Macro-propagation of native cane (*Arundinaria* spp.) in central Kentucky and restoration out-plantings in western Tennessee and southern Alabama.

by

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A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Auburn, Alabama
August 1, 2015

Keywords: *Arundinaria*, canebrake, ethnobotany, macro-propagation, restoration, rhizome

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Abstract

Canebrakes, monodominant stands of native bamboo (*Arundinaria* spp.; hereafter cane), are a critically endangered ecosystem in the Southeastern United States. Canebrakes have declined to <2% of their former range from overgrazing by livestock, land conversion, habitat fragmentation, and fire suppression. Canebrakes are important for wildlife habitat, riparian buffers, and Native American ethnobotany. In cane macro-propagation trials, I investigated mother plant collection site and time-since-transplantation effects on rhizome production. Additionally, I assessed the effects of collection site and container type on propagule survival, growth rate, and final aboveground growth. In out-planting trials, I investigated the effects of shade, mulch, and fertilizer on survival and growth of propagules. My results indicate that an interaction between time and collection site affected rhizome production. Propagule survival was affected by collection site and final size was affected by collection site and container type. I suggest using macro-propagation for small-scale canebrake restoration (<10 ha).

Acknowledgments

I would like to thank The Nature Conservancy and the USDA NRCS for project funding, particularly Jeff Fore for his help and interest and David Elliott for his help with the Atmore site coordination. I thank the Poarch Band of Creek Indians for their support and interest in cane ethnobotany. Thank you to Jim Hamlington and the Tennessee Wildlife Resources Agency for their help with coordinating the Tennessee plantings and site preparation.

I would like to thank my advisor, Dr. Mark Smith, for his guidance and mentoring. Without his help and direction, this project and manuscript would not have come to fruition. I would like to thank my committee members, Dr. Nancy Loewenstein and Dr. Christopher Anderson, for their contributions in helping me become a better writer and scientist. I thank Dr. Todd Steury for his statistical guidance, without which this manuscript would not be the same. I thank Dr. John Kush for his ample supply of obscure literary resources that helped me along the way.

I would like to thank my family for their continuous support and integral advice throughout my time at Auburn. I would also like to thank my partner, Michael Murray, for his constant encouragement, assistance with my field research, and dedication to seeing me succeed. Thanks to all the student workers, both undergraduate and graduate, who helped with this project, in particular Brandon Craft, Rachel Dedman, and Chase Kinsey.

Table of Contents

List of Tables

List of Figures

Chapter I: Macro-propagation of native cane (*Arundinaria* spp.) in central Kentucky

ABSTRACT

Canebrakes, monotypic stands of native bamboo (*Arundinaria* spp.; hereafter cane), are critically endangered ecosystems in the United States. Canebrakes have declined to <2% of their former range from livestock overgrazing, land conversion, habitat fragmentation, and fire suppression. Although canebrake conservation is a priority throughout its range, successful restoration depends on propagule availability. Macro-propagation, which involves the use of rhizome cuttings (propagules) from potted mother plants, has the greatest potential for large-scale restoration. My objectives were to 1) investigate the effects of mother plant collection site and time-since-transplantation on production of suitable rhizomes for out-planting and 2) assess the effects of site and container type (pot vs. plug) on propagule survival, growth rate, and final cumulative aboveground size in the nursery. I harvested propagules in 2013 ($\bar{x} = 21.3$ propagules/mother plant, SE = 0.94, $N = 7{,}211$) and 2014 ($\bar{x} = 10.0$ propagules/mother plant, SE = 0.45, $N = 4{,}158$). Propagules grew from March to September under a 60% shade house and were monitored from June to September. I observed an interaction between site and age on rhizome production ($\chi^2_6 = 45.96$, $P \leq 0.001$), with rhizome production differing across sites in all age classes except Age 4 plants ($\chi^2_2 = 2.68$, $P = 0.26$). Propagule survival ($F_{10,35} = 21.65$, $P = \leq 0.001$) and final size ($F_{9,123} = 9.01$, $P \leq 0.001$) differed by site; however, growth rate did not ($F_{10,2} = 4.51$, $P = 0.20$).

Propagules planted in plug containers were 2.22 (± 0.24; 95% CI) times more likely to survive than those in pots ($F_{1,1} = 101.44$, $P = 0.06$). Propagule growth rates were similar between container types ($F_{1,790} = 0.80$, $P = 0.37$); however, final size was 2.63 cm (± 0.92; 95% CI) greater for plugs ($F_{1,254} = 7.49$, $P = 0.007$). Further refinement of macro-propagation techniques will improve canebrake planting stock production.

KEYWORDS *Arundinaria*, cane, canebrake, macro-propagation, native bamboo, restoration, rhizomes.

INTRODUCTION

Cane (*Arundinaria* spp.) is the only native bamboo genus of the Southeastern United States. Monotypic stands of cane growing in a contiguous area, called a canebrake, are now considered to be a critically endangered ecosystem having declined >98% from their historical extent (Noss et al. 1995, Platt and Brantley 1997, Judziewicz et al. 1999). Currently, canebrakes exist from Florida to eastern Texas, and northward to southeastern Missouri and Virginia (Marsh 1977, Judziewicz et al. 1999). Historically, canebrakes occurred throughout the Southeastern United States, co-occurring with Southeastern mixed forests (Harper 1958, Platt and Brantley 1997, Judziewicz et al. 1999, Stewart 2007). The largest canebrakes occurred on the natural levees in alluvial floodplains that were often referred to as “cane ridges” (Delcourt 1976, Platt and Brantley 1997). However, in today’s highly altered landscapes, cane is commonly found growing diffusely under forest canopies or forming small stands in forest gaps, along forest edges, and in riparian areas (Marsh 1977, Gagnon and Platt 2008*a*). Canebrakes have been greatly reduced in extent and abundance from their historic range due to overgrazing by livestock, land conversion to agriculture and closed canopy forests, and alterations of disturbance regimes such as tree gap dynamics, beaver herbivory, floods, and fire suppression (Hughes 1951, Platt and Brantley 1997, Stewart 2007, Gagnon and Platt 2008*b*, Klaus and Klaus 2011).

Interest to restore canebrakes for wildlife habitat (Brantley and Platt 2001), ethnobotanical uses by Native Americans (Platt et al. 2009), and to improve sediment reduction (Schoonover et al. 2006), nutrient attenuation (Schoonover and Williard 2003, Blattel et al. 2005, Schoonover et al. 2005), and stream bank stabilization via riparian buffers (Sexton et al. 2002, Zaczek et al. 2004, Andrews et al. 2011) has increased among federal, state, and non-governmental agencies. For example, Schoonover and Williard (2003) found that 10-m cane buffers along no-till agricultural fields reduced nitrates incoming to streams by 99%. Furthermore, Schoonover et al. (2005, 2006) found 10-m cane buffers reduced surface runoff of nutrients and agricultural sediment by 100%. As a result, cane has been considered as a candidate in multispecies riparian buffer zone restoration designs (Schoonover and Williard 2003).

In spite of its recognized importance, a paucity of information exists regarding canebrake ecosystems, ecological processes, restoration, and most importantly, cost-effective cane propagation methods for producing propagules for large-scale restoration. Like most bamboos, cane flowers gregariously and sporadically in an irregular pattern of up to 30-50+ year intervals that are typically followed by massive vegetative die-offs (Hughes 1951, Marsh 1977, Liese 1987, Keeley and Bond 1999). Along with the infrequent flowering of cane, low seed viability due to putative self-incompatibility, inviable pollen, and limited compatible pollen sources are detrimental to successful fruiting (Koshy and Jee 2001). Sexual reproduction of canebrakes is limited, as a result, seed germination cannot be relied upon to produce a consistent supply of viable propagules. Natural canebrake expansion relies heavily upon asexual, rhizomatous growth. Therefore, an entire canebrake may be a single genotype, further lessening the probability of effective pollination and successful sexual reproduction (Hughes 1966, Marsh 1977, Platt and Brantley 1993).

Several methods of cane propagation exist, including clump division (McClure 1966), collection and germination of seed (Gagnon and Platt 2008*a*), micro-propagation using plant tissue cultures (Baldwin et al. 2009), and macro-propagation via rhizome sections (Baldwin et al. 2009). Clump division involves transplanting a soil clump containing culms and rhizomes collected from a donor canebrake (McClure 1966). Although this technique has a high success rate (96.3%; Baldwin et al. 2009), it is labor intensive and cost-prohibitive for most restoration projects. Collection of seed is difficult and unpredictable given the infrequency and inconsistency of cane flowering and subsequent fruiting. Micro-propagation techniques, although promising, have thus far failed to successfully regenerate root tissue (Baldwin et al. 2009). Currently, macro-propagation holds the most immediate promise for producing propagules for restoration projects (Baldwin et al 2009).

In macro-propagation, rhizomes are extracted from potted mother plants from donor canebrakes, clipped into multiple-internode sections, and planted in containers with a nursery growing media combination of peat moss, sand, and/or shredded pine bark (Baldwin et al. 2009). Baldwin et al. (2009) demonstrated that individual mother plants have the potential to yield up to 400 viable clones when harvesting single-node rhizome clippings and have achieved up to 88.3% rhizome shooting success when harvesting 2- to

3-node rhizome clippings. However, to create a sufficient supply of propagules for cost-effective, large-scale projects, macro-propagation protocols must be further refined and the resulting information and technology transferred to private producers. Development of a more precise temporal schedule and more thorough guidelines for cane macro-propagation are needed. Particularly, optimal propagation timing, overwintering start dates and facility standards, optimal rhizome planting orientations and container types, and increased mother plant genetic and geographic diversity are necessary. Currently, macro-propagation is a costly method that needs streamlining to become commercially viable.

