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RESEARCH ARTICLE

Propagation, physiology, and biomass of Giant Cane (*Arundinaria gigantea*) for conservation and restoration

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Abstract *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. Our research goals were to; 1) examine methods for greenhouse propagation for restoration; 2) examine the physiology of cane at one of the only canebrakes on public land in SW MO, greenhouse propagated cane, and field planted cane; and 3) develop an allometric equation to estimate biomass of the canebrake. We used the number of shoots produced 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. We recorded 100% propagation success from every rhizome with culm cut at 2nd internode with regular watering on 8 x 15.6-inch pots having soil-mix/perlite media. Leaf chlorophyll 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. We estimated 12,359.508 kg of biomass which is equivalent to 5.8 metric tons of carbon stored by a 0.17 ha canebrake at Mincy Conservation Area in SW MO. Our research provides baseline data for understanding the role of cane and canebrakes in ecosystem functioning in existing canebrakes, and habitats where cane could be restored.

Keywords: biomass, biomass model, carbon sequestration, photosynthetically active radiation, propagation

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Introduction

Bamboo is a woody grass with 1,250 species. 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). Giant cane (*A. gigantea*) is a native to 22 states in the U.S. (USDA., 2021). Canebrakes were usually located on bluffs, natural levees, and in mixed cane savannas located along waterways and in backwater areas and floodplains (Platt and Brantley., 1997) 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 and Brantley., 1997). Recovering a historic landscape feature by reestablishing canebrakes to the bottom-land hardwood forest mosaic can provide habitat for a variety of wildlife and restore ecosystem functions. It appears that restoration and management for *A*.

Fig 1. Historic giant cane distribution in USA.

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 and Platt, 2008). Therefore, propagation through rhizomes is the method that will need to be utilized to restore canebrakes.

Numerous older 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). 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 and 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. Rivercane populations may be responding to canopy gap and disturbance related openings in the canopy (Gagnon *et al*., 2007; Gagnon and Platt, 2008 and as an evergreen, continue to photosynthesize during winter months.

Despite these results, there still seems like a lack of the best method for propagation and no research has been done to study the physiology of cane used for restoration or its status once propagated. 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). 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 and 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. The photosynthetic processes are limited by the reduction of the radiant energy on which the fitness success of a plant depends (Fitter and Hay., 2002).

The root pressure is common in bamboo species and the occurrence of root pressure is important for woody bamboo to maintain diurnal water balance (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 and Köhl., 2015). Almost 52% of the bamboo culm constitute parenchyma cells (Liese., 1998), which could potentially serve as a large storage for water. 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. 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). 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 method for restoration of giant cane (2) to develop a model (biomass equation) to estimate biomass and carbon based on culm diameter and height.

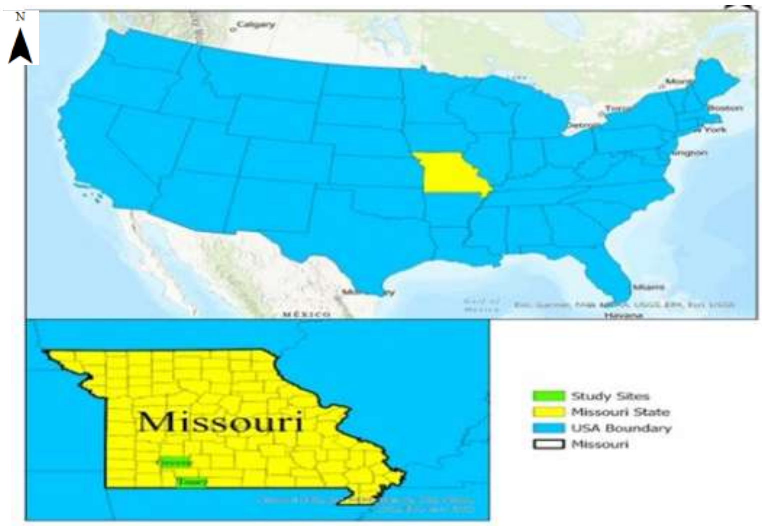

Fig 2. The area of giant cane in Mincy Conservation Area in Taney County, Missouri, USA where cane is available. The Rockspan farm in Greene County, Missouri, USA where establishment of cane was initiated and greenhouse at Missouri State University, Greene County, Missouri, USA.

