

THE EFFECTS OF AGEING ON THE HYDRAULIC CONDUCTIVITY OF *GALAX URCEOLATA* (POIR.)

A Thesis

by

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ABSTRACT

THE EFFECTS OF AGEING ON THE HYDRAULIC CONDUCTIVITY OF *GALAX URCEOLATA* (POIR.).

(December 2002)

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Galax urceolata (Poir.) is a rhizomatous evergreen herb distributed throughout the understory of deciduous forests of the southern Appalachian mountains. Galax leaves persist for approximately three years, decreasing in their ability to assimilate carbon as they age. The focus of this study was to examine the hydraulic architecture of Galax and to determine if ageing petioles decrease in their ability to transport water to the leaf blades which might contribute to their decline in carbon assimilation. Rhizomes had the shortest vessel lengths $(1.32 \pm 0.15 \text{ cm}, \text{mean} \pm \text{SE})$, followed by petioles $(4.20 \pm 0.31 \text{ cm})$, and then runners $(10.83 \pm 0.64 \text{ cm})$. Galax petiole vessel diameters in the 20 μ m size class were the most frequent, comprising 35% of the vessels and were predicted to contribute 42% to the total hydraulic conductance (k_h) . Runner vessel diameters in the25 μ m size class were the most frequent, comprising 31% of the vessels and contributing 24% of the k_h . The maximum hydraulic conductivity (max k_h) of current and one-year-old Galax

petioles were determined using a Sperry apparatus. Older petioles averaged a slightly higher max k_h than younger petioles (0.406 ± 0.027 vs. 0.379 ± 0.044 mg mm s⁻¹ kPa⁻¹) but were not statistically different. The susceptibility of different age classes to waterstress-induced xylem cavitation was also studied using two separate methods, a centrifugal force method and a pressure sleeve method. No significant differences were found in the different age classes of *Galax* petioles in their ability to transport water, even at low xylem water potentials. These results suggest that some other factor, most likely age-related degradation of the chloroplasts, leads to the decline of carbon assimilation in *Galax* leaves over time.

Cavitation in *Galax* petioles does not begin until water potentials drop below -2.0 MPa. At -2.5 MPa water potential, the percent loss of max k_h is only 10%, while at -4.0 MPa *Galax* still maintains a k_h that is 50% of max k_h . Since leaf water potential of *Galax* in the field has not been observed to decrease below -1.6 MPa, formation of drought-induced embolisms is probably uncommon. However, *Galax* is rooted shallowly in the leaf litter of deciduous forests and the litter is prone to dessication if rain events are infrequent. During severe droughts, soil water potentials have been measured as low as -2.5 MPa, which would result in only a 10% loss of k_h . Thus the cavitation-resistant hydraulic architecture of *Galax* lends itself to be well-suited to the intermittently dry habitat in which it is found.

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INTRODUCTION

Evergreeness is a habit found in plants in which at least some leaves are maintained on a plant throughout the year. Broad-leaved evergreens predominate at tropical latitudes and conifers predominate at higher latitudes (Chabot and Hicks 1982). Even in deciduous forests evergreen trees, shrubs, and herbs are usually quite common. Evergreeness can be achieved by producing short-lived leaves that last less than one year, or by producing leaves that persist for at least a year or more (Kikuzawa 1991). It is likely that leaf lifespans are under selective pressure and thus reflect an evolutionary strategy that may maximize carbon gain for the plant (Kikuzawa and Ackerly 1999). When the cost of maintaining a leaf offsets the maximum net gain of the leaf per unit time, the leaf will be discarded (Chabot and Hicks 1982; Kikuzawa 1989, 1991).

A decrease in photosynthetic capacity as leaves age is characteristic of evergreen species (Reader 1978; Chabot and Hicks 1982; Nilsen 1986; Sobrado 1994; McCarron 1995). It has been proposed that degradation of the chloroplast membranes within the mesophyll of the leaf is responsible (Yoshie and Kawano 1986, Yoshie and Yoshida 1987, Bao and Nilsen 1988, Nilsen *et al.* 1988). Nilsen et al. (1988) found a correlation between the breakdown of the chloroplast lamellae and an accumulation of plastiglobuli, lipids formed from the degradation of the chloroplasts' thylakoid membranes, in *Rhododendron maximum* L. leaves as they age. Also, *R. maximum* leaves decrease in longevity with an increase in light exposure (Nilsen 1986). In temperate regions evergreens must also contend with damage from photoinhibition caused by freezing temperatures and high irradiance which disrupts electron transport and may ultimately destroy photosynthetic pigments (Powles 1984). Therefore, decreases in the ability of chloroplasts to assimilate carbon may lead to leaf senesence, although the exact mechanism is still poorly understood.

