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Establishment methods of *Arundinaria* species for restoration purposes

By

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Establishment methods of *Arundinaria* species for restoration purposes

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Rivercane, *Arundinaria gigantea,* is the native woody evergreen grass that has exhibited rapid population decline since European colonization of North America. Agriculture and urban expansion have reduced this important ecosystem type to remnant populations. This poses challenges to current restoration efforts by minimizing genetic diversity and limiting healthy host sites for propagation. Objectives of this research were to test four methods of establishment that would promote the greatest survivability and growth of propagules. Non-irrigated field studies indicated greatest rivercane growth response when planted in increased shade (60 - 85% light reduction). Monthly plantings indicated that February offered the greatest probability of survival. Application of slow release 19-6-12 fertilizer (33.3 g) enhanced growth, but fertilizer applications are not recommended without adequate soil moisture. Halosulfuron (72.6 g a.i./ha) applications for weed control showed no damage to rivercane plants compared to control.

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CHAPTER I

INTRODUCTION

Three species from the genus *Arundinaria* are the only bamboo species native to the United States; including two evergreen species and one deciduous species. Evergreen species include rivercane or giantcane (*A. gigantea* (Walter) Muhl.), and switchcane (*A. tecta* (Walter) Muhl.). Hillcane (*A. appalachiana* (Triplett, Weakley, and L.G. Clark)) is the third and the only deciduous species (Triplett et al. 2006). All three are indigenous to the southeastern U.S. and belong to the division *Bambusoideae* (bamboo), family *Poaceae* (grasses). Rivercane, the most widely distributed species of this genus, is found growing south from Florida to east Texas and north from central Missouri to Virginia. Switchcane flourishes from the lower portions of Louisiana and east to central Florida, while occupying wetter niches in the southern portion of rivercane's native range. (Marsh 1977; Platt and Brantley 1997) Hillcane, which occupies only a small portion of the southern region, is prominent throughout the southern Appalachian mountain region and into the Blue Ridge/Piedmont Escarpment (Marsh 1977).

Large canebrakes composed of rivercane once stretched across many hectares of the southeastern U.S., but were quickly reduced after European settlers traveled west and cleared vast amounts of southern prairie and forest for farmland (Platt and Brantley 1997). Today farming practices, infrequent flowering events, and other anthropogenic activities continue to augment the decline of this critically endangered ecosystem (Noss et al. 1995). Typically found in monotypic stands, rivercane now occupies no more than two percent of its original range (Noss et al. 1995; Platt and Brantley 2004). Large stands that were once abundant are now reduced to small, highly isolated and disconnected brakes that are unlikely to cross pollinate, a requirement for the perenniation of the species (Baldwin et al. 2009).

Naturally found in riparian zones, rivercane thrives in disturbed areas and along woodland edges where competition is minimal (Marsh 1977). Spread by a network of woody rhizomes; shoots and roots arise from underground nodes that benefit the related ecosystem by improving habitat, water quality, and soil stabilization (Schoonover et al. 2006). Re-establishing rivercane in its disturbed, native environment has become a main goal of many conservation and land restoration projects. Healthy canebrakes create ideal habitat for an array of fauna, some of which are endemic to this ecosystem. Well-established brakes create excellent filter strips, preventing soil erosion (Schoonover et al. 2003, 2006). However, low success rates following the initial establishment of rivercane has now become a primary concern and point of focus for this current research.

Studies have focused on aspects of rivercane growth that directly affect survival, not only of container grown plants, but more importantly, in-field establishment (Russell et al. 2010, 2011; Hamlington et al. 2011). Re-establishing rivercane in its disturbed, native environment has become a main goal of many conservation and land restoration projects, and is important to the continuation of this ecosystem. Understanding initial establishment methods of *Arundinaria* would be useful in growing healthier, more productive stands.

Knowledge of the plant's native habitat and structure is a step toward implementing future studies. The goal of plant establishment and survival, as affected by time of year (rainfall and transpirational loss), should be kept in mind as future studies are structured. Likewise, the evaluation and understanding of light requirements (testing under different light regimes to determine the light level required for maximum growth), cane ecology, and local distribution (leptomorphic nature), is critical to locating suitable restoration sites. Observations of soil characteristics found in naturally occurring brakes would suggest moisture and fertility needs, allowing future experiments to evaluate the forms and necessity of supplemental fertilizer. Inorganic forms, emulating similar soil nutrient requirements, may offer a growth enhancement necessary for initial establishment. Fertility should be tested (Cirtain et al. 2009; Zaczek et al. 2010) to determine the most favorable supplement for plants. Another natural process of succession is competition; therefore studies should be established to address this issue. To aid in competition control, comparison analysis of cultural management techniques and herbicide use will indicate best management practices to ensure plant success.

Due to low success rates and limited literature, the goal of this study is to evaluate planting methods and cultural techniques to insure the most successful and efficient ways to establish rivercane into new field situations. Some of these evaluations and objectives include, but are not limited to, light requirements, soil fertility, control of weed competition, time of planting, and management techniques. Healthy canebrakes are a beneficial conservation tool that provides riparian buffers (Schoonover et al. 2010), soil stabilization, intercepts pollutants (Schoonover et al. 2006), increases percolation, and improves water quality (Schoonover et al. 2003). Future studies are critical to the life and ecology of many remaining canebrakes across the Southeast.

CHAPTER II

LITERATURE REVIEW

Native grasses and related ecosystems have experienced extensive decline since the pre-settlement era and colonization of the eastern United States by Europeans. Prior to the 16th Century, when Native Americans inhabited the land and regularly managed rangelands with fire, grassland prairies thrived and supported diverse populations of flora and fauna (Platt and Brantley 1997). One of the key native species supported was rivercane (*Arundinaria gigantea*), a woody, evergreen grass that provides primary habitat for a variety of game and non-game species. Rivercane has been a valuable species for several reasons, such as an important cultural resource for Muskogean-speaking Native Americans, habitat for wildlife, soil stabilization, water quality, and a food source for wildlife and livestock (Platt and Brantley 1997; Marsh 1997).

In light of canebrake restoration today, methods are still being developed and tested. Current methods are labor-intensive, with continued research in the areas of micro-propagation, seed production, and macro-propagation. Successful methods include both seedling transplants and crown divisions. Dattilo and Rhoades (2005) tested the method of crown division by digging clumps, averaging 45 cm in diameter and 3-8 culms, from donor canebrakes and transplanting to test sites. Clump survival proved to be 98% successful after amending the soil with two rates of organic (200 kg N/ha, 53 kg

P/ha, and 176 kg K/ha; and 2,900 kg N/ha, 770 kg P/ha, and 2,550 kg K/ha) and one inorganic (196 kg N/ha, 66 kg P/ha, and 168 kg K/ha) fertilizer. Cirtain et al. (2004) showed that seedling transplants were also successful. Seedlings in this study were grown from seed (Summertown, TN) to an average height of 5 to 15 cm. Seedlings were then treated with three levels of moisture regimes, along with two levels of nitrogen fertilization. Test results indicated that well-drained soils provided the best environment for seedling growth. In light of current propagation techniques, seedling transplants may seem easier, but development is extremely slow. Current efforts have transplanted single rhizome cuttings and ramets from donor stands with some success, but only limited data are available.

History and Background

Historical accounts of rivercane indicate that reduction occurred in equal proportion with that of bison (*Bison bison* L.), elk (*Cervus elaphus* Nelsoni), and black bear (*Ursus americanus* Pallas) that once thrived in the region. The propagation and spread of this particular species of bamboo was encouraged mostly by the cultural practices of Native Americans and natural disturbances. Indigenous people utilized cane in nearly every aspect of their lifestyle, using this resource for housing material, hunting, fishing, and tool implements, jewelry and personal adornment, baskets, and musical instruments (Platt and Brantley 2009). During this time, large expanses of canebrakes were common, providing cover and food to many animal species that today are either very rare or not found at all. Today canebrakes still provide an important resource for the livelihood of Native American tribes and habitat for an array of wildlife. Remnant canebrakes provide shelter for wildlife such as the swamp rabbit (*Sylvilagus aquaticus* Bachman), canebrake rattlesnake (*Crotalus horridus* L.), Louisiana black bear (*Ursus americanus* L.), white-tailed deer (*Odocoileus virginianus* Zimmerman), and the endangered Swainson's warbler (*Limnothlypis swainsonii* Audobon). Studies have shown that established cane stands, called brakes, along riparian systems significantly reduced sediment loads from agricultural watersheds (Schoonover et al. 2006). However, the large brakes described in historical accounts are now only small remnant patches (Noss et al. 1995), limiting a key component in ecosystem biodiversity.

As concerns mount, the importance of rivercane is being understood and promoted by natural resource and environmental advocates (USDA 2011). Restoration activities are necessary to allow for healthy canebrakes to perform their ecological role. Nevertheless, there remain questions regarding plant propagation and re-establishment.

Rivercane (*Arundinaria gigantea*) is one of three bamboo species native to the United States. From the Poaceae family, this evergreen woody perennial grass is a component of bottomland and riparian forest ecosystems throughout the Southeast (Marsh 1977; Simon 1986). Classified as a facultative wetland plant, *Arundinaria gigantea* occurs in wetlands 66-99% of the time (USDA 2011). Other species include *A. tecta* and *A. apalachiana*. These species differ somewhat in regard to physiographic regions and growth habit. Switchcane (*Arundinaria tecta*) is commonly found in wetter conditions and whose range is primarily restricted to the Coastal Plains of the Southeast from southern Maryland to Mississippi (McClure 1973; Hitchcock 1951). Hillcane (*Arundinaria appalachiana*) is mostly found on mesic slopes and upland hardwoods in the foothill regions of the Apalachian Mountain range (Triplett et al. 2006).

Arundinaria is a monopodial bamboo, having erect culms which arise from nodes along linear underground stems called rhizomes. Culm growth is initiated each spring and in ideal habitat, healthy stands may have culms reaching heights of 8m with a diameter of 2cm (Meanley 1972; Judziewiez et al. 1999; Hughes 1951). The rhizomes from which they sprout are usually found within the upper 15 cm of soil and range in diameter from two to 20 mm (McClure 1966). The culms they produce are of an equal or slightly larger diameter, sprouting approximately every 50 cm (McClure 1966). Dense colonies of these mature culms may or may not represent single genetic individuals. Studies in North Carolina have found that at least 12% of samples between two remote stands exhibit genetic diversity. Of these two brakes, 12 clones were found with no distinct genotypes between stands, indicating that genetic diversity is influenced by fragmentation resulting from habitat loss (Mathews et al. 2009).

Before an era of increasing habitat loss, William Bartram (Van Doren 1928) noted healthy canebrakes during his four-year journey from Philadelphia through Mississippi and Florida in 1773. During his travels he cites, "Now at once opens to view, perhaps, the most extensive Cane break (sic) that is to be seen on the face of the whole earth,... there appears no bound but the skies." Today these sites are rare and brakes have been reduced to dislocated, remote, and limited stands.

The stands previously mentioned primarily reproduce asexually from the continued culm growth along its extended rhizomatous network. *Arundinaria* can also propagate sexually, but seed production is sporadic and is characterized by low viability (Platt and Brantley 1997; Farrelly 1984). Bamboo exhibit three modes of flowering: gregarious, sporadic, and continuous (McClure 1966), the first of which is typical of the woody bamboos native to the U.S. This type of flowering occurs across large geographic areas after long periods of vegetative growth and subsequently results in the death of the plant (monocarpy) (Platt and Brantley 2004). The hypotheses of Kelly (1994) and Taylor et al. (1991) attempts to explain the evolutionary significance of this flowering method. It was noted that this mass flowering event helps to ensure successful cross-pollination within the specific bamboo species. Furthermore, the die-offs of the parent plant proved to increase seedling survival by providing the smaller plants the opportunity to grow and fill an open canopy. These cycles are described as irregular and unpredictable, with some stands flowering repeatedly every year and others only once before dying. Platt and Brantley (2004) further noted that the majority of the flowering stands across the southeastern states between 1986 and 2002 were gregarious. However, during this period, they concluded that observed *A. gigantea* also flowered sporadically between gregarious flowering episodes. Sporadic flowering is often irregular and unpredictable in occurrence and may or may not lead to the death of the plant. Sporadic flowering has been observed on highway right-of-ways, and may be influenced by mowing and hormone-mimicking herbicides used to control weeds (Baldwin pers. comm.). Furthermore, these observations led them to believe that *A. gigantea* may exhibit two modes of flowering. Mathews et al. (2009) suggested that certain genotypes occurring within unrelated brakes may flower alternately from other parent plants. This reasoning may explain why only portions of certain stands exhibit sporadic or gregarious flowering. Nevertheless, the reproductive phenology of cane is still poorly understood and additional long-term research is needed.