Materials and methods

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 (37^0 14' N latitude, 93^0 23' W longitude) and the Missouri State University Biology Department greenhouse in Springfield MO. MCA has a 1,720 m^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).

Data Collection

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. We 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 we eventually only used that pot size. Pots were placed in a heated greenhouse under a misting regime during daylight hours and misting cycle was every 25 minutes. Different methods were used including regular watering within 2 days interval and non-regular watering (once in two-week period), rhizome alone, rhizome with culm cut at 2^{nd} internode, rhizome with entire culm, and different length rhizome. Watering was done heavily to soak all soil-mix or perlite based upon (Zaczek *et al.*, 2004). We used the appearance of new shoot as an indicator of success and counted all the new shoots above 5 cm from the soil-mix or perlite surface.

We 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. 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). We recorded new shoots from rhizome with culm between Mincy to Rockspan Farm (*n = 5*) and Mincy to greenhouse and later to Rockspan Farm (*n = 4*).

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.

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).

Water potential of *A. gigantea* was measured using a Scholander pressure bomb. Leaves were collected haphazardly. Leaves without any deformation or visible stress were used. Measurements were done during summer in day light hour on 06/14/2022 at 1:00 PM.

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 120^0 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.

$$\text{Dry matter content (DMC)} = \text{(Dry Weight/Fresh Weight)} * 100$$

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) (Oli., 2006).

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. 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.

Using the *A. gigantea* canebrake at Mincy Conservation Area we estimated the carbon sequestration potential of the stand as biomass is related to carbon sequestration (IPCC., 2006). An allometric model was developed to measure the biomass of the culm based upon its height and diameter. Google Earth was used to extract the image of the study area and creating sample plots. ArcGIS Pro was used to analyze the image and area was drawn by the edit tool and the total area (approx. 1720 m^2) was calculated. 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).

Data Analysis

The R (v.3.6.1. R Core Team 2022) was used for data analyses. 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

Propagation and Field Plantation

Rhizomes with culm cut at the second internode had a greater propagation success (100%) than transplanting rhizomes without culm (25%) based upon appearance of new shoots. 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 (Table 1 & 2).

Table 1. Different methods used to propagate *A. gigantea* collected from a canebrake in MO (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.

Propagation Trial	*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 *A. gigantea* collected from a canebrake (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.

Propagation Trial	*Plant Part*	*Propagation Success*	*Mean ± SE Number of New Shoots per Pot/Rhizome*
1	Rhizome alone	25 %	0.25 ± 0.16
1	Rhizome with culm cut at 2nd internode	100 %	2.77 ± 0.4
2	Rhizome with culm cut at 2nd Internode	100 %	4.33 ± 1.75
2	Rhizome with culm not cut	0 %	0
3	Rhizome alone	100 %	2.33 ± 0.33
3	Rhizome alone	80 %	0.83 ± 0.16
4	Rhizome alone	100 %	1.33 ± 0.16
4	Rhizome alone	25 %	0.25 ± 0.16
4	Rhizome with culm cut at 2nd internode	100 %	2.66 ± 0.44
4	Rhizome with culm cut at 2nd internode	100 %	2.75 ± 0.45

Regular watering of the rhizome alone had a 100% success. Non-regular watering on rhizome alone had only 25% success with fewer number of mean new shoots. However, 100% success was found from regular or non-regular watering on rhizome with culm cut at 2nd internode. We found longer rhizome alone produced a greater number of new shoots. The 35cm rhizome alone resulted in the greatest number of mean new shoots. The 20 cm rhizome alone produced a smaller number of mean new shoots which were all regularly watered. The number of new shoots after 5 months from field-to-field planting (Mincy to Rockspan) plantation of 5 rhizomes with culm were 12/13, 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.

