estimated relative biomass of below-ground and above-ground material. They found the below-ground material down to 25cm depth is 68% of above-ground material. The biomass stock density of a sampling plot was converted to carbon stock densities after multiplication with the default carbon fraction of 0.47 (IPCC 2006).

2.3 Data Analysis

ArcGIS Pro and R (v.3.6.1. R Core Team 2022) were used for data analyses. Propagation success was determined based upon the new shoots. As the response variable consists of counts, it was assumed to have a Poisson distribution. Multiple regression was applied to build the biomass model in R. Two biomass models were developed one for poles, and the other for leaf and branches. MuMin package in R was used for step and dredge code functions. These code functions were used to generate the models. The *AICc* and delta *AICc* was considered for selection of the best model. Pearson correlation was performed between height, diameter, biomass, density, photosynthesis, chlorophyll, rhizome diameter, and culm height.

RESULTS

3.1 Propagation and Field Plantation

Rhizomes with culm cut at 2nd internode and rhizomes alone were compared for propagation success (Fig. 4; Tables 1 & 2), which was indicated by a new shoot that grew in pots. Rhizomes with culm cut at the second internode had a better propagation success 100 % rather than transplanting rhizomes without culm 25 % based upon new shoots that grew in pots which were not regularly watered. Rhizomes with culm cut at second internode had better propagation success 100 % compared to rhizomes with culm not cut 0 % (i.e., left entire culm) which were all regularly watered.

Regular and non-regular watering were done on pots. Regular watering rhizome alone had a 100 % success based upon new shoots from each pot and higher number of mean new shoots. Non-regular watering on rhizome alone had only 25% success with fewer number of mean new shoots. However, 100% success from regular or non-regular watering on rhizome with culm cut at 2nd internode. They both produced similar mean number of new shoots. I found longer rhizome alone produced greater number of mean new shoots. The 35 cm rhizome alone produced a greater number of mean new shoots. 20 cm rhizome alone produced a smaller number of mean new shoots which were all regularly watered (Fig. 4; Tables 1 & 2).

Direct field to field propagation of *A. gigantea* from Mincy to Rockspan Farm ($n = 5$) had a comparatively lower success compared to Mincy to greenhouse ($n = 4$) and to the field based upon the new shoots. Number of new shoots after 5 months from field-to-field plantation of 5 rhizomes with culm were 12 and one planted culm dead, while the success of field to greenhouse and to the field was comparatively high, approximately 24 new shoots from 4 rhizomes with culm and all survived.

3.2 Relationships between Growth, Environment and Physiology

I found a mean difference in chlorophyll content of sun and shade leaves as a function of time of year (summer and fall). Shade leaves had a higher chlorophyll compared to sun leaves. A variation in chlorophyll range was observed in these time periods (Fig. 6; Table 3). *A. gigantea* leaves chlorophyll was 30 % higher in leaves sampled at the Mincy Conservation Area canebrake compared to greenhouse, Rockspan Farm had got 25% higher compared to greenhouse, with the lowest content found in greenhouse propagated cane (Fig. 7; Table 3).

I found that fully expanded mature leaves had the high mean chlorophyll content compared to expanding or newly initiated leaves. However, the expanding and newly initiated leaf had the similar mean chlorophyll (Fig. 8; Table 3). Chlorophyll measurement at the different positions of culm was performed: upper, middle, and lower on the same day and same time, expanded leaves were selected for measurement. No significant difference in the chlorophyll was observed at these different parts (Fig. 9; Table 3).

A higher assimilation rate was observed in Mincy compared to the Rockspan Farm and greenhouse (Fig. 11; Table 4). Different PARs were applied to examine photo-saturated assimilation rates with rates that would be found in leaves throughout the canopy. Higher assimilation was observed at 1400 PAR compared to 700 PAR and 150 PAR (Fig. 12). See (Appendices A & B) for R code used to plot. Photosynthesis was observed similar at different times of year summer and fall 2022. No correlation was obtained between chlorophyll and photosynthesis (Fig. 10), ($r=0.38$, $P>0.05$). Mean water potential of -1.8 MPa water was observed in cane leaves during summer and percentage of water in young shoot and rhizome was calculated based upon fresh and dry weight (Table 5).

It took 20 days for emergence of new shoots from cane rhizomes and found a mean height of 25 cm after 10 days, 60cm after 25 days, and 75 cm after 1 month. No difference in height was observed from similar diameter rhizomes. After one-month, height of cane did not show a rapid growth and a mean height of 110 cm was observed after five months. Cane shows rapid growth at first and then slower growth after 1 month.

Propagated rhizome diameter was measured and found more the diameter, higher the new shoots height. Correlation was observed between the propagated rhizome diameter and the new shoots height ($r=0.92$). In June 2022 from Mincy, four 0.25 m^2 plots were harvested in June 2022 from four 8-17 culms were collected and within 5 months period November 2022, 6-12 new shoots were growing from that harvested plot.

3.3 Relationships Between Biomass, Culm Diameter and Height

Biomass models were developed to estimate the biomass of culm (Fig. 13). Dry matter content (DMC) was calculated to for the relationship between fresh weight and dry weight and for model development, DMC for pole was 54%, while leaf and branch was 78%.

Pole biomass = 5.942 + 0.23*D^2*H, (R^2 = *0.931, AICc = 103.8*).

Leaf and branch biomass = -2.804 + 13.6*D, (R^2 = *0.6236, AICc = 161.4*).

Diameter of a culm is represented by D and height is represented by H. The model was selected based upon *AICc* (Fig. 13; Tables 6 & 7). See (Appendices C & D) for R code used to plot. For pole biomass, the R^2 was 93.1% and prediction error was 3%, which verified the validity of the model. For leaf and branch biomass, R^2 was 62.36%. The prediction error was 5%, validating the model.

Data obtained from each sample plot from Mincy for biomass estimation, (Table 8) was scaled to get the overall biomass of 1720m^2cane plot. Approximate total above ground biomass was 7,356.85 Kg. 5,002.65 below ground biomass, so total biomass was 12,359.508Kg (Fig. 14; Table 9). Percentage of Pole biomass obtained was 16%, leaf and branch biomass was 44% and below ground biomass was 40%. Per m^2 pole biomass obtained was 1.11kg, while leaf and branch was 3.16 kg. Total carbon sequestered was estimated to be 5,808.96 Kg (5.8 metric ton) in Mincy conservation area (Approx. 1720m^2 giant cane plot) which was obtained after multiplying biomass with 0.47. Culm density found was 50,086culms/1720m^2 (182 culms in 6.25m^2).

Air dry matter content (DMC) of the pole was 66%, while for leaf and branch was 91%. Pole air dry biomass = 2.66 + 0.308*D^2*H. (R^2 *= 0.9863, AICc = 83.4*). Leaf and branch air dry biomass = - 3.146 + 15.94*D. (R^2*= 0.6311, AICc = 167.5*). For Pole biomass, the R^2 was 98.6% and prediction error was less than 5%, which verified the validity of the model. For leaf and branch biomass, R^2 was 63.11% and prediction error was less than 5%, which verified the validity of the model. Pearson correlation was performed to examine the correlation between variables. Correlation was performed based upon the sample plots data generated for biomass estimation. No correlation between density and height ($p>0.05$), or density and diameter ($p>0.05$). However, there was a correlation between the height and diameter ($r= 0.83, p<0.05$)

DISCUSSION

4.1 Propagation and Field Plantation

Rhizomes collected from the field and transplanted is the most applied method of propagation for *A. gigantea* (Zaczek et al. 2009). The propagation success helps to establish *A. gigantea*. However, the role of water in propagation of rhizome alone or rhizome with culm cut has never been compared. *A. gigantea* needs regular watering based upon the method I applied, rhizomes with buds are needed for propagation (Singh et al. 2018) and rhizome with culm cut at 2nd internode has a greater success rate compared to rhizome alone based upon new shoots grown on pots. I tried to propagate through the seeds, but germination was not successful, which may be due to less viable period or seeds were in dormancy. Given the historical accounts of propagation through seed, this needs to be examined further. Culm cutting was performed to examine if there was possibility of growth from the culm of the young immature *A. gigantea*, however no growth was observed from the culm cutting.