Arundinaria plays an important role in both early and late-successional plant communities, persisting in either natural or anthropomorphic disturbances. Early successional growth historically took place in fields where rotational fires of the Mississippian Culture of Native Americans and other disturbances maintained the natural integrity of the plant community (Platt and Brantley 1997). These fields were mainly located along the low, flat riverine systems that naturally provided irrigation to crops. The largest canebrakes currently are found in the more mesic, fertile soils on elevated terraces where flooding is still likely, but not common (Judziewicz et al. 1999). These fields, once abandoned, provided ideal early to mid-successional habitats. Consequently, rivercane persists as an understory species in late-successional plant communities.

Response to Light Conditions

Studies have suggested that the spreading, clonal nature of canebrakes enable them to persist beneath forest canopy, allowing rapid, positive response to large-scale wind, or anthropogenic (logging) disturbances (Gagnon et al. 2007). In an area where rivercane persisted, new culm production rates in tornado-blowdown areas were 2.5 times greater than in forested areas. Even though the number of young culms increased, the data indicated an inverse relation in light levels to plant height (plants grew taller in the shade than sun).

In a similar experiment to assess the impact of light intensity on rivercane growth, Cirtain et al. (2009) conducted both field and laboratory studies which exposed both an established, forested canebrake and potted seedlings to various light intensities. Overstory trees in established stands were girdled to remove 60% canopy. Post-treatment photosynthetic active radiation (PAR) readings showed an average of 32 $\mu mol/m^2/s$ in the control site and 781 $\mu mol/m^2/s$ in the thinned site. In field tests, there were increases of new culm growth during the first year after thinning, but then slowed in the following two years. New shoot diameter was also greater in rivercane stands receiving increased light. In the laboratory tests, similar light reductions were made exposing the shaded plants at 50-100 µmol/m^2/s PAR and the control at 200-400 µmol/m^2/s PAR. Potted plants in this experiment also receive supplemental nitrogen fertilizer. The results of laboratory tests indicated that overall seedling growth, biomass, and leaf and shoot numbers increased in the full light as compared to shaded (Cirtain et al. 2009). When considering canebrake restoration, data from these experiments suggest increased light availability to promote optimal plant growth.

Response to Fertility

Along with proper light requirements, nutrient availability may be an additional component in successful growth, distribution, and establishment, of rivercane. Previous studies however, have not conclusively confirmed any specific fertility antidote, as most experiments have produced varied results. Cirtain et al. (2009) conducted an experiment where ammonium nitrate was added to seedlings at a concentration of 0.000005% (0.4 µM) once per week. This 2004 experiment indicated that nitrogen fertilization did not significantly affect the growth of rivercane seedlings. Researchers concluded that the lack of significant seedling growth was potentially a result of (1) applied nitrogen levels were too low (2) *A. gigantea* has a wide tolerance of nitrogen levels, or (3) nitrogen was not the limiting growth factor (Cirtain et al. 2004). Kozlowski and Pallardy (1997) would disagree, stating both light and nitrogen typically are limiting growth factors of C_3 grasses. Romney (1989) and others reported that small amounts of slow-release fertilizer enhance plant establishment, but negative results occurred when “recommended rates”, too high for most native shrubs, were applied. Nonetheless, a follow-up study was conducted by Cirtain and others (2009) using increased rates of ammonium nitrate (NH_4NO_3).

A two-factor experimental design used treatments that included five levels of ammonium nitrate (0, 0.5, 5.0, 25.0, and 100.0 g/L), along with two treatments of light intensity (200 $\mu mol/m^2/s$ PAR) and (50-100 $\mu mol/m^2/s$ PAR). The standard commercial field application was the lowest treatment of 0.5 g/L of ammonium nitrate, and the highest of 100.0 g/L was used to mimic agricultural and livestock runoff concentrations (Carpenter et al. 1998, Schoonover and Willard 2003). Two seedlings per pot were planted in a soil mixture of 60% sand and 40% commercial topsoil. Seedlings from 50 pots (½ shaded and ½ non-shaded) were then collected eight weeks after initiation along with shoot and root length, number of shoots, and number of leaves. Combined with full light, the nitrogen treatments of 0, 0.5, and 5 g/L increased shoot numbers and shoot biomass. Increased root and rhizome biomass occurred, but only at 0 and 0.5 g/L of nitrogen (Cirtain et al. 2009). Treatments exceeding 0.5 g/L tended to decrease seedling growth and survivorship. These results however appear to indicate that the observed growth may be primarily due to full light exposure rather than nitrogen treatments.

Survival rates have also been in question when adding fertilizer to initial planting. Yamashita and Manning (1995) reported a slightly negative effect on survival the first year when planting saltbush (*Atriplex canescens*) in arid regions of California, but observed no decrease the second year. Many have questioned whether these outcomes are due to water availability or time of planting, as fertilizer applications have been known to decrease survival of unirrigated shrubs. Results have indicated that fertilizer does not decrease transplant survival if there is enough available water (Yamashita and Manning 1995), suggesting proper soil moisture may be necessary.

Still little is known about rivercane physiology and its need, or tolerance, of agricultural byproducts. Rivercane may not be the only plant that benefits from added nutrients. While enhancing conditions for rivercane growth, side effects of competition are likely. Experiments should evaluate these associations to determine if fertility is a limiting factor for rivercane growth and establishment.

Weed Competition

If rivercane is the target species, management of competition may be necessary for successful establishment. Constraints are imposed upon initial field establishment however, due to lack of research on this species. Nevertheless, when establishing either ramets or seedlings, particularly in native habitat for conservation purposes, control of weed competition is essential (Sweeney et al. 2002).

Research is limited on the use of herbicide application among established or newly-planted rivercane, but reactions are likely to be similar to that of other cool-season grasses. When considering competition, both herbaceous and woody species become a factor. In 2009 an experiment was conducted by Nathan and Joyce Klaus (2011) to test the tolerance of switchcane (*A. tecta*) to four commonly used woody herbicides. Herbicides included hexazinone (Velpar® L, DuPont Crop Protection), glyphosate (Razor Pro®, Nufarm Specialty Products), triclopyr (Garlon® 3A, Dow AgroSciences LLC), and imazapyr (Chopper®, BASF Specialty Products) and were applied per manufactures' instructions. Damage or topkill of rivercane culms were observed with glyphosate, imazapyr, and triclopyr treatments, but not in the control or hexazinone treatments (Klaus 2011). Imazapyr and glyphosate had significantly more damage to switchcane than the other treatments. Triclopyr and hexazinone had the least negative affect on cane health after 15 months. The herbicides, along with 2,4-D and Trimec® used are known to effectively control woody competition such as privet (*Ligustrum sinense*) and hardwood seedlings such as maple (*Acer spp.*) and water oak (*Quercus nigra*) (Klaus and Klaus 2011). These herbicides also exhibit different modes of action and therefore effectiveness is dependant on environmental conditions.

Other studies have shown successful rivercane growth in areas pre-treated with herbicide to reduce weed competition, but do not address injury or rivercane tolerance. In an experiment conducted by Mills (2011) at Mississippi State University, a randomized block, split-plot design was established which contained two sub-plot factors: soil tillage and herbicide application. These factors were both incorporated and applied separately two weeks prior to planting on two plots, one dominated by exotic weeds and the other by native species. The herbicide, glyphosate (Eraser®, Control Solutions Inc.) was used to kill existing native and non-native competition. Pendimethalin (Prowl, BASF® Specialty Products) was also sprayed among experimental units requiring herbicide in order to prevent the emergence of weed seed. Results indicated that rivercane growing amongst exotic weed species responded favorably to herbicide-sprayed treatments vs non-sprayed treatments by increasing both spread and diameter. Rivercane in the exotic-dominated plot also showed an increase in shoot diameter in treatments that used a combination of tillage and herbicide (Mills 2011). When observing plant height, rivercane in native-dominated plots grew taller in treatments not containing herbicide vs those that were sprayed. This may be due to adjacent plants' competition for light. When comparing types of competition, either native or exotic, results indicate that site preparation by herbicide application does not enhance growth of rivercane grown among native competition. However among exotic competition, growth of rivercane was increased as demonstrated by plant growth index, radial spread, and shoot diameter (Mills 2011).

As these studies indicate, selective herbicides may be used without harm to rivercane and pre-treating areas could be beneficial in establishment. However, in order to address additional competition issues of rivercane for establishment, cultural management practices may provide mutual control.

Use of Fire as a Management Practice

Previous experiments by Zaczek et al. (2010) utilized fertilization in conjunction with prescribed fire on a four year old rivercane stand. After initiating a restoration project in 2001 in a bottomland cleared for agriculture, an experimental design was set up which tested four treatment combinations of fire and fertilization. In 2005, 20 plots of one meter wide by 14 meters long were established. The four-year old planting was tested by combining the use of prescribed fire with inorganic nutrient applications (N, P, and K). A granular (12-12-12) nutrient mix was applied to the soil surface at a rate of 56.1 kg N/ha. Vegetation sampling was taken between January and April each year. Both plots were burned on the same day in February one year later. The first notable results indicated that prior to burning, there were greater than three times the number of dead culms in unfertilized plots compared to plots receiving fertilization, suggesting fertilization may increase survivorship. Supporting these results, increases in culm density, height, and cover were found in plots receiving fertilizer without burning during the second growing season. Fire treatments affected rivercane by decreasing the culm height and diameter, but not density and spread. Increased density was not seen in unburned plots. When culm stands were more dense, both height and diameter were reduced (Zaczek et al. 2010). Li et al. (1998) attributes this phenomenon to reductions in carbohydrates after burning when new shoot growth is initiated. Initially the use of fire seems to reduce height and cover of culms, but it is favorable in canebrake longevity. Results from these data seem to confirm historical accounts that the use of fire is beneficial.

Gagnon and Platt (2008) also confirmed that rivercane ramet densities nearly doubled in stands subjected to windstorm and fire disturbance in ten months. Data indicated that during a single growing season, rivercane stands more than replaced all burned culms, suggesting that established understory canebrakes respond rapidly to large-scale disturbances (Gagnon and Platt 2008). Large openings similar to the ones established in natural disturbances are known to promote the earlier successional habitat. However, research has shown that disturbances occurring greater than ten years may deplete rhizome stores (Hughes 1951, 1966; Gagnon and Platt 2008), suggesting management of disturbance return time is needed.

CHAPTER III

MATERIALS AND METHODS

Response to Light Reduction

Light regime is a critical component of all plant growth. Light is often a limiting factor of native rivercane stands. An experiment was conducted during the summer of 2010 and 2011 on the R.R. Foil Plant Science Research Facility (North Farm) at Mississippi State University. The objective of this study was to assess effects of four levels of shade, plus full sunlight, on the growth of individual rivercane seedlings. This was conducted by reducing ambient sunlight by 30, 50, 60, and 85%, plus 0% (control). This study was arranged in a randomized complete block (RCB) design with subsampling. This design consisted of six replications, each of which contained the four light reduction treatments and the control. Experimental units (EU) were three plants of the same treatment within each block.

Plant material were derived from seed collected from a flowering stand in May of 2009, Fayette County, Kentucky (38.04° N, 84.51° W). After germination, seedlings were grown in a greenhouse approximately eight months prior to field planting. Ninety seedlings were transplanted into 22.8 cm diameter pots (Classic 550, Nursery Supplies Inc.) on June 22, 2010 and randomly assigned to each of the five shade treatments in each block. Shade structures were constructed using Garden Plus® galvanized poultry netting

(24" wide) and four thicknesses of light-reducing mesh. The five treatments included: poultry netting structure without wire screening (0% light reduction), a single layer of screening (Phifer Inc.® Charcoal Aluminum) (30% light reduction), a double layer of the same material (50% light reduction), a single layer of screening (Phifer Inc.® Charcoal Fiberglass) (60% light reduction), and a double layer of the same material (85% light reduction). Galvanized poultry netting was rolled and cut, creating a cylindrical frame 60 cm tall and with a diameter large enough to fit over each pot (23cm diameter and 60 cm in height). For the 30, 50, 60, and 85% light reduction, appropriate screening combinations were wrapped around the wire frame (control treatments had no screening).