Relationships between Growth, Environment and Physiology

We 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. *A. gigantea* leaves chlorophyll was 30 % higher in leaves sampled at the Mincy Conservation Area canebrake compared to greenhouse, Rockspan Farm had 25% higher chlorophyll compared to greenhouse, with the lowest content found in greenhouse propagated cane.

We 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 similar chlorophyll concentrations (Fig. 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 (Table 3).

Table 3. Mean (±SE) chlorophyll content (umol/m^2) and categories of *A. gigantea* leaves based upon location of measurement. All leaves are fully expanded and sunny unless otherwise noted.

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

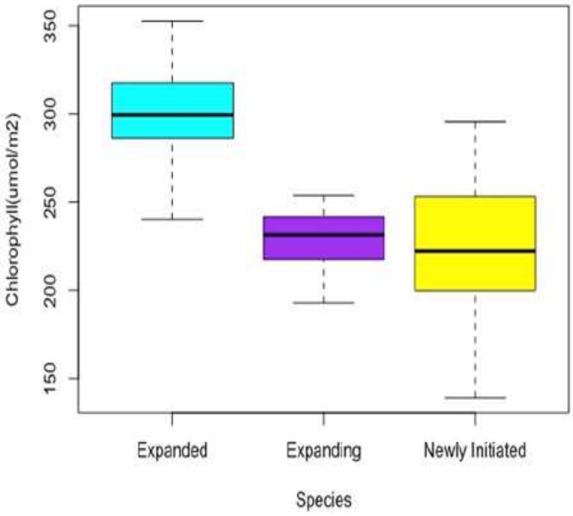

Fig 3. Leaf chlorophyll content of *A. gigantea* at different age of leaves based upon the regular judgement. The middle dark line is the median and outer line are the range. Points outside the boxplots are outliers.

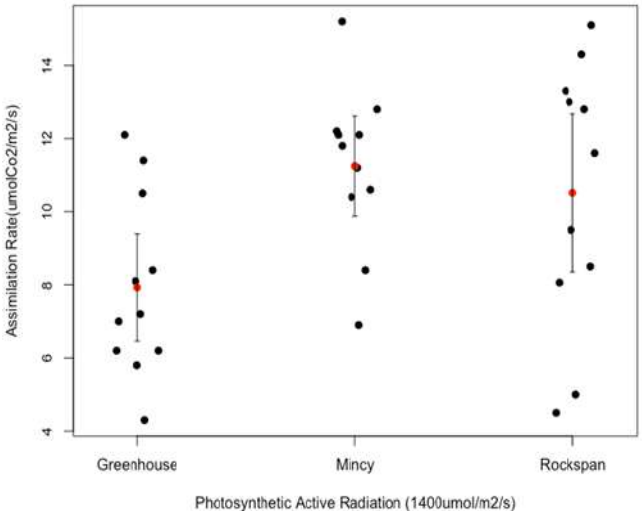

Fig 4. Relationship between the photosynthetic active radiation (PAR) and assimilation rate of *A. gigantea.* Same PAR (1400 umol/m^2/s) was applied to see the effect on different sites. Dots represent the individual measurement. Middle red point represents the mean and the line represent the standard deviation.

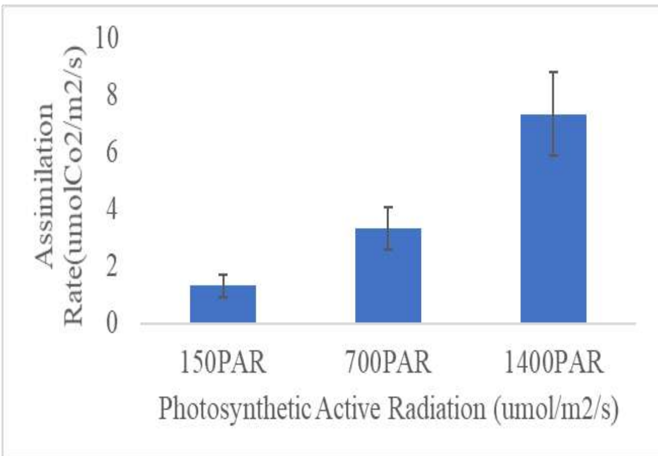

Fig 5. Relationship between assimilation rate and photosynthetic active radiation (PAR). Different PARs were applied to see the assimilation rate of *A. gigantea.*