4.2 Physiology and Growth

Chlorophyll, growth, effect of light on assimilation rate and effect of rhizome diameter on the culm height has never been simultaneously investigated for *A. gigantea*. Greenhouse propagated *A. gigantea* requires higher fertilization rates compared to the canebrake or planted along the Sac River based upon the chlorophyll data obtained from three study locations. Site with abundant light availability is required for *A. gigantea* growth. The results of the different PARs on assimilation rate verified cane needs light for the growth. A site with light availability or open canopy is needed for cane propagation (Cirtain et al. 2009). Chlorophyll content in *A. gigantea* was relatively high compared to adjacent species suggesting *A. gigantea* shows a fast growth. A variation in chlorophyll range was observed in different time summer or fall, which indicates it contains variation in chlorophyll during the hot and cold season. Singh et al. (2018) studied the effect of rhizome on propagules however the effect of rhizome diameter on culm height was never done. I found the similar diameter rhizome gave similar height of new shoots; this may be due to same collection site. I found the correlation between the rhizome diameter and new shoots height. The more the diameter of rhizome the bigger was the new shoots height. It opens future research on the effect of site on the new shoot height. An *A. gigantea* shows fast growth at beginning and slow growth; however, Xu et al. (2011) showed the growth rate of moso bamboo is slow fast slow.

4.3 Biomass Model and Biomass of Canebrake

Oli (2006) developed a biomass model for *Bambusa tulda*; however, no biomass model has ever been developed for cane. Singh et al. (2018) developed an allometric equation for cane to estimate the viable propagules based upon the rhizome length and buds. However, no allometric equation was developed for *A. gigantea*. I developed the biomass model for *Arundinaria gigantea* to estimate the biomass of the existing stand and to predict the future stand biomass. I found the total above ground biomass 7,357 Kg and the density 50,086 number/1720 m^2 of the canebrake at Mincy. Comparing aboveground biomass results for this study to a study by (Schoonover et al. 2005) on a canebrake in southern Illinois, their estimate biomass of 36,000 kg/ha was somewhat lower as our 42,772 kg/ha, but their estimate for the culm density of 328,003 culms/ ha was higher to our results of 291,198 culms/ha. I recorded a culm density that was similar to that reported by Sing et al. (2018). Wastler (1952) measured stem density at 151,408 culms/ha and estimated that *A. gigantea* in Louisiana produced 40,000 kg/ha of aboveground biomass. Southern Illinois is the northern extent of *A. gigantea* distribution, it's not surprising that biomass estimates from southern states would be higher (McClure 1973). The culm density found in previous studies was much lower. This was consistent with my data, where an increase in culm diameter was accompanied by lower stand density (Hoffman 2010). Bamboo can be used for furniture, food, equipment, and natural benefits, the biomass models may provide useful information on above ground biomass to forest user groups, forestry professionals, bamboo growers, and other interested parties. Although the biomass estimation is confined to the species, this can be applied to other sites where *A. gigantea* is available.

Air drying was done for the valuation of the culm: pole and greenhouse. *A. gigantea* pole is used for different furniture, fence, equipment, and leaves along with branches can be used for mulch. This model can be used for *A. gigantea* grower to predict the value before cut and weight.

4.4 Limitations of Research

4.4.1 Site Availability. Lack of study sites is an obvious limitation to my study as I could not compare the different success rate among sites. This is also a limitation for interpreting biomass, propagation, chlorophyll, PAR, and growth. *A. gigantea* as a critically endangered species, it limits rhizome harvest to the minimum, so it won't destroy the entire habitat at Mincy Conservation Area.

CONCLUSION

The *A. gigantea* restoration effort is important to preserve the canebrakes as they are home to many wildlife species including migratory birds, reptiles, moths, and butterflies (Platt et al. 2001). Platt et al. (2001) reported that due to the loss of canebrake habitat, over 50 species of wildlife are at risk. However, the restoration of canebrakes is a relatively difficult process because its propagation depends on number of viable rhizomes used in the restoration process.

The physiology, propagation methods and biomass study help the grower to select site with abundant light, water, and nutrient availability. Propagation methods applying regular watering, rhizomes with culm cut at 2nd internode, and longer rhizomes with more buds has a better success rate. The global warming is the major issue of current world, with restoration of *A. gigantea* we can sequester more carbon and provide benefits to the ecosystem by providing habitat to wildlife. The non-destructive method I found will be helpful for *A. gigantea* growers to know the biomass and forest managers to know the biomass and carbon of the canebrakes.

Table 1. Different methods used to propagate cane collected from the Mincy site. Five different methods with varying rhizome length, part of plant and watering regimes were examined. Sample size refers to the number of pots. See (Table 2) for percent of pots that produced new shoot, and the total number of shoots across all pots.

Experiment	***Rhizome Length***	***Plant Part***	***Watering Regime***	***Sample Size***	***Dates***
1	20 cm	Rhizome alone	Not regularly watered	8	June 2022
1	20 cm	Rhizome with culm cut at 2nd internode	Not regularly watered	9	June 2022
2	20 cm	Rhizome with culm cut at 2nd internode	Regularly watered	6	August 2022
2	20 cm	Rhizome with culm not cut	Regularly watered	6	August 2022
3	35 cm	Rhizome alone	Regularly watered	6	September 2022
3	20 cm	Rhizome alone	Regularly watered	6	September 2022
4	20 cm	Rhizome alone	Regularly watered	9	November 2022
4	20 cm	Rhizome alone	Not regularly watered	8	November 2022
4	20 cm	Rhizome with culm cut at 2nd internode	Regularly watered	9	November 2022
4	20 cm	Rhizome with culm cut at 2nd internode	Not regularly watered	8	November 2022

Table 2. Different methods used to propagate cane collected from the Mincy site. Percent of pots that produced new shoot, and the total number of shoots across all pots. See (Table 1) for sample size and watering regime.

Experiment	***Plant Part***	***Propagation Success***	***Mean ± SE Number of New Shoots***	***Symbols on Figure 4***
1	Rhizome alone	25 %	0.25 ± 0.16	NWRA_J
1	Rhizome with culm cut at 2nd internode	100 %	2.77 ± 0.4	NWRCL_J
2	Rhizome with culm cut at 2nd Internode	100 %	4.33 ± 1.75	WRCL_S
2	Rhizome with culm not cut	0 %	0	WREC_S
3	Rhizome alone	100 %	2.33 ± 0.33	35cm_RA_A
3	Rhizome alone	80 %	0.83 ± 0.16	20cm_RA_A
4	Rhizome alone	100 %	1.33 ± 0.16	WRA_N
4	Rhizome alone	25 %	0.25 ± 0.16	NWRA_N
4	Rhizome with culm cut at 2nd internode	100 %	2.66 ± 0.44	WRCl_N
4	Rhizome with culm cut at 2nd internode	100 %	2.75 ± 0.45	NWRCl_N

Table 3. Mean (±SE) chlorophyll content (umol/m^2) and categories of leaves based upon location of measurement.