I used methodology of Baldwin et al. (2009) and Hamlington and Smith (Auburn University, unpublished report) to further investigate cane macro-propagation. The objectives of this study were to determine if 1) rhizome production was influenced by mother plant collection site and time-since-mother-plant-transplantation, 2) differences in propagule survival, growth rate, or final cumulative aboveground size were influenced by mother plant collection site, and 3) container type (pot vs. plug) influenced propagule survival, growth rate, or final cumulative aboveground size.

STUDY AREA

Research was conducted in two stages: 1) mother plant collections from donor canebrakes in northern, central, and southern Alabama and western Tennessee (2010-2013) and the potting and storage of these mother plants at Roundstone Native Seed, LLC in Upton, Kentucky (2010-2015), and 2) rhizome harvest (macro-propagation) from mother plants stored at Roundstone Native Seed, LLC (2013-2015). Mother plants were collected in a previous cane propagation study in 2010-2012 (Hamlington and Smith, unpublished report) and from my study in 2013 (Fig. 1.1).

Mother Plant Collection Sites

Jackson County (*JONES, SIMMS, SWAIM, and WHITAKER*).—Mother plants were harvested from four canebrakes in Jackson County, Alabama, USA, located in the northeastern corner of Alabama. Jackson County is in the United States Department of Agriculture (USDA) temperature zones 7a and 7b and the Jackson County Mountains district of the Cumberland Plateau physiographic section (Neilson 2007). The mean annual temperature of Jackson County is 15.7° C with a mean annual maximum of 21.7° C and mean annual minimum of 10.0° C. The mean annual precipitation of Jackson County is 144.78 cm with 7.62 cm of precipitation from snowfall (CLRsearch [CLR] 2012). The JONES site was located northwest of the intersection of US Hwy 72 and State Hwy 65 on private property with soils classified as Melvin silty clay (Web Soil Survey [WSS] 2015). The SIMMS and SWAIM sites were located on the Skyline Wildlife Management Area with soils classified as Egam silty clay loam and Huntington silt loam, respectively (WSS 2015). The WHITAKER site was located on the Roy B. Whitaker Paint Rock River Preserve north of US Hwy 72 between the towns of Paint Rock, AL and Gurley, AL with soils classified as Melvin silty clay (WSS 2015).

Dallas County (*HABA*).—Mother plants were collected from one site in Dallas County (HABA) in central Alabama in the Old Cahawba Archaeological Park along the Cahaba River where the soils are classified as udifluvents (WSS 2015). Dallas County is located in the USDA temperature zone 8a in the Coastal Plain physiographic region (Neilson 2007). The mean annual temperature of Dallas County is 19.72° C, with a mean annual maximum of 25.0° C and a mean annual minimum of 13.89° C. The mean annual precipitation of Dallas County is 162.56 cm with 2.54 cm of precipitation from snowfall (CLR 2012).

Conecuh National Forest (*CONECUH and DIXON*).—Mother plants were collected from three canebrakes in Conecuh National Forest in Covington County of southern Alabama. Covington County is located in USDA temperature zone 8a in the Coastal Plain physiographic region (Neilson 2007). The mean annual temperature of Covington County is 19.72° C with a mean annual maximum of 25.0° C and a mean annual minimum of 13.89° C. The mean annual precipitation of Covington County is 162.56 cm with 2.5 cm of precipitation from snowfall (CLR 2012). In 2012, CONECUH mother plants were collected from a canebrake in Conecuh National Forest with soils classified as Troup fine sands (WSS 2015). In 2013, mother plants were collected from two canebrakes (DIXON) at the Auburn University Solon Dixon Forestry Education Center in Andalusia, Alabama, USA. The soils there are classified as Troup fine sands (WSS 2015).

Western Tennessee (*BATEMAN, HATCHIE, TULLY1, and TULLY 2*).—Mother plants were collected from Lauderdale, Fayette, and Haywood Counties in western Tennessee. Lauderdale County is located in the USDA temperature zone 7b in the Mississippi Alluvial Valley physiographic region (Etnier and Starnes 1993). The mean annual temperature of Lauderdale County is 16.83° C with a mean annual maximum of 22.22° C and a mean annual minimum of 11.11° C. The mean annual precipitation of Lauderdale County is 132.08 cm with 12.7 cm of precipitation from snowfall (CLR 2012). Mother plants were collected in 2011 and 2012 from Lauderdale County at John

Tully Wildlife Management Area (TULLY1 and TULLY2), with soils there classified as Robinsonville silt loam (WSS 2015).

Fayette and Haywood Counties are located in the USDA temperature zone 7b in the Inner Coastal Plain physiographic region (Etnier and Starnes 1993). The mean annual temperature of Fayette and Haywood Counties is 16.83° C with a mean annual maximum of 22.22° C and a mean annual minimum of 11.11° C. The mean annual precipitation of Fayette and Haywood Counties is 132.08 cm with 12.7 cm of precipitation from snowfall (CLR 2012). Mother plants were collected from a site located on Bateman Road near the bridge of the Wolf River on Wolf River Wildlife Management Area in Fayette County (BATEMAN) with soils there classified as Waverly silt loam (WSS 2015). Mother plants were collected on Hatchie National Wildlife Refuge 6.4 km south of Brownsville in Haywood County (HATCHIE) with soils there classified as Routon silt loam (WSS 2015)

Roundstone Native Seed, LLC

Macro-propagation, which involves the use of rhizome cuttings from potted mother plants, occurred at Roundstone Native Seed, LLC, a commercial producer and supplier of native seed and plants in Upton, Kentucky, Hardin County, USA. Hardin County is in the USDA temperature zone 6b, located in the Knobs region on the western side of the Outer Bluegrass Region physiographic section (Elbon 2015). The mean annual temperature of Hardin County is 13.39° C with an annual maximum average of 18.89° C and an annual minimum average of 7.78° C. The mean annual precipitation of Hardin County is 111.76 cm with 40.64 cm of precipitation from snowfall (CLR 2012).

METHODS

Mother Plant Collection

In order to facilitate genetic diversity of planting stock during macro-propagation trials, a mother plant collection of multiple genotypes from various locations was assembled (Table 1.1). I used mother plants from a previous study (Hamlington and Smith, unpublished report) along with new mother plants collected during this study to create this diverse source of genetics. Because mother plants were collected over several years, mother plant age-since-transplantation, or number of years since being extracted from a donor canebrake and potted, ranged from 1-4 years.

During mid- to late-winter 2013, I selected portions of canebrakes that contained multiple culms (aboveground stems) <2 m tall in an approximately 35-cm diameter area for mother plant collection. I extracted mother plants using round point shovels that were inserted with the blade perpendicular to the ground to a depth of approximately 25 cm. I extracted a 35-cm diameter clump of soil and rhizosphere containing >3 culms from the ground by inserting the shovel blade beneath the cane clump and pushing downward around the clump, then prying upward. Immediately following extraction, I placed cane clumps into 55-cm diameter, 0.05-mm thick white plastic Polypipe® (Chicot Irrigation, Lake Village Arkansas) irrigation tubes. I sealed the tubes at one end to create bags with 35.6-cm zip ties and added approximately 1 L of water to each Polypipe bag to reduce transpiration loss and embolism. Lastly, I sealed the opposite end of the bag with another 35.6-cm zip tie (Baldwin et al. 2009). I transported the sealed Polypipe bags containing cane clumps from the collection site within 48 hours of extraction to Roundstone Native Seed, LLC in Upton, Kentucky using a 7.3-m U-Haul truck.

I removed mother plants from the Polypipe bags 48-72 hours after extraction and placed them into 35-cm diameter, 24.66-L liner pots. I added a 1:1 mixture of sand and peat moss and additional granular micronutrients (MicroMax®, Everris NA, Inc.) to the intact, native soil clump within each pot. I placed the liner pots on the ground in 4- to 7-pot wide rows, touching each other, along the east-facing side of a barn to shade and protect the plants from the wind, thereby preventing desiccation. For a week following transportation, a watering crew hand-watered plants daily until the soil in the pots was saturated. Pots were watered every other day for the remainder of the growing season (March to September). During the winter season, mother plants remained on the east-facing side of the barn and were hand-watered as needed. I placed hay and mulch between the rows for insulation in the colder months.

Rhizome Harvest

During mid-March of each year (2013, 2014) I extracted all mother plants by hand from their liner pots and exposed rhizomes were clipped with hand pruners from the outer soil clump edges. I left ≥3 rhizome nodes per culm on the mother plant to sustain future growth. If mother plants had >10 culms/pot, or rhizomes were protruding from the bottom of the pot, they were considered root bound and were divided into multiple clumps following rhizome harvest, placed into new liner pots, and returned to the east-facing side of the barn. I further divided rhizomes into 3- to 5-node sections (hereafter propagules) and soaked them overnight in 18.9 L Ziploc® bags with a solution containing 0.20 g of Strike® 50 WDG fungicide (OHP Inc., Mainland, PA) per 3.8 L of water. I conducted this process separately for each of the 11 mother plant collection sites and recorded the number of 3- to 5-node rhizome sections harvested from each mother plant. A portion of mother plants did not produce any propagules.