Pot bottoms were removed from each of the pots so that when planted, the soil both inside and outside of the pot would be in direct contact with each other, allowing capillary movement and water adsorption to the plants. This also allowed for both the rhizomes and roots to be semi-contained within the pot sides during the study period. The soil type was a Leeper silty clay loam. Leeper soils are typically found in flood plains and are somewhat poorly drained with 0-2% slopes. Bottomless pots were set into the ground 20 cm, with approximately 3 cm left above soil line. The removed soil was used as planting medium for seedlings. All shade cages of the various light reduction levels were lowered over each pot approximately 10 cm and excess soil was packed against the outside to hold cages in place. After planting, 12.5g of Osmocote ® 19-6-12 was added to each potted seedling and watered in with approximately 2 liters per pot. Seedlings received the same amount of water every other day, as needed, for approximately three weeks. All cages were removed at arboreal leaf senescence in autumn (November 19, 2010) and replaced during spring leafing (March 31, 2011).

Five times throughout the study period (Initial-June 16, 2010; November 19, 2010; June 22, 2011; November 23, 2011; May 22, 2012), measurements were taken of shoot height, canopy diameter, and number of culms to determine plant growth index (PGI). PGI is a non-destructive measure of plant canopy volume (cm^3). This was determined by first, taking the plant's longest cross section measurement (XS_1), followed by the subsequent perpendicular cross section measurement (XS_2). These cross sections were averaged to get the mean diameter of the rivercane plant. After dividing by two to get the mean radius (r), and acquiring the height of the tallest shoot (h), PGI was measured by the equation: $[\pi (((XS_1 + XS_2)/2)/2)^2]\ h$.

We also calculated differences in PGI between measurement dates to determine a trend in growth rate over the two year study period. Initial measurements were recorded and subtracted from each of the five month measurements, resulting in the first period of PGI differences. The five month measurements were subtracted from 12 month, 12 month from the 17 month, and the 17 month from the final 23 month measurements, respectively. Initial measurements were then subtracted from the final to determine total mean PGI over time. The same procedure was conducted on the recorded number of culms from each plant throughout the duration of the experiment.

At the conclusion of the study, biomass accumulation (dry weight) was used to measure actual photosynthetic accumulation. This was a destructive indicator of plant growth, in which we accounted for all plant material both above and below ground. Plants were dug from the study site, ensuring all living tissue remained intact. They were then hand washed and all remaining soil was removed before separating plant parts by roots, rhizomes, stems, and leaves. Allocation of plant growth was determined by the dry weight measurement of each plant component. Plant parts were then placed in paper bags and oven dried at 60°C for at least 48 hours. Statistical analysis was conducted using PROC GLM ($p \leq 0.05$). Shade treatment, PGI, and shade treatment by PGI interactions were compared for each measurement time. Shade treatment, dry weight (by plant part and total), and shade treatment by weight interactions were also analyzed.

Fertilization Study

Literature is sparse on fertility requirements to maximize rivercane growth, but Zaczek (2010) reports that applications of fertilizer, particularly 12-12-12, significantly reduces mortality and increases growth of established rivercane stands. Our study, testing fertilizer rates, was installed January 2011, and a second in May. The objective was to compare different formulations of slow-release fertilizer in order to optimize growth. Eight slow-release fertilizer formulations were used (Table 1). To test phosphorus' effect, nitrogen was held constant across the eight fertilizers at 4 g, while phosphorus differed according to the brands' respective rate. Likewise, to test nitrogen's impact, phosphorus rate was held constant at 2 g across the eight fertilizer treatments; while the nitrogen varied according to the brands' respective formulation (Table 2). With the addition of two randomly assigned control treatments (no fertilizer), each study contained 18 fertilizer treatments. The constant rates of nitrogen and phosphorus were chosen based on the recommended amounts of slow release fertilizer brands most commonly used.

Table 1 Fertilizer treatments with nitrogen rates held constant. Applied to rivercane (*Arundinaria spp.*) seedlings and ramets on North Farm Research Facility on January 21 and May 10, 2011.

Trt #	Brand (analysis)	Manufacturer & Location	Rate (g/plant)	Total N (g)	Total P (g)
P1	Miracle-gro® tree & shrub* [a] (15-5-10)	Scotts Inc., Marysville, OH	26.7	4	1.3
P2	Miracle-gro® fruit & citrus* [b] (10-15-15)	Scotts Inc., Marysville, OH	40.0	4	6
P3	High Yield® (12-6-6)	Growers Special™, Bonham, TX	33.3	4	2
P4	Jobe's® tree and shrub (15-3-3)	Easy Gardener Inc., Waco, TX	26.7	4	1.9
P5	Agriform® tablets* [c] (20-10-5)	Scotts Inc., Marysville, OH	20.7	4	2.1
P6	Osmocote® (19-6-12)	Scotts Inc., Marysville, OH	21.1	4	1.3
P7	Ross® (8-16-16)	Easy Gardener Inc., Waco, TX	23.5	4	4
P8	Garden Club Select® * [d] (16-2-14)	Enviro-Safe Laboratories Inc., Sarasota, FL	25.6	4	0.5
P9	Control		0	0	0

* indicates fertilizers with micronutrients

[a] 16% sulfur, 0.1% iron, and 0.05% manganese

[b] 9.0% sulfur, 0.1% iron, and 0.05% manganese

[c] 3.3% calcium, 0.7% magnesium, 2.0% sulfur, 0.04% boron, 0.05% copper, 0.9% iron, 0.07% manganese, and 0.05% zinc

[d] 2.41% magnesium, 0.05% copper, 2.94% sulfur, 0.47% iron, 0.23% manganese, and 0.05% zinc

Table 2 Fertilizer treatments with phosphorus rates held constant. Applied to rivercane (*Arundinaria spp.*) seedlings and ramets on North Farm Research Facility on January 21 and May 10, 2011.

Trt #	Brand (analysis)	Manufacturer & Location	Rate (g/plant)	Total N (g)	Total P (g)
N1	Miracle-gro® tree & shrub* [a] (15-5-10)	Scotts, Inc., Marysville, OH	40.0	6	2
N2	Miracle-gro® fruit & citrus* [b]	Scotts Inc., Marysville, OH	13.3	1.3	2
N3	High Yield® (12-6-6)	Growers Special™, Bonham, TX	33.3	4	2
N4	Jobe's® tree and shrub(15-3-3)	Easy Gardener Inc., Waco, TX	66.7	4.3	2
N5	Agriform® tablets* [c] (20-10-5)	Scotts Inc., Marysville, OH	20.7	4.1	2
N6	Osmocote® (19-6-12)	Scotts Inc., Marysville, OH	33.3	6.3	2
N7	Ross® (8-16-16)	Easy Gardener Inc., Waco, TX	28.4	2.3	2
N8	Garden Club Select® * [d] (16-2-14)	Enviro-Safe Laboratories Inc., Sarasota, FL	100.0	16	2
N9	Control		0	0	0

* indicates fertilizers with micronutrients

[a] 16% sulfur, 0.1% iron, and 0.05% manganese

[b] 9.0% sulfur, 0.1% iron, and 0.05% manganese

[c] 3.3% calcium, 0.7% magnesium, 2.0% sulfur, 0.04% boron, 0.05% copper, 0.9% iron, 0.07% manganese, and 0.05% zinc

[d] 2.41% magnesium, 0.05% copper, 2.94% sulfur, 0.47% iron, 0.23% manganese, and 0.05% zinc

Upon completion, (May 2012) PGI and culm counts were made to determine changes in growth. Total biomass accumulation (a destructive harvest) was also made to determine actual plant growth. Dry weights of roots, rhizomes, stems, and leaves were recorded. End of experiment growth assessment (PGI), mean biomass (total, and each plant part), and interactions between treatments and propagule types were analyzed using PROC GLM. Differences were considered significant at $p \leq 0.05$.

Herbicide Study

The control of plant competition is another important factor to consider when establishing rivercane. During the summer of 2010 and 2011 studies were conducted to assess herbicides as an aid to initial establishment. Studies were installed on June 15, 2010 at the North Farm and June 20, 2011 at the Leveck Animal Research Center (South Farm), both located on the campus of Mississippi State University.

A RCB design with subsampling was constructed for the study and its repeat. Each of the four blocks included nine EUs. Each EU included five seedling plants. Treatments (eight herbicides plus control) were randomly assigned to the EUs across each block. The five plants within each EU were planted in a row with 0.3 meter spacing. There was a distance of 1.5 meters between both the EUs and blocks.

One hundred and eighty seedlings (Kentucky genotype) were maintained under greenhouse conditions for seven months, in planting media consisting of 75% pine bark mulch and 25% sand (v:v). Experimental plots were located within two soil types. Soils from the experimental area of North Farm consisted of Leeper silty clay loam. Soils for the June 2011 study on the South Farm included Catalpa silty clay loam.

One month after planting, the herbicide treatments were applied. Herbicides were chosen based on label specifications, the need to control specific species, and the hypothesis of injury. Herbicides were applied at the median label rate. A spray adjuvant (nonionic surfactant) was added at 0.25% v/v rate. Therefore 5ml of adjuvant was added to each 2L herbicide treatment. The applied herbicide and the rates for each (Table 3) are as follows: trifloxysulfuron (Envoke®; Syngenta Crop Protection, Inc., Greensboro, NC), nicosulfuron (Accent®; DuPont Crop Protection, Wilmington, DE), nicosulfuron 56.2%, metsulfuron 15% (Pastora®; DuPont Crop Protection, Wilmington, DE), imazapic (Plateau®; BASF Specialty Products, Research Triangle Park, NC), halosulfuron (Sedgehammer®; Gowan Co., Yuma, AZ), imazaquin (Image®; BASF Specialty Products), sethoxydim (Vantage®; Dow AgroScience, Indianapolis, IN) and fluazifop (Fusilade®; Syngenta Crop Protection, Inc., Greensboro, NC). Trifloxysulfuron was applied at 10.5g a.i./ha, nicosulfuron at 52.52g a.i./ha, nicosulfuron with metsulfuron was at 51.16g a.i./ha, imazapic at 0.138L a.i./ha, fluazifop at 0.2147L a.i./ha, halosulfuron at 72.6g a.i./ha, imazaquin at 0.084L a.i/ha, and sethoxydim at 0.33L a.i/ha.

Each herbicide was applied only once at the beginning of the study. Each herbicide treatment was separately contained and sprayed from a separate two-liter plastic bottle using a CO_2 pressured, banded spray applicator (nexAir, Memphis, TN). This was conducted in the early morning of July 13, 2010 (North Farm) and July 20, 2011 (South Farm) under still air conditions, reducing the risk of drift. Data collection did not focus on the specific grassy weeds controlled. Rather, herbicide damage to individual rivercane seedlings was assessed based on a 1-5 rating scale; 5 representing a rivercane seedling with no damage (Table 4). Seedlings assigned to the control treatment were the standard by which herbicide damage was assessed. The PGI of each seedling was also taken on the day of application. Ratings were taken of each seedling beginning the day of application, and continued every seventh day until the end of four weeks.

Table 3 Herbicides and application rates used in rivercane (*Arundinaria gigantea.*) used in 2010 herbicide trial. Herbicide rates were applied at one-half the recommended rate.

Chemical	Trade Name	Recommended Rate	Application Rate	Target Species
Fluazifop	Fusilade	0.43L a.i./ha	0.2147L a.i./ha	Grasses
Halosulfuron	Sedgehammer	145.2g a.i./ha	72.6g a.i./ha	Broadleaf & Nutsedge
Imazapic	Plateau	0.276L a.i./ha	0.138L a.i./ha	Grasses
Imazaquin	Image	0.168L a.i./ha	0.084L a.i./ha	Broadleaf & Nutsedge
Nicosulfuron + Metsulfuron	Pastora	102.32g a.i./ha	51.16g a.i./ha	Selective Broadleaf & Grasses
Nicosulfuron	Accent	105.04g a.i./ha	52.52g a.i./ha	Selective Grasses
Sethoxydim	Vantage	0.66L a.i./ha	0.33L a.i./ha	Selective Grasses
Trifloxysulfuron	Envoke	21g a.i./ha	10.5g a.i./ha	Selective Broadleaf & Grasses

Table 4 Herbicide damage to rivercane seedlings for 2010 and 2011 herbicide trial. Scale is based on a 1 to 5 rating with 1 describing rivercane seedlings with very severe damage and 5 describing rivercane seedlings with no damage.