Categories	***Sample Size***	***Mean ± SE***	***Range***	***Location***	***Description of Leaf***	***Time***
Expanded Leaves	13	292.9 ± 13.1	240-352	Greenhouse	Sun leaves	August 2022
Expanding Leaves	19	233.2 ± 6.2	193-254	Greenhouse	Sun Leaves	August 2022
Newly Initiated Leaves	13	222.2 ± 12.8	139-296	Greenhouse	Sun Leaves	August 2022
Sun leaves	11	228 ± 12.1	180-286	Mincy	Expanded	June 2022
Shade Leaves	11	263.7 ± 5.2	241-300	Mincy	Expanded	June 2022
Sun Leaves	24	329.7 ± 6.4	277-383	Mincy	Expanded	October 2022
Shade Leaves	16	354.4 ± 6.9	306-403	Mincy	Expanded	October 2022
Upper Canopy Leaves	12	262.4 ± 12.1	188-333	Mincy	Expanded	August 2022
Middle Canopy Leaves	12	265.8 ± 18.1	177-370	Mincy	Expanded	August 2022
Lower Canopy Leaves	12	270.5 ± 11.5	220-378	Mincy	Expanded	August 2022
Mincy Leaves	39	339.4 ± 5.2	290-403	Mincy	Sun and Expanded	November 2022
Greenhouse Leaves	50	234.5 ± 8.4	74-317	Greenhouse	Sun and Expanded	November 2022
Rockspan Farm Leaves	11	315.8 ± 5.7	287-353	Rockspan Farm	Sun and Expanded	November 2022

Table 4. Mean (±SE) photo-saturated photosynthetic rates in leaves of *A. gigantea* measured in three different study locations.

Treatment	***Assimilation Rate(umolCo2/m²/s) in PAR=1400 umol/m²/s***
Mincy	11.2± 0.66
Rockspan	10.51± 1.10
Greenhouse	7.93± 0.74

Table 5. Mean (±SE) relevant physiological variables collected from the canebrake at Mincy Conservation Area and greenhouse propagated cane at MSU.

Variables Measured	***Field Values***	***Greenhouse Values***
% Water in young 25 cm shoots	85±4%* and 84±8%	89±3%
% Water in leaves	48±2%	53±5%
% Water in rhizomes	49±3%	58±5%
Water Potential (MPa)	-1.88 ± 0.36	NA

Table 6. Models tested to estimate the biomass of pole (Fig. 13) from diameter and height of cane collected at Mincy site. Model selection for actual estimation was based upon lowest *AICc* and delta.

Models	***Biomass***	***LogLik***	***AICc***	***Delta***	***Weight***
M1	Y = 5.94 + 0.23D^2*H	- 47.971	103.8	0	0.281
M2	Y = - 34.6 + 0.643D^2 + 13.91*H	- 47.214	105.8	1.97	0.105
M3	Y= - 1.87 + 0.27*D^2 + 0.15*D^2*H	- 47.305	105.9	2.15	0.096
M4	Y = 29.08 + 5.5*D + 0.15*D^2*H	- 47.339	106	2.22	0.093
M5	Y = - 90.09 + 15.5D	- 49.308	106.5	2.67	0.074
M6	Y = 16.32 + 0.25D^2*H – 6.53*H	- 47.657	106.6	2.86	0.067

Table 7. Models tested to estimate the biomass of leaf and branches (Fig. 13) from diameter of *A. gigantea* collected at Mincy site. Model selection for actual estimation was based upon lowest *AICc* and delta.

Models	***Biomass***	***LogLik***	***AICc***	***Delta***	***Weight***
M1	Y = - 2.804 + 13.6D	- 76.79	161.4	0	0.264
M2	Y = 65.5 + 0.66D^2	- 77.01	161.9	0.48	0.208
M3	Y= - 642 – 6.7 D^2 + 145.7 D	- 75.54	162.4	1.01	0.159
M4	Y = 1.2 + 16.1D – 11.9H	- 76.721	164.8	3.37	0.049
M5	Y = - 1008 - 10.82D^2 + 237.7D - 58.540H	- 74.32	164.1	2.70	0.068
M6	Y = 36.78 + 38.290H	- 78.172	164.2	2.79	0.066

Table 8. Data used to scale individual cane variables to estimate canebrake biomass at Mincy. Mean (± SE) for illustration.

Variables	***Mean ± SE***	***Range***
Pole Biomass(g)	37.27 ± 1.89	6.1-124.68
Leaf and Branch Biomass(g)	105.67± 2.39	24.396-187.59
Height(m)	1.67 ± 0.05	0.2-3.3
Diameter(mm)	8.01± 0.17	2-14
Density (#/.25m^2)	7.28 ± 1.05	1-17

Table 9. Total pole, below ground up to 25 cm deep, and leaf and branch biomass of canebrakes at Mincy (Approx. 1720m^2).

***Biomass in 1720*$\mathbf{m^2}$**	***Kilogram(kg)***
Pole	1918.4
Leaf and Branch	5438.45
Below Ground	5002.65

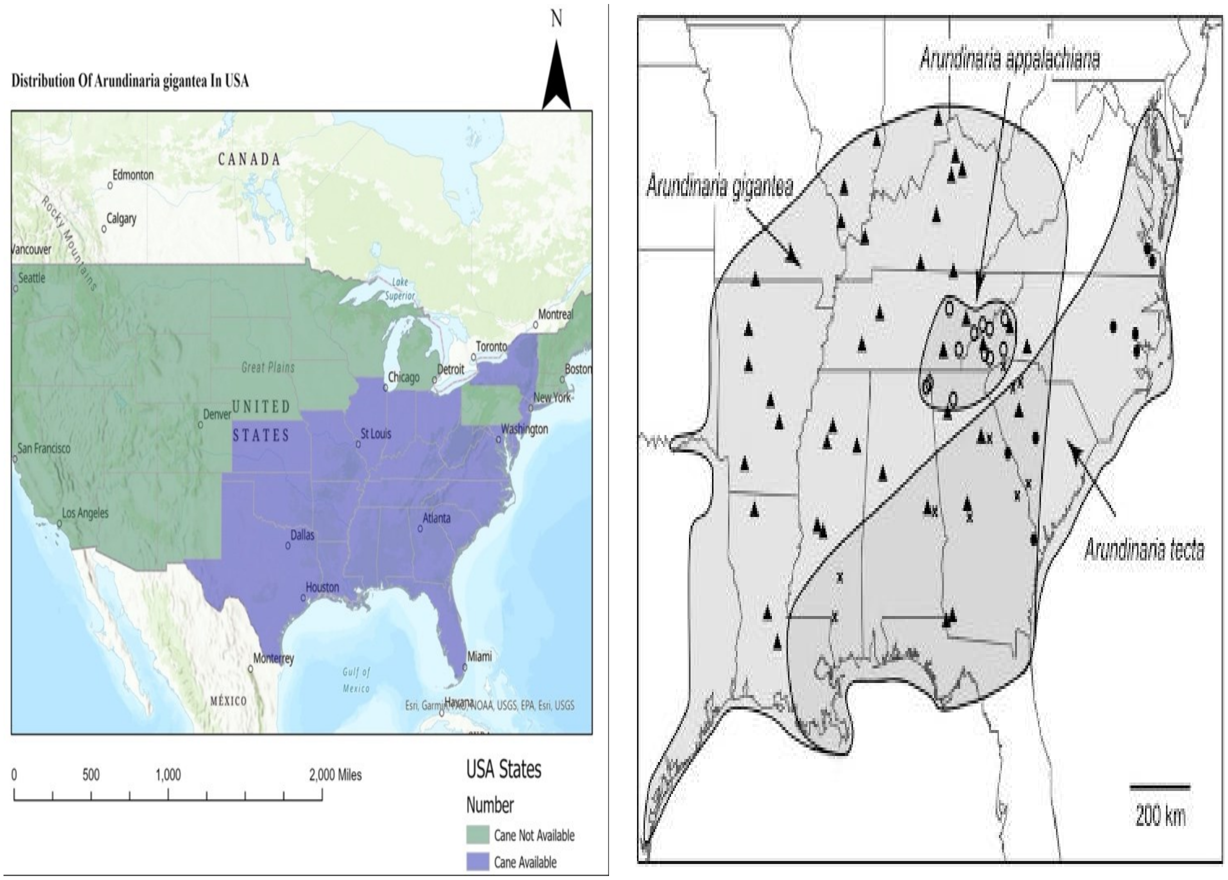

Figure 1. Historic *A. gigantea* distribution in North America (figure on left). Figure on right illustrates collecting locations in a 2010 study analyzing the North American *A. gigantea* species complex and is taken directly from the source (Triplett et al. 2010).

