

Sap flow dynamics of a tropical, woody bamboo:
Deductions of physiology and hydraulics
within *Guadua angustifolia*

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Abstract.

Despite considerable investigation into water transport and use in trees, research on grass hydraulics remains lacking. In this study, sap flow densities in 14 culms of an individual of the tropical, sympodial bamboo *Guadua angustifolia* were recorded using Granier heat dissipation sensors. Variations in sap flow dynamics were analyzed across the variables of culm age, size, height of measurement, and tissue type. Dynamics and trends were used to deduce conclusions about hydraulics in this species. Neither total daily sap flow nor maximum instantaneous sap flow correlated with culm size. Sap flow was found to occur predominantly between 23:00 and 09:00, suggesting positive root pressure as the primary mechanism for hydraulic movement in *G. angustifolia*. Sap flow measurements at different heights and a general lack of substantial diurnal hydraulics furthermore suggest that nocturnally-pumped sap is stored within internodal cavities (lacunas) for daily use. In addition, differences among tissue sap flow profiles add that root pressure-induced sap flow is mediated by internodal tissue, while subsequent distribution to leaves and branches is carried out by nodal tissue.

Introduction.

Photosynthesis and consequently overall plant growth are intimately tied to plant hydraulics (Goldstein *et al.* 1998; Phillips *et al.* 2003). Sap flow in trees has been extensively studied (Cermak and Prax 2001; Cienciala *et al.* 1999; Granier *et al.* 1996; Granier *et al.* 2000; Phillips *et al.* 1996), leading to a robust understanding of tree physiology and its relation to sap flow (see Cruziat *et al.* 2002 or Tyree and Ewers 1991). Often, sap flow is directly correlated with the rate of transpiration, as per the Cohesion-Tension theory (Dixon and Joly 1894). However, contemporary work has shown that roots may likewise play a role in fluid movement through both hydraulic lift and root pressure. Hydraulic lift, the passive movement of soil water via plant roots from deep soil to sub-surface regions, adds moisture to otherwise dry soils, dramatically altering the micro-environment and consequently influencing plant community composition (Jackson *et al.* 2000; Meinzer *et al.* 2001).

Yet root systems have also recently been demonstrated to be capable of inducing fluid movement within the plant xylem themselves, with both dicotyledonous and monocotyledonous species exhibiting the phenomenon. Among dicots, prominent examples include the sugar maple (*Acer saccharum*: Sperry *et al.* 1988), walnut tree (*Juglans regia*: Améglio *et al.* 2001), oak tree (*Quercus robur* and *Q. petraea*: Steudle and Meshcheryakov 1996), and birch tree (*Betula cordifolia*: Sperry 1993; *B. lenta* L. and *B. populifolia* Marsh.: Miller-Rushing and Primack 2008), as well as several important agricultural dicots such as the common grape (*Vitis vinifera*: Sperry *et al.* 1987), tomato plant (*Solanum lycopersicum*: White 1938), kiwifruit tree (*Actinidia* spp.: Clearwater *et al.* 2007), and the sunflower (*Helianthus annuus*: Dustmamatov *et al.* 2004). Thus, far from a rare phenomenon, root pressure appears to be a widespread if not common factor in sap flow. However, in many of the above dicots (Sperry *et al.* 1987; Sperry *et al.* 1988; Sperry 1993; Miller-Rushing and Primack 2008; Améglio *et al.* 2001; Clearwater *et al.* 2007) the observed root pressure was seasonal, being synced with the onset of

Spring. Because xylem cavitations are largely a product of winter freezing (Cobb *et al.* 2007), this seasonal occurrence of root pressure is believed to function as a mechanism to repair freezing-induced cavitation.

As a corollary to this postulate, it is logical that monocots and other plants with reduced secondary growth would both be more likely to exhibit root pressure and to employ it with higher (even daily) frequency. Unable to grow new xylem, these plants would be especially reliant on the prevention of xylem dysfunction due to cavitation, and thereby likely to utilize a repair mechanism such as root pressure. Indeed, a group of recent studies found root pressure in 61 of 109 tropical vine-like species despite the lack of freezing temperatures, suggesting this to be a regular if not daily occurrence (Fisher *et al.* 1997, Ewers *et al.* 1997); both monocotyledonous and dicotyledonous vines evinced the phenomenon. In addition, significant daily root pressure has been observed in herbaceous dicots (Milburn and McLaughlin 1974; Kramer and Kozlowski 1979), palms (Davis 1961), and banana (Davis 1961; Lu *et al.* 2002). Root pressure therefore appears to be intimately linked to the hydraulics of plants with reduced or absent secondary growth. Grasses in particular have widely demonstrated a daily pattern of root pressure, with examples including sugarcane (*Saccharum* spp.: Neufeld *et al.* 1992; Tyree *et al.* 1986), corn (*Zea mays*: Tyree *et al.* 1986; Miller 1985), rice (*Oryza sativa*: Stiller *et al.* 2003), rhodesgrass (*Chloris gayana* Kunth.: Ogata *et al.* 1985), the vine-like bamboo *Rhipidocladum racemiflorum* (Cochard *et al.* 1994), and several others (*Phleum pretense* and *Festuca pratensis*: Macduff and Bakken 2003).

Yet at this time little work has been undertaken to explore the relationship between root pressure and the associated organism-level hydraulics in these plants. Even disregarding the other notable examples, the common occurrence of root pressure in the grass family alone merits a further understanding of this phenomenon. In addition to being one of the most common plant families, grasses are considered among the most important plants for mankind, with members such as rice, corn, and sugarcane serving as worldwide staple food grains and cereal crops. Thus, if for no other reason, a robust understanding of root pressure as it relates to sap flow is a matter of global agricultural concern. However, despite this no attempts have been made to date into an investigation of the role of root pressure in whole-plant sap flow or its influence upon the temporal aspect of sap movement.

The present study is an attempt to examine these questions, utilizing the tropical, woody bamboo *Guadua angustifolia* as a model species.

Methods.

Species of interest

Guadua angustifolia (family *Poaceae*, subfamily *Bambusoideae*; *la caña de guadua* in Spanish) is a member of the grass subfamily bamboo, a group of giant arborescent grasses distinguishable by their fast-growing stems (called culms), enlarged underground rhizomes, and generally vegetative (i.e. clonal) reproduction. An individual bamboo plant is composed of a number of culms connected subterraneously via rhizomes, by

which water and nutrients are shared. *G. angustifolia* is classified into the subgrouping of sympodial bamboo, also known as pachymorphic, determinate, or “clumping” and delineated by rhizome growth which produces individuals composed of discrete clumps of bamboo culms (as opposed to monopodial or “running” bamboo).

G. angustifolia is a woody, tropical bamboo native to the Ecuadorian coastal plain, where it is abundant along riverbanks and floodplains. In addition, it is cultivated within the region as a lightweight building material with high loadbearing ability per unit weight and a fast harvest time, being harvestable within 4 to 6 years of seeding. Among American woody bamboos, *G. angustifolia* is one of the largest both in terms of height (up to 30 m) and diameter (15-22 cm in diameter) (Judziewicz *et al.* 1999).