Rating	Description
1	Very severe herbicide damage; herbicide application caused almost total seedling death (greater than 90% rivercane seedling is dead).
2	Severe herbicide damage; rivercane seedling tissue is mostly dead but some green tissue is present (greater than 75% of seedling is dead).
3	Average herbicide damage; slight damage to rivercane seedling (about 50% of rivercane seedling is dead).
4	Minimal herbicide damage; chlorosis or necrosis of seedling is minimal (up to 25% of rivercane seedling is dead).
5	No visible herbicide damage; rivercane seedling is 100% green.

Proc GLM procedure (SAS Version 9.2, SAS Institute, Cary, NC) was used to examine the effect of the herbicide treatment on rivercane seedlings over time. From this data we were able to determine herbicide effect on seedlings on a per-week basis throughout the study period.

Planting Date: Rivercane Growth and Survival

In addition to establishment methods, its important to consider the month, or season, of planting to increase the likelihood of survival. Understanding *Arundinaria's* physiology and C_3 response, our hypothesis would suggest planting during cooler and wetter times such as fall or early spring.

A two-study experiment was conducted, which utilized 24 rivercane seedlings in a monthly planting date test to determine the optimal month, or season, for increased field survivability. The initial experiment began in February 2011. It utilized seedlings from germination testing of Kentucky seed source. Seed were germinated six months prior to every consecutive planting date to ensure a consistent seedling age. The repeat study began in December 2011. It utilized the remaining Kentucky seedlings from Study 1 and others of similar age. All seedlings were kept in the greenhouse in 10.16 cm diameter pots (Classic C150, Nursery Supplies Inc.) and irrigated on an as-needed basis.

On the 15th day of each month, 24 potted seedlings were transplanted to a field at the R.R. Foil Plant Science Research Facility (North Farm). Test plots were located in a Leeper, silty clay loam soil, which is typically found in flood plains, and are somewhat poorly drained, with 0-2% slopes. The experiment was arranged as a Completely Randomized (CR) Design. Individual seedlings were planted in a plot arrangement of six columns and four rows with one meter between each plant.

In addition to planting date, two treatments were applied: cut (top growth removed) or no cut (top growth remained). Since removing top growth at planting reduces transpirational loss (Baldwin et al. 2009), each cutting treatment was randomly assigned to half of the 24 plants. One liter of water was applied to each plant only once. In addition to survival/mortality, plant growth index (PGI), and number of culms were recorded for each plant upon installation. PGI is a non-destructive measure of plant canopy volume (cm^3). This was determined by first, taking the plant's longest cross section measurement (XS_1), followed by the subsequent perpendicular cross section measurement (XS_2). These cross sections were averaged to get the mean diameter of the rivercane plant. After dividing by two to get the mean radius (r), and acquiring the height of the tallest shoot (h), PGI was measured by the equation: $[\pi (((XS_1 + XS_2)/2)/2)^2]$ h.

The initial study ended August 2011 (seven months) when all of the consecutively-germinated seedlings had been utilized. The second, repeat, study began in December 2011 and ended April 2012. Plants of Study I were collected on November 10, 2011 and plants for Study II were collected on May 22, 2012. Upon completion of each study; PGI, culm counts, and total number of survivors were recorded. Survival analysis was based on plant mortality determined from the initiation of the experiment to the final collection time for each study treatment. A plant was considered alive if any part of the above-ground growth remained green. Change in growth over time was also determined by subtracting ending PGI and culm numbers from initial recordings. This analysis would indicate any positive cumulative mean growth over time.

Proc GLM was used to test differences in mortality, PGI, number of culms, and top growth removal on plant growth. Pairwise comparisons were conducted to analyze the effects of treatment on mortality, PGI, number of culms, and top growth removal. Differences were significant at $p \leq 0.05$.

CHAPTER IV

RESULTS AND DISCUSSION

Response to Light Reduction

Plant Growth Indices

Initial objectives of this study were to determine how light reduction affected initial establishment of rivercane and to develop protocol for future restoration projects. Upon evaluation of our findings, analysis of variance (ANOVA) indicated interactions between treatments and time, regarding plant growth index (PGI), number of culms, and biomass. The significance of shade treatments, as indicated by total mean PGI across time, indicated variation (Table 5). Beginning with initial measurements at planting (June 2010), greatest plant growth was represented by the 30% light reduction treatment (1,624 cm^3). Growth, as measured by PGI, was not significantly greater than plants within 0% (full sun) treatment (1,347 cm^3), but was greater than 85% (1,155 cm^3), 60% (927 cm^3), and 50% (809 cm^3), respectively. Because there were differences in mean PGI across treatments initially, all comparisons were made on change in PGI over time (Table 6).

Table 5 Mean plant growth index (cm^3) of rivercane (*Arundinaria gigantea*), indicating interaction over time (June 2010 - May 2012) and by shade treatment. Results are from the light reduction study.

	Time									
Treatment (% light reduction)	Initial		5 month		12 month		17 month		23 month	
	- - - - mean PGI (cm^3) of seedlings - - - -									
85%	1155	C[†] bc[‡][¶]	5126	C ~~a~~	19,339	B ~~a~~	16815	B ~~a~~	28835	A ~~ab~~
60%	927	C c	2933	C ~~b~~	17,162	B ~~ab~~	15517	B ~~ab~~	36436	A ~~a~~
50%	809	B c	1154	B ~~c~~	5,027	AB ~~c~~	5254	AB ~~abc~~	8320	A ~~b~~
30%	1624	CD a	1149	D ~~c~~	6,181	AB ~~bc~~	4382	BC ~~bc~~	7401	A ~~b~~
0%	1347	A ab	392	A ~~c~~	1,858	A ~~c~~	1021	A ~~c~~	1470	A ~~b~~

[†] Values followed by the same UPPERCASE letter within a row (changes over time) are not significantly different at the 0.05 level.

[‡] Values followed by the same lowercase letter within a column (differences between light treatments) are not significantly different at the 0.05 level.

[¶] Strikethrough indicates invalid data comparison due to initial variation between treatments.

Table 6 Mean change in plant growth index (cm^3) of rivercane (*Arundinaria gigantea*) within a time period (June 2010 - May 2012), due to shade treatments. Results are from the light reduction study.

Treatment (% light reduction)	Time									
	June 2010 - Nov. 2010		Nov. 2010 - June 2011		June 2011 - Nov. 2011		Nov. 2011 - May 2012		Initial - Final collection	
	- - - - mean PGI (cm^3) of seedlings - - - -									
85%	4,227	a[‡]	14,213	a	-3,575	a	13,070	a	27,671	ab
60%	1,930	b	11,777	a	-2,938	a	19,367	a	35,448	a
50%	314	c	1,718	b	-1,086	a	3,816	a	7,589	bc
30%	-425	c	2327	b	-2,773	a	3,020	a	5,722	bc
0%	-894	c	73	b	-837	a	449	a	-511	c

[‡] Values followed by the same lowercase letter within a column (differences between light treatments) are not significantly different at the 0.05 level.

As we observed initial change over the first five months, indicated by PGI difference from June 2010 - November 2010, 85% (4,227 cm^3) light reduction treatment produced significantly greater growth than any other treatment. Plants within the 60% (1,930.3 cm^3) light reduction treatment had the second greatest growth during this time period, and was significantly more than plants within 50% (314 cm^3), 30% (-425 cm^3), and 0% (-894 cm^3) light reduction treatments, respectively. There were no PGI differences between these remaining three treatments.

Analysis of change in PGI during the second time period (November 2010 - June 2011) indicated that treatments 85% (14,213 cm^3) and 60% (11,777 cm^3) produced the greatest change in plant growth. Like the previous measurement period, shade treatments

30% (2,327 cm^3), 50% (1,718 cm^3), and 0% (73 cm^3) produced significantly less growth, respectively.

The next measurement period (June 2011 - November 2011) indicated a net loss in mean growth across every shade treatment. During this time period, there were no shade treatments which produced growth significantly different from another. Measurements from the following time period (November 2011 - May 2012) indicated positive growth from every shade treatment. However, like the previous measurement period , there were no single shade treatments which produced plant growth significantly different from another.

Upon evaluation of the total change in plant growth (initial planting - final collection) over time, by treatment, data indicated that shade treatments 60% (35,448 cm^3) and 85% (27,671 cm^3) produced the greatest PGI. Shade treatments 50% (7,589 cm^3) and 30% (5,722 cm^3) resulted in the next greatest growth over time, respectively, but did not result in significantly less plant growth than treatment 85%. Plant growth within treatment 0% (-511 cm^3) was the only treatment which produced a net loss in plant growth over the study duration.

Our results do not agree with previous field studies (Russell et al. 2010), which indicated that rivercane seedlings had greatest response from full sun conditions. In this previous study, scheduled irrigation was utilized throughout the experiment. Findings by Cirtain and others (2009) also differed from ours. Light reduction experiments on existing forest brakes indicated positive growth response to an opened overstory canopy, suggesting light was a limiting factor (Cirtain et al. 2003). In both these experiments however, soil moisture was not limiting. Regarding our latest field experiment, plants were only watered as needed for the first three weeks. Results indicated that when moisture is lacking during establishment, seedling plants compensate under increased shade (60% light reduction) by increasing leaf canopy, amplifying PGI. Field applications of rivercane propagules would benefit from an overstory canopy that reduces light by 60% - 85%, and minimizes competitive vegetation.

We then analyzed treatments separately and used ANOVA to indicate changes in PGI over time (Table 5). Beginning with the 85% light reduction treatment, we observed an increase in PGI over time. We first noticed significant growth 12 months after planting (June 2011). From June 2011 to November 2011 data indicated a reduction in PGI, (19,339 cm^3 to 16,815 cm^3), but the difference was not significant. During the final measurements (May 2012) we noted another significant increase in PGI (28,835 cm^3), which was the greatest growth within treatment 85% to date. We noted, that the significant increase from November 2011 to May 2012 was a 71.4% increase in PGI.

We then observed change in PGI over time within the 60% light reduction treatment. Here, we noted, the same trend and level of significance as growth in treatment 85%. Again, growth increased during all points of measurement, except between June 2011 and November 2011 (17,162 cm^3 to 15,517 cm^3). The final measurement (May 2012) indicated a significant spike in PGI from November 2011, that was a 134.8% increase in rivercane growth.

Variance in plant growth over time was not as evident in the 50% light reduction treatment. Despite another increasing PGI trend, the first significant increase in growth was not seen until May 2012, 23 months after planting. PGI during this time was not significantly greater than growth at the June 2011 and November 2011 measurement dates. Unlike treatments 85% and 60% which indicated a decline in PGI from June 2011 to November 2011, plants within treatment 50% gradually increased. Data indicated a 4.5% increase in PGI from June 2011 to November 2011 and a 58.3% increase from November 2011 to May 2012.

Regarding changes in PGI over time within the 30% light reduction treatment, fluctuating plant growth was evident. Inconsistency was observed between every measurement date for plants within this treatment. Although not significant, there was a numerical drop in PGI from the initial measurement date (June 2010) to November 2010 (-475 cm^3). We noted that mean PGI decreased at every measurement which followed the summer period (June - November). Conversely, PGI increased at every measurement which followed the winter period, suggesting a typical C_3 photosynthesis response. As noted from previous treatments, the first significant increase in growth was seen 12 months after planting; a gain of 4,557 cm^3 from planting date. Growth declined once more from June 2011 to November 2011, however the change was not significant. Significant growth was seen from November 2011 to the final measurement (May 2012), a 68.8% increase. Despite the undulating trend, after 23 months, the increase in mean PGI was significant in the 30% light reduction treatment.

Based on ANOVA, an increase in plant growth, as measured by PGI, from the 0% light reduction treatment (full sun) was constant over time. There was an increase in plant growth (cm^3) over time, but change was not significant (Table 4a). The first five months actually resulted in a drop in PGI, but it then increased from November 2010 to June 2011, a trend also seen in treatment 30%. From June 2011 to November 2011 PGI declined again; a -831 cm^3 PGI loss. By the final measurement date, plant growth had once again recovered 43.9% from six months prior.

As observed in other shade treatments, increases in PGI during the winter months is evidence for rivercane's most active growth. A reduction in above-ground growth may appear during summer months and environmental stresses (i.e. soil moisture loss), but the typical C$_3$ photosynthesis response curve rebounds with cooler temperatures. Adequate soil moisture may also contribute to growth during this period, as survival studies have suggested (Hamlington et al. 2011). As Cirtain and others (2009) found from field experiments, rivercane maintains a net photosynthetic response during the temperate dormant season. Therefore, an ideal restoration site would benefit rivercane propagules if light is reduced by 60% and adequate soil moisture is available.