Figure 2. Area of *A. gigantea* at Mincy Conservation Area represented by two polygons. Variation in dots color represents the density of culms in 0.25m^2 sample plot. Bottom Figure represents the proposed site for *A. gigantea* plantation at Rockspan Farm.

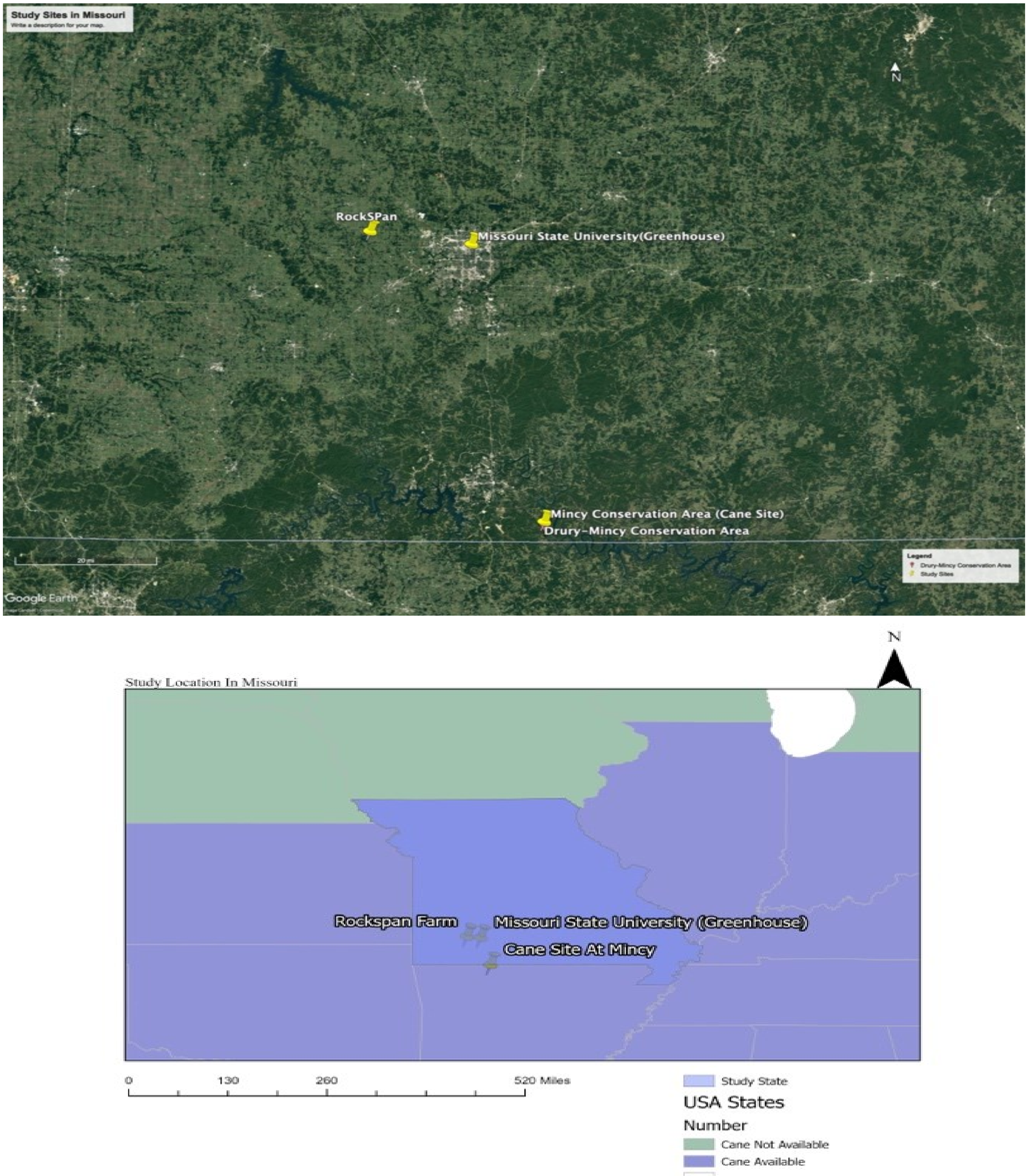

Figure 3. The area of *A. gigantea* in Mincy Conservation Area in Taney County, Missouri where cane is available. The Rockspan farm in Greene County, Missouri where establishment of cane was initiated.