The following morning, I removed the sheaths covering the axial buds of each propagule by hand and planted the propagules in containers. In 2013, I used either 25-cell IP 200 Rigipot ™ plastic nursery plug trays with 4.75-cm cell diameter and 12-cm deep plug cavities or trays with 18 individual 10.16-cm × 10.16-cm square pots. I planted propagules in plug trays vertically to accommodate the container shape with the axial buds facing upward and the most distally harvested bud (tips point opposite direction of mother plant culm) exposed to sunlight. I planted propagules in pot trays horizontally and covered the propagules with approximately 0.5 cm of growing media. In 2014, only IP 200 Rigipot plastic nursery trays were used. In 2013 and 2014, I filled all trays with a 4:3:3 ratio of fresh, finely shredded pine bark, peat moss, and sand. This mixture facilitated adequate drainage and rhizome growth (Cirtain et al. 2009). I marked the upper right corner of each tray to identify trays by mother plant collection site and individual tray number.

I completed all statistical tests with SAS 9.4 for Windows (SAS Institute, Inc., Cary, NC). Because rhizome production was a count, I used a negative binomial regression (PROC GENMOD, SAS 9.4) to test differences in the number of propagules yielded from mother plants (mean rhizome production). Mother plant collection site, age-since-transplantation, and the interaction between collection site and age were considered fixed effects. If I observed an interaction, I conducted separate negative binomial regression models for each age class (Age 1 to 4), with mother plant collection site as the fixed effect due to the collinearity of age-since-transplantation and mother plant collection site. Where differences ($P < 0.05$) occurred, I used a Wald chi-square test to compare mean rhizome production among collection sites and reported the corresponding Z-test statistic. Only rhizome-producing mother plants were included in the negative binomial regression models ($n = 752$). Additionally, I conducted a simple regression (PROC REG, SAS 9.4) on mean rhizome production of distance of collection sites (in km) from Roundstone Native Seed, LLC to test differences between the number of propagules yielded from mother plants harvested from different distances from the propagation site.

Propagule Survival and Growth

In April 2013, the month following rhizome harvest, I transported all trays from the east-facing side of the barn to an outdoor 30.5-m × 6.1-m shade house with 60% shade cloth (GEMPLER's; Janesville, Wisconsin) on the top, east, and west-facing sides where they were held until being out-planted in early 2014. In 2014, I placed propagules in the shade house immediately following macro-propagation. Trays were arranged randomly by tray number within the shade house and watered by hand with a garden hose every day for 2 weeks following transplantation and every other day for the remainder of the growing season (March to September). Propagules began sprouting approximately 8 weeks following propagation and monthly propagule survival and growth data were collected from June to September.

I recorded all propagules as alive or dead and sprouting or non-sprouting. I considered a propagule alive if the rhizome section was green (photosynthetic) or if it was actively sprouting. I calculated overall survival using a binary code, with an individual propagule receiving a 1 if alive or a 0 if not alive. Survival was calculated each month as the number of alive propagules divided by the total number of planted propagules. I considered a propagule sprouting if ≥1 green, living shoot was present. Due to the parameters I used to determine a propagule being alive, it was possible that increased sprouting throughout the data collection period could have led to increased survival over the growing season. Data were collected on cumulative monthly aboveground growth from a random sample of sprouting propagules (*n* = 1,502). I measured initial size in mid-June 2013 and final size in mid-September 2013. I measured (to the nearest 0.1 cm) from the sprouting bud to the base of the terminal leaves using a meter stick for every sprout on each propagule. I calculated the total cumulative aboveground size during each month by the sum of all sprout measurements on each propagule. If a sprout died during the growth data collection it was no longer measured and was considered a decrease in cumulative aboveground size. Growth rate was determined by the difference in total cumulative aboveground size (cm) among monthly measurements.

Because propagule survival was binary (i.e. “alive” or “dead”), I used a generalized linear mixed model with a binomial distribution (PROC GLIMMIX, SAS 9.4) to test differences in final propagule survival among mother plant collection sites. Collection site was considered a fixed effect while individual propagules nested within trays was a random block effect. I used a linear mixed effects model with repeated measures and a linear mixed effects model (PROC MIXED, SAS 9.4) to test for differences between propagule growth rates and final propagule aboveground size among mother plant collection sites, respectively. I considered repeated measurements on individual propagules as a random effect when testing growth rate whereas I considered individual propagules as a random effect when testing final size. Where differences (*P* = < 0.05) occurred, I determined the least-squares means and conducted pairwise t-tests, using a Tukey's p-value adjustment, to compare differences among mean survival, growth rate, and final size among collection sites. I only included 2013 propagule survival and growth data due to extreme winter weather in 2014 that resulted in markedly low propagule survival, sprouting, and cumulative aboveground size for propagules from the 2014 macro-propagation. For growth rate, I only used measurements from trials 2-4 (July to September); therefore, I only included propagules that survived for >1 trial period (*n* = 1,052). For comparing final cumulative aboveground size among collection sites I only used measurements from trial 4 (September); therefore, I only used propagules that survived for the entire data collection period (*n* = 866). Additionally, I conducted a simple regression (PROC REG, SAS 9.4) on mean propagule survival with distance to site of mother plant collection (in km) from Roundstone Native Seed, LLC.

Comparison of Pots and Plugs

In 2013, I simultaneously tested differences in propagule survival, growth rate, and final size between two container types by planting a subsample (*n* = 1,258) of propagules from two study sites (TULLY 1, Age 2; TULLY 2, Age 1) in 10.16-cm × 10.16-cm square pots. I planted pot rhizome sections horizontally in trays containing 18 individual pots. I covered pot rhizome sections entirely with approximately 0.5 cm of growing media. I planted the remainder of the rhizome sections from TULLLY 1 and TULLY 2 (*n* = 3,897*)* vertically in 25-cell IP 200 Rigipot nursery trays oriented with the axial buds facing upward and the most distally harvested bud exposed to sunlight.

Because propagule survival was binary, I used a generalized linear mixed model with a binomial distribution (PROC GLIMMIX, SAS 9.4) to test differences in propagule survival between container types. Container type was considered a fixed effect whereas propagules nested within a tray was considered a random block effect. I used a linear mixed effects model with repeated measures and a linear mixed effects model (PROC MIXED, SAS 9.4) to test differences in propagule growth rate and final aboveground size between container types, respectively. For growth rate, I only used measurements from trials 2-4 (July to September). For comparing final cumulative aboveground size among collection sites I only used measurements from trial 4 (September). Container type was considered a fixed effect in both models, while I considered the repeated measures of individual growth trial measurements on propagules as a random effect when testing growth rate. I considered the blocking effect of individual propagules as a random effect when testing final size. Where differences ($P = < 0.05$) occurred, I determined the least-squares means and conducted pairwise t-tests, using a Tukey's p-value adjustment, to compare differences in mean survival, growth rate, and final size between container types. For growth rate, I only used measurements from trials 2-4 (July to September; therefore, I only included propagules that survived for >1 trial period (n = 792). For final cumulative aboveground size, I only used measurements from trial 4 (September); therefore, I only included propagules that survived for the entire data collection period (n = 434).

RESULTS

In 2013 and 2014, respectively, 76% and 63% of mother plants produced harvestable rhizomes. The percentage of producer mother plants varied by site in 2013

(Range: 43-95%) and 2014 (Range: 0-89%). During the 2013 rhizome harvest, all but three sites (BATEMAN, CONECUH, and SWAIM) had >70% of mother plants producing harvestable rhizomes, whereas only two sites (HABA and TULLY 1) had >70% of mother plants producing harvestable rhizomes in 2014. In 2013, I harvested 7,211 rhizome sections from 440 mother plants ($\bar{x} = 21.30$, SE = 0.94 rhizome sections per mother plant) whereas in 2014 I only harvested 4,158 rhizome sections from 641 mother plants ($\bar{x} = 10.02$, SE = 0.45 rhizome sections per mother plant).

Rhizome Harvest

I observed an interaction between age since mother plant transplantation and mother plant collection site ($\chi^2_6 = 45.96$, $P < 0.001$) on mean rhizome production. Rhizome production differed among mother plant collection sites within Age 1 plants ($\chi^2_3 = 105.84$, $P \leq 0.001$), Age 2 plants ($\chi^2_5 = 113.42$, $P \leq 0.001$), and Age 3 plants ($\chi^2_6 = 17.31$, $P = 0.008$), but not Age 4 plants ($\chi^2_2 = 2.68$, $P = 0.26$). For Age 1 mother plants, the mean number of harvested rhizomes from CONECUH ($\bar{x} = 7.83$, SE = 1.08) and DIXON ($\bar{x} = 6.87$, SE = 0.60) were similar ($Z_{3,221} = 0.81$, $P = 0.81$) and the lowest number of rhizomes per producer mother plant. HABA ($\bar{x} = 22.35$, SE = 2.69) and TULLY 2 ($\bar{x} = 25.10$, SE = 2.44) were similar ($Z_{3,221} = -0.75$, $P = 0.45$) and yielded the most rhizomes per producing mother plant (Table 1.3). Yield from both CONECUH and DIXON was lower than yield from HABA ($Z_{3,221} = -5.74$, $P \leq 0.001$; $Z_{3,221} = -1.18$, $P \leq 0.001$) and TULLY 2 ($Z_{3,221} = -6.92$, $P \leq 0.001$; $Z_{3,221} = -1.30$, $P \leq 0.001$). For Age 2 mother plants, TULLY 1 yielded the most rhizomes per mother plant ($\bar{x} = 27.22$, SE =1.80) and differed from all other sites (Table 1.3). Additionally, TULLY 2 ($\bar{x} = 7.97$, SE = 0.79) yielded the fewest rhizomes and differed from CONECUH ($\bar{x} = 11.98$, SE = 1.43;