Culm Growth

In addition to PGI, culm production, another indicator of rivercane growth, was observed during the study period. As in PGI data, the significance of shade treatments, as indicated by total mean difference in the number of culms across time, indicated variation (Table 7). However, because there were differences in mean number of culms across treatments initially, all comparisons were made based on change in number of culms over time (Table 8).

As we observed initial change in the first five months (initial planting to November 2010), plants within treatment 85% (1.62) indicated a greater increasing change in the number of culms per plant, than plants within treatment 50% (0.07) and

30% (0.68). Culm differences within treatments 60% (0.71) and 30% (0.68) were not significantly different than treatment 85% (Table 8).

Table 7 Mean number of rivercane (*Arundinaria gigantea*) culms per seedling, as reported for each collection date (June 2010 - May 2012) according to shade treatment. Results are from the light reduction study.

	Time									
Treatment (% light reduction)	Initial		5 month		12 month		17 month		23 month	
	- - - - mean number of culms per seedling - - - -									
85%	2.22	D[†] ~~a~~[‡] [¶]	3.93	C ~~a~~	7.50	B ~~a~~	7.13	B ~~a~~	10.43	A ~~ab~~
60%	2.55	C ~~a~~	3.42	C ~~ab~~	6.91	B ~~ab~~	6.45	B ~~ab~~	14.40	A ~~a~~
50%	1.61	B ~~b~~	1.78	B ~~c~~	3.37	AB ~~ab~~	3.33	AB ~~ab~~	5.28	A ~~bc~~
30%	1.61	D ~~b~~	2.31	CD ~~bc~~	4.11	BC ~~ab~~	5.14	AB ~~ab~~	6.28	A ~~bc~~
0%	1.55	A ~~b~~	1.75	A ~~c~~	3.00	A ~~b~~	3.00	A ~~b~~	2.00	A ~~c~~

[†] Values followed by the same UPPERCASE letter within a row (changes over time) are not significantly different at the 0.05 level.

[‡] Values followed by the same lowercase letter within a column (differences between light treatments) are not significantly different at the 0.05 level.

[¶] Strikethrough indicates invalid data comparison due to initial variation between treatments. time (Table 5b).

Table 8 Mean change in number of rivercane (*Arundinaria gigantea*) culms per plant within a time period (June 2010 - May 2012), due to shade treatments. Results are from the light reduction study.

	Time									
Treatment (% light reduction)	June 2010 - Nov. 2010		Nov. 2010 - June 2011		June 2011 - Nov. 2011		Nov. 2011 - May 2012		Initial - Final collection	
	- - - - mean number of culms per seedling - - - -									
85%	1.62	a[†]	3.56	a	-0.66	a	3.33	ab	8.12	ab
60%	0.71	ab	3.08	a	-0.81	a	7.40	a	11.60	a
50%	0.07	b	1.2	a	-0.66	a	2.50	ab	3.42	bc
30%	0.68	ab	1.11	a	0.28	a	1.14	b	4.28	bc
0%	0.00	b	1.0	a	0.00	a	-1.00	b	0.00	c

[†] Values followed by the same lowercase letter within a column (differences between light treatments) are not significantly different at the 0.05 level.

Throughout the duration of the second measurement period (June 2010 to November 2010), data indicated no differences in culm growth between any shade treatment. However, within every shade treatment, plants did show positive change in growth, suggesting benefits from winter months.

From June 2011 to November 2011, treatments 85% (-0.66), 60% (-0.81), and 50% (-0.66), resulted in net losses in the mean number of culms per plant. Despite the reduction in the mean number of culms over time, there were no significant differences in change between any shade treatment during this period. Between November 2011 and May 2012, 60% light reduction treatment had the greatest increase in mean number of culms (7.40). However, change in mean culm number from treatments 85% and 50% were not significantly less than treatment 60% (Table 8).

Upon analysis of total change in the mean number of culms per plant across shade treatments, we observed results that were similar to total PGI across time. From these results, data indicated that 60% shade treatment (11.6) produced greater number of culms over time than any other shade treatment except for 85% (8.12) light reduction.

This data suggests that culm production is a component of PGI, and increases proportionately over time. Our results however do not agree with Gagnon et al. (2007) and Cirtain et al. (2009), whose field experiments found increased culm production in open forest canopies, rather than increased shade. A major difference in our study is establishment. Previous research was conducted on established brakes persisting underneath forest canopy. Our results regarding culm growth suggest increased shade (60%) is necessary for best growth during initial establishment.

We then analyzed treatments separately and used ANOVA to indicate changes in culm growth over time (Table 7). Beginning with the 85% light reduction treatment, we observed an increasing culm production trend over the duration of the experiment. Like the mean number of culms per plant in the 60% light reduction treatment, after 23 months, culm production was significantly greater than data from any other measurement date.

Culm production also indicated an increasing trend within the 50% and 30% light reduction treatments after 23 months. However, culm production from these treatments had less variation, meaning production was not as rapid as plants within treatments 60% and 85% light reduction.

Culm production from plants within the 0% light reduction indicated the least change over time, compared to all other treatments. There was no significant growth between any measurement date, suggesting once again, full sun does not promote adequate growth and culm production during initial establishment.

Regarding culm production specifically, light reduction treatments 85%, 60%, 50%, and 30% all promote increased production, indicating findings similar to Gagnon and others (2007). When clonal spread of established stands were compared between different light intensities (16.1% and 88.3% mean total transmitted light), they found the rate of expansion was similar. Their findings suggests clonal spread was not disturbance-dependant. Our results imply both 85% and 60% light reduction is suggested to promote greatest culm production during initial establishment.

Biomass Accumulation

Based on findings from previous studies (Russell et al. 2010), biomass accumulation has been an accurate indicator of actual plant growth verses solely PGI. Separated by plant parts, ANOVA indicated variation between most plant parts and its interaction by shade treatment (Table 9). Root biomass was the only plant component that was not significantly affected by shade treatments. Although not significantly different from any other, treatment 60% had the highest numeric mean dry weight root biomass with 8.8 g.

Table 9 Mean dry biomass accumulation by rivercane (*Arundinaria gigantea*) plant part as affected by shade treatments from June 2010 through May 2012. Dry weights taken May 2012. Results are from the light reduction study.

	Rivercane Plant Biomass by Part									
Treatment (% light reduction)	Roots		Rhizomes		Stems		Leaves		Total	
	- - - - mean dry weight (g) - - - -									
85%	5.68	a[†]	9.06	ab	5.22	ab	5.25	ab	25.2	ab
60%	8.80	a	16.20	a	9.40	a	7.40	a	41.8	a
50%	3.92	a	5.07	b	3.71	ab	2.28	ab	15.0	ab
30%	3.00	a	3.00	b	2.21	b	1.14	b	9.3	b
0%	2.00	a	0.50	b	0.50	b	0.50	b	3.5	b

[†] Values followed by the same lowercase letter within a column (treatment effects) are not significantly different at the 0.05 level.

Regarding rhizome biomass, treatments 60% (16.2 g) and 85% (9.06 g) produced the greatest amount of rhizome biomass according to dry weight. Remaining treatments, 50% (5.07 g), 30% (3.0 g), and 0% (0.5), produced significantly less rhizome biomass after 23 months than treatment 60%, suggesting increased light percentages may not be ideal for initial establishment especially when soil moisture is limited. These results are in opposition to findings from a previous study (Russell et al. 2010), where rivercane biomass results indicated greatest growth within 0% light reduction (full sun). Biomass derived from above ground growth (stems and leaves) showed identical effects regarding light reduction percentages. Differences in stem and leaf biomass were not as great as the difference in rhizome growth across treatments. However, both above-ground components did have the greatest biomass within treatment 60%, like rhizomes. Mean stem biomass totaled 9.4 g, while leaf biomass totaled 7.4 g within treatment 60%.

Growth from 60% light reduction does not agree with previous studies (Cirtain et al. 2009; Gagnon et al. 2007; Russell et al. 2010). Field experiments by Cirtain and Gagnon observed growth responses of existing stands to changes in forest canopy, rather than initial planting. Their experiment suggested increased light was directly related to increased growth, but there were no root establishment or soil moisture issues. Since light is a limiting factor in the growth of rivercane and other C_3 plants (Cirtain et al. 2003), results indicating greatest biomass under full sun conditions would not have been surprising. Instead, low amounts of underground biomass and soil moisture, likely increased mortality. Water availability was the difference between these studies. The initial shade study (Russell et al. 2010) utilized regular, scheduled irrigation. This, the repeat study planted in June 2010, was only watered during the first three weeks. After 23 months, higher light treatments caused high moisture stress, which resulted in low biomass and mortality. Similar results were found by Yamashita and Manning (1995) in their restoration experiments in California. Research conducted on established stands suggest increased light may prove beneficial, but partial shade (60%) seemed to aid in initial rivercane seedling establishment.

Fertilization Study

January 2011

Analysis of variance (ANOVA) indicated significant interaction between the two studies (January and May), therefore each was analyzed separately. An overall assessment of the field experiment, planted January 2011, indicated 68% mortality, leaving 46 rivercane propagules to evaluate (Table 10). The remaining live plants were analyzed for PGI and total biomass. Analysis of PGI indicated significant differences between fertilizer treatments ($p \leq 0.05$). The greatest increase in PGI occurred with treatment N6 (Osmocote® 19-6-12; 1,039,101 cm^3) and indicated significantly greater above-ground growth than all other treatments, except for N1 (347,693 cm^3), N9 (control; 335,703 cm^3), P3 (662,643 cm^3), and P6 (555,852 cm^3). It should be noted that treatment N6 is the same fertilizer brand as P6; also among the greatest PGI. There were no significant differences in mean PGI of plants between any of the remaining treatments.

Mortality percentages from this study were also substantial. While plants in the N6 treatment had the greatest PGI, it contained a single surviving ramet propagule, limiting our ability to make accurate assumptions. Treatments P2 and P4 had the lowest numerical mortality, both with 37.5% (Table 10). However, neither of these treatments' mortality was different than all others, except N8 (100% mortality). Variability found in results suggests repeat studies with higher reps may be necessary to have more viable data.

An assessment of total biomass accumulation after 23 months indicated actual growth of each plant by accounting for above and below ground plant parts. Plant components were separated and included: dry weight of roots, rhizomes, stems, and leaves. Analysis of variance showed substantial variance around the mean of plant components, therefore clear separation due to treatment was difficult.

Table 10 Mean plant growth index (PGI) and mortality results of rivercane (*Arundinaria spp.*) from January 2011 fertilization study. Data indicates interaction effects of fertilizer treatment.

Fertilizer Treatment*	N (live)	Mean PGI (cm^3)		Mortality (%)	
P1	2	126,573	b[†]	75	ab
P2	5	117,215	b	37.5	ab
P3	4	662,643	ab	50	ab
P4	5	195,957	b	37.5	ab
P5	2	218,422	b	37.5	ab
P6	1	555,852	ab	87.5	ab
P7	3	215,82	b	62.5	ab
P8	4	143,397	b	50	ab
P9 (control)	3	62,648	b	62.5	ab
N1	1	347,693	ab	87.5	ab
N2	4	195,783	b	50	ab
N3	1	137,637	b	87.5	ab
N4	2	71,548	b	75	ab
N5	3	73,282	b	62.5	ab
N6	1	1,039,101	a	87.5	ab
N7	1	308	b	87.5	ab
N8	0	-	-	100	a
N9 (control)	4	335,703	ab	50	ab

* Specific formulations can be found in Tables 1a and 1b

[†] Values followed by the same letter within a column (treatment effects) are not significantly different at the 0.05 level.

- Denotes values that could not be calculated due to 0 plant growth index (100% mortality).

Since literature (Hughes 1966; Cirtain 2004) and observations from previous field studies have indicated underground plant stores to be the source of survival and rejuvenation for rivercane, roots and rhizomes were an obvious target for analysis. When comparing root biomass across treatments, ANOVA indicated significant variance around the mean (Table 11). Data showed that the greatest root production occurred in treatment P5 (59.0 g), suggesting 20.7 g per plant of this nutrient formulation, was most ideal for root production. This treatment (Agriform® tablets 20-10-5) produced significantly greater root biomass than all other treatments, except P2 (17.2 g), P3 (31.5 g), P4 (16.8 g), P6 (58.0 g), N2 (19.5 g), N4 (17.0 g), and N6 (46.0 g).