A higher assimilation rate was observed in plants in the canebrake (Mincy) compared to those transplanted to Rockspan Farm and propagated in the greenhouse (Fig. 4). Different PARs were applied to examine photo-saturated assimilation rates with rates that would be found in leaves throughout the canopy in a canebrake. As expected, higher assimilation was observed at 1400 PAR compared to 150 PAR, but 1400 PAR was also higher than 700 PAR (Fig. 5). Photosynthesis was observed to be similar at different times of year: summer and fall 2022. No correlation was obtained between chlorophyll and photosynthesis, (r=0.38, P>0.05). Mean water potential of -1.8 MPa water was observed in cane leaves during a hot dry summer.

Between Biomass, Culm Diameter and Height

Biomass models were developed to estimate the biomass of culms. 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. 6; Tables 4 & 5). 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, was scaled to get the overall biomass of 1720 m^2 cane 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. 7). 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.

Table 4. Models tested to estimate the biomass of pole (Fig. 5) from diameter and height 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 = 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 5. Models tested to estimate the biomass of leaf and branches (Fig. 5) 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

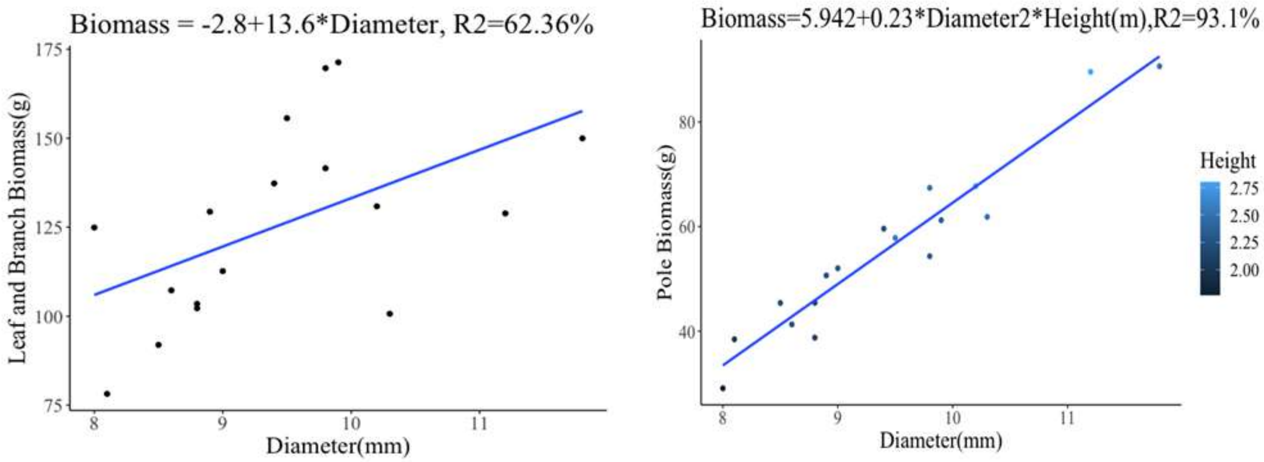

Fig 6. Relationship between culm biomass as a function of culm diameter and height of *A. gigantea*. Left figure represents the leaf and branches biomass as a function of diameter, while the right figure represents pole biomass as a function of diameter and height. The individual points represent the 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