Variables of Interest

The primary variables under investigation in this study were culm age, height, height of measurement, and tissue type (nodal vs. internodal). Ages were assigned to three categories: sprout, juvenile, or mature. Sprouts were defined as any culm lacking photosynthetic leaves, including those that had begun branching. Mature culms were distinguished from juveniles based on the presence of lichens on the culm; older culms exhibit an abundance of lichen associations, taking on a white, mottled appearance (Peggy Stern, personal communication).

Heights were likewise qualitatively organized into small, medium, and large culms. The clump height was measured with a clinometer to be roughly 22 meters on average, making that the approximate height of medium culms. Within a given clump, height variance is insignificant, with maximum variation between medium and small or medium and large culms estimated at 3 meters.

Environment and Site Location

The study was conducted at Hacienda Margarita, located near Patricia Pilar, Ecuador, at kilometer 35 on the road from Santo Domingo to Quevedo. Elevation was measured to be 350 m. The climate of the region is typical of the coastal plain. The average daily temperature, humidity, and transpiration throughout the study were 26.6 degrees Celsius, 83%, and 3.1 mm, respectively. Rainfall ranged from 0 mm to 45 mm in a day, averaging 10.9 mm daily.

The primary determinant in site choice within the farm was proximity to a water source, so as to remove water limitation as a variable. The secondary determinant was the presence of medium and large sprouts within the clump, as this age class was the least represented. The chosen site was located along the bank of a stream and among an orchard of African oil palm. This palm grows to a lower overall height than *G. angustifolia* and requires considerable space between plants. As a result, effects from external shading on the data were likewise minimized.

The study was carried out from April 8th to April 28th, 2008, in partial fulfillment of the requirements of the Ecuador: Comparative Ecology and Conservation program, SIT Study Abroad. Data was collected from April 10th to April 28th, resulting in 10 days of

useable data once equipment failures (discussed below) and sensor relocation are taken into account.

Granier sensors

Sap flow measurements were made using the methodology and sensor equipment originally proposed by A. Granier (Granier 1985). A Granier sensor consists of two cylindrical probes (20 mm long, 2 mm in diameter) containing copper-constantan thermocouples. The top probe is heated at a constant rate via the Joule effect and covered with an aluminum tube to minimize heating perturbations. The heat transfer between the top thermocouple and the aluminum is aided by a thermal conducting paste applied at the junction between the two. The lower “reference” probe remains unheated. The two probes are electrically connected and the temperature difference between them is measured as a voltage difference, typically in the range of 0.1 – 1.2 mV. As sap circulates, it cools the heated sensor; thus the observed temperature difference decreases with increasing sap flow. When no sap is flowing all heat from the top sensor dissipates into the nearby sap and the largest temperature difference is observed.

Setup Power

The setup was powered by a single Bosch 66FE 12 V, 70 Ah car battery, with a second charging while the first was in use. Battery switches occurred roughly every two days. Power was directly connected from the battery to the datalogger at 12 V, however power to the Granier sensors first passed through power regulation circuits, built by members of the Phillips Ecophysiology Lab of Boston University, to fix the delivered amperage at 134 mA.

Data Collection

Data were recorded by a Campbell Scientific CR10X datalogger (Campbell Scientific Inc., Logan, USA) with Campbell Scientific AM16/32 multiplexer attachment to increase the number of available data ports. Temperature differences were automatically measured every 30 seconds by the datalogger. These were then averaged and recorded as a single data point every 2 minutes, resulting in 720 data points per sensor per day. Data were downloaded in the field to a Campbell Scientific SM4M storage module and from there transferred to a laptop for analysis.

Sensor Installation Process

Sensors were fabricated on site from pre-made probes (from the Phillips Ecophysiology Lab) and belden cable. Probe connections were soldered and electrically taped to produce a Granier sensor, which was then soldered to the end of a stripped belden cable to increase the sensor’s range.

The power setup and datalogger were housed in the field as a single unit within a large crate box covered by plastic tarp to prevent rain damage. Once the box had been optimally placed among the clump to maximize access to a variety of culm ages and sizes, individual culms were selected for study and a sensor was run between the power/data box and the culm of interest. A 2.38 mm hole was then drilled into the culm at approximately 1.3 m height by means of a cordless drill, taking care to avoid nodal

tissue, and a 1.59 mm hole was drilled 10 cm directly below the first.* Next, an aluminum tube was inserted into the larger hole. Thermal conducting paste was applied to the heated probe, which was then inserted into the aluminum-lined upper hole. The reference probe was inserted below. The belden cable was stapled to the culm to relieve tension on the sensor wires, taking care not to staple directly above or below the sensor. A reflective aluminum sheet was stapled to the culm to protect the sensor from solar irradiation. Lastly, the other end of the belden cable was then wired into a power card circuit and datalogger port, and information on the port number, age, and height of the selected culm were recorded.

*For nodal and internodal sensors the heated probe was placed in the desired position and the reference was placed in the opposite medium one-half node below. For example, a nodal sensor would have the heated probe in the node and the reference in the internode directly below.

Data Monitoring

Data were downloaded every one to two days and directly imported into Microsoft Excel to look for anomalies and malfunctioning equipment. Although the data was still in the form of voltage differences, this directly correlates with sap flow and so trends among data were the same; converting the data into sap flow would have been unnecessarily time-consuming for the purpose of data monitoring.

First, empty columns of data were removed, as were placeholder -6999 values. Sensor data was graphed in two main ways. First, sensor values were plotted against hourly time so that data across days would overlap. This was done to look for cyclic, repeating data indicative of a properly functioning sensor. Additionally, data were plotted against a “full time” column made up of the sum of the numeric date (e.g. 112 for April 21, 114 for April 23) and the daily fractional time (time in hours and minutes divided by 2400). For example, noon on April 20th would be 111.5. Plotting the data in this way as one continuous datastream over days made large anomalies easier to identify. Finally, data from similar culms (medium juveniles, for example) were sometimes compared to look for agreement.

Data Analysis

Throughout the data monitoring process, irregular and non-repeating sections of data were removed. Once data collection was completed, all data from a given age and height category were compiled to form an averaged, 24-hour profile of the given culm type. This archetypal voltage profile was then converted into a sap flow density profile for each culm category with the BaseLiner program developed by Yavor Parashkevov of Duke University (see bibliography for further information) and based upon the empirical Granier calibration formula:

$$Fd=119 \times [(\Delta T_{max}-\Delta T)/\Delta T]^{1.23}$$

where Fd is sap flow density in g/m²/s (BaseLiner Help File, 2001). The sap flow densities were then compared with respect to the aforementioned variables. Direct

comparisons of sap flow densities (as opposed to sap flow) is justified in that culm diameter and consequently cross-sectional conducting area was not found to vary greatly between categories of interest (average radius = 6.08 cm, standard deviation = 0.64). Consequently, this paper assumes equal cross-sectional conducting area across all investigated variables. Total daily sap flow was computed by summing all sap flow measurements for a given culm type and multiplying by 120 seconds (the time between data points), which was then unit converted to kg/m².

Experimental Difficulties and Recommendations.