Rhizome biomass, a collection of underground stems and lateral buds, did not indicate a variation as wide a range as root biomass. There were no other fertilizer treatments which produced rhizome biomass equal to N6 (118.0 g). Treatment N1 (Miracle-gro® tree & shrub 15-5-10) produced the next best rhizome biomass (50.0 g), but was only significantly greater than N4 (2.0 g) and N7 (0.5 g).

Stems and leaf biomass were then analyzed, and indicated data similar to rhizomes. Rivercane stem data indicated only one treatment produced a dry weight as significant as N6 (82.0 g). Treatment P6, which is a decreased rate of the same fertilizer (Osmocote® 19-6-12), produced an accumulated stem dry weight of 54.0 g. This treatment was in the same grouping as treatment N6, but with variance to group it with all other treatments, except P7 (4.0 g), N4 (10.0 g), and N7 (0.5 g). Analysis of leaf biomass accumulation, indicated there were no fertility treatments which produced leaf biomass as great as Osmocote® (N6) at the 0.05 significance level.

Table 11 Mean dry biomass accumulation of rivercane (*Arundinaria spp.*) from January 2011 fertilization study by plant part as affected by fertilizer treatment. Dry weights taken May 2012.

Fertilizer Treatment	Rivercane Plant Biomass by Part									
	Roots		Rhizomes		Stems		Leaves		Total Biomass	
	- - - - mean dry weight (g) of plant parts - - - -									
P1	5.0	cd[†]	11.2	bc	19.0	bc	15.0	bc	50.2	bc
P2	17.2	abcd	16.8	bc	20.4	bc	12.9	bc	67.3	bc
P3	31.5	abcd	34.0	bc	38.5	bc	29.0	bc	133.0	bc
P4	16.8	abcd	19.8	bc	22.5	bc	13.0	bc	72.1	bc
P5	59.0	a	23.2	bc	37.0	bc	20.0	bc	139.2	bc
P6	58.0	ab	44.0	bc	54.0	ab	30.0	bc	186.0	ab
P7	2.6	d	4.6	bc	4.0	c	3.33	b	14.6	c
P8	15.5	bcd	14.0	bc	18.1	bc	10.6	bc	58.2	bc
P9 (control)	6.8	cd	11.5	bc	12.1	bc	7.6	bc	38.1	bc
N1	14.0	cd	50.0	b	18.0	bc	34.0	b	116.0	bc
N2	19.5	abcd	30.1	bc	22.6	bc	15.1	bc	87.3	bc
N3	8.0	cd	24.0	bc	36.0	bc	12.0	bc	80.0	bc
N4	17.0	abcd	2.0	c	10.0	c	5.0	bc	34.0	c
N5	6.0	cd	9.3	bc	11.3	bc	6.0	bc	32.6	c
N6	46.0	abc	118.0	a	82.0	a	66.0	a	312.0	a
N7	2.0	d	0.5	c	0.5	c	0.5	c	3.5	c
N8	-	-	-	-	-	-	-	-	-	-
N9 (control)	11.5	cd	22.1	bc	24.5	bc	17.2	bc	75.3	bc

[†] Values followed by the same letter within a column (treatment effects) are not significantly different at the 0.05 level.

- Denotes values that could not be calculated due to 0 plant growth index (100% mortality).

Upon evaluation of total plant biomass accumulation, the greatest mean dry weight of plants came from treatment N6 (312.0 g) (Table 11). Total dry weights from treatment P6 (186.0 g) was the only other treatment not significantly different, suggesting Osmocote® 19-6-12 as the most likely product to promote rivercane growth. However, treatment N6 and P6 had only one surviving plant, preventing us from making accurate fertility suggestions. P6 was similar to all other treatments, except P7 (14.6 g), N4 (34.0 g), N5 (32.6 g), and N7 (3.5 g). These had lower total mean biomass weights than N6 and P6.

Osmocote® 19-6-12 was the same product used by Yamashita and Manning (1995) to establish shrubs in California. In this experiment, fertilizer (10 g) increased growth, especially during the second year with adequate soil moisture. Our experiment produced greatest growth from 33.3 g (N6), which is higher than nitrogen rates suggested by Cirtain et al. (2009). However, Cirtain's experiment used ammonium nitrate (NH_4NO_3), a form either lost or utilized rather quickly. Osmocote® 19-6-12, extended release, provided nutrients over a longer period (four months) as suggested by previous research (Judziewicz et al. 1999). The addition of adequate soil moisture from January planting likely aided in plant establishment and fertilizer uptake.

An assessment of propagule type, as indicated by mean total biomass from each plant component, confirmed our hypothesis that rivercane ramets resulted in higher establishment rates and increased growth (Cirtain et al. 2004). Data from January 2011 planting (January 2011 - May 2012) indicated that ramets maintained significantly higher biomass accumulation (g) than seedlings across all plant parts (Table 12). For this reason, Feeback and Luken (1992) found that rhizome cuttings were a preferred transplanting method for establishing stands. We suspect the difference is due to propagule type instead of species, but we can not definitively make that statement.

Table 12 Mean dry biomass accumulation of rivercane (*Arundinaria spp.*) from January 2011 fertilization study after 17 months of growth, indicating effects of propagule type on rivercane plant part.

	Rivercane Plant Biomass by Part									
Type (ramet or seedling)	Roots		Rhizomes		Stems		Leaves		Total Biomass	
	- - - - total mean dry weight (g) of plant parts - - -									
Ramet	23.6	a[†]	29.4	a	30.6	a	20.3	a	104.1	a
Seedling	4.3	b	2.1	b	3.7	b	2.9	b	13.1	b

[†] Values followed by the same letter within a column (propagule type) are not significantly different at the 0.05 level.

May 2011

The second fertility study, planted in May 2011, experienced excessive mortality. At the conclusion of the field experiment (May 2012), plant mortality was so great; biomass data collection was impossible. An overall mortality of 82.6% (Table 13), left only 25 of 144 rivercane propagules to assess. We did however, assess remaining data by treatment, noting the impact of fertility treatment on mortality. Both control treatments (P9 and N9) had the least mortality. There was only a single rivercane plant which died among the 18 plants in the two treatments (Table 13). Treatment P9 indicated a 12.5% mortality, while N9 had 0% (100% survivability). This leads us to believe that fertilizers may be more beneficial to established stands rather than new propagules. Adequate soil moisture is necessary for establishment, and May is not the ideal time of planting.

Analysis of applied fertilizer treatments indicated different results between the two studies, suggesting soil moisture, due to date of planting, may be a limiting factor. Based on data, primarily the January 2011 study, the N6 treatment of Osmocote® 19-6-12 promoted the greatest mean PGI and total biomass accumulation in rivercane. However, this is based on a single plant in each Osmocote® treatment (P6 and N6). Due to low survival of plants within this treatment population, we cannot make conclusive statements about Osmocote®. Though, at a rate of 33.3 g per plant (N6), 6.3 g total nitrogen was the most sufficient at increasing plant growth. Treatment N6 also promoted more growth than the varied rate of the same brand (P6) where Osmocote® 19-6-12 was applied at 4 g of total nitrogen per plant. Trends detected within these two treatments of Osmocote® denote reason to do more investigations. Despite using the same brand, our study was limited to more than the one-third the "recommended rate" Yamashita and Manning (1995) used in a fertility study using saltbush (*Atriplex canescens*) in Owens Valley, California. Our most effective fertilizer, a polymer-coated slow release (Osmocote®), is labeled to last four months, providing nutrients to plants without burning. As per product specifications, this fertilizer was most likely depleted before the onset of plant growth the following year. We suspect mortality results may be due to planting date, soil moisture, or propagule type, rather than effects of fertilizer, because both control treatments (no fertilizer) had the lowest mortality percentage.

Table 13 Field study indicating mortality trends of rivercane (*Arundinaria spp.*) observed in May 2011 fertilization study. Dry weights taken May 2012.

	Rivercane Plant Mortality by Treatment	
Fertilizer Treatment	N (live)	Mortality (%)
P1	1	87.5
P2	1	87.5
P3	1	87.5
P4	-	100
P5	1	87.5
P6	-	100
P7	2	75
P8	-	100
P9 (control)	7	12.5
N1	-	100
N2	2	75
N3	-	100
N4	-	100
N5	2	75
N6	-	100
N7	-	100
N8	-	100
N9 (control)	8	0

- Denotes values that could not be calculated due to 0 plant growth index (100% mortality).

As results from fertility studies suggests, planting date, as it impacts soil moisture, is an important factor in successful rivercane establishment. We observed 68% mortality from the initial study planted in January and 82.6% mortality from the May planting. We suspect the addition of fertilizers upon planting may have added undue stress on immature, unestablished plants. In a scenario where fertilizers are placed within the same planting hole as rivercane roots, despite slow-release tendencies, fertilizer salts bind available water, further reducing osmotic potential near plant roots. This is more likely to occur in situations where mean temperatures have increased transpirational demand, drought conditions are more frequent, and C_3 plant growth is reduced; in this case, a May planting date. Results from this study and others have indicated that fertilizer does not decrease transplant survival if there is enough available water (Russell et al. 2010; Yamashita and Manning 1995). Based on results from our May 2011 study, all treatments containing fertilizer had a mortality percentage at, or greater than, 75%. However, within the two control treatments (no fertilizer), 15 of the 16 plants survived; a combined 6.25% mortality (Table 13).

Based on data from our January 2011 planting, propagule type also affects growth (Table 12). Biomass data indicated, significantly greater dry weight (g) accumulations occurred with the use of ramets (all $p \leq 0.05$). Upon initial planting, ramets' advantage is most likely due to larger, more robust underground biomass (personal observation). This would likely reduce water stress, but amelioration is minimal as mortality remained high. Studies suggest (Zaczek et al. 2009) that containerized rhizomes grown in the greenhouse for more than three months promote a more transplant-conditioned planting stock, able to confer a survival advantage over less intensively cultured plants (i.e. seedlings).

Herbicide Study

Attempts were made to first pool the data from 2010 and 2011 by running a two-way ANOVA procedure to determine if the herbicide treatments and time influenced visual ratings of rivercane seedlings. Data did indicate significant variation ($p \leq 0.05$) among herbicide treatments by year during the 28 DAT observation. These significant differences among data suggested possible external (environmental) influences on treatment results. Therefore, data was compared separately using PROC GLM. Seedling response from control treatment was the gauge by which these environmental factors were observed.

North Farm 2010

Proc GLM indicated a change in response among rivercane seedlings between weekly observations (Table 14). We did not evaluate the impact on weed control, as these results are known. Rather, we were investigating any potential damage the herbicide may have had on rivercane. A rating of one indicated seedlings were almost completely dead from severe herbicide injury, while rating five indicated no visible herbicide damage and nearly 100% green tissue growth.

In 2010, seven days after herbicide application, seedlings treated with fluazifop (2.75) and nicosulfuron+metsulfuron (2.80) had a mean rating greater than control plants (2.0), halosulfuron (2.05), and trifloxysulfuron (1.80). There was no significant difference among seedlings treated with sethoxydim (2.40), imazapic (2.30), imazaquin (2.30), and nicosulfuron (2.30) (Table 14).

Table 14 Mean herbicide ratings of rivercane (*Arundinaria gigantea*) by collection dates across treatments, from North Farm - 2010 herbicide study. Rating results reported mean rating every seven days after treatment until 28 days after treatment. Scale is based on a 1 to 5 rating with 1 describing rivercane seedlings with very severe damage and 5 describing rivercane seedlings with no damage.

	Duration of Treatment							
Herbicide	**7 DAT**		**14 DAT**		**21 DAT**		**28 DAT**	
Control	2.0	b[†]	1.6	c	1.4	c	1.8	ab
Fluazifop	2.75	a	2.55	ab	2.3	a	2.15	a
Halosulfuron	2.05	b	2.0	bc	1.65	bc	1.85	ab
Imazapic	2.3	ab	1.8	c	1.65	bc	1.45	bc
Imazaquin	2.3	ab	2.1	bc	1.8	abc	1.75	ab
Nicosulfuon + Metsulfuron	2.8	a	2.7	a	2.0	ab	1.7	abc
Nicosulfuron	2.3	ab	2.15	abc	1.8	abc	1.55	bc
Sethoxydim	2.4	ab	2.1	ab	2.05	ab	1.8	ab
Trifloxysulfuron	1.8	b	1.6	c	1.6	bc	1.25	c

[†] Values followed by the same letter within a column (treatment effect) are not significantly different at the 0.05 level.