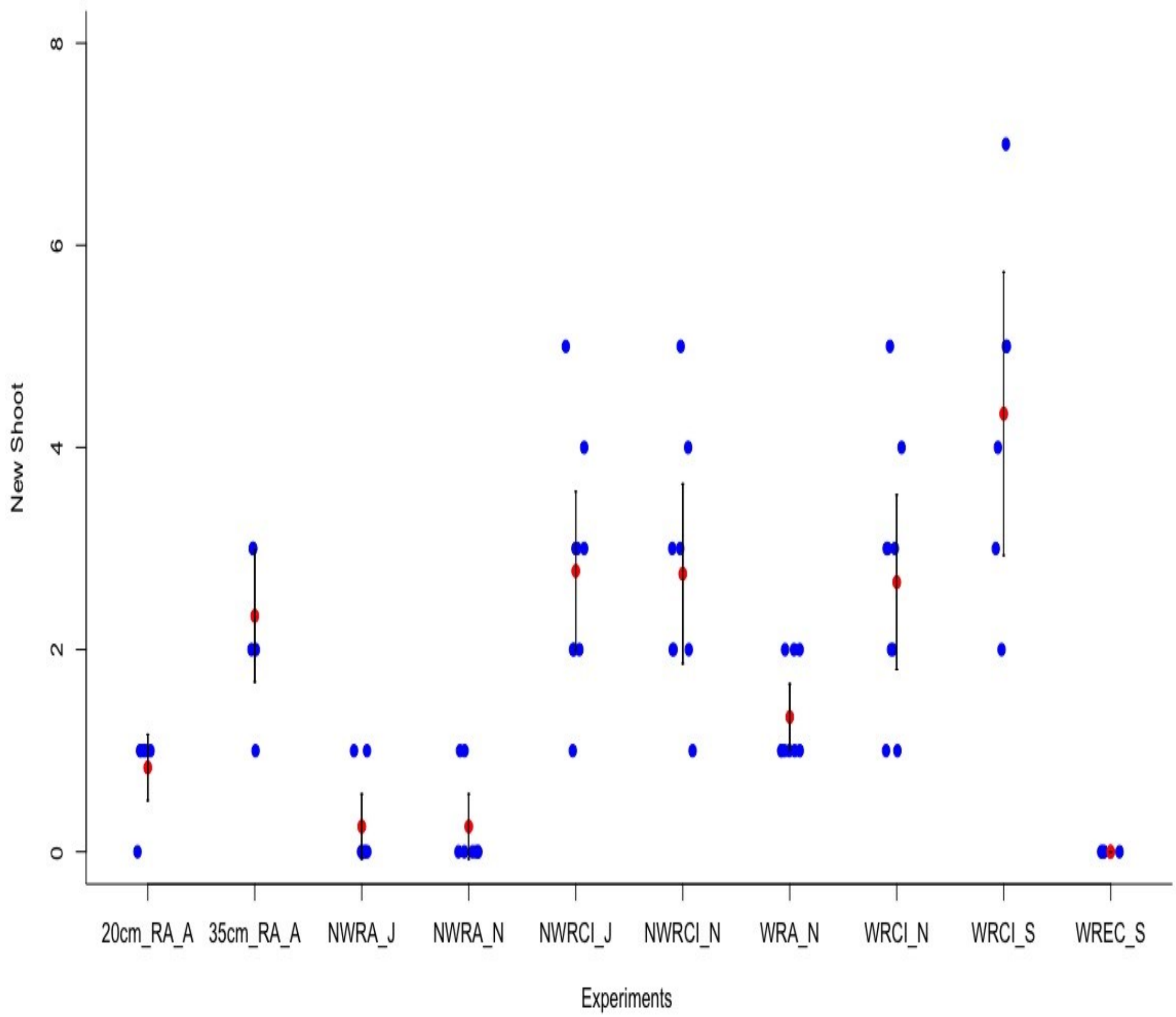

Figure 4. Mean (±SE) of number of new shoots based upon the experiments. 20cm_RA_A represents 20cm rhizome alone collected in August, 35cm_RA_A represents 35cm rhizome alone collected in August, NWRA_J represents non regular watering rhizome alone collected in June, NWRA_N represents non regular watering on rhizome alone collected in November, NWRCl_J represents non regular watering on rhizome with culm cut at 2nd internode collected in June, NWRCl_N represents non regular watering on rhizome with culm cut at 2nd internode collected in November, WRA_N represents regular watering on rhizome alone collected in November, WRCl_N represents regular watering on rhizome with culm cut at 2nd internode collected in November, WRCl_S represents regular watering on rhizome with culm cut at 2nd internode collected in September and WREC_S represents regular watering on rhizome with entire culm collected in September. For further details (Tables 1 & 2).

Figure 5. Photographs illustrating propagation success of *A. gigantea* at greenhouse where cane is planted in perlite and soil-mix. Planted rhizome with culm at Rockspan Farm (bottom right).

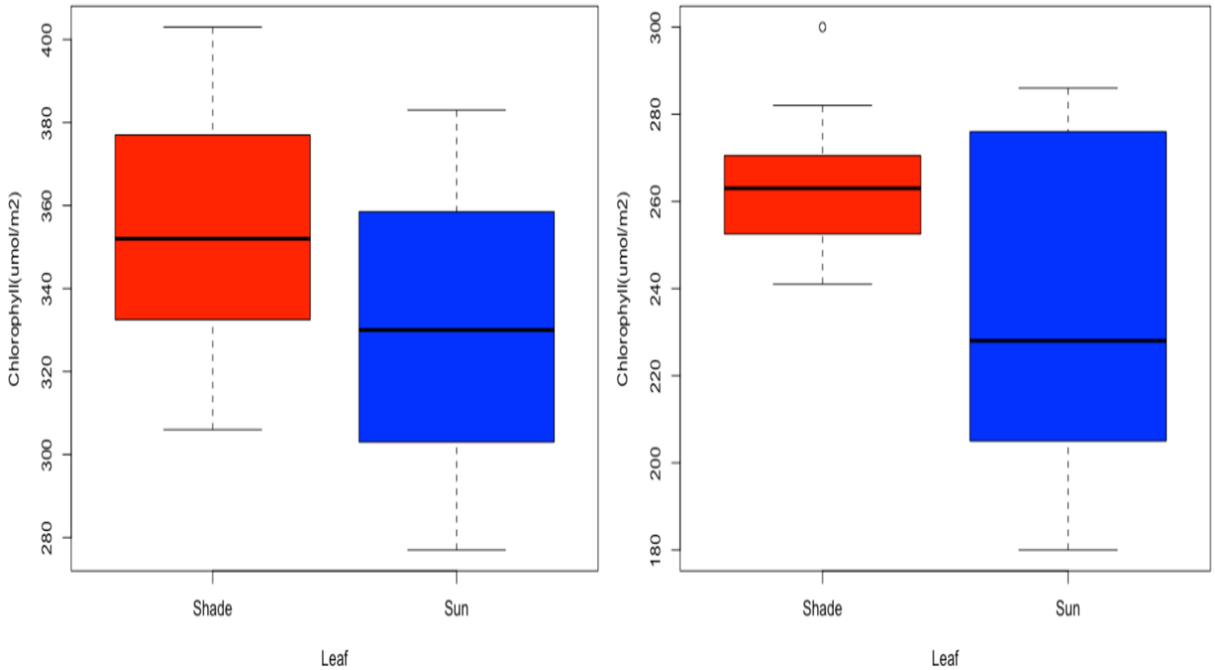

Figure 6. Leaf chlorophyll content of sun (n = 35) and shade (n = 27) leaves of *A. gigantea* at different seasons (fall – left figure and summer – right figure). Measurement was done on same day and fully expanded green leaf was selected. Sun and shade leaves were determined based upon the light they receive. The middle dark line is the median. Points outside the boxplots are outliers.

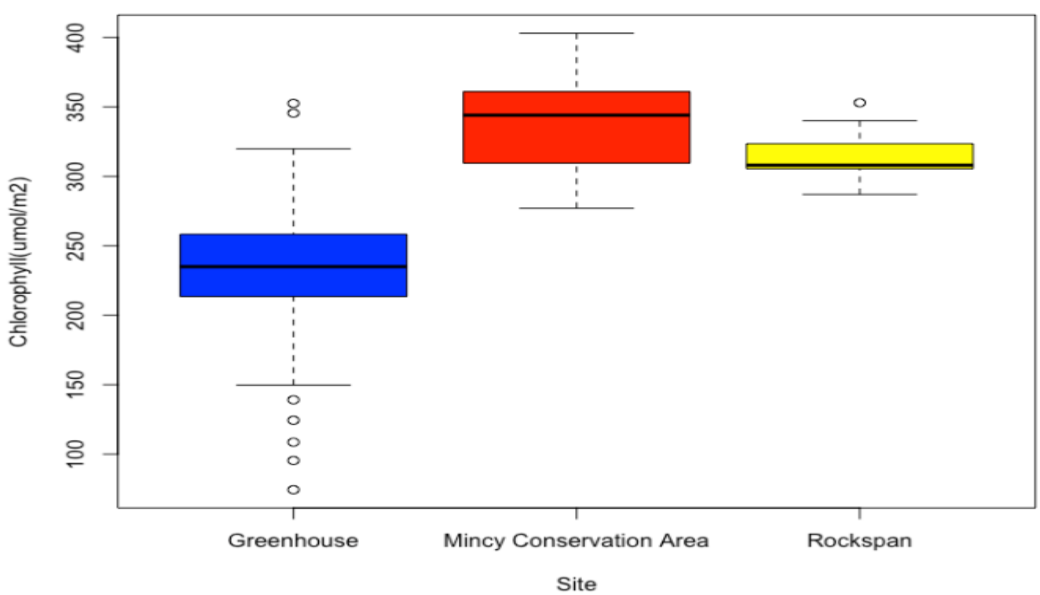

Figure 7. Leaf chlorophyll content of *A. gigantea* at different "sites". Fully expanded and sun leaves were selected for the measurement. The middle dark line is the median. Points outside the boxplots are outliers.