$Z_{5,297} = 2.62$, $P = \leq 0.001$) and HABA ($\bar{x} = 11.56$, SE = 1.51; $Z_{5,297} = 2.26$, $P = 0.02$). For Age 3 mother plants, HATCHIE ($\bar{x} = 3.80$, SE = 1.74) differed from all other sites (Table 1.3). WHITAKER ($\bar{x} = 20.63$, SE = 4.31) yielded the most rhizomes and differed from BATEMAN ($Z_{6,189} = -2.33$, $P = 0.02$), HATCHIE ($Z_{6,189} = 0.50$, $P \leq 0.001$), SIMMS ($\bar{x} = 6.17$, SE = 2.45; $Z_{6,189} = -2.69$, $P = 0.007$), and TULLY 1 ($\bar{x} = 12.05$, SE = 0.92; $Z_{6,189} = -2.41$, $P = 0.02$), but not SWAIM ($\bar{x} = 17.67$, SE = 9.34; $Z_{6,189} = -0.27$, $P = 0.78$), or JONES ($\bar{x} = 11.31$, SE = 2.93; $Z_{6,189} = 1.81$, $P = 0.08$). For Age 4 mother plants, there was no difference in rhizome production among JONES ($\bar{x} = 7.80$, SE = 1.97), SIMMS ($\bar{x} = 18.00$, SE = 8.93), and WHITAKER ($\bar{x} = 11.05$, SE = 1.25; Table 1.3). Additionally, there was no relationship between rhizome production and distance from Roundstone Native Seed, LLC ($t_{1,9} = -1.01$, $R^2 = 0.10$, $P = 0.34$).

Propagule Survival and Growth

In mid-September 2013, 33% ($n = 2,363$) of the 7,211 propagules remained alive whereas in 2014 only 4.5% ($n = 185$) of the 4,158 propagules remained alive (Table 1.4). Propagule survival in 2013 (Range: 0.0-0.41) differed by mother plant collection site ($F_{10,35} = 21.65$, $P \leq 0.001$; Table 1.5). TULLY 1 ($\bar{x} = 0.40$, SE = 0.01) had the greatest survival while DIXON ($\bar{x} = 0.01$, SE = 0.01), SWAIM ($\bar{x} = 0.02$, SE = 0.02), BATEMAN, ($\bar{x} = 0.03$, SE = 0.02), and CONECUH ($\bar{x} = 0.05$, SE = 0.01) were similar and had the lowest survival (Table 1.5). There was no trend between propagule survival and distance from Roundstone Native Seed, LLC ($R^2 = 0.19$, $P = 0.18$).

Propagule growth rates in 2013 (Range: -4.80 to 32.24 cm; Table 1.6) were similar among mother plant collection sites ($F_{10,2} = 4.51$, $P = 0.20$). There were differences between propagule initial and final size in 2013 and 2014 propagules (Table

1.7). In 2013, propagule final size differed among mother plant collection sites ($F_{9,123}$ = 9.01, $P \leq 0.001$; Table 1.8) with HABA ($\bar{x}$ = 32.24, SE = 1.17) yielding the largest plants and BATEMAN ($\bar{x}$ = 12.40, SE = 10.91) yielding the smallest plants.

Comparison of Pots and Plugs

Propagules planted in plugs ($\bar{x}$ = 0.41, SE = 0.01) were 2.22 (± 0.24; 95% CI) times more likely to survive than those in pots ($\bar{x}$ = 0.25, SE = 0.01; $F_{1,1}$ = 101.44, P = 0.06; Table 1.9). However, growth rates between plugs ($\bar{x}$ = 0.75 cm, SE = 0.15) and pots ($\bar{x}$ = 0.99 cm, SE = 0.28) were similar ($F_{1,790}$ = 0.80, P = 0.37). Final size was 2.63 cm (± 0.92; 95% CI) greater for plugs than pots ($F_{1,254}$ = 7.49, P = 0.007).

DISCUSSION

These results suggest that mother plant collection site strongly influences rhizome production. Although mother plant collection sites were not tested for individual genotypes, all collection sites were located >10 km apart and were likely not the same clone. Differences in survival and growth among cane and bamboo have been demonstrated when propagules arise from different genotypes and from ontogenetically (level of development/age) unique canebrakes (Bell 2000, Brendecke and Zaczek 2008). Effect of mother plant collection site on rhizome production was likely due to genetic differences among sites and possibly the proximity of the site to the location of propagation (i.e., more production from mother plant collection sites closer to the macro-propagation facility). Although neither relationship in this study was significant, a negative trend may exist between rhizome production and propagule survival as distance from macro-propagation site increases in other studies. Subsequently, distance from mother plant collection sites to the macro-propagation facility may need to be considered in restoration efforts (i.e., local restoration with local plants/genotypes). It should be noted that the potential collinearity between mother plant collection site and age-since-transplantation may have influenced mean rhizome production results by inflating the standard errors, thereby creating more conservative estimates of differences in rhizome production among mother plant collection sites.

Mother plants potted for less years yielded more rhizomes sections than mother plants that had been potted longer (Table 1.3). Zaczek et al. (2009) hypothesized that older stands of cane typically have rhizomes with fewer buds at the nodes than younger stands. Therefore, rhizome sections harvested from more recently potted mother plants, or mother plants that are younger upon extraction, may have a greater probability of producing surviving culms. In greenhouse trials, Brendecke and Zaczek (2008) found rhizomes from younger plants had greater survival, more buds, and a greater probability of a bud producing a culm.

My results regarding mother plant collection site’s effect on rhizome production among age classes also suggest that rhizome production, and therefore feasible planting stock, may taper with age. This result suggests that restoration projects should annually harvest new mother plants, particularly at collection sites with demonstratively greater mean yields, for maximal rhizome production from fresh rhizomes. Although annual harvest will increase the cost of production, it is worth re-harvesting mother plants due to the dramatic decrease in rhizome production over time. However, developing ways to increase rhizome production in subsequent years from the same mother plants could offset this increase in cost. Future research regarding aboveground biomass (i.e. culm height and density) effect on rhizome production should be conducted.

Due to the effect of mother plant collection site on propagule survival, it would be advantageous to select harvest sites with demonstrated greater propagule survival percentages. In this study, western Tennessee and northern and central Alabama propagules had consistently greater survival and final size than southern Alabama propagules (Table 1.5; Table 1.7). Dalzotto (2013) found that larger propagules have a greater likelihood of survival when out-planted. Furthermore, Zaczek et al. (2009) found that propagules that had taller culms in the greenhouse prior to out-planting had greater survival five years after out-planting. Mother plant collection site did not have an effect on growth rate, but did have an effect on final cumulative aboveground size. Therefore, when making recurrent collections, it would be advantageous to select for collections sites based on the final size of propagules and overall propagule survival. I hypothesize the difference in final aboveground size was due to collection sites differing in their initial sprouting dates. Earlier initial sprouting dates would theoretically result in larger, more competitive cane propagules upon out-planting.

Greater propagule survival in plugs may be due to the rhizome's vertical planting orientation and/or increased depth of container allowing for additional root development. Although container type did not affect propagule growth rate, propagules planted in plug containers produced taller propagules. Zaczek et al. (2004) found that exposure of rhizomes to sunlight increased the number of culms produced. The earlier sprouting in the plug containers may be due to exposure of a portion of the rhizome section to sunlight allowing photosynthesis or bud break to occur faster than in the buried, horizontal rhizome sections in pots (Sexton et al. 2003, Zaczek et al. 2004). Greater survival percentages and larger propagules, along with the fact that plug containers are easier to out-plant due to their compactness, better shape for planting tools and handling, and potential for machine planting suggests favoring their use (Zaczek et al. 2004, Zaczek et al. 2009).

The conspicuously low propagule survival in 2014 suggests that much of the mother plants' rhizomatous tissue harvested during March 2014 propagation was actually dead. I attribute this mortality to sustained below-average winter temperatures from November 2013-February 2014 at Roundstone Native Seed, LLC. Propagules and mother plants experienced below-freezing temperatures 14 days in November, 20 days in December, 26 days in January, and 21 days in February (Western Kentucky University Kentucky Mesonet [WKU KM] 2015). Average monthly temperatures were substantially lower than the previous two winters. Specifically, average temperatures in November 2013 were 0.0° C and 3.55° C colder than November 2011 and 2012 while December 2013 was 2.78° C and 1.5° C colder than December 2011 and 2012. Additionally, January 2014 was 5.11° C and 5.83° C colder than January 2012 and 2013 while February 2014 was 2.05° C and 4.55° C colder than February 2012 and 2013 (WKU KM 2015). Furthermore, mother plants and therefore 2014-derived propagules in winter 2013-2014 experienced below-average minimum temperatures compared to the winters of 2011-2012 and 2012-2013. Between November 2013 and February 2014, plants experienced 20 days of -9.44° C (15° F), and one day below -17.78° C (0° F) as opposed to only two days of -9.44° C in winters 2011-2012 and 2012-2013. Observations indicated that native cane growing in the same Kentucky location experienced die back from the hard freezes as well (John Seymour, personal communication). Historically, below-average winter temperatures have resulted in the die back of cane (Winterringer

1952, Marsh 1977). This is further indicated by substantially lower sprouting, cumulative aboveground size, mean propagule final size in the 2014 propagules compared to the 2013 propagules, and the complete mortality of potted mother plants in early 2014. Although mother plant pots were surrounded by hay bales and mulch, it may be beneficial to house mother plants in a pot-in-pot array for improved insulation and protection from extreme winter temperatures (Baldwin et al. 2009). Additionally, propagules should be housed indoors (i.e., greenhouse) during the winter months to further protect planting stock from extreme cold.