Alternatively, decreases in the ability of leaves to take up water could also lead to a suppressed photosynthetic capacity. This could lead to reductions in stomatal conductance (g_s) caused by direct linkeages between g_s and hydraulic conductivity (k_h) of the xylem (Sperry *et al.* 1993). If the xylem in an evergreen plant degrades over time, this could also lead to reduced rates of xylem sap delivered to the leaves. Cytokinins, which suppress leaf senesence, are produced in roots and are transported through the transpiration stream to the leaves (Thimann 1977). A decrease in xylem sap flow due to a reduction of the conducting area of xylem may lower the amount of cytokinins reaching the leaves, inducing senesence. Also, photosynthetic rates may diminish over time due to declines in the amount of water or essential solutes transported in the xylem sap and entering the leaves. However, little is known about the ability of xylem tissue in the petioles of ageing evergreen leaves to maintain flow over long periods of time.

Plants take up water from the soil through their roots and distribute it to the shoots and leaves. Water conduction in angiosperm xylem tissue occurs primarily in tube-like cells called vessel elements and tracheids, both of which are dead at maturity (Esau 1977). Vessel members are restricted almost entirely to angiosperms and are stacked end-to-end

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forming long pipe-like structures. Each vessel member is attached to another by a perforation plate where primary and secondary cell walls are absent and water flow is practically unimpeded. Tracheids, considered precursors to vessel members, are thick-walled, narrow, tapered cells that are found throughout most vascular plant taxa. Water may be moved laterally from one vessel or tracheid to another through pits, which are thin areas where the secondary cell wall is absent.

The currently accepted mechanism for water transport in plants is the cohesiontension theory of sap ascent (Dixon and Joly 1894). As water transpires from the surface of a leaf the water potential of the transpiring cells is lowered, causing water to move into these cells from the xylem. Since water is cohesive and forms a continuous column from the leaves to the roots, it is pulled through the xylem conduits as it leaves the transpiring cells. This creates a tension (negative pressure) on the water column in the xylem that is transmitted all the way into the soil via the roots.

When the water within the xylem conduits is placed under extreme tension, e.g, by severe water-stress, the water column can break or cavitate. This results in an embolism, an air-filled vessel or tracheid. The current understanding of the mechanism of drought-induced embolism formation is illustrated by the air-seeding hypothesis developed by Oertli (1971) and reintroduced by Zimmerman (1983). As tension in the water column increases, air is eventually aspirated into a vessel or tracheid via an adjoining air space (Zimmerman 1983) or a previously embolized vessel or tracheid (Crombie *et al.* 1985) causing a cavitation event. An air-water meniscus forms in the pores of the pit membranes between embolized and non-embolized vessels and/or tracheids, which limits the embolism

to a single cell or vessel. The meniscus will remain intact as long as the tension on the water column remains below the pressure gradient required to break the air-water interface (Tyree and Ewers 1991). If the meniscus is broken, a cavitation will occur and an embolism will result.

The expansion of gas within the xylem segment occurs with enough force to create an audible pop. Cavitation events have even been detected acoustically using both low frequency audio detection (Milburn and Johnson 1966) and ultrasonic detection (Tyree and Dixon 1983; Tyree *et al.* 1984; Neufeld *et al.* 1992). The pressure gradient (Δp in MPa) required to break the air-water meniscus is dependent on the surface tension (T in N/m⁻², where N = Newtons) of the xylem water and the diameter of the largest pore in the pit membrane (D in µm) and is expressed by the following capillary equation (Sperry and Tyree 1988):

$$\Delta p = 4 \left(T / D \right) \tag{1}$$

The larger the pore size (D), the lower the pressure gradient or tension required to break the air-water meniscus. Therefore, the larger the pores the more vulnerable the vessel to drought-induced cavitation (Sperry and Tyree 1988, 1990; Cochard *et al.* 1992; Sperry and Sullivan 1992; LoGullo and Salleo 1993).