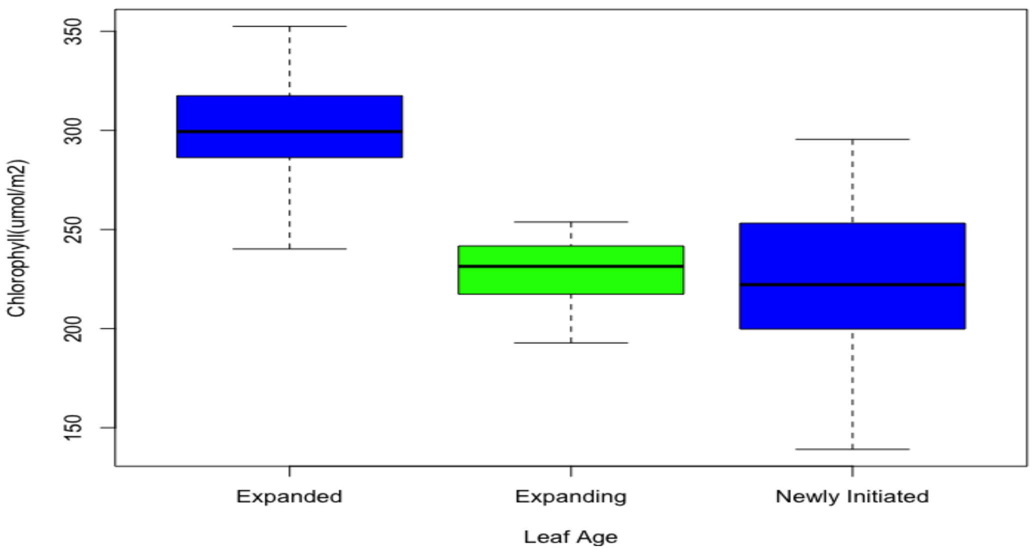

Figure 8. Leaf chlorophyll content of *A. gigantea* at different age of leaves based upon the regular judgement. The middle dark line is the median. Points outside the boxplots are outliers.

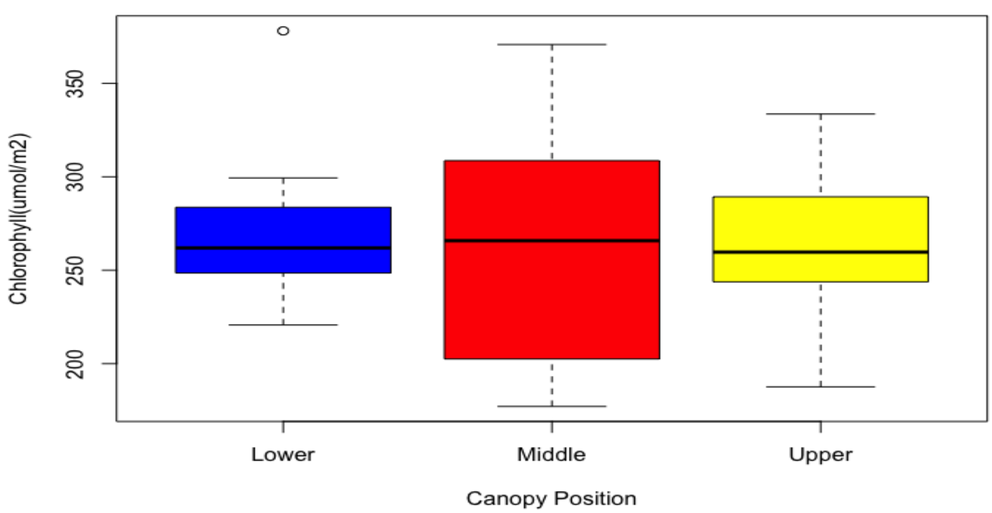

Figure 9. Leaf chlorophyll content of *A. gigantea* at different position based upon canopy. Fully expanded leaves were selected for measurement. The middle dark line is the median. Points outside the boxplots are outliers.

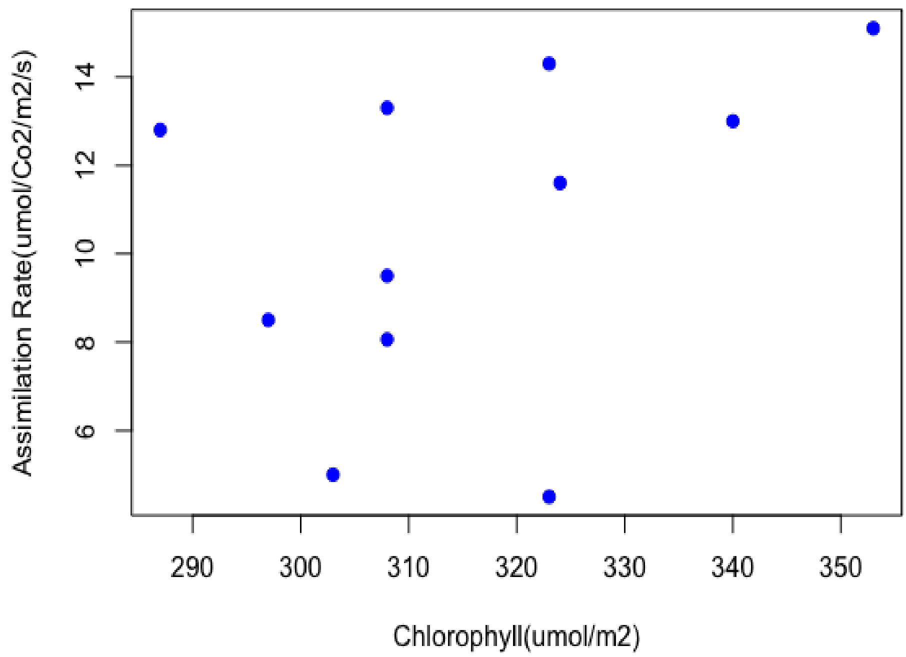

Figure 10. Relationship between carbon assimilation rate and chlorophyll of *A. gigantea* leaf measured at 1400PAR at Mincy Conservation Area.

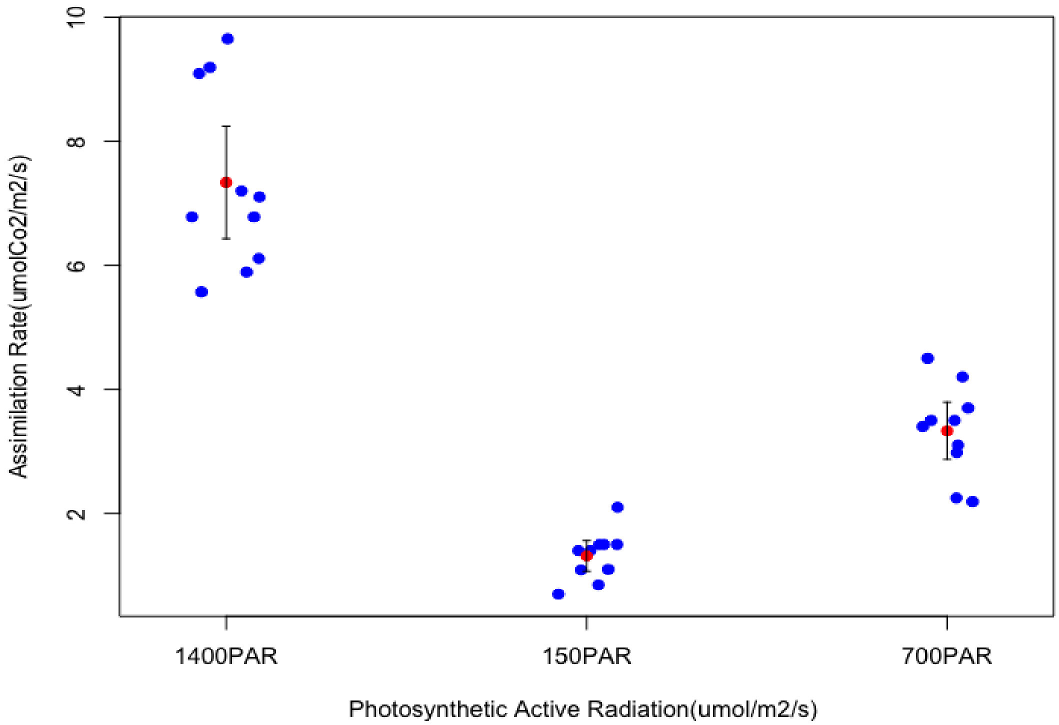

Figure 11. Relationship between assimilation rate and photosynthetic active radiation (PAR). Different PARs were applied to see the assimilation rate. Dots represent the individual measurement. Middle red point represents the mean and the line represent the standard deviation.