Future research should examine propagation containers for a size and shape that improves propagule survival and growth. Whereas these results suggest that plugs were better containers than pots for production of viable and larger cane propagules, different plug cavity sizes and dimensions may yield even greater differences in survival and growth. Smaller diameter plugs may provide greater plug soil and root cohesion and maximize the amount of plugs per tray.

MANAGEMENT IMPLICATIONS

Due to limited planting options, many private landowners are restricted to planting bottomland hardwoods, particularly oaks, in riparian areas and cane is often forgotten as an option for restoration projects (Allen 1997). If a large-scale, cost-effective macro-propagation process for cane is developed, private landowners and natural resources agencies will have an additional option for planting. In addition, cane could be used for government programs such as the Agriculture Conservation Easement Program’s Wetland Reserve Easements and could contribute to wildlife habitat improvement and diversity, water quality improvement, and the conservation of a declining, native plant community. Multiple studies indicate that macro-propagation may be used to generate plantable stock for cane, thereby increasing the feasibility, rapidity, and success of restoration efforts (Sexton et al. 2002, Zaczek et al. 2004, Zaczek et al. 2009). Plug containers show greater promise for cane macro-propagation due to increased propagule survival, greater propagule final size, and a more practical shape for handling and planting. Furthermore, more ergonomic trays or container types may be useful to simplify and expedite the out-planting process. For example, in this study, trays only contained 1-6 viable plugs. If using trays with individual, interchangeable or removable plug cells rather than fixed position plug cells, individual successful plugs could be consolidated into the same tray which may minimize transport space. Additionally, frequent (annual) mother plant harvest from collection sites with demonstrative superior rhizome harvest production, propagule survival, and propagule final size should be facilitated while maintaining genetic and geographic diversity (i.e., harvesting from multiple mother plant collection sites in multiple USDA plant zones). If a canebrake's life history is known, targeting younger, more vigorous donor canebrakes with dense culms may increase potential rhizome production.

Based on these results, I would suggest using cane macro-propagation rather than clump division for restoration projects due to a potentially continuous supply of propagules and less labor-intensive methodology. However, current macro-propagation technology is still cost-prohibitive and labor intensive for restoration projects greater than 10 ha. In this study, 441 mother plants produced 7,211 rhizome sections of which ~2,300 survived to be viable, plantable propagules. At 3.6-m x 3.6-m spacing, 750 propagules are needed per ha for a total of 7,500 propagules to restore 10 ha. Future research should focus on development of micro-propagation technology via cane tissue cultures for large-scale, range-wide restoration requiring 10,000s-100,000s propagules.

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Table. 1.1. Mother plant collection site by year collected and area collected, 2010-2014.

Collection Site	Year	Area
Bateman	2011	Western Tennessee
Conecuh	2012	Southern Alabama
Dixon	2013	Southern Alabama
Haba	2012	Central Alabama
Hatchie	2011	Western Tennessee
Jones	2010	Northern Alabama
Simms	2010	Northern Alabama
Swaim	2010	Northern Alabama
Tully 1	2011	Western Tennessee
Tully 2	2012	Western Tennessee
Whitaker	2010	Northern Alabama

Table 1.2. Number of mother plants and mean number of 3- to 5-node rhizome sections (propagules) harvested per mother plant by collection site and year, Roundstone Native Seed, LLC, Upton, KY, 2013-2014.

	2013			2014		
Site	*n*	$\bar{x}$	SE	*n*	$\bar{x}$	SE
Bateman	7	8.29	2.23	2	3.50	0.50
Conecuh	35	7.83	1.06	43	11.98	1.83
Dixon	n/a	n/a	n/a	89	6.87	0.69
Haba	40	22.35	2.09	36	11.56	1.33
Hatchie	21	10.10	1.76	5	3.80	0.97
Jones	13	11.31	1.80	5	7.80	1.77
Simms	6	6.17	1.35	1	18.00	0.00
Swaim	3	17.67	8.69	0	0.00	0.00
Tully 1	130	27.22	1.54	148	12.05	0.85
Tully 2	61	25.10	2.57	66	7.97	1.06
Whitaker	19	20.63	4.25	22	11.05	1.43

Table 1.3. Cane propagules per mother plant (least-squares means) comparisons by age class (1-4) across collection sites, Roundstone Native Seed, LLC, Upton, KY, mid-September 2013 and mid-September 2014.

	Age 1		Age 2		Age 3		Age 4	
Site	LSMEANS	SE	LSMEANS	SE	LSMEANS	SE	LSMEANS	SE
Bateman			8.29[a]	2.53	3.50[a]	2.56		
Conecuh	7.83[a]	1.08	11.98[b]	1.43				
Dixon Center	6.87[a]	0.60						
Haba	22.35[b]	2.69	11.56[b]	1.51				
Hatchie			10.10[a]	1.75	3.80[b]	1.74		
Jones					11.31[c]	2.93	7.80[a]	1.97
Simms					6.17[d]	2.45	18.00[a]	8.93

Swaim					17.67^{c}	9.34		
Tully 1			27.22^{c}	1.80	12.05^{d}	0.92		
Tully 2	25.10^{b}	2.44	7.97^{a}	0.79				
Whitaker					20.63^{e}	4.31	11.05^{a}	1.25

[a] lack of common superscript signifies significant differences ($P < 0.05$).

Table 1.4. Cane propagule survival (%) by month and collection site at Roundstone Native Seed, LLC, Upton, KY, June to September 2013, 2014.

	2013					2014				
Site	*N*[a]	Jun	Jul	Aug	Sep	*N*[a]	Jun	Jul	Aug	Sep
Bateman	60	0.22	0.08	0.03	0.03	9	0.11	0.00	0.00	0.00
Conecuh	256	0.70	0.43	0.13	0.06	500	0.05	0.02	0.01	0.01
Dixon	108	0.46	0.27	0.07	0.01	900	0.42	0.15	0.07	0.04
Haba	875	0.79	0.47	0.35	0.30	400	0.16	0.07	0.03	0.01
Hatchie	185	0.52	0.30	0.17	0.14	16	0.31	0.31	0.13	0.13
Jones	144	0.31	0.23	0.19	0.18	37	0.24	0.19	0.05	0.03
Simms	25	0.48	0.32	0.28	0.20	21	0.00	0.00	0.00	0.00

Swaim	50	0.12	0.02	0.06	0.02	0	0.00	0.00	0.00	0.00
Tully 1	3594	0.73	0.52	0.45	0.41	1575	0.29	0.17	0.09	0.06
Tully 2	1561	0.67	0.49	0.43	0.39	500	0.23	0.15	0.05	0.03
Whitaker	353	0.73	0.52	0.40	0.34	200	0.16	0.07	0.02	0.02
Total[b]	7211	0.67	0.45	0.37	0.33	4158	0.26	0.15	0.07	0.04

[a] initial number of propagules as of March at time of harvest

[b] not weighted

Table 1.5. Percent survival of cane propagules from different mother plant collection sites (least-squares means) in descending order, Roundstone Native Seed, LLC, Upton, KY, September 2013.

Site	LSMEANS	SE
Tully 1	0.40^{abd}	0.01
Whitaker	0.34^{abcd}	0.03
Tully 2	0.31^{bcd}	0.01
Haba	0.29^{bcd}	0.02
Simms	0.20^{abcdef}	0.08
Jones	0.18^{def}	0.03
Hatchie	0.14^{defh}	0.03
Conecuh	0.05^{gh}	0.01
Bateman	0.03^{gh}	0.02
Swaim	0.02^{fgh}	0.02
Dixon	0.01^{gh}	0.01

[a] lack of common superscript signifies significant differences ($P < 0.05$).

Table 1.6. Cane propagule net growth rates (mean and SE) in cm/month by collection site, Roundstone Native Seed, LLC, Upton, KY, July-September 2013.

	Jul		Aug		Sep	
Site	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
Bateman	0.10	n/a	1.00	n/a	1.20	n/a
Conecuh	-4.80	0.92	-0.13	0.33	1.00	0.20
Dixon	-3.85	0.94	-1.05	2.52	0.00	n/a
Haba	-3.45	0.73	-0.43	0.69	1.24	1.66
Hatchie	-1.11	0.81	2.28	0.71	-0.19	0.70
Jones	0.02	0.53	1.21	0.45	0.34	0.19
Simms	1.05	1.52	4.30	1.50	2.90	2.14
Swaim	-0.17	0.27	-0.43	0.47	0.90	n/a
Tully 1	-1.56	0.25	1.94	0.22	2.03	0.34
Tully 2	-0.81	0.35	2.30	0.39	1.45	0.49
Whitaker	0.85	0.89	1.31	0.44	2.19	1.39

Table 1.7. Mean initial and final size (cm) of propagules by year and mother plant collection site, Roundstone Native Seed, LLC, Upton, KY, June-September 2013, 2014.