Two weeks after herbicide application, visual ratings indicated that nicosulfuron+metsulfuron (2.70) rated significantly better than all other herbicide applications except fluazifop (2.55) and nicosulfuron (2.15). The others, imazaquin (2.10), sethoxydim (2.10), halosulfuron (2.0), imazapic (1.8), control (1.6), and trifloxysulfuron (1.6), respectively, rated less. During this second week of observations, the control treatment made a noticeable decline in visual mean rating, falling from 2.0 during the first week to 1.6 in the second. The control did not change significantly from week two to week three, but visual improvement was made in seedlings treated with fluazifop.

By week three (21 DAT) seedlings treated with fluazifop (2.3) still exhibited the highest rating (Table 14). This rating was significantly better than halosulfuron (1.65), imazapic (1.65), trifloxysulfuron (1.6), and control (1.4). Compared to fluazifop, there was no significant difference among seedlings treated with sethoxydim (2.05), nicosulfuron+metsulfuron (2.0), imazaquin (1.8), and nicosulfuron (1.8). Also during week three, we observed the seedlings' response to environmental factors as the control treatment resulted in the lowest mean rating of all seedlings.

In the fourth and final week (28 DAT) of the study, we observed that seedlings treated with fluazifop, once again, represented the highest mean rating at 2.15, but was only significantly better than nicosulfuron (1.55), imazapic (1.45), and trifloxysulfuron (1.25). Seedlings treated with halosulfuron (1.85), sethoxydim (1.8), control (1.8), imazaquin (1.75), and nicosulfuron+metsulfuron (1.7) were not significantly different than fluazifop.

Across a 28 day treatment, fluazifop (Fusilade®) applied at 0.2147 L a.i./ha, seems to have smallest negative impact to rivercane propagules. As a foliar-applied graminicide, a herbicide which controls weedy grasses, rivercane exhibited tolerance. Fluazifop is an amino acid synthesis inhibitor, unlike hexazinone (Velpar®), a photosynthesis inhibitor, which has also found success in rivercane. Nathan and Joyce Klaus (2011) have found success in rivercane when hexazinone was applied at a 3.1 mL/m^2 rate. They found this herbicide had damage equal to control treatments (no application). However, hexazinone, a photosynthesis inhibitor, can be soil and foliar applied, unlike fluazifop which is active by foliar applications. Mixed results suggests additional studies are necessary.

South Farm 2011

Proc GLM data from replication of the 2010 study again indicated differences in herbicide treatments and weekly observations. Visual observations, which began 7 days after treatment, indicated seedlings treated with trifloxysulfuron (2.9) and the control (2.9) only rated significantly higher than halosulfuron (2.45) (Table 15). There were no significant differences in ratings between seedlings treated with fluazifop (2.75), nicosulfuron (2.7), imazaquin (2.7), nicosulfuron+metsulfuron (2.7), imazapic (2.6), and sethoxydim (2.6).

Observations taken 14 days after herbicide application indicated that halosulfuron (3.1) rated significantly better than all treatments except nicosulfuron+metsulfuron (2.85), control (2.8), and sethoxydim (2.7). Remaining herbicides which rated respectively less included imazaquin (2.55), nicosulfuron (2.55), fluazifop (2.45), and imazapic (2.4). Seedlings treated with trifloxysulfuron (2.35) indicated a significantly lower rating than all others.

Table 15 Mean herbicide ratings of rivercane (*Arundinaria gigantea*) by collection date across treatments from South Farm - 2011 herbicide study. Rating results reported mean rating every seven days after treatment until 28 days after treatment. Scale is based on a 1 to 5 rating with 1 describing rivercane seedlings with very severe damage and 5 describing rivercane seedlings with no damage.

	Duration of Treatment							
Herbicide	**7 DAT**		**14 DAT**		**21 DAT**		**28 DAT**	
Control	2.9	a[†]	2.8	abc	2.7	abc	2.8	ab
Fluazifop	2.75	ab	2.45	bcd	2.25	d	2.15	de
Halosulfuron	2.45	b	3.1	a	3.0	a	3.1	a
Imazapic	2.6	ab	2.4	cd	2.2	d	2.1	e
Imazaquin	2.7	ab	2.55	bcd	2.6	abcd	2.75	abc
Nicosulfuon + Metsulfuron	2.7	ab	2.85	ab	2.8	ab	2.55	bcd
Nicosulfuron	2.7	ab	2.55	bcd	2.4	bcd	2.35	cde
Sethoxydim	2.6	ab	2.7	abcd	2.25	d	2.2	de
Trifloxysulfuron	2.9	a	2.35	d	2.35	cd	2.35	cde

[†] Values followed by the same letter within a column (treatment effect) are not significantly different at the 0.05 level.

Halosulfuron remained constant into the third week of observations, only dropping to a rating of 3.0. These seedlings rated significantly better than all treatments except nicosulfuron+metsulfuron (2.8), control (2.7), and imazaquin (2.6). Control and imazaquin-treated seedlings rated better, but not significantly more than nicosulfuron (2.4) and trifloxysulfuron (2.35). Seedlings treated with sethoxydim (2.25), fluazifop (2.25), and imazapic (2.2) rated significantly less than all other seedlings 21 days after treatment. There wasn't significant change during the fourth week of observations, because halosulfuron (3.1) again rated significantly better than all treatments except control (2.8) and imazaquin (2.75). Ratings from seedlings treated with nicosulfuron+metsulfuron (2.55) dropped one from the previous week to indicate a significant difference from those aforementioned. Seedlings treated with trifloxysulfuron (2.35) and nicosulfuron (2.35) were not significantly different from each other, but were significantly better than seedlings response from sethoxydim (2.2), fluazifop (2.15), and imazapic (2.1). In 2011, seedlings in the control treatment were never rated less than the highest rating.

Results from various herbicide treatments applied to field-planted rivercane seedlings have produced mixed findings over years. When considering the mean rating between 2010 and 2011 studies, fluazifop and halosulfuron, exhibited the least damage over 28 days. Numerically, fluazifop rated the same during the 28 day observation in both studies, 2010 and 2011. However, in the 2011 study, halosulfuron affected rivercane substantially less. Regarding field restoration sites, this may not be an ideal solution due to costs. At the date of publication, a cost estimate of halosulfuron herbicide would total approximately $80.00 USD per hectare, not including the cost of application. Additional variables to consider would be: date of planting, rainfall amounts, and herbicide mode-of-action. Our planting date occurred in June during both years, which is later than ideal based on other studies in this work.

Total rainfall amounts varied by 7.6 cm, between years (Figure 1). The results of this difference may be observed in the control treatment of each study. Mean ratings of the control treatment across the duration of 2010 and 2011 study periods were 1.97 and

2.8, respectively. Since ratings are compared to the control, accurate recommendations of specific herbicide should be based on 2011 data, and not 2010 data.

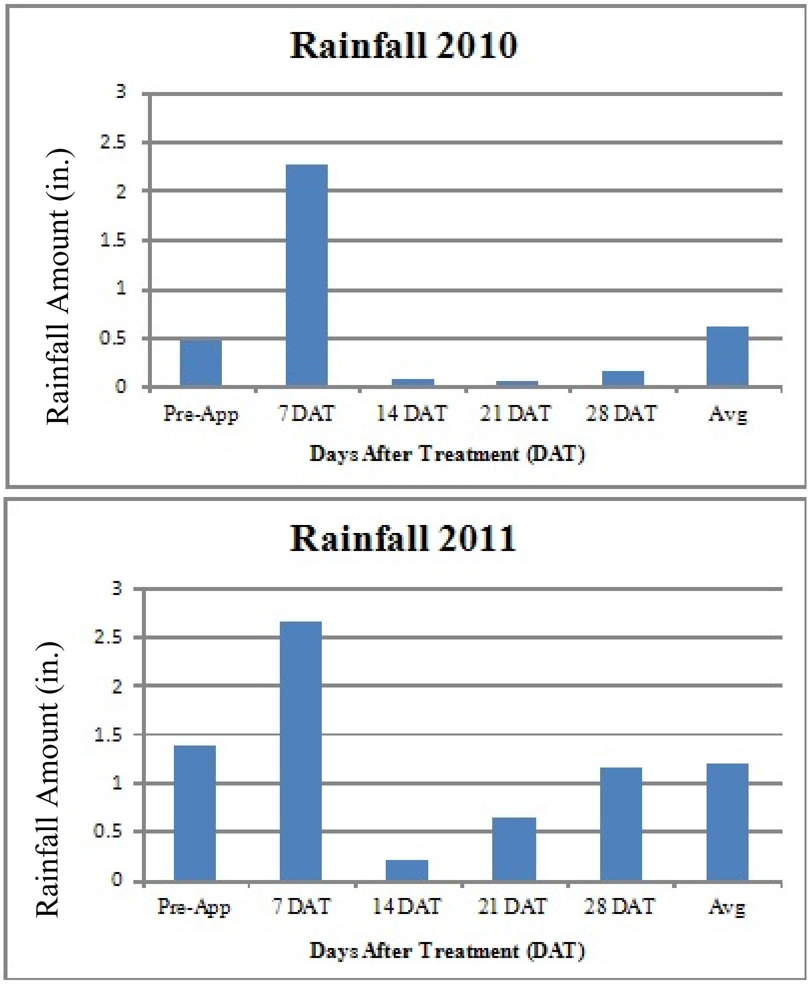

Figure 1 Differences in rainfall amounts (in.) between 2010 and 2011 at Mississippi State University, where 2010 and 2011 herbicide studies on rivercane (*Arundinaria gigantea*) were conducted. The graph indicates rainfall amounts between each study's observation date and an overall average from the study period.

Interpreting this data indicates that if increased rainfall amounts also constitute increased growth, fluazifop may have caused injury as engineered. Fluazifop is a post emergent, phenoxy herbicide that inhibits the acetyl-coA carboxylase (ACCase) enzyme once the chemical is absorbed rapidly through leaf surfaces, especially during periods of increased growth. This would have likely occurred during the 2011 study period when rainfall was 1.9 times more than in 2010. To be effective, herbicides have to be taken up by actively growing plants. Limited rainfall in 2010 is believed to have limited the lethal effects of some of the herbicides, not seen in 2011.

Data from field experiments have also indicated that additional herbicides aid in establishment. Pre-emerge applications of mitotic inhibitor such as pendimethalin (Prowl®) on tilled plots have proven to suppress weed seed emergence without causing injury to rivercane transplants (Mills 2011). Broadleaf herbicides like 2,4-D+MCPA+dicamba (Trimec®) may be applied as a post-emergent herbicide to reduce broadleaf competition. Preliminary research suggests that combining these two preventative and control measures increases the likelihood of successful rivercane establishment. However, findings from this same study (Mills 2011) also suggests rivercane growth is promoted by native, perennial grasses, a plant regime usually desired in restoration sites.

The clump-forming nature of native grass species seem to exhibit a “nurse plant” effect to others (Franco and Nobel 1989), such as rivercane. The leptomorphic nature of cane, verses the deep root strucure of warm season grasses (*Andropogon spp.* L., *Sorghastrum nutans* L., etc.), exhibit contrasting levels of water and nutrient demands that actually benefit both. This is opposed to weedy, annual grasses that extract valuable water and nutrients in the same root zone as rivercane; competition that is targeted by herbicides. Based on findings from our previous shade study, plant associations with clump-forming grasses may also provide a percentage of light reduction (60% - 85%) that is desired for optimal rivercane growth. Regarding these mutualisms (Boucher et al. 1982; Bertness and Callaway 1994), herbicide applications may not be necessary. Still the greatest challenge is annual grasses in a rivercane plot.

Planting Date: Rivercane Growth and Survival

Study I: North Farm

Understanding rivercane requirements for successful establishment involves several components. Regarding soil moisture, a limiting factor observed in previous studies (Russell et al. 2010; Yamashita and Manning 1995), proper planting date has a direct relationship with the amount of water available to established plants. In our efforts to determine proper time of planting, results from our field studies (Study I and II) varied. Because the two studies were not exact repeats of each other, analysis of variance (ANOVA) indicated significant interaction between the two studies, therefore each was analyzed separately.