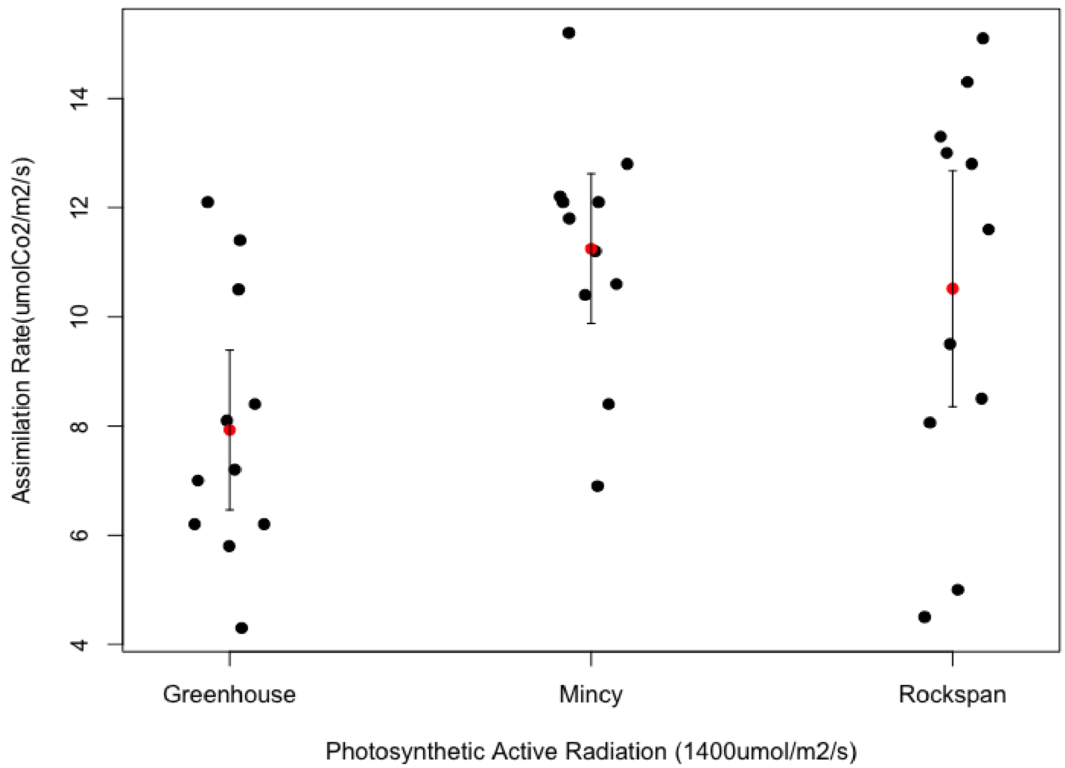

Figure 12. Relationship between the photosynthetic active radiation (PAR) and assimilation rate. Same PAR (1400 umol/m^2/s) was applied to see the effect on different sites. Dots represent the individual measurement. Middle red point represents the mean and the line represent the standard deviation.

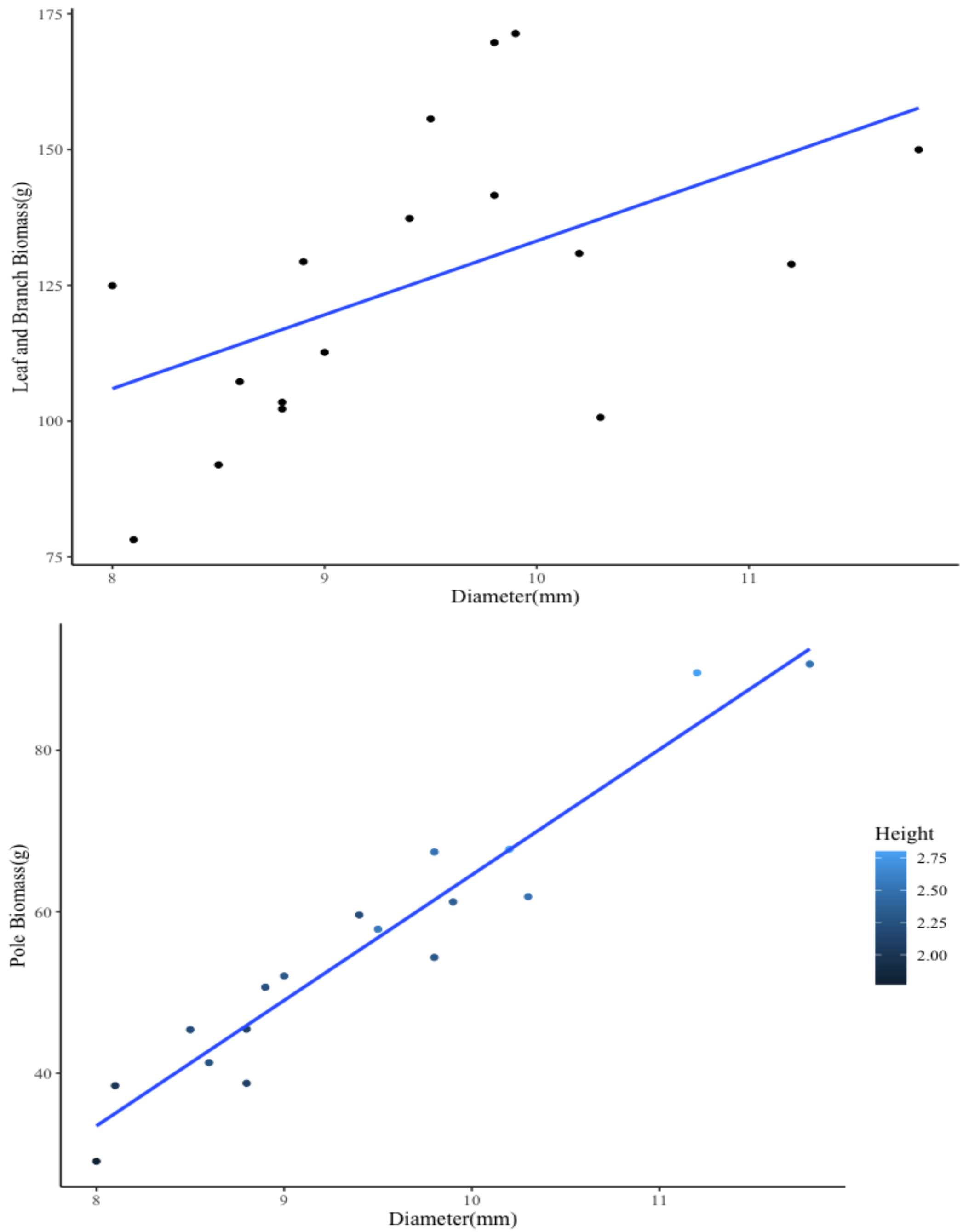

Figure 13. Relationship between culm biomass as a function of culm diameter and height. Top figure represents the leaf and branches biomass as a function of diameter, while the bottom figure represents pole biomass as a function of diameter and height. The individual points represent the biomass of leaf and branch, and the line represents the biomass relationship with diameter. Variation in color of points in pole biomass is represented by height of pole. Biomass is in gram, height in meter and diameter in mm.