No correlation has been found between conduit diameter and susceptibility to drought-induced cavitation among taxa (Tyree and Dixon 1986; Sperry and Sullivan 1992) or among individuals of the same species (Sperry and Saliendra 1994). There is, however, significant evidence that within an individual or genotype, larger diameter vessels embolize prior to smaller diameter vessels (Tyree and Dixon 1986; Salleo and LoGullo 1986, 1989; Sperry and Tyree 1988, 1990; Lewis *et al.* 1994; Sperry and Saliendra 1994). This suggests that within a single plant, larger vessels have larger pores in their pit membranes. The reason for this is either developmental, i.e., larger vessels within an individual simply have larger pores (Tyree and Sperry 1989) or statistical, i.e., larger vessels within an individual will contain more pores, increasing their probability of having larger pores (Hargrave *et al.* 1994).

It would seem logical that if larger vessels or tracheids within an individual plant are more susceptible to water-stress-induced cavitation, that xylem tissue should consist entirely of small, highly resistant vessels and tracheids. However, there is a tradeoff between the diameter of a xylem element and the amount of water that can flow through per unit pressure gradient. The Hagen-Poiseuille equation (Zimmerman and Brown 1971) defines the volume flux (ν) of water passing through an ideal capillary as:

$$v = \pi r^4 (\Delta p)^2 8 \eta^{-1} l^{-1}$$
(2)

in which r = radius of the capillary (m), $\Delta p =$ pressure gradient (N / m² where N (newton) = kg m sec⁻¹), $\eta =$ dynamic viscosity of the fluid (g m⁻¹ sec⁻¹), and l = length of the capillary (m). In essence, the mass flow through a capillary is proportional to the fourth power of the radius (Calkin *et al.* 1984; Hargrave *et al.* 1994). Reducing the radius in half decreases mass flow by 16 times while a doubling increases it by the same amount. Therefore, while smaller xylem elements may be more resistant to drought-induced cavitation than larger xylem elements within an individual, the smaller elements are unable to transport as much water as even slightly larger elements. A mixture of both large and small conduits ensures optimal water conduction when the water supply is plentiful and continued water transport through times of mild waterstress. Narrow vessels or tracheids are used primarily as auxiliary pathways for rerouting water from larger vessels or tracheids as they become embolized by drought stress (Carlquist 1982). During drought, narrow conduits play an essential role in allowing water transport to continue, albeit at a lower rate due to the increased resistance to flow through the narrower, non-embolized vessels (Hargrave *et al.* 1994). Embolism formation by water-stress, therefore, is of common occurrence in the xylem tissue of many plant species. Recent studies suggest that the occurrence of embolisms in larger vessels and rerouting water through narrower vessels may occur diurnally and not just during periods of drought (Neufeld *et al.* 1992; Zwieniecki and Holbrook 1998). Therefore, narrow conduits may play a more important role than previously thought.

Hydraulic conductivity $(k_h \text{ in } m^4 \text{ mPa sec}^{-1})$ can be defined as the mass flow of water (m) through a plant segment per unit pressure gradient (Δp) per length (l) (Sperry *et al.* 1988a):

$$k_h = m \, \Delta p^{-1} \, l^{-1} \tag{3}$$

Cavitation reduces the conducting area which lowers k_h . It is thought that when plants are functioning optimally they operate at a water potential just below the level that would induce cavitation, thus maintaining maximum hydraulic conductance (Kikuta *et al.* 1997; Tyree and Sperry 1988).

Plants regulate water loss primarily through their stomata. Stomatal closure reduces $\triangle P$ and *m*, preventing runaway cavitation. However, this can limit the diffusion of

 CO_2 into the leaf, resulting in a lowering of the internal CO_2 and decreasing the rate of photosynthesis (Tyree and Sperry 1988). It is now thought that maximum stomatal conductances are limited by k_h in plants (Sperry 2000).