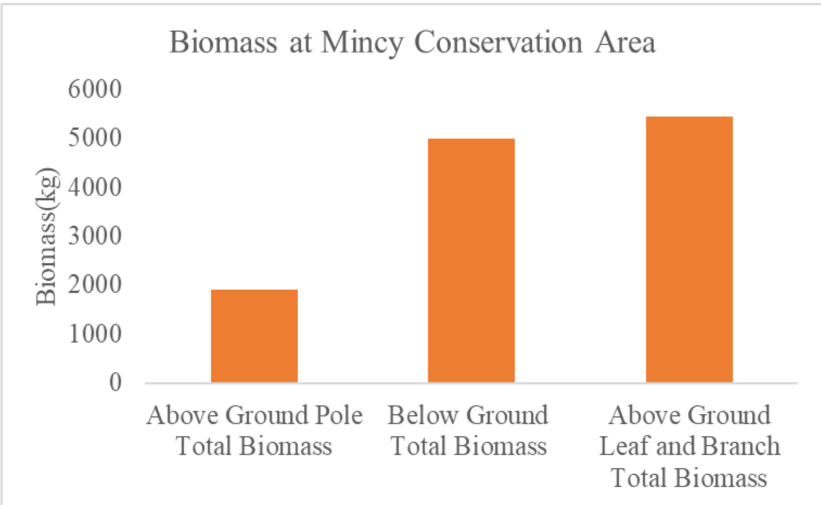

Fig 7. Total pole, leaf and branch, and below ground biomass of canebrake at Mincy Conservation Area. Y axis represents the biomass value in kg.

Total carbon sequestered was estimated to be 5,808.96 kg (5.8 metric ton) in Mincy conservation area (Approx. 1720 m^2 giant canebrake) which was obtained after multiplying biomass by 0.47. Culm density was 50,086culms/1720 m^2 (182 culms in 6.25 m^2). 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

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.* Research was done on the basis of rhizome alone. However, comparison between the propagation success of the rhizome and rhizome with culm cut at 2nd internode or with entire culm was lagging. The role of water in propagation of rhizome alone or rhizome with culm cut has not been assessed. *A. gigantea* needs regular watering based upon the methods we 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 appearing. We found out that the greenhouse propagated cane showed a higher success rate compared to direct field planted which aligned to the result shown by (Zaczek *et al*., 2004). It can be extrapolated that the green house propagated cane develops more resistant to outside for survival compared to direct field to field plantation. As indicated by the (Zaczek *et al.,* 2004) initial application of fertilizer has a greater chance of shoot emergence and period application of fertilizer will increase the number of shoots and plant vigor. However, too much application will cease the growth. I tried to propagate through the seeds, as there was not any attempt to propagate through seeds. However, the germination was not successful, which may be due to less viable period or seeds were in dormancy. This coincides with the reports by Baldwin (Baldwin *et al*., 2009) suggest that river cane seed has a variable and low germination rate of 6% to 58% depending on temperature and the specific population sampled. Other research by Gagnon and Platt (2008) reported similarly variable seed production, but high germination rates, up to 95%. This research suggests that a system of seed-based mass propagation of river cane would be subject to high uncertainty in seed availability and viability. Given the historical accounts of propagation through seed, this needs to be examined further.

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 to maintain chlorophyll levels found in a canebrake or planted along the Sac River Site; with abundant light availability being required for *A. gigantea* growth and carbon assimilation. The results of the different PARs on assimilation rate verified cane needs high light to photo saturate. 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. During mild drought in summer 2022 it maintained the water potential of -1.8 MPa which shows that the culm store water so they can use during drought as indicated by (Liese., 1998). Singh *et al*. (2018) studied the effect of rhizome on propagules however the effect of rhizome diameter on culm height was never done. We found the similar diameter rhizome gave similar height of new shoots; this may be due to same collection site.

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*. We developed the biomass model for *Arundinaria gigantea* to estimate the biomass of the existing stand and to predict the future stand biomass. We 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. We 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 our 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 one canebrake, this can be applied to other sites where *A. gigantea* is available.

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. 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 propagation methods, physiology 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 based upon the result we found. 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 one canebrake, this can be applied to other sites where *A. gigantea* is available.

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