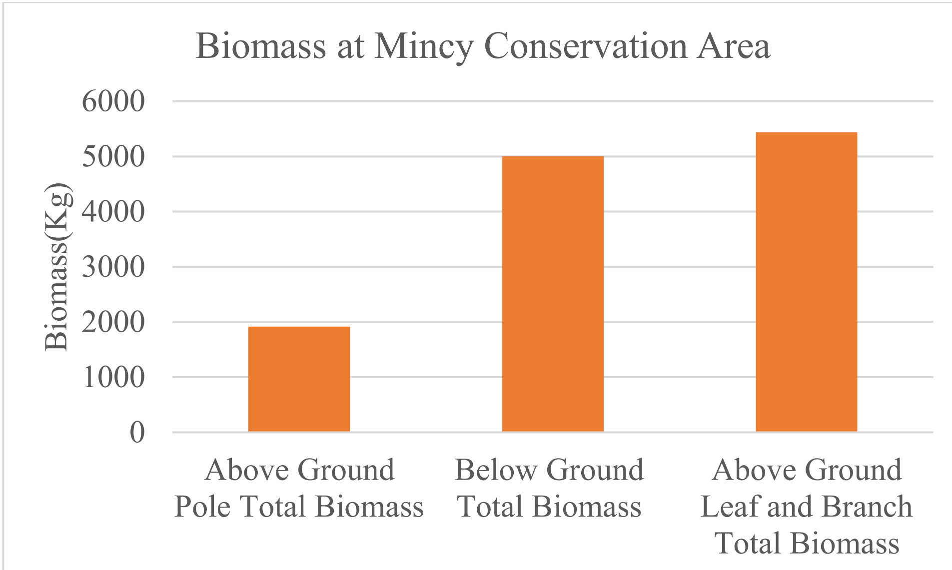

Figure 14. Total pole, leaf and branch and below ground biomass at Mincy Conservation Area (Fig. 15 for morphology). Y axis represents the biomass value in kg.

Figure 15. Morphology of *A. gigantea*.

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APPENDICES

Appendix A. R Code for PAR and Assimilation Rate at Same Site.

```
Photo<-read_excel("Desktop/Thesis data/Light.xlsx") ## Data location
names(Photo)
View(Photo)
par(mar=c(4,4,1,1))
stripchart(Photosynthesis~Treatment, data = Photo,
    vertical=TRUE,
    method="jitter",
    pch=19,
    col="blue",
   ylab=" Assimilation Rate(umolCo2/m2/s ",
   Tick=FALSE,
   Font="Times in New Romain",
   size= 18,
   cex.lab=1.5,
   cex.axis=1.5)

mtext("Photosynthetic Active Radiation(umol/m2/s) ", side = 1, line = 3, font = 1, cex = 1.5)
groupMeans<-aggregate(Photo$Photosynthesis~Photo$Treatment, FUN=mean)[,2]
groupSDs<-aggregate(Photo$Photosynthesis~Photo$Treatment, FUN=sd)[,2]
groupNs <- rep(NA,length(unique(Photo$Treatment)))
```

```
for(i in 1:length(unique(Photo$Treatment))){
 groupNs[i] <- sum(Photo$Treatment==unique(Photo$Treatment)[i])}
groupNs<-c(10,10,10)
UpperLimit <- groupMeans + 1.96 * groupSDs/sqrt(groupNs)
LowerLimit <- groupMeans - 1.96 * groupSDs/sqrt(groupNs)
points(1:3,
       groupMeans,
       pch=19,
       col="red")
segments(1:3, #Vertical line
         LowerLimit,
         1:3,
         UpperLimit)
segments(seq(0.99,2.99,1), #horizontal upper
         UpperLimit,
         seq(1.01,3.01,1),
         UpperLimit)
segments(seq(0.99,2.99,1), #horizontal lower
         LowerLimit,
         seq(1.01,3.01,1),
         LowerLimit)
```

Appendix B. R Code for PAR and Assimilation Rate at Different Sites.

```
S<- read_excel("Desktop/Thesis data/Photosynthesis.xlsx") ## Data location
names(S)
stripchart(Photosynthesis~Treatment,
           data = S,
           vertical=TRUE,
           pch=19,
           method='jitter',
           ylab="Assimilation Rate(umolCo2/m2/s",
           Font="Times in New Romain",
          size= 12,
          cex.axis= 1.5,
          cex.lab=1.5,
          bty='L')
mtext("Photosynthetic Active Radiation 1400umol/m2/s", side = 1, line = 3, font = 1, cex = 1.5)
groupMeans<-aggregate(S$Photosynthesis~S$Treatment, FUN=mean)[,2]
groupSDs<-aggregate(S$Photosynthesis~S$Treatment, FUN=sd)[,2]
groupNs <- rep(NA,length(unique(Photo$Treatment)))
for(i in 1:length(unique(Photo$Treatment)){
 groupNs[i] <- sum(Photo$Treatment==unique(Photo$Treatment)[i])}
 groupNs<-c(11,10,11)
UpperLimit <- groupMeans + 1.96 * groupSDs/sqrt(groupNs)
LowerLimit <- groupMeans - 1.96 * groupSDs/sqrt(groupNs)
```

```
points(1:3,
       groupMeans,
       pch=19,
       col="red")
segments(1:3, #Vertical line
         LowerLimit,
         1:3,
         UpperLimit)
segments(seq(0.99,2.99,1), #horizontal upper
         UpperLimit,
         seq(1.01,3.01,1),
         UpperLimit)
segments(seq(0.99,2.99,1), #horizontal lower
         LowerLimit,
         seq(1.01,3.01,1),
         LowerLimit)
```

Appendix C. R Code for Pole Model and Plot.

```
Pole <- read_excel("Desktop/Thesis data/Leaf.xlsx")## Data available location
names (Pole)
diameter<-Pole$Diameter^2
DL<-Pole$Diameter^2*Pole$Height
p3<-lm(C_Final~Height*Diameter*DL*diameter,
    data = Pole) ##Multiple regression
summary(p3)
library(ggiraphExtra)
library(moonBook)
library (MuMIn)
step(p3)
options (na.action = na.fail)
dredge(p3) ## model selection
##For Plot:
library(ggplot2)
ggplot(Pole, aes(y=C_Final, x=Diameter, color=Height)) + geom_point() +
stat_smooth(method="lm",se=FALSE) + theme_classic() + labs(title =
"Biomass=5.942+0.23*Diameter2*Height(m),R2=93.1%") + xlab("Diameter(mm)") +
ylab("Pole Biomass(g)") + theme(text = element_text(size=12, family = "Times New Roman"))
```

Appendix D. R Code for Leaf and Branch Model and Plot.

```
Leaf <-read_excel("Desktop/Thesis data/Leaf.xlsx")## Data location
names(Leaf)
p1<-lm(L_Final~Height*Diameter*diameter,
   data = Leaf)## Multiple regression
summary(p1)
library(MuMIn)
step(p1)
options(na.action = na.fail)
dredge(p1)## Model selection
library(ggplot2)
ggplot(Leaf, aes(y=L_Final, x=Diameter)) + geom_point() +
stat_smooth(method="lm",se=FALSE) + theme_classic() + labs(title ="Biomass = -
2.8+13.6*Diameter, R2=62.36%") +
  xlab("Diameter(mm)") + ylab("Leaf and Branch Biomass(g)") + theme(text =
element_text(size= 18, family = "Times New Roman"))
```