	2013						2014					
	Initial Size			Final Size			Initial Size			Final Size		
Site	*n*	$\bar{x}$	SE	*n*	$\bar{x}$	SE	*n*	$\bar{x}$	SE	*n*	$\bar{x}$	SE
Bateman	2	9.35	0.75	1	12.40[a]	n/a	0	0.00	n/a	0	0.00	n/a
Conecuh	56	17.59	1.24	3	19.17	2.40	1	7.00[a]	n/a	1	11.20[a]	n/a
Dixon	25	14.58	1.19	0	0.00	n/a	6	4.00	0.23	1	5.50[a]	n/a
Haba	198	29.99	1.25	87	32.24	1.53	0	0.00	n/a	0	0.00	n/a

Hatchie	25	13.81	1.35	16	17.45	1.47	2	12.25	1.75	2	15.70	1.30
Jones	22	11.51	1.12	15	13.56	1.21	0	0.00	n/a	0	0.00	n/a
Simms	5	9.72	1.46	4	19.40	3.44	0	0.00	n/a	0	0.00	n/a
Swaim	5	12.30	1.96	1	18.50[a]	n/a	0	0.00	n/a	0	0.00	n/a
Tully 1	771	17.57	0.38	486	22.36	0.48	22	4.39	0.61	5	11.48	1.79
Tully 2	318	18.51	0.56	204	23.91	0.75	1	4.50[a]	n/a	1	4.00[a]	n/a
Whitaker	75	17.66	1.09	49	23.06	1.33	4	19.50	2.72	3	27.73	2.71
Total	1502	19.15	0.31	866	23.47	0.39	36	6.51	0.96	13	14.82	2.38

[a] Value is not a true mean

Table 1.8. Propagule final size (least-squares means; in cm) comparisons in descending order among mother plant collection sites, Roundstone Native Seed, LLC, Upton, KY, September 2013.

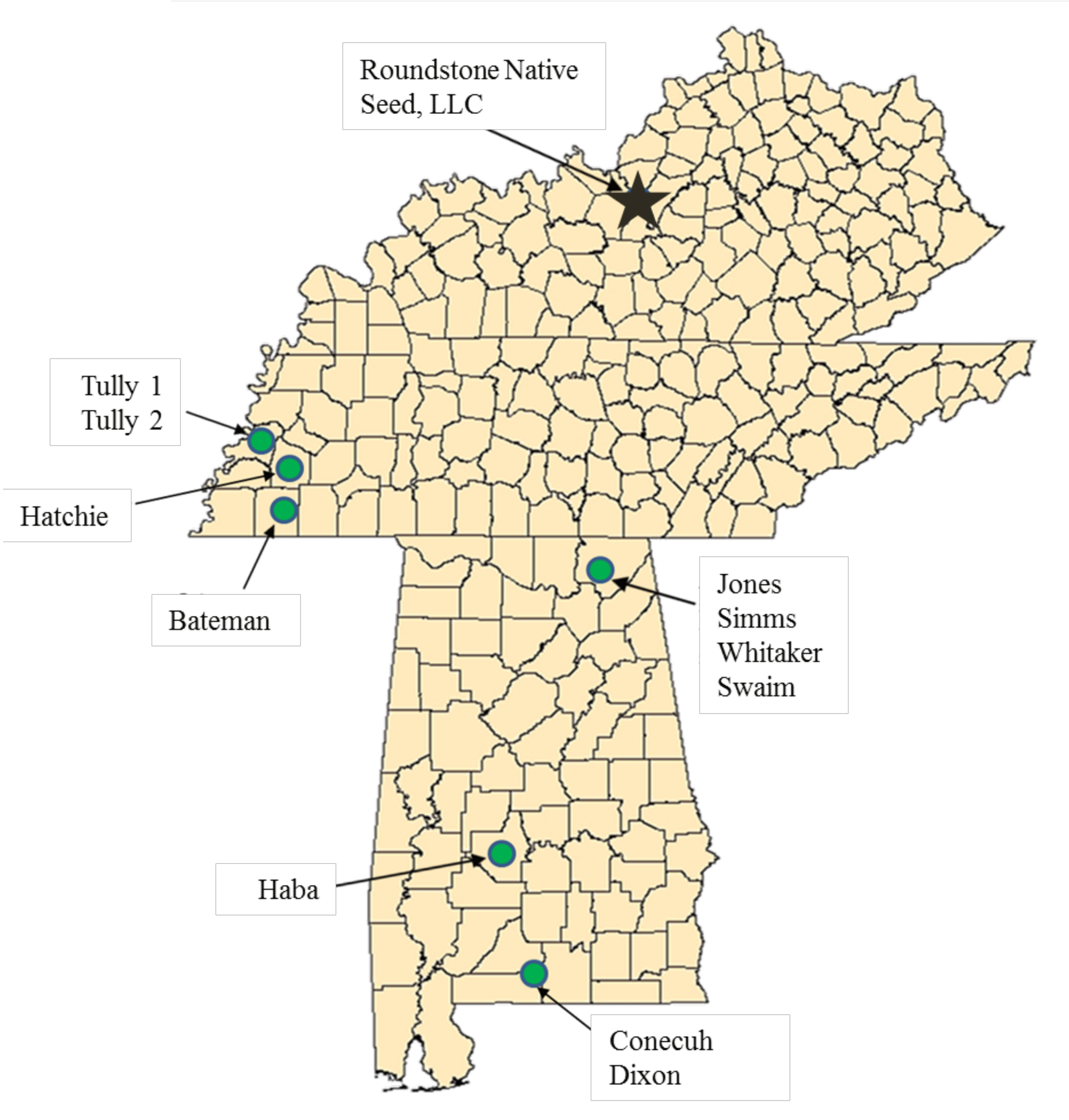
Site	LSMEANS	SE
Haba	32.24^{ae}	1.17
Tully 2	23.91^{bcde}	0.76
Tully 1	22.36^{bcdef}	0.50
Whitaker	23.06^{bcdef}	1.56
Simms	19.40^{bcdefg}	5.46
Conecuh	19.17^{bcdefg}	6.30
Swaim	$18.50^{abcdefg}$	10.92
Hatchie	17.45^{cdefg}	2.73
Jones	13.56^{defg}	2.82
Bateman	$12.40^{abcdefg}$	10.91

[a] lack of common superscript signifies significant differences ($P < 0.05$).

Table 1.9. Survival (% and SE) of propagules planted in pots and plugs by month, Roundstone Native Seed, LLC, Upton, KY, 2013.

	Jun			Jul			Aug			Sep		
Container	*n*	$\bar{x}$	SE	*n*	$\bar{x}$	SE	*n*	$\bar{x}$	SE	*n*	$\bar{x}$	SE
Plug	4132	0.71	0.01	2834	0.51	0.01	2286	0.44	0.01	2049	0.41	0.01
Pot	669	0.53	0.01	418	0.33	0.01	373	0.30	0.01	314	0.25	0.01

Figure 1.1. Cane mother plant collection sites (circles; 2010-2013) and cane macro-propagation site (star; 2013-2014)



Chapter II: Canebrake (*Arundinaria* spp.) restoration out-plantings in western Tennessee and southern Alabama.

ABSTRACT

Canebrakes, monodominant stands of native bamboo (*Arundinaria* spp.*;* hereafter cane), are critically endangered ecosystems in the United States that have been reduced to <2% of their former range. Canebrakes are dependent on disturbances that are most often human-influenced such as prescribed fire and periodic flooding. Therefore, it is unlikely that canebrakes will return without human intervention in modern landscapes. Recently, interests in restoring canebrakes has increased. My objectives were to conduct a specialty crop out-planting in southern Alabama and a restoration out-planting in western Tennessee in early spring 2014. A 3-factor split-plot containing 3 shade cloth treatments (0%, 50%, 80%), 2 fertilizer treatments (presence, absence), and 2 pine bark mulch treatments (presence, absence) at 0.6 m × 0.6 m spacing was used at the Alabama site. Planting at the Tennessee site was on a 3.66-m × 3.66-m spacing. In February, I planted 768 and 1,200 propagules at the Alabama and Tennessee site, respectively. Complete mortality occurred 5 months following the 2014 out-planting at both locations due to propagules having experienced below-average winter temperatures in central Kentucky prior to out-planting. Measures of out-planting survival and growth will be key to improving canebrake restoration in the future.

KEYWORDS *Arundinaria*, cane, canebrake, ethnobotany, native bamboo, out-planting, restoration.

INTRODUCTION

Canebrakes are monotypic stands of native bamboo (*Arundinaria* spp.; hereafter cane). Canebrakes are the only native bamboo genus to North America and are critically endangered ecosystems. Canebrakes have declined to <2% of their former range since the early 1800s (Noss et al. 1995). Cane has been documented in 24 states, encompassing the Southeastern United States and extending into southern New England and the southern Midwest (Krayesky and Chmielewski 2014, United States Department of Agriculture Natural Resources Conservation Service [USDA NRCS] 2015). Historically, canebrakes occurred along nearly every southeastern waterway. However, in today's heavily altered landscape, cane only occurs diffusely along field edges, stream banks, and forest gaps (Marsh 1977, Gagnon and Platt 2008*a*). The loss of canebrakes is predominately attributed to overgrazing by livestock, land conversion to agriculture and closed forests, and anthropogenic alterations to historic fire and flood regime frequencies (Hughes 1951, Platt and Brantley 1997, Judziewicz et al. 1999, Stewart 2007, Gagnon and Platt 2008*b*).

In the last 20 years, interest in restoring canebrakes as a specialty crop for Southeastern Native American basketry (Hill 1997) and wildlife habitat, particularly for neo-tropical migrant songbirds, has increased (Platt et al. 2009). Currently, the main limitation to cane restoration is the lack of efficient mass production of cane planting stock (i.e., propagules; Baldwin et al. 2009). Little is known about cane sexual reproduction due to the infrequency and unpredictability of synchronized flowering events (Gagnon and Platt 2008*a*). Cane, like most bamboos, flowers gregariously and sporadically at intervals as frequently as every 2-3 years to as infrequently as every 50 years (Liese 1987). This irregularity makes collecting seed difficult without frequent field observations and travel. Therefore, most canebrake restoration relies upon asexual propagation via clump division (McClure 1966) or macro-propagation (Baldwin et al. 2009) for planting stock. Clump division is both labor and time intensive (Datillo and Rhoades 2005) requiring intense, manual labor to extract and translocate clumps with hand tools. Studies have determined macro-propagation to be the most promising immediate promising method for large-scale restoration (Zaczek et al. 2004, Baldwin et al. 2009). Macro-propagation involves harvesting multiple-node sections of rhizomes from containerized mother plants and planting the rhizomes similarly to stem cuttings in containers or bareroot directly into the field site's native soil (Baldwin et al. 2009, Zaczek et al. 2009).