Xylem cavitation can also result from the freezing and subsequent thawing of xylem sap. Water molecules form a lattice as freezing occurs and because gases are insoluble in ice, they are forced out of solution (Hammel 1967). If the bubble is small enough and/or tension is minimal during thawing, the bubble will simply redissolve into the solution. If, however, the bubble is large and there is sufficient tension on the water column during thawing, the bubble will grow, resulting in an embolism (Hammel 1967). A bubble will redissolve into solution only if the internal pressure (P_b) of that bubble exceeds the vapor pressure of water (Davis *et al.* 1999). P_b is dependent on the radius of curvature of the bubble (R), surface tension of the xylem sap (T), and xylem sap pressure (P_x):

$$P_{\rm b} = 2 T / (R + P_{\rm x})$$
 (4)

The larger the bubble the larger the radius, ergo, the greater the possibility of nucleation of the air bubble. Since a larger vessel will contain a greater volume of water it will contain a greater amount of dissolved gas. Thus, larger conduits will produce larger bubbles, increasing their susceptibility to freeze-induced cavitation as thaw occurs (Yang and Tyree 1992; Sperry *et al.* 1994). Susceptibility to freeze-induced cavitation, unlike drought-induced cavitation, has been found to be strongly correlated with vessel size (Ewers 1985; Cochard *et al.* 1992; Sperry and Sullivan 1992; LoGullo and Salleo 1993; Davis *et al.* 1999).

Embolisms accumulated by freezing or drought must be dissolved back into the water stream to maintain maximum hydraulic capacity. In order for this to occur, the xylem pressure potential (Ψ_{xp}) must be equal to or greater than atmospheric pressure (101.3 kPa) (Sperry *et al.* 1988b; Tyree and Yang 1992):

$$\Psi_{xp} \ge 0 \text{ MPa}$$
 (5)

This has been found to occur with root pressure on both a seasonal and daily basis (Milburn 1979; Sperry *et al.* 1987, 1988b; Cochard *et al.* 1994). Recent evidence suggests embolism dissolution can also occur while the transpiration stream is still under tension (Salleo *et al.* 1996; Canny 1997; McCully *et al.* 1998; Zwieniecki and Holbrook 1998).

The exact mechanism that results in the dissolution of embolisms while under tension is still poorly understood. Recently, however, a method for embolism dissolution that can operate under tension during active transpiration was proposed (Holbrook and Zwieniecki 1999, Zwieniecki and Holbrook 2000) For this to occur, embolized and nonembolized vessels must be hydraulically isolated from one another. Lignin, found in the secondary cell wall of the vessel elements, creates low permeability in the cell wall and tension from the water column cannot be transferred through it. Living cells adjacent to embolized vessels generate a driving gradient that draws water into the lumen of the vessel. The structure of the bordered pits retains hydraulic isolation until the lumen has been completely filled. Once filled, the previously embolized vessel unites hydraulically with the actively conducting vessels. Therefore, embolism dissolution can occur even under the rather large tensions found when transpiration rates are high. The hydraulic architecture of a plant can be defined as the structure and arrangement of xylem tissue in relation to the leaf area it must supply with water (Zimmermann 1978). Although plant taxa differ greatly in their hydraulic architectures, there must be a balance between carbon allocation in leaves and the conducting area of the xylem tissue (Zimmermann 1983; Salleo and LoGullo 1986; Sperry 1986; Tyree and Sperry 1988; Meinzer *et al.* 1992). If there are more leaves on a tree than can be supplied water by the xylem conduits, cavitation events would be uncontrollable. Therefore, the amount of leaves able to be supported by a plant is dependent on the amount of xylem tissue present within the plant (Zimmermann 1978). As evergreen leaves age, their ability to assimilate carbon decreases (Nilsen 1992, Neufeld and Young 2003), hence, older leaves are discarded for younger, more productive leaves maintaining a balance between leaf area and conducting area of the xylem tissue.

Galax urceolata (Poir.) Brummitt, formally *Galax aphylla* (L.), is a rhizomatous evergreen herb found in the understory of deciduous forests in the southern Appalachian mountains. Each rhizome produces up to three, thick waxy leaves per year. Leaves normally persist for three years (McCarron 1995) but can live occasionally up to five (A.T. and B.G. Hallowell, personal communication). *Galax* is in the family Diapensiaceae and is distributed throughout the eastern part of North America, from Georgia to New York. It is most abundant in the nutrient poor soils of xeric, oak-hickory forests in the southern Appalachian mountains (Whittaker 1966), and is commonly found in association with *Kalmia* and *Rhododendron* (Baldwin 1941). At first glance, the habitat in which *Galax* grows does not appear to be a stressful environment, since even the more xeric forests of the southern Appalachians receive plentiful rainfall, with drought occurring infrequently. *Galax*, however, is shallowly rooted in the leaf litter, and this top layer of soil can dry rapidly. Thus, in contrast to more deeply rooted shrubs and trees, *Galax* must tolerate occasional severe drought conditions.