Throughout the course of this study various difficulties were encountered, chiefly related to sensor building, spine-related sensor installation problems, rain protection for the power/data box, and battery issues. Ways to avoid such complications and likewise to improve upon the employed methodology follow.

Sensor Building

The Granier sensors were unassembled upon arrival, necessitating that the first two days be spent soldering the connections between probes, cutting and stripping the belden cable, soldering the sensors to the cable, and testing the sensor connections to make sure they worked. The result was two days of lost field time. In addition, a wireless soldering iron was used, which proved slow and ineffective. Being wireless, the soldering iron took considerably more time to heat up and reached an overall lower temperature than a corded model would. This lower temperature made the melted solder “stick” to the iron, consequentially making connection soldering difficult. It would be advisable to build and test the Granier sensors and fabricate their belden cable connections prior to arriving at the research site. Additionally, use of a wired soldering iron is strongly recommended for future studies.

Spine-Related Problems

The genus *Guadua* is notorious for the abundance and sharpness of its spines, which grow prolifically on basal branches from ground level to above head height. These spines, capable of tearing pants and piercing rubber boot soles, make access to the associated culm nearly impossible without their removal. In this study a machete and pocket knife were used to remove basal branches and allow culm access, however neither proved effective. The flexibility and light weight of the branches limited the success of machete-based efforts, while removal with a pocket knife proved tremendously tedious and time-consuming. Hedge clippers or pruning shears and work gloves are recommended for future studies involving *Guadua*. In addition, the tangles of spiny basal branches made running the belden cable and attached Granier sensors difficult, with the fragile sensors often snagging on spines. Taping the sensor probes to the belden cable during sensor and cable placement would reduce such difficulties. Likewise, the presence of another person to assist in passing the cable through these tangles would be of benefit. Both methodological changes would reduce the required setup time and the potential for snag-related connection breaks and the need for their subsequent repair.

Rain Protection

Two large, thin, plastic tarps were utilized to protect the power box from rain. Although these worked well initially, tears quickly developed as a result of the aforementioned basal branch spines. Even with the minimal movement of removing and replacing the tarp to download data, within three or four days the tarps were damaged enough that it became necessary to tape up the tears and carefully orient the tarp above the box to avoid the holes. However rainwater still accumulated above the box and in high volumes would cause a bowing of the tarp downwards, putting stress on the repaired holes and exposing those that had been strategically oriented so as not to be above the box. The temporary solution was to place a support (in this case a machete) across the center of the box to prevent rainwater accumulation and the resultant bowing. This worked well and is advisable for future studies in areas with heavy precipitation. To further minimize the danger of water damage it is also advised to place the power/data box below tree cover, as opposed to within more readily accessible gaps. Above all though, use of a durable, tear-resistant tarp is highly recommended.

Battery Issues

The choice to power the setup with a car battery, recharging a replacement at the same time, worked well. However, difficulties were encountered when the recharged batteries failed to last a reasonable amount of time. Although the batteries powered the setup for over two days each on their initial charge, subsequent recharges to the original 12.4 V resulted in battery death and a loss of power to the setup within roughly 8 hours. It was later learned that 12 V batteries should be recharged to 14 V. Following this recommendation, no further instances of power loss were encountered and the batteries again provided two days power without failure.

Category	Total Daily Sap Flow (kg/m ²)	Sap Flow, 09:00 to 23:00	Sap Flow, 23:00 to 09:00	Percent Sap Flow from 23:00 to 09:00
Small Juveniles	1225.967	470.857	755.110	61.593
Medium Sprout	1104.374	783.637	320.737	29.042
Medium Juvenile	523.882	17.639	506.243	96.633
Medium Juvenile (0.52 m)	627.839	66.367	561.472	89.429
Medium Mature	1047.705	146.846	900.859	85.984
Large Juvenile	1219.851	36.076	1183.775	97.043
Large Mature	1172.399	405.653	766.747	65.400
Internodal (Medium Mature)	620.187	75.688	544.499	87.796
Nodal Type 1 (Medium Mature)	815.937	558.026	257.911	31.609
Nodal Type 2 (Medium Mature)	808.547	674.766	133.781	16.546
Average	916.669	323.555	593.113	66.108
Average for Developed Size/Age Classes at 1.3 m	1037.961	215.414	822.547	81.331

Table 1. Sap Flow Data for Categories of Interest

Results.

Fourteen of the 28 functioning sensors provided consistent data, recording sap flow information at nodes and internodes in a medium mature, at 0.52 m on a medium juvenile, and for 6 of the height and age categories of interest at 1.3 m: medium sprouts, small juveniles, medium juveniles, large juveniles, medium matures, and large matures.

General Trends

Across all height classes within developed culms (juveniles and matures) sap flow was greatest pre-dawn, peaking between 04:00 and 05:00 in the range of 25 to 50 $\text{g}/\text{m}^2/\text{s}$ depending on age and height; only medium sprouts did not fit this trend (Fig. 1 and 2; see Appendix for larger versions).

Excepting medium sprouts, sap flow was much higher during the night than during the day, with a dramatic increase in hydraulic activity beginning near 23:00 and subsiding at approximately 9:00, henceforth termed the nocturnal hydraulic event (NHE). Sap flow during this period averaged 81.3% of the total daily sap flow at 1.3 m for developed culms (Table 1, nodal tissue not included). Sap flow from 9:00 to 23:00 was greatly reduced and less variable. For medium juveniles, large juveniles, and medium matures diurnal activity was insignificant (0-5 $\text{g}/\text{m}^2/\text{s}$). In comparison, the other two classes—small juveniles, and large matures—underwent a secondary rise in sap flow activity soon after the termination of the NHE, reaching a second, lower maximum within two hours and maintaining this level of activity until the onset of the NHE at 23:00. Medium sprouts showed similar behavior.

Sap Flow vs. Culm Height

Juveniles sap flow activity did not directly correlate with height (Fig. 1). Large juvenile nocturnal activity peaked at 52 $\text{g}/\text{m}^2/\text{s}$, while medium juvenile and small juvenile peaks were 25.4 $\text{g}/\text{m}^2/\text{s}$ and 32.8 $\text{g}/\text{m}^2/\text{s}$, respectively. The time of peaking as well as the onset and termination of the NHE were the same among all juveniles size classes. Only small juveniles showed significant diurnal activity, beginning at roughly 11:30 and remaining constant at 12 $\text{g}/\text{m}^2/\text{s}$ throughout the day. Total daily sap flow in small, medium, and large juveniles was 1226.0 kg/m^2 , 523.9 kg/m^2 , and 1219.9 kg/m^2 , respectively.

Peak sap flow in matures likewise did not correlate with height (Fig 1). Medium matures and large matures peaked at 44.6 $\text{g}/\text{m}^2/\text{s}$ and 33.4 $\text{g}/\text{m}^2/\text{s}$, respectively. The time of

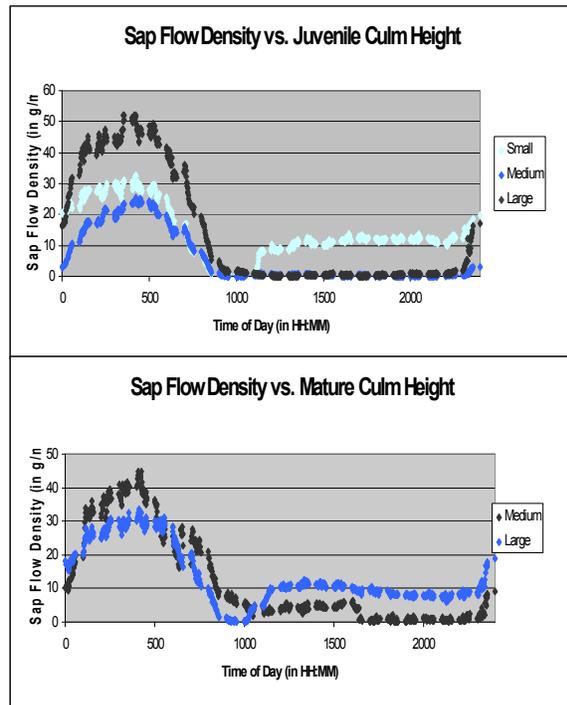


Figure 1. Sap flow density at 1.3 m within juvenile and mature culms as a function of culm height.

peaking and onset of the NHE were the same, however the termination of the NHE was less well-defined for medium matures than large matures. Sap flow in large matures decreased to zero near 9:00, followed by a rise at 10:30 to 12 g/m²/s and subsequent diurnal activity diminishing to 8 g/m²/s by 22:00. Total daily sap flow was 1047.7 kg/m² in medium matures and 1172.4 kg/m² in large matures.

Sap Flow vs. Culm Age

Sap flow among medium matures was greater than that of medium juveniles, with peak NHE values of 44.6 g/m²/s and 25.4 g/m²/s, respectively (Fig. 2). Neither showed significant diurnal activity. Medium sprouts exhibited no NHE but did show a marked decrease in sap flow in the 2.5 hours prior to the typical time of NHE termination noted in other classes (i.e. 9:00). Hydraulic activity in medium sprouts increased significantly from 09:00 to 11:00 to a steady-state sap flow of 17.5 g/m²/s, declining again at 23:00. Total daily sap flow in medium sprouts was 1104.4 kg/m², 523.9 kg/m² in medium juveniles, and 1047.7 kg/m² in medium matures.

Nodal vs. Internodal Sap Flow in Medium Matures

Internodal hydraulic dynamics showed the presence of a typical NHE, followed by minimal diurnal activity (Fig 3). Two types of nodal hydraulics were observed. Each occurred in at least two sensors and was repeated across days; thus it is unlikely that either is an experimental anomaly. Nodal type 1 is characterized by a minimum in activity at 09:00, followed a daily peak at 11:45 and thereafter a slow decline until the following 09:00. Consequently this nodal type shows relatively little diurnal variation. Nodal type 2 is characterized by a large, broad peak beginning at 07:00 and terminating at 18:00. A sharply-defined maximum occurs at 12:30. From 18:00 to 07:00 sap flow is minimal. Neither nodal type exhibits an NHE. Total daily internodal sap flow was 620.2 kg/m² while total daily nodal sap flow was 815.9 kg/m² in nodal type 1 and 808.5 kg/m² in nodal type 2.

Sap Flow vs. Sensor Height in Medium Juveniles

Sap flow differed notably between measurements at 1.3 m and 0.52 m, with greater sap flow at 0.52 m than 1.3 m on average. The temporal percent difference in sap flow with respect to the 0.52 m measurement is plotted in Figure 4. The calculated averaging percent difference was 26.1%, however the data is subject to a great deal of noise from 10:00 to 18:00 and this value may in fact be much larger. The percent difference appears to plateau near 80-100% from 10:00 to 23:00, quickly thereafter dropping to 0 for the period 23:00 to 4:30. From 4:30 to 10:00 the percent difference in sap flow increased non-linearly to a maximum of 99.9%. Total daily sap flow at 0.52 m was greater than at 1.3 m, measuring 627.8 kg/m² and 523.9 kg/m², respectively.

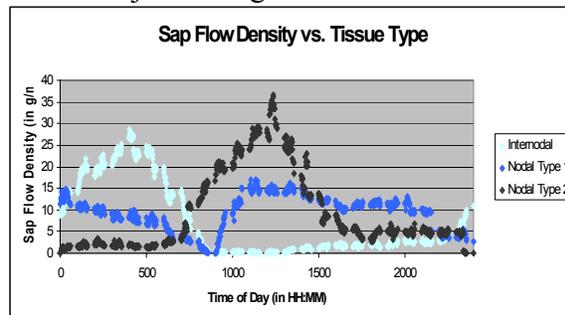


Figure 2. Sap flow density at 1.3 m within medium culms as a function of culm age.

Discussion.

Height Comparison

No consistent trend in sap flow was found among juveniles of various heights (Fig. 1). Although peak NHE sap flow was indeed greater in large juveniles than in smaller size classes, small juvenile peak NHE sap flow exceeded that of medium juveniles. In addition, the substantial diurnal activity in small juveniles resulted in approximately equal total daily sap flow in small juveniles and large juveniles (1226.0 kg/m^2 vs. 1219.9 kg/m^2), while both were far in excess of the total daily sap flow in medium juveniles (523.9 kg/m^2). The reasons for these trends remain unclear, though the equivalent sap flow in small and large juveniles suggests that hydraulic needs are not strongly correlated to height in juveniles. The height comparison in matures (Fig. 1) lends credence to this hypothesis. Although sap flow dynamics in medium and large matures differ, total daily sap flow is similar (1047.7 kg/m^2 and 1172.4 kg/m^2 , respectively). Thus it appears that across both developed age categories other variables than height take precedence in determining the magnitude of sap flow. Sap movement is intimately tied to photosynthetic transpiration, and therefore a logical hypothesis would be that hydraulic activity is correlated with total leaf area.

Age Comparison

While trends among the two developed age classes are similar, sprouts dynamics differed markedly (Fig. 2). Medium sprouts were the only medium age class to show no evidence of an NHE. In addition, they were the only medium age class to evince significant diurnal activity. Indeed, the majority of the medium sprout hydraulic activity occurred from 10:00 to 24:00. Most notably, the onset (9:00) and onset of decline (23:00) of this activity coincide well with the termination and beginning of the NHE, suggesting a connection between the two (see below).

Nocturnal Hydraulic Event

The most surprising of the results is the general trend among developed culms to display significant nocturnal activity. In contrast, most studied plants have demonstrated a strong temporal correlation between sap flow and photosynthesis, resulting in primarily diurnal hydraulics (Lu *et al.* 2002; Phillips *et al.* 2003). However, several instances of nocturnal sap flow have been documented. Goldstein *et al.* (1984) found stored water in plants of the dicot genus *Espeletia* was refilled during the night, while Milburn and McLaughlin (1974) noted nocturnal root pressure functioning to repair embolisms in the herbaceous dicot genus *Plantago* and furthermore speculated that this may in fact be a common occurrence among herbaceous plants and cereals. Indeed, root-pressured induced nocturnal sap flow has been seen in several grasses, including rice (Stiller *et al.* 2003), the vine-like bamboo *Rhipidocladum racemiflorum* (Cochard *et al.* 1994), sugarcane, and

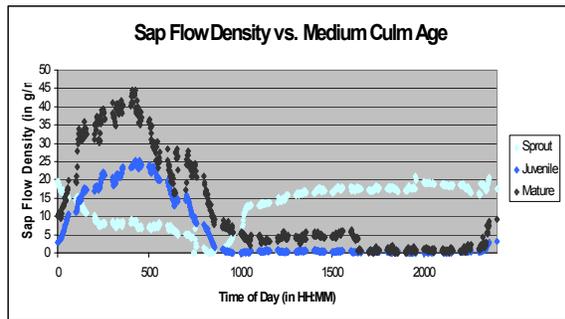


Figure 3. Sap flow density at 1.3 m within medium mature culms as a function of tissue type.

corn (both Tyree *et al.* 1986). In many of these instances, xylem pressures were high enough to theoretically force sap to the top of the plant. Beyond these examples though, the only other documented occurrences of nocturnal sap flow to the author's knowledge are in the monocot vine *Smilax rotundifolia* (Cobb *et al.* 2007) and a report of restored turgor in banana leaves overnight (Lu *et al.* 2002). In addition, not all grasses have shown nocturnal sap flow; the tussock grass *Stipa tenacissima* L. (Ramírez *et al.* 2006) and *Phleum pretense* and *Festuca pratensis* (both Macduff and Bakken 2003) demonstrated typical flow diurnal patterns.

As specifically related to this study, four bamboos have been shown to be capable of generating positive root pressure (*Rhipidocladum racemiflorum* and *Bambusa arundinaceae*: Cochard *et al.* 1994; *Chusquea ramosissima* and *Merostachys clausenii*: Saha *et al.* 2009), and the data suggest that this is the principal hydraulic mechanism in *G. angustifolia*. For these documented instances of root pressure as well as those above, it has been found to occur only when transpiration rates are low (i.e. night or rain events), explaining well the time and defined nature of the onset and termination of the NHE. In addition, the consistency in these onset and termination times lends support to the idea of the NHE being induced by root pressure, as all surveyed culms were part of the same individual and therefore shared the same root system. The nodal vs. internodal findings imply that NHE activity is principally mediated via internodal vessels, with internodal activity from 23:00 to 09:00 mirroring typical NHE sap flow (Fig. 3). In contrast, nodal activity during this time is lower and does not appear related to the NHE.

Anecdotal evidence abounds as to the presence of water within bamboo nodal cavities (called lacunas), often in large quantities, and indeed throughout the sensor installation and removal processes water spouting from the holes was a common occurrence. It is therefore likely that sap pumped up from the roots is stored within lacunas for use throughout the day. Furthermore, sap flow difference data at 0.52 m and 1.3 m suggest that sap is stored in top nodes first. Sap flow between 0.52 m and 1.3 m was approximately equal from the onset of the NHE until 4:30, during which time sap is postulated to be stored in upper lacunas. From 4:30 until 10:00 the difference in sap flow between these sensors increased non-linearly as lacunas between 1.3 m and 0.52 m filled, until no sap flow was recorded in the 1.3 m sensor. From 10:00 to 23:00 sap flow in both sensors was minimal (see Appendix, Fig. 5). By utilizing stored water, the daily period of maximum transpiration can be extended, the apparent resistance to sap flow between the roots and the leaves is decreased, and fluctuations in water availability (as might occur during times of high transpiration or low soil moisture) are dampened (Goldstein *et al.* 1998), making this an adaptive pairing of physiology and behavior.

This hydraulic strategy, nocturnally-based and induced by positive root pressure, differs notably from that of other large flora. Although the reasons for this difference are not known, within grasses they are likely related to the vast physiological differences between grasses and most woody plants. It may be that the lack of secondary growth in bamboos results in a smaller cross-sectional conducting area, making on-demand sap flow an impossibility. At the same time, this inability to grow new xylem vessels necessitates the ability to repair cavitation, making root pressure a critical survival

mechanism which happens to serve a dual purpose in pumping sap to the lacunas. Another possibility is that the presence of storage vessels (lacunas) within bamboo might offer an evolutionary advantage, making nocturnal pumping and daily storage less energetically costly than on-demand hydraulics. Indeed, previous studies have shown water storage to be important in permitting further plant growth (Phillips *et al.* 2003) and higher overall transpiration rates (Goldstein *et al.* 1998). These two hypotheses are not, of course, mutually exclusive, and bamboo physiology may in fact be the adaptive compromise for reduced cross-sectional conducting area in favor of increased water storage capabilities.

Diurnal Sap Flow Variations

This hydraulic hypothesis not only explains the presence of the NHE in developed culms but may account for the presence of additional diurnal activity in various classes. The limited storage capacity of the lacunas may be insufficient to contain certain culms' total daily water needs. As a result, in these culms additional sap flow is necessary throughout the day. These proposed hydraulics may explain the observed sap flow activity of medium sprouts as well. Sprout water and nutrients are provided by the already established rhizome and root network. The lack of photosynthetic leaves in developing culms not only removes the need for temporal synchrony between sap availability and irradiation levels, but likewise the typical method of diurnal sap movement. Although positive root pressure exists, it is possible that the lacunas or transport tissue is not thoroughly developed, hence explaining the lack of an NHE.

While this theory seems logical, the lack of a satisfactory mechanism for diurnal sap flow is troubling. This activity is unlikely to be caused by positive root pressure. Not only is root pressure believed to be generated for only a portion of the day, but the large drop to zero sap flow at 9 am following the termination of the NHE and prior to diurnal activity cannot be explained if the mechanism for both the NHE and diurnal sap movement is assumed to be the same.

Furthermore, the interconnected root system would imply that this diurnal trend should be found in all surveyed culms if it is related to root pressure. This activity is likewise unlikely to be caused by transpiration-induced turgor pressure. Although this might explain diurnal sap flow in developed culms, it cannot explain this sap flow within sprouts, which lack the necessary photosynthetic leaves. Sprout diurnal activity is identical to that in small juveniles and large matures and thus it is reasonable to assume that they are mediated by the same mechanism.

In addition to the lack of a mechanism for diurnal activity, its constancy is puzzling. Photosynthesis and consequently water use varies throughout the day as a function of solar radiation. Thus, one would assume that sprout diurnal hydraulics would vary

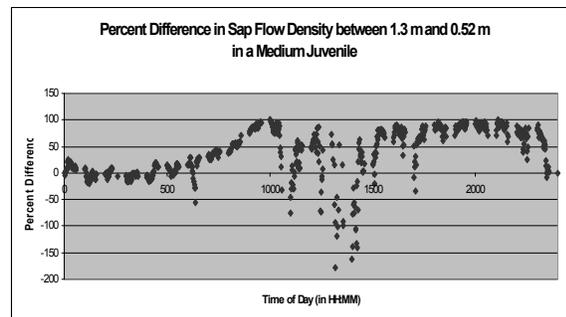


Figure 4. Percent sap flow at 1.3 m as a function of sap flow at 0.52 m in the same medium juvenile.

similarly. Even if this sap flow is functioning to replace nodal fluid loss, temporal variance associated with photosynthetic rates would be expected. Further study into the underlying mechanisms for *G. angustifolia* diurnal sap flow and its function are necessary.

Internodal vs. Nodal Activity

Differences between nodal and internodal dynamics further illuminate hydraulics within *G. angustifolia*. As stated previously, the NHE appears principally mediated via internodal vessels, with insignificant internodal activity from 09:00 to 23:00. In contrast, nodal sap flow increases substantially as internodal activity is diminishing, suggesting that diurnal hydraulics and the distribution of water to leaves and branches is primarily regulated by nodal tissue. The time of peak sap flow for both nodal types is between 12:00 and 12:30, which is also the time of greatest irradiation and transpiration, lending further support to this claim. Of the two nodal types, type 2 matches almost perfectly with irradiation levels, first increasing at 06:00 and finally decreasing to close to its minimal value at 18:00. Thus nodal type 2 seems to be responsible for sap flow to leaves. In contrast, nodal type 1 is quite similar in form to the diurnal activity noted in small juveniles, large matures, and medium sprouts. Medium matures were not seen to display this behavior in internodal tissue (Fig. 1), suggesting that diurnal hydraulic movement may in fact occur in other (potentially all) age and size classes, however in some it may be directed internodally in some and in others via nodal tissue.

Suggestions for Future Research

Time limitations and technical difficulties hindered this study, resulting in a small sample size across only six of the nine size and age combinations of interest, excluding small sprouts, large sprouts, and small matures. A larger-scale replication including several replicates of each size/age category would be beneficial to confirm these preliminary findings. In addition, sap flow measurements of a sprout transitioning to juvenile may provide interesting insight into physiological development of the lacuna and the onset of the NHE. Looking at sap flow among culms of different leaf area might also prove enlightening, helping to clarify whether differences in total sap flow and maximum sap flow are indeed correlated with total leaf area. In addition, further research into the mechanism and regulation of diurnal hydraulics would be of benefit, especially regarding differences in nodal tissue sap flow and the presence of different nodal types. Any or all of these findings may differ based on bamboo rhizomal type (leptomorph vs. pachymorph), genera, or even species. Research in other bamboo and non-bamboo grasses is therefore also necessary. Finally, anecdotal support is strong among farmers and bamboo cultivators for a correlation between bamboo hydraulics and the lunar cycle, with stored water decreasing substantially to coincide with full moons. Research into this claim and, if true, its underlying mechanism could be quite interesting.

Conclusion.

The magnitude of sap flow in *G. angustifolia* did not show a strong correlation with size class. Larger culms did not consistently demonstrate a greater maximum instantaneous sap flow than smaller culms. Additionally, total daily sap flow was unrelated to size. Sap flow in developed culms (i.e. juvenile and mature) was found to be predominantly nocturnal and concentrated between 23:00 and 09:00, suggesting that positive root pressure serves as the chief mechanism for sap movement. Only medium sprouts did not experience significant nocturnal activity. Small juveniles, large matures, and medium sprouts also evinced notable diurnal sap flow, beginning between 09:00 and 11:00 and lasting until 23:00, though the mechanism remains unclear. The time of the large nocturnal hydraulic event, along with internodal data and trends on differences in sap flow at different sensor heights, advance the belief that sap brought up by positive root pressure is stored within lacunas for use throughout the day. Nodal data indicate that this stored water is distributed to leaves and branches primarily via nodal tissue throughout the day. Based upon this and findings in other studies, it is not illogical to conceive that sap flow in grasses may differ from the typical Cohesion-Tension theory advanced among non-grasses.

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Bibliography.

Améglio, T, FW Ewers, H Cochard, M Martignac, M Vandame, C Bodet, and P Cruiziat. 2001. Winter stem xylem pressure in walnut trees: effects of carbohydrates, cooling and freezing. *Tree Physiology* 21: 387-394.

BaseLiner Help File. 2001. C-H₂O Ecology Lab Group. Nicholas School of Environmental and Earth Sciences, Duke University.

Cermak, J and A Prax. 2001. Water balance of a southern Moravian floodplain forest under natural and modified soil water regimes and its ecological consequences. *Annales des Sciences Forestières* 58: 15-29.

Cienciala, E, J Kucera, and A Lindroth. 1999. Long-term measurements of stand water uptake in Swedish boreal forest. *Agricultural and Forest Meteorology* 98-99: 547-554.

Clearwater, MJ, P Blattmann, Z Luo, and RG Lowe. 2007. Control of scion vigor by kiwifruit rootstocks is correlated with spring root pressure phenology. *Journal of Experimental Botany* 58 (7): 1741-1751.

Cobb, AR, B Choat, and NM Holbrook. 2007. Dynamics of freeze-thaw embolism in *Smilax rotundifolia* (Smilacaceae). *American Journal of Botany* 94: 640-649.

Cochard, H, FW Ewers, and MT Tyree. 1994. Water relations of a tropical vine-like bamboo (*Rhipidocladum racemiflorum*): root pressures, vulnerability to cavitation and seasonal changes in embolism. *Journal of Experimental Botany* 45 (8): 1085-1089.

Cruziat, P, H Cochard, and T Améglio. 2002. Hydraulic architecture of trees: main concepts and results. *Annals of Forest Science* 59: 723-752.

Davis, TA. 1961. High root-pressure in palms. *Nature* 192: 227-278.

Dixon, HH and J Joly. 1894. On the ascent of sap. *Philosophical Transactions of the Royal Society of London B* (186): 563-576.

Dustmamatov, AG, VN Zholkevish, and VV Kuznetsov. 2004. Water pumping activity of the root system in the process of cross-adaptation of sunflower plants to hyperthermia and water deficiency. *Russian Journal of Plant Physiology* 51 (6): 822-826.

Ewers, FW, H Cochard, and MT Tyree. 1997. A survey of root pressures in vines of a tropical lowland forest. *Oecologia* 110: 191-196.

Fisher, JB, GA Angeles, FW Ewers, and J López-Portillo. 1997. A survey of root pressure in tropical vines and woody species. *International Journal of Plant Science* 158 (1): 44-50.

Goldstein, G, JL Andrade, FC Meinzer, NM Holbrook, J Cavelier, P Jackson, and A Celis. 1998. Stem water storage and diurnal patterns of water use in tropical forest canopy trees. *Plant, Cell, and Environment* 21: 397-406.

Goldstein, G, F Meinzer, and M Monasterio. 1984. The role of capacitance in the water balance of Andean giant rosette species. *Plant, Cell, and Environment* 7: 179-186.

Granier, A. 1985. A new method to measure the raw sap flow in the trunk of trees. *Annales des Sciences Forestières* 42: 193-200.

Granier, A, P Biron, N Breda, JY Pontallier, and B Saugier. 1996. Transpiration of trees and forest stands: short and long-term monitoring using sapflow methods. *Global Change Biology* 2: 265-274.

Granier, A, P Biron, and D Lemoine. 2000. Water balance, transpiration and canopy conductance in two beech stands. *Agricultural and Forest Meteorology* 100: 291-308.

Jackson, RB, JS Sperry, and TE Dawson. 2000. Root water uptake and transport: using physiological processes in global predictions. *Trends in Plant Science* 5: 482-488.

Judziewicz, EJ, LG Clark, X Londoño, and MJ Stern. 1999. American Bamboos. Smithsonian Institution Press, Washington, DC.

Kramer, PJ and TT Kozlowski. 1979. The Physiology of Woody Plants. Academia Press: Orlando.

Lu, P, KC Woo, and ZT Liu. 2002. Estimation of whole-plant transpiration of bananas using sap flow measurements. *Journal of Experimental Botany* 53 (375): 1771-1779.

Macduff, JH and AK Bakken. 2003. Diurnal variation in uptake and xylem contents of inorganic and assimilated N under continuous and interrupted N supply to *Phleum pretense* and *Festuca pratensis*. *Journal of Experimental Botany* 54 (381): 431-444.

Meinzer, FC, MJ Clearwater, and G Goldstein. 2001. Water transport in trees: current perspectives, new insights and some controversies. *Environmental and Experimental Botany* 45: 239-262.

Milburn, JA and ME McLaughlin. 1974. Studies of cavitation in isolated vascular bundles and whole leaves of *Plantago Major L.* *New Phytologist* 73: 861-871.

Miller, DM. 1985. Studies of root function in *Zea mays*: III. Xylem sap composition at maximum root pressure provides evidence of active transport into the xylem and a measurement of the reflection coefficient of the root. *Plant Physiology* 77: 167-167.

Miller-Rushing, AJ and RB Primack. 2008. Effects of winter temperatures on two birch (*Betula*) species. *Tree Physiology* 28 (4): 659-664.

Neufeld, HS, DA Grantz, FC Meinzer, G Goldstein, GM Crisosto, and C Cristosto. 1992. Genotypic variability in vulnerability of leaf xylem to cavitation in water-stressed and well-irrigated sugarcane. *Plant Physiology* 100: 1020-1028.

Ogata, S, H Saneoka, and K Matsumoto. 1985. Nutritional-physiological evaluation of drought resistance of warm season forage species: comparative studies on root development water and nutrient absorption of forage species at various soil moisture levels. *Journal of the Japanese Society of Grassland Science* 31: 263-271.

- Phillips, N, R Oren, and R Zimmerman. 1996. Radial patterns of xylem sap flow in non-, diffuse- and ring-porous tree species. *Plant, Cell, and Environment* 19: 983-990.
- Phillips, NG, MG Ryan, BJ Bond, NG McDowell, TM Hinckley, and J Cermak. 2003. Reliance on stored water increases with tree size in three species in the Pacific Northwest. *Tree Physiology* 23: 237-245.
- Ramírez, DA, F Valladares, A Blasco, and J Bellot. 2006. Assessing transpiration in the tussock grass *Stipa tenacissima* L.: the crucial role of the interplay between morphology and physiology. *Acta Oecologica* 30: 386-398.
- Saha, S, NM Holbrook, L Montti, G Goldstein, and GK Cardinot. 2009. Water relations of *Chusquea ramosissima* and *Merostachys clausenii* in Iguazu National Park, Argentina. *Plant Physiology* 149: 1992-1999.
- Sperry, J S. 1993. Winter xylem embolism and spring recovery in *Betula cordifolia*, *Fagus grandifolia*, *Abies balsamea* and *Picea rubens*. In M. Borghetti, J. Grace, A. Raschi, [eds.], Water transport in plants under climatic stress, 87-98. Cambridge University Press, Cambridge, UK.
- Sperry, JS, JR Donnelly, and MT Tyree. 1988. Seasonal occurrence of xylem embolism in sugar maple (*Acer saccharum*). *American Journal of Botany* 75: 1212-1218.
- Sperry, JS, NM Holbrook, MH Zimmermann, and MT Tyree. 1987. Spring filling of xylem vessels in wild grapevine. *Plant Physiology* 83: 414-417.
- Stedle, E and AB Meshcheryakov. 1996. Hydraulic and osmotic properties of oak roots. *Journal of Experimental Botany* 47: 387-401.
- Stiller, V, HR Lafitte, and JS Sperry. 2003. Hydraulic properties of rice and the response of gas exchange to water stress. *Plant Physiology* 132: 1698-1706.
- Tyree, MT and FW Ewers. 1991. The hydraulic architecture of trees and other woody plants. *New Phytologist* 119: 345-360.
- Tyree, MT, EL Fiscus, SD Wullschleger, MA Dixon. 1986. Detection of xylem cavitation in corn under field conditions. *Plant Physiology* 82: 597-599.
- White, PR. 1938. Root pressure—an unappreciated force in sap movement. *American Journal of Botany* 25: 223-227.

Appendix.

Table 2. Sensors Used for Categories of Interest

Category of Interest	Sensors Used
Small Juvenile	DL16
Medium Sprout	DL1, DL2
Medium Juvenile	DL4, DL25
Medium Juvenile (0.52 m)	DL30
Medium Mature	DL8, DL9
Large Juvenile	DL10, DL12
Large Mature	DL11
Internodal	DL21
Nodal Type 1	DL20 (4/13-4/16), DL22
Nodal Type 2	DL20 (4/18-4/28)

Figure 1.

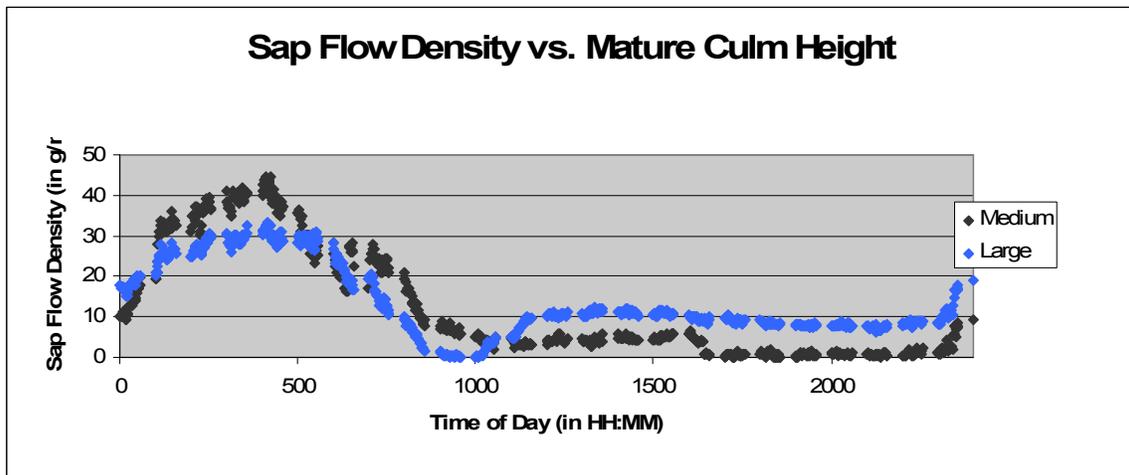
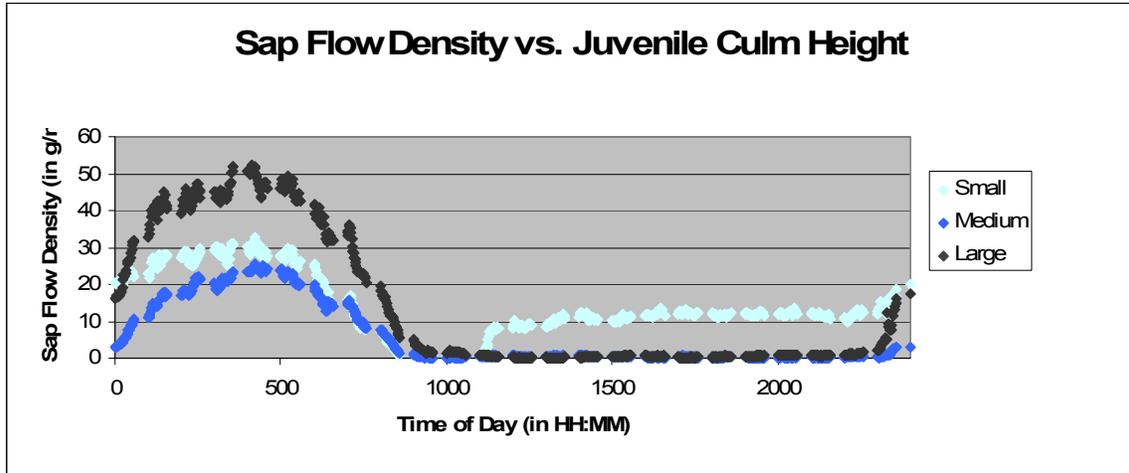


Figure 2.

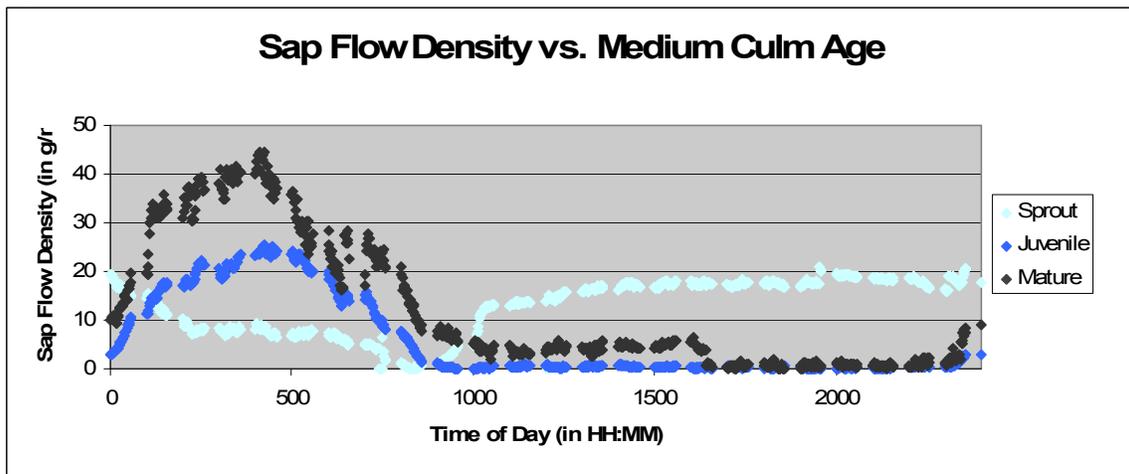


Figure 3.

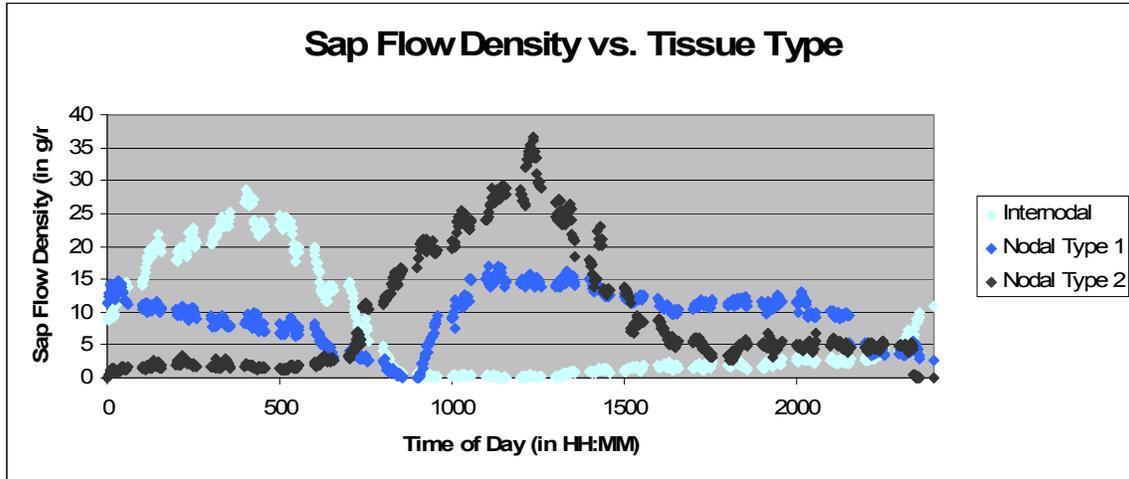


Figure 4.

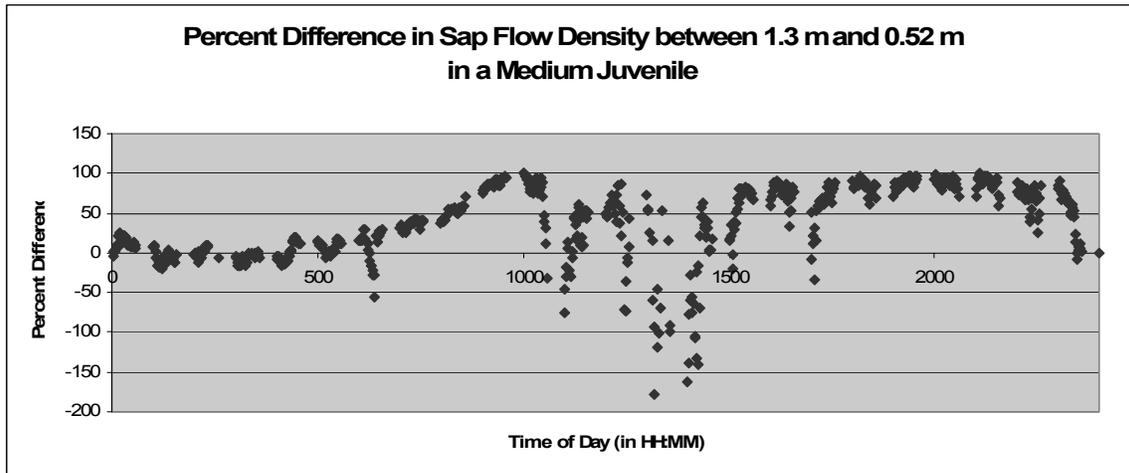


Figure 5.