Specialty crop restoration is of interest to many Native American tribes throughout the Southeast due to cane's ethnobotanical value (Hill 1997, Platt et al. 2009). Cane was used extensively in Cherokee, Creek, and Choctaw culture as the main source of basket materials, housing materials, fishing and cooking vessels, flutes, and in various ceremonial traditions (Hill 1997, Platt et al. 2009). Swanton (1946) considered cane to be one of the most important plant resources for Southeastern tribes. Because cane was pervasive in tribal life, it was considered appropriate for Southeastern tribes to be designated as a "bamboo society" (Anderson 1993, Platt et al. 2009). Canebrakes were valued hunting lands of Native Americans and were burned once every 7-10 years to maintain and expand canebrakes by eliminating competing woody vegetation (Brantley and Platt 2001). The adoption of an open range grazing system for Native American livestock such as swine, cattle, and horses (Van Doren 1928) was largely due to the availability and exceptional nutritional qualities of cane (Hill 1997). Cane served as excellent forage and shelter throughout the winter, a quality that most co-occurring forages could not provide (Platt and Brantley 1997, Stewart 2007). Cane was regarded so highly as a livestock forage, that war ensued between Euro-American settlers and Native Americans on multiple occasions due to settler's livestock over-grazing canebrakes (Platt et al. 2002). Interest to restore cane has been rejuvenated by the Mississippi Band of Choctaw Indians' Pearl River Demonstration Project by Mississippi State University, the Cherokee Preservation Foundation in conjunction with Western Carolina University, and the Friends of the Cache River Watershed in Illinois (Mississippi State University Rivercane [MSU] 2008) among others.

Restoration of canebrakes for wildlife habitat has been attempted throughout cane's natural range by state and federal agencies, non-governmental agencies, and private individuals. The Nature Conservancy has designated cane as a target species for restoration in the Upper East Coast priority area (Zaczek et al. 2009). Canebrakes are documented to provide habitat for >70 species of wildlife including the Swainson's warbler (*Limnothlypis swainsonii*) and swamp rabbit (*Sylvilagus aquaticus*), and previously provided nesting and roosting habitat for the now extinct Bachman's warbler (*Vermivora bachmanii*) and passenger pigeon (*Ectopistes migratorius*; Smart et al. 1960, Platt et al. 2013). Canebrakes serve as larval hosts and food sources to 7 species of cane-dependent butterflies, including the newly discovered Lepidopteran, *Cherokeea attakullakulla*, that depends solely on *Arundinaria appalachiana* in the southern Appalachians as its larval host (Platt et al. 2001, Quinter and Sullivan 2014). Canebrakes served as natural corridors for megafauna such as bison (*Bison bison*), black bears (*Ursus americanus*), cougars (*Puma concolor*), and white-tailed deer (*Odocoileus virginianus*; Platt et al. 2001, Platt et al. 2013).

During canebrake restoration, proper site selection is critical to in-field planting success and survival. The United States Department of Agriculture, Natural Resources Conservation Services' (USDA NRCS) Plants Database (2015) currently lists cane as a Facultative Wetland Plant (FACW); however, Tanner et al. (2011) suggests that this is a misclassification due to its frequent presence on numerous non-wetland sites. Tanner et al. (2011) suggest that an upland (UPL) or facultative upland (FACU) classification is more appropriate and further suggested restoring canebrakes in well-drained, sandy soils.

Aside from site selection, many factors such as competition, shade, soil moisture, and weather conditions affect in-field cane success (Datillo and Rhoades 2005, Cirtain 2009, Osland et al. 2009, Klaus and Klaus 2011). For example, Chinese privet (*Ligustrum sinese*) is a common competitor with cane and sites containing privet should be avoided unless herbicide treatments will be implemented pre- and post-cane planting (Osland et al. 2009, Klaus and Klaus 2011). A number of studies have investigated in-field planting success factors that largely center on moisture and rhizome susceptibility to desiccation (Platt and Brantley 1993, Zaczek et al. 2004, Datillo and Rhoades 2005, Osland et al. 2009, Zaczek et al. 2009). Klaus and Klaus (2011) found that the best indicator of transplantation success for cane was the Keetch-Byram Drought Index (KBDI) value, a calculation based on rainfall and temperature that estimates soil moisture deficiency on a scale of 0-800. Specifically, the lower the KBDI values (i.e., early spring in the Southeast) leading up to and during the day of transplantation, the greater the out-planting success. Gagnon (2006) suspected observations of increased cane seedling establishment in Louisiana were due to the presence of leaf litter moderating local moisture and temperature regimes. Transplanted cane clumps demonstrated a positive effect of a hardwood mulch and manure combination application treatment (40% increase in new culms compared to clumps treated with mulch alone), likely from more consistently moist soil and reduced herbaceous competition (Datillo and Rhoades 2005). Nutrient addition has been recommended to accelerate aboveground production following cane transplantation for decades (Hughes 1951).

My objectives were to 1) out-plant a high-density, specialty crop restoration site in southern Alabama on the Poarch Band of Creek Indian Reservation for the purposes of public education and eventual, sustainable harvest, 2) out-plant a low-density, habitat restoration site at the John Tully Wildlife Management Area in western Tennessee for the purposes of wildlife habitat improvement and land conversion to native flora, and 3) monitor out-planted propagule survival and aboveground growth at both sites.

STUDY AREA

This study was conducted in 3 stages: 1) cane macro-propagation at Roundstone Native Seed, LLC in Upton, Kentucky (March 2013 to March 2015), 2) out-planting of cane propagules at the Poarch Band of Creek Indian Reservation, Alabama (February 2014 and January 2015), and 3) out-planting of cane propagules at John Tully Wildlife Management Area, Tennessee (February 2014 and March 2015).

Roundstone Native Seed, LLC

Macro-propagation, including mother plant and propagule storage occurred at Roundstone Native Seed, LLC, a commercial producer and supplier of native seed and plants in Upton, Kentucky, USA in Hardin County from March 2013 to March 2015. The mean annual temperature of Hardin County is 13.39° C with a mean annual maximum of 18.89° C and a mean annual minimum of 7.78° C. The mean annual precipitation of Hardin County is 111.76 cm, with 40.64 cm of precipitation from snowfall (CLRsearch [CLR] 2012). Hardin County is in the USDA temperature zone 6, located in the Knobs region on the western side of the Outer Bluegrass Region physiographic section (Elbon 2015).

Poarch Band of Creek Indian (PBCI) Reservation, Alabama

Propagules were out-planted near the town of Atmore in Escambia County, Alabama, USA on the Poarch Band of Creek Indian (hereafter PBCI) property. The planting site was located on Aplin Road, adjacent to Hog Branch Creek, north of Highway 14 and I-65, at 91 m above sea level. The 0.4-ha site was previously pastureland, and densely populated with blackberry (*Rubus* spp.), toothache grass (*Ctenium aromaticum*), broomsedge bluestem (*Andropogon virginicus*), panic grass (*Dichanthelium*) spp., reed (*Juncus* spp.), bog cheetoo (*Polygala lutea*), sweetbay magnolia (*Magnolia virginiana*), and sparse cane (*Arundinaria* spp.). The site was adjacent to a 4.0-ha pitcher plant bog (*Sarracenia leucaphylla*) with remnant longleaf (*Pinus palustris*) burned on a 3-year rotation. The soil is classified an Atmore silt loam with a pH of 4.7 (Web Soil Survey [WSS] 2015). The mean annual temperature is 19.72° C with a mean annual maximum of 25° C and a mean annual minimum of 13.89° C. The mean annual precipitation is 162.56 cm, with 2.54 cm of precipitation from snowfall (CLR 2012). Escambia County is in the USDA temperature zone 8b, located in the Coastal Plain physiographic region (Neilson 2007).

John Tully Wildlife Management Area (TULLY), Tennessee

Propagules were out-planted on the 863-ha John Tully Wildlife Management Area (TULLY) near the town of Ripley in Lauderdale County, Tennessee, USA. Lauderdale County is bordered by the Mississippi, Hatchie, and Forked Deer Rivers in the Mississippi watershed (Toplovich 2010). John Tully WMA objectives include alluvial bottomland hardwood restoration and preservation, timber production, and hunting and fishing lands. The site is currently owned by the Tennessee Wildlife Resources Agency (TWRA), but was previously planted in soybeans by commercial farmers. The planting site is an approximately 2-ha dormant agricultural field, bordered to the West by a stream, 75 m above sea level. The site is susceptible to annual flooding from the adjacent stream in the late winter or early spring. The surrounding fields were in soybean production or hardwood plantings. The soil is classified as a Commerce silt loam with high levels organic matter and a pH of 6.3 (WSS 2015). The mean annual temperature of Lauderdale County, TN is 16.83° C, with a mean annual maximum of 22.22° C and a mean annual minimum of 11.11° C. The mean annual precipitation of Lauderdale

County, TN is 132.08 cm with 12.7 cm of precipitation from snowfall (CLR 2012). The western portion of Lauderdale County, encompassing the out-planting site, is located in the USDA temperate zone 7b, in the Mississippi Alluvial Valley physiographic region (Etnier and Starnes 1993).