Plant Growth Indices and Mortality

Across all months of planting, no single month indicated positive cumulative mean growth, as measured by taking the difference between PGI at the collection date (November 10, 2011) and planting dates (Table 16). Mortality was 100% for May and July. Mean PGI differences in March planting (-1797 cm^3), August planting (-2087 cm^3), and April planting (-2822 cm^3) were significantly less than plants from the month of February (-6743cm^3). February also had the lowest mortality percentages across both treatments (Table 17), indicating that the high PGI during this month resulted from greatest number of remaining plants. Since this study duration spanned the summer months of 2011, a decrease in PGI was observed due to low soil moisture, as seen in previous shade study. The mean PGI difference for June planting (-3861 cm^3) was not significantly different from any other month.

To reduce mortality due to moisture stress, we tested applications of top-growth removal (Baldwin et al. 2009). Pairwise comparisons of treatment application (cut or no cut) and month of planting was conducted to evaluate mortality (Table 17). Analysis of variance indicated that February and August were the only months where application of treatment significantly affected mortality. We noted that February had the lowest mortality within both treatments, but leaving top-growth at planting does significantly reduce mortality. Because of high mortality from other months of planting, a repeat (Study II) was conducted.

Table 16 Mean change in rivercane (*Arundinaria gigantea*) plant growth index over time (February - August 2011), as affected by date of planting within Study I: North Farm (2011).

	Rivercane PGI by Month of Planting			
Month (2011)	Initial	Final	Difference	
	- - - - mean PGI (cm^3) by month of planting over time- - -			
February	6,932	588	-6743	b[†]
March	2,931	1,003	-1797	a
April	5,722	1,814	-2822	a
May	3,234	-	-	-
June	2,718	425	-3861	ab
July	2,198	-	-	-
August	2,677	592	-2087	a

[†] Values followed by the same lowercase letter within a column (planting date effect) are not significantly different at the 0.05 level.

- Denotes values that could not be calculated due to 0 plant growth index (100% mortality)

Table 17 Percentage of mortality of rivercane (*Arundinaria gigantea*) as affected by treatment across planting dates for Study I: North Farm (2011).

	Treatment and Planting Date Effect on Mortality			
Month (2011)	Cut		Not Cut	
	- - - - difference in % mortality between measurement dates- - -			
February	66.6	A[†]	25	B
March	91.6	A	75	A
April	83.3	A	58.3	A
May	100	A	100	A
June	100	A	75	A
July	100	A	100	A
August	100	A	83.3	B

[†] Values followed by the same UPPERCASE letter within a row (treatment effect) are not significantly different at the 0.05 level.

Culm Growth

There were no significant differences in the mean number of culms between any month ($p \leq 0.05$) (Table 18). Additionally, there was no significant effect by treatment (cutting vs. not cutting) according to a pairwise comparison of data on culm difference. June planting with remaining top-growth was the only month by treatment that indicated a net mean loss in the number of culms (-0.33) (Table 18). Mortality was 100% during this month when top-growth was removed. Planting in August indicated no change in the number of culms (0.00). Planting in April (1.20), March (0.83), and February (0.69) all indicated positive, but not significant, culm growth. Across the study period, analysis of treatments indicated that the uncut treatment exhibited a mean culm number difference of

0.904 while the difference in mean culm number under the cutting treatment was 0.00 (no change). Although no significant differences were observed, repeat studies (Study II) were necessary for an accurate judgement on top-growth removal.

Table 18 Mean differences in number of rivercane (*Arundinaria gigantea*) culms per plant, indicating effects of treatment and planting date (February - August 2011) within North Farm Study I.

	Treatment and Planting Date Effect on Culms			
Month (2011)	Cut		Not Cut	
	- - difference in mean number of culms between measurement dates- -			
February	0	A[†] a[‡]	1.0	A a
March	0	A a	1.6	A a
April	0	A a	1.5	A a
May	-	a	-	a
June	-	a	-0.3	a
July	-	a	-	a
August	-	a	0	a

[†] Values followed by the same UPPERCASE letter within a row (treatment effect) are not significantly different at the 0.05 level.

[‡] Values followed by the same lowercase letter within a column (effect of planting date) are not significantly different at the 0.05 level.

- Denotes values that could not be calculated due to 0 plant growth index (100% mortality)

Study II: North Farm

Plant Growth Indices and Mortality

Among the planting months of December 2011 - April 2012, ANOVA indicated significant interaction between planting date and treatment effects on mean PGI. Mortality numbers overall, were lower during this experiment which led to more readily accepted results, compared to the previous study. When considering the differences in mean PGI across the study period, February planting (3,256 cm^3) resulted in the only positive PGI difference compared to all other monthly planting (Table 19). February planting also resulted in a significantly greater mean PGI than any other month of planting. March planting had the next best mean PGI difference (-409 cm^3), which indicated a change only significantly better than an April planting (-3,845 cm^3). Planting in January (-1,557cm^3) and December (-3,022cm^3) did not result in significant mean PGI differences. A notable observation was that, leaving top-growth in February did substantially affect rivercane growth by positive mean growth (Table 19).

Analysis of data, indicated how the treatment application affected seedling mortality (Table 20). December and January were the only months of planting where treatment application (not cutting), actually affected mortality. Leaving top-growth in every month except January resulted in 0% mortality (100% survival). It seems adequate soil moisture may have benefitted survival of plants more than application of treatment, agreeing with findings of previous studies (Russell et al. 2010). However, because of high mortality in January, future restoration studies may consider limiting their planting date when temperatures regularly fall below freezing 0°C (32°F), as this seems to decrease survival. Mean temperatures between 1.1°C (34°F) and 14.4°C (58°F) (range of

Mississippi mean temperatures in February) seem to promote rivercane survival. When plants remained intact, a February planting produced significantly greater growth than any other month of planting. However, of the cutting treatment, February also had the lowest mortality percentage (8.3%) of all other months of planting. Planting in December resulted in the next lowest mortality where plants were exposed to temperatures between 1.1°C (34°F) and 13.3°C (56°F). The highest mortality was actually seen in a January planting, but still indicated significant growth when top growth was removed (Table 20). Before the end of the month, our January planting experienced three days below -1.1°C (30°F), which, we presume, was the cause for increased mortality. Planting in March (5.0°C (41°F) - 18.8°C (66°F)) and April (10°C (50°F) - 23.3°C (74°F)) had the same amount of plant loss (25%) when top-growth was removed. These results are different from what Baldwin and others (2009) suggested.

Table 19 Mean change in rivercane (*Arundinaria gigantea*) plant growth index over time (December 2011 - April 2012), indicating date of planting and treatment effects, within North Farm Study II.

	Treatment and Planting Date Effect on PGI Differences			
Month (2011 - 2012)	Cut		Not Cut	
	- - - - difference in mean PGI (cm^3) between measurement dates- - -			
December	-4,592	B[†] b[‡]	-1,713	A b
January	-1,888	A a	-1,391	A b
February	-221	B a	6,443	A a
March	-1,344	A a	292	A b
April	-6,790	B b	-1,636	A b

[†] Values followed by the same UPPERCASE letter within a row (treatment effect) are not significantly different at the 0.05 level.

[‡] Values followed by the same lowercase letter within a column (effect of planting date) are not significantly different at the 0.05 level.

Table 20 Percentage of rivercane (*Arundinaria gigantea*) mortality as affected by treatment across planting dates (December 2011 - April 2012) for Study II: North Farm.

	Treatment and Planting Date Effect on Mortality			
Month (2011 - 2012)	Cut		Not Cut	
	- - - - difference in % mortality between measurement dates- - -			
December	16.6	A[†]	0	B
January	58.3	A	16.6	B
February	8.3	A	0	A
March	25	A	0	A
April	25	A	0	A

[†] Values followed by the same UPPERCASE letter within a row (treatment effect) are not significantly different at the 0.05 level.

We suspect the immediate loss of top-growth at planting halted any potential photosynthesis from occurring, thus hindering the plant's ability to store chemical energy. The lack of stored energy consequently would have prevented re-growth from a depleted root system, especially after freezing temperatures. However, because of the overall increase in survival compared to the previous study (Study I), more accurate conclusions can be made. That is, based on mortality results from both studies, a planting in February (1.1°C (34°F) to 14.4°C (58°F)) without removing top-growth, would contribute to plant survival. As previously observed (Russell et al. 2010, Yamashita and Manning 1995), available soil moisture may be more of a limiting factor.

Culm Growth

When assessing culm production, another component of rivercane growth, ANOVA indicated significant differences in culm growth between months of planting and by treatment effect (Table 21). As observed in PGI and mortality data, February planting when top-growth remained, indicated the only significant difference in mean number of culms (1.6). Regarding the cutting treatment, planting in December produced significantly more culms (0.7) than any other month except February (-0.3) when top-growth was removed. Similar to our light reduction study, where greatest growth was observed from November to June, February planting produces significantly greater number of culms than all other months of planting.

These findings reflect what Simon (1986) and Bell (2000) concluded in their studies considering collection and propagation dates. They noted that making collections in late winter and early spring resulted in higher culm production and survivability. Zaczek and others (2004) found an even greater increase in culm production from rhizomes collected in early spring, reinforcing the success we found from a February planting. Our original hypothesis, based on prior field observations, was that limited soil moisture, contained roots, and excessive transpiration, were likely when transferring seedlings from a greenhouse and into field conditions, especially in the months following February-March. This led to the removal of above-ground growth. In a month where C_3 growth increases, rivercane may benefit greater from available soil moisture (Platt and Brantley 1997) rather than removing top growth. Despite preventing transpirational loss (Baldwin et al. 2009) by the removal of top-growth, a February planting seems to combat negative effects of initial establishment.

Table 21 Mean differences in number of rivercane (*Arundinaria gigantea*) culms per plant, indicating effects of treatment and planting date (December 2011 - April 2012) within North Farm Study II.

	Treatment and Planting Date Effect on Culms			
Month (2011 - 2012)	Cut		Not Cut	
	- - difference in mean number of culms between measurement dates- -			
December	0.7	A[†] a[‡]	0.2	A b
January	-0.3	A b	-0.7	A b
February	0.1	B ab	1.6	A a
March	-0.5	A b	-0.6	A b
April	-0.3	A b	0.08	A b

[†] Values followed by the same UPPERCASE letter within a row (treatment effect) are not significantly different at the 0.05 level.

[‡] Values followed by the same lowercase letter within a column (effect of planting date) are not significantly different at the 0.05 level.

CHAPTER V

CONCLUSIONS

Applications of rivercane establishment methods have proved challenging, but do lend themselves for future use in restoration sites. Data would suggest establishment of rivercane from ramet, not seedling, propagules, and in a limited irrigation environment, to be planted in 85% light reduction to ensure greatest chance of survival. After 23 months, less shade (60%) is required for successful growth. Field sites providing these conditions would include filtered light from forest canopy gaps, or among native, warm season grasses where mutual plant growth benefits occurred. Containing the root mass of transplants without irrigation, negatively impacted establishment and survival, producing results different from prior shade study work. Transplant sites located on moist, but well-drained levees where brakes naturally occur, would facilitate rhizome growth and establishment.

Fertilizer applications may promote rivercane establishment, but adequate soil moisture is necessary for maximum growth. Within the parameters of this study, a slow release formulation of 19-6-12, provided the best growth within the first year. A rate of 33.3 g of product per plant produced greater results than a lower rate of 22.1 g. However, because of high mortality, fertilization at planting cannot be advised. If adequate soil moisture is not continuously available, fertilizer applications to rivercane plantings should be avoided.

The month of February seemed to be the best planting time in Mississippi for maximum field survival. Mean temperatures between 1.1°C (34°F) and 14.4°C (58°F) aided in high rivercane survival percentages and culm production by minimizing the likelihood of freezing. Removal of top-growth at planting, as an attempt to prevent transpirational loss (Baldwin et al. 2009), is not necessary in February. Propagules are also more successful when transplanting rhizome cuttings verses seedlings.

Results of rivercane response varied from herbicide application and year. Initial study results indicated fluazifop (Fusilade®) at a 0.214 L a.i./ha rate, damaged rivercane the least at 28 days after treatment. However, results from our repeat study suggested that fluazifop was lethal to rivercane, and halosulfuron (Sedgehammer®) at a 72.6 g a.i./ha rate, had no negative effects on rivercane compared to the control. Because of excessive mortality and potential high costs, direct application of these findings must be limited. Future studies are necessary in order to make more accurate claims.

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