Galax must also deal with low light conditions throughout a considerable portion of the year. Since it is found in the understory of deciduous forests, much of the sunlight is intercepted by the trees above it once the leaves flush out. The evergreen habit of *Galax* probably evolved in response to the low light levels found in the understory. Leaf life spans are directly related to the cost of producing a leaf versus the leaf's ability to offset construction costs via carbon assimilation (Chabot and Hicks 1982). The considerably sclerophyllous, ovate leaves of *Galax* range from 3 to 10 cm in length (Radford *et al.* 1964) and the carbon cost of producing such a thick, waxy leaf probably exceeds the carbon gain of that leaf in a single growing season. Consequently, it takes more than a single year to assimilate enough carbon to pay back the construction costs.

I decided to learn more about the hydraulic architecture of this evergreen understory herb, *Galax*. McCarron (1995) found that the rates of carbon assimilation decrease as *Galax* leaves age. Nilsen (1992) and Nilsen *et al.* (1988) found *Rhododendron* leaves also decrease in their ability to photosynthesize as they age as do other understory herbs (Neufeld and Young, 2003). Despite the ubiquity of this trend, little is known about why an ageing evergreen leaf decreases in its ability to assimilate carbon. One possibility is a reduction in the ability of xylem conduits to provide adequate water and nutrients to the ageing leaf blade. Xylem degradation may result in occlusions that clog pit membranes and disrupt water flow, ultimately lowering hydraulic conductivity. In this study, I attempted to determine if the petioles of *Galax* leaves decreased in their ability to conduct water as they aged. I also wanted to determine if the petioles became more susceptible to xylem cavitation with age. A reduction in the ability of a petiole to conduct water and nutrients over time might be the proximate cue that triggers programmed leaf senesence.

MATERIALS AND METHODS

Plant Materials

Galax urceolata possesses a woody rhizome measuring up to 20 cm in length. The rhizome grows apically with the addition of one to three leaves per year. Leaf blades are ovate, measuring 3 to 10 cm in width and are attached to a 3 to 25 cm long petiole (Radford *et al.* 1964). Belowground runners, referred to hereafter simply as runners, emerge from the older portion of the rhizome. These runners grow through the leaf litter away from the parent plant, form a new rhizome at their tip and eventually a new ramet develops. Within a plot of *Galax*, several ramets may be found interconnected with belowground runners.

Galax plants were collected from naturally occurring clumps in the understory of a deciduous forest near Boone, North Carolina ($81^{\circ}43' W 36^{\circ}' N$). The collection site was on a south-facing slope of ~20° at an elevation of 1005 m. In the spring of 1997, 500 newly emerging *Galax* leaves were tagged with numbered, blue, plastic labels. At that same time, 500 leaves that had survived the winter were tagged with numbered, red, plastic labels, establishing two age classes of *Galax* leaves.

Galax plants were harvested from these clumps to perform vessel size measurements and to collect hydraulic conductivity data throughout the next year. Entire Galax plants, including the rhizome, were collected so as not to introduce any new embolisms into the petioles or rhizomes. Plants were then placed into tightly-sealed, black, plastic bags so dehydration and subsequent cavitation events would be minimal. All plant materials were used within three days of harvesting.

Vessel Measurements: Maximum Vessel Lengths

To gain a general understanding of vessel structure in Galax, maximum vessel lengths for petioles, rhizomes, and runners were determined. After Galax plants were brought back to the lab at Appalachian State, the portion of the plant being studied (either the petiole, rhizome, or runner) was cut underwater from the rest of the plant. A piece of Tygon tubing was placed over the cut end of the plant part being examined and attached to a tank of compressed air equipped with a flow regulator. The plant part was submerged underwater and 55 kPa of pressure was applied. At such a low pressure, air could not pass through the pits or end walls of the vessels (Zimmerman and Jeje 1981). The other end of the plant part was trimmed away underwater until a stream of air bubbles was seen perfusing from the cut end. Only after both of the end walls had been cut from a single vessel would air be able to pass freely through the plant material being used. Therefore, when air bubbles were first seen the plant material was removed and its length was measured and recorded as an approximation of maximum vessel length (Greenidge 1952). Maximum vessel lengths were generated for 30 each of Galax petioles, rhizomes, and runners.

Vessel Measurements: Vessel Diameters

Vessel diameters of *Galax* were measured at Duke University in Dr. John Sperry's lab using a Microplan II Bit Pad (Donsanto Inc., Natuck, Massachusettes). Vessels were measured by viewing cross-sections of either a petiole or runner under a compound microscope. The lumen diameter for each vessel was measured and recorded for a total of five petioles and five runners. Rhizomes were not measured because of the presence of multiple leaf traces, which made accurate readings of vessel diameters impossible.

Theoretical hydraulic conductivities for each vessel and for each petiole and rhizome as a whole were calculated using a computer program written by Dr. John Sperry which incorporated the Hagen-Poiseulle equation (Zimmerman and Brown 1971). Vessel diameters were also grouped into size classes and the contribution of each size class to the overall water conduction was calculated.

Maximum Hydraulic Conductivity: Petioles

A comparison of maximum hydraulic conductivities between age classes of *Galax* petioles was accomplished through the use of a modified Sperry apparatus (Figure 1) (Sperry *et al.* 1988a). Due to the low conductivities found in *Galax* petioles, a vacuum was applied to pull water through the apparatus in place of the normal employment of positive pressure. Petioles were cut from the rest of the *Galax* plant underwater and subsequently trimmed to 10 cm sections. It was determined from the maximum vessel length measurements that 10 cm was more than twice the average maximum vessel length found in *Galax* petioles. They were then inserted through holes in the center of rubber

stoppers. Three of the petioles of each of the two age classes were then loaded into the Sperry apparatus. Petioles were flushed of any existing embolisms by exerting 103 kPa of pressure into the system via a captive air tank (see Figure 1). Petioles were pressurized for 15 minutes to ensure all existing embolisms were flushed out.



Figure 1. Schematic representation of modified Sperry apparatus used to obtain hydraulic conductivity estimates from the petioles of *Galax*.

The Tygon tubing used in the Sperry apparatus was allowed to relax for approximately 15 minutes after flushing to ensure that the constriction of the tubing had ceased before measurements of maximum hydraulic conductivities were attempted. Hydraulic conductivity readings were taken by turning a three-way stopcock to direct flow from a microbalance (Sartorius R 200 D) through the petiole being examined. A vacuum pressure of 460 kPa was routinely applied to the system to pull water through the petiole from the balance. The change in weight over 10 second intervals was recorded by a computer (Toshiba 1200 laptop) attached to the balance, using a Pascal program written by Dr. John Sperry that calculated hydraulic conductivity per unit length. An average of six conductivity readings was recorded and tabulated into the hydraulic conductivity equation. Background readings were made before and after each hydraulic conductivity reading in the same manner, however, the stopcocks were turned so as to allow flow to pass freely from the vacuum to the balance when no vacuum pressure was applied. Thus, any changes in weight on the microbalance due to background noise, such as constriction of the tubing after pressurization, were factored into the hydraulic conductivity equation. Hydraulic conductivity values were corrected for altitude, latitude, temperature, and displacement effects of the pipette in the vial of water on the balance. The hydraulic conductivity reading was immediately calculated and recorded upon the completion of the final background noise measurement. The maximum hydraulic conductivities of 18 petioles for each of both age classes were measured and recorded.

Susceptibility to Water-Stress-Induced Cavitation: Centrifuge

Two methods were utilized to determine if differences in susceptibility to droughtinduced embolism formation existed between the two age classes of *Galax* petioles. The first method, employing centrifugal force (Alder *et al.* 1997; Holbrook *et al.* 1995; Pockman *et al.* 1995), was carried out at Duke University on March 5, 1998. *Galax* plants were collected on March 2, 1998 and brought to Duke University in a sealed, black, plastic bag. Petioles were cut from the rest of the plant underwater and trimmed to 12.9 cm so as to fit into the centrifuge setup. The petioles were flushed with pressurized distilled water and their maximum hydraulic conductivities measured and recorded as previously described.

The petioles were then placed in L-shaped, plastic reservoirs containing deionized water (Alder *et al.* 1997). The six reservoirs which held three petioles at once were placed within a specially designed rotor, housed within a Sorvall RC-5C Plus centrifuge (Kendro Laboratory Products, Newton, Connecticut). Petioles were spun at a force creating -1 MPa water potential within the petiole. This pressure was held for approximately seven minutes. When spun in this manner, tension is created on the water column within the xylem tissue of the petiole just as it would be if the plant was experiencing water stress. Then the petioles were removed from the centrifuge and their hydraulic conductivities remeasured. This process was repeated, decreasing the water potentials incurred by the petioles by 1 MPa each time, down to -5 MPa. Due to petiole breakage at high rpms, only six petioles from the younger age class and four petioles from the older age class withstood spinning to -5 MPa.

Susceptibility to Water-Stress-Induced Cavitation: Pressure Sleeve

Susceptibilities to drought-induced cavitation between the two age-classes of *Galax* petioles were also examined using a pressure sleeve (Salleo *et al.* 1992; Sperry and Saliendra 1994) coupled with a modified Sperry apparatus (Figure 2). The following experiment was conducted in the ASU physiology lab from March 15 through April 11, 1998. A pressure sleeve was built in the ASU machine shop using schematics provided by Jonathan Comstock at the Boyce Thompson Institute for Plant Research, located on the

campus of Cornell University in Ithaca, New York.

Before being placed in the pressure sleeve, petioles were trimmed underwater to 10 cm and flushed for 15 minutes at 103 kPa in the Sperry apparatus used previously. A single petiole was then placed within the pressure sleeve and the end caps secured. The Sperry apparatus, modified to hold a single petiole while inserted in the pressure sleeve, was attached to the petiole. This modified Sperry apparatus allowed us to take hydraulic conductivity readings without removing the petiole from the pressure sleeve (see Figure 2). A maximum hydraulic conductivity reading was then measured and recorded as described previously.



Figure 2. Schematic representation of pressure sleeve with modified Sperry apparatus for measuring loss of hydraulic conductivity as drought-stress embolism formation occurs.

A pressure gauge and a tank of compressed air were attached separately to the pressure sleeve and pressure was gradually applied to the outside of the petiole and held at 0.5 MPa for five minutes. Applying pressure to the petiole via the pressure sleeve may force air from previously embolized vessels and/or air spaces into adjacent nonembolized vessels, reducing hydraulic conductivity. The effect of this positive pressure in embolism formation is comparable to what would occur if an equivalent tension (negative pressure) was placed on the water column through drought stress. The pressure was then gradually reduced to zero through a pressure release after five minutes of pressurization. The petiole was allowed to rest for five minutes before its hydraulic conductivity was retaken. This was repeated in 0.5 MPa increments up to 4.0 MPa. Five petioles from each age class were measured.

Maximum Hydraulic Conductivity: Petiole-Rhizome Junctions

A single *Galax* rhizome will normally possess at least two age-classes of leaves. After measuring maximum hydraulic conductivity (max k_b) on isolated, detached petioles, I chose to determine if *Galax* decreased in its ability to move water through the petiolerhizome junction as leaves aged. This was accomplished by modifying the Sperry apparatus to house a cut portion of rhizome on the vacuum side of the apparatus. Up to four petioles, two from each age class, could be inserted into the microbalance portion of the system. Under this arrangement water that was pulled through the petiole had to first go through the rhizome. The following experiment was carried out from June 5 through June 18, 1998 in the physiology lab at ASU.

Galax plants containing one or two leaves from both age classes were collected

from the study site. Leaf blades were cut from the petioles underwater and the petioles subsequently trimmed to 6 cm, as measured from the rhizome-petiole junction to the cut end. The rhizome was then cut back underwater to 1.5 cm and inserted into the vacuum end of the apparatus while the petioles were inserted into stoppers with holes in them and then into the other end of the apparatus. The plant segments were then flushed of embolisms for 15 minutes at 103 kPa. Maximum hydraulic conductivity readings were taken for each individual petiole emerging from the rhizome. The experiment was repeated for 11 petioles from each age class.

Statistics Used

A one-way ANOVA and Duncan's multiple range test was performed on the maximum vessel length data. Differences in the frequency distribution of vessel diameters between *Galax* petioles and runners were tested using a chi-square analysis. Two-sample t-tests were used to determine differences in susceptibility to water-stressed induced cavitation between current year and one-year old *Galax* petioles. A paired t-test was used to test for differences between current year and one-year old *Galax* petioles hydraulic