METHODS

Macro-propagation

In March 2013, I harvested rhizome sections from potted cane mother plants collected from 2010-2013 from locations in northern, central, and southern Alabama and western Tennessee (Fig. 2.1). I clipped 3- to 5-node rhizome sections (hereafter propagules) from mother plants, soaked them overnight in a fungicide, and planted them with the most distally harvested bud (tips face opposite direction of culm) upright in 25-cell IP 200 Rigipot™ plug trays or planted them horizontally under approximately 0.5 cm of growing media in 10.16-cm × 10.16-cm square pots. In 2013, all trays were housed outdoors under a 60% shade house from April until out-planting in February 2014. For more detailed macro-propagation methodology refer to Chapter I.

Site Preparation

I coordinated different site preparations on the PBCI and TULLY sites for cane plantings. In 2013, a land management crew hand-sprayed Cornerstone® (Winfield Solutions, LLC; 41% glyphosate) herbicide in November at a rate of 2.34 L/ha at the PBCI site, followed by a dormant season burn, and disking one day prior to planting in February 2014. A crew bush-hogged the TULLY site two weeks prior to planting in February 2014.

Soil Testing—A soil sample was collected from each out-planting site using a round point shovel. The vegetative matter was scraped from the top of the soil to exclude it from soil testing. Approximately 10-15 small soil samples were collected from the top 20 cm of soil from different locations within the out-planting site and placed them in a paper bag. I sent the comprehensive soil sample to the Auburn University Alabama Cooperative Extension System's Soil Testing Laboratory for a routine soil analysis for general soil conditions, pH, and soil amendment prescriptions.

Shade House Construction— At the PBCI site, I constructed 8 shade houses (four, 50% shade houses, four, 80% shade houses) from treated pine lumber along with 4 open plots as a shade cloth control treatment (0% shade) for a total of 12 plots at the PBCI site. I constructed shade houses from 2.4 m long, 10.16-cm × 10.16-cm posts sunk into the ground approximately 45-60 cm. I used a 2-person earth auger to achieve this post depth. I constructed the shade house framing from 5.08-cm × 10.16-cm treated lumber of various lengths. Shade houses measured approximately 6 m × 12 m at completion with the north and south-facing walls open. I secured a single piece of shade cloth, measuring 6 m × 18 m with roofing nails on the roof and east and west-facing walls. I constructed kickers of scrap 5.08-cm × 10.16-cm material for the upper corners following shade cloth application to increase shade house stability. I measured each plot grid using a tape measure and marked individual plant locations with pin flags.

Out-planting

In February 2014, I transported propagules from the Roundstone Native Seed, LLC facility to out-planting sites using a 7.3-m U-haul truck. A planting crew planted cane propagules using hand trowels and dibble bars at the PBCI site whereas at the TULLY site a crew planted propagules with dibble bars and JIM-GEM® KBC planting bars (Forestry Suppliers, Inc., Jackson, Mississippi). Temperatures were freezing at Roundstone Native Seed LLC when plants for the PBCI site were picked up. Trays were "quick-thawed" by spraying water from a garden hose directly on the plants one day prior to arrival at the planting site.

PBCI—A crew planted a 0.4-ha specialty crop plot at a high density (0.6 m × 0.6 m). I implemented a 3-factor split-plot design of treatments: 1) whole-plot factor of shade (0%, 50%, 80%), 2) sub-plot factor of mulch (presence, absence), and 3) sub-sub plot factor of composted manure fertilizer (presence, absence). I constructed 12 whole-plot factor shade plots: four, 50% shade houses, four, 80% shade houses, and four, 0% shade control plots. I constructed 4 sub-plot-factor mulch plots per shade plot for a total of 48 mulch sub-plots. Half of the mulch sub-plots received an 8-cm layer of aged pine bark mulch. Furthermore, I constructed 2 sub-sub-plot factor fertilizer plots per mulch sub-plot, 8 per shade house plot, for a total of 96 fertilizer sub-sub plots. Half of the fertilizer sub-sub-plots received an approximately 10-cm radius, 5-cm deep layer of composted cow manure around the base of each plant. Eight plants occurred per fertilizer sub-sub plot, 16 plants occurred per mulch sub-plot, and 64 plants occurred per shade plot for a total of 768 plants.

I monitored in-field survival and aboveground size for every plant three months following the planting, then every two months during the growing season (March to September). Mortality was determined by a complete lack of green (photosynthetic) coloration in the culms and leaves. I used a binary code for survival where dead plants received a 0 and alive plants received a 1. Percent survival was determined by the sum of all plants receiving a 1 dividing by the total number of plants out-planted. I measured aboveground size by measuring culm height to the nearest 0.1 cm with a meter stick for every sprout. I then summed the measurements of the sprouts for a total aboveground measurement for each plant for each month measured. Growth rate was determined by the difference in total aboveground growth measurements between months. All eleven mother plant collections sites from Chapter I were included in this out-planting and were randomly planted throughout the split-plot design.

TULLY—I out-planted a 4.0-ha, low-density (3.66 m × 3.66 m) restoration plot. I divided the planting grid with mason line and marked individual plant locations with pin flags. Three months following planting, I hammered 2-m pieces of conduit into the ground at the base of 100 randomly selected plants for survival and aboveground size measurements. I monitored in-field survival and aboveground size of this sample of plants three months after the out-planting then every two months during the growing season (March to September). All eleven mother plant collections sites from Chapter I were included in this out-planting and randomly stratified throughout the site.

I completed all statistical tests with SAS 9.4 for Windows (SAS Institute, Inc., Cary, NC). Because plant survival was binary, I used a generalized linear mixed model with a binomial distribution (PROC GLIMMIX, SAS 9.4) to test differences in survival among mother plant collection sites at the TULLY site. At the PBCI site, I tested differences in survival among the whole and sub-plot factors of shade, mulch, and fertilizer plots. I analyzed a split-split-plot design with fertilizer nested within mulch nested within shade considered as fixed effects. I used a linear mixed effects model with repeated measures (PROC MIXED, SAS 9.4) to test differences in plant growth among data collection periods. At the TULLY site, mother plant collection site was considered a fixed effect, while repeated growth measurements of individual plants were considered random effects. At the PBCI site, shade, mulch, and fertilizer were considered fixed effects and the repeated growth measurements of individual plants were considered random effects.

RESULTS

In February 2014, 768 propagules were out-planted at the PBCI site and 1,200 propagules were out-planted at the TULLY site. Three months following the out-planting only 5 propagules (PBCI: *n* = 2; TULLY: *n* = 3), remained alive. All propagules from the PBCI site and the TULLY site were dead by July.

DISCUSSION

The cane propagules experienced below-average winter temperatures for an extended period of time prior to out-planting in Kentucky and massive temperature changes during out-planting that likely caused mortality. Propagules experienced below-freezing temperatures 14 days in November, 20 days in December, 26 days in January, and 21 days in February while in Kentucky. Between November 2013 and February 2014, propagules experienced 20 days of -9.44° C (15° F), and 1 day below -17.78° C (0° F) as opposed to 2 days of -9.44° C in winters 2011-2012 and 2012-2013 each. Average monthly temperatures were substantially lower than the previous two winters (Western

Kentucky Unversity Kentucky Mesonet [WKU KM]). Observations of native cane growing in the same Kentucky location indicated die back from the hard freeze (John Seymour, personal communication). Cane can withstand freezing temperatures; however, prolonged below-average winter temperatures have resulted in the die back of cane (Winterringer 1952, Marsh 1977). Additionally, aboveground, containerized cane would be more susceptible to cold weather than underground, field cane. The PBCI site plants were quickly thawed with water prior to out-planting which may have additionally contributed to plant stress and mortality. One day following out-planting, the TULLY site experienced a winter storm with freezing ground temperatures and snowy/icy precipitation. Frozen soil during out-planting at both sites was another issue that may have led to less root to soil contact and less water absorption than desirable for successful planting.

I would recommend mean temperatures be taken into detailed account upon out-planting and directly following out-planting. Ideally, cane would be planted in well-thawed ground in the early spring during the dormancy period, with well-thawed containers and intact soil clumps. Another factor to consider when out-planting is the Keetch Byram Drought Index (KBDI) the day of planting and preceding week, regardless of the time of year out-planting occurs. A lower KBDI value may be an indicator of improved out-planting success (Klaus and Klaus 2011). Klaus and Klaus (2011) suggest that the conditions preceding cane out-planting are much more important than the conditions following out-planting. Although my study experienced mass mortality, other studies have had success with cane out-planting via macro-propagation/rhizome sections

(Zaczek et al. 2004, Brendecke and Zaczek 2008, Zaczek et al. 2009). Currently, canebrake restoration is limited by a lack of available planting stock and rudimentary field establishment processes (Zaczek et al. 2009).

MANAGEMENT IMPLICATIONS

The extant literature on canebrakes and their restoration is minimal, underdeveloped, and often leave more questions than answers. Small contributions to the knowledge of cane restoration can make large improvements, particularly, if they advance mass production of cane propagules or in-field planting success. Despite the lack of success in this study's out-plantings, I would recommend macro-propagation over clump division for small scale (<10 ha) canebrake restoration projects. The continual production of propagules and less cost-prohibitive nature of macro-propagation makes it the only immediate promise for canebrake restoration (Baldwin et al. 2009). However, future research should focus on the development of micro-propagation methodology via tissue cultures for large-scale, range-wide canebrake restoration.

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Figure 2.1. Cane mother plant collection sites (circles; 2010-2013) and cane macro-propagation site (star; 2013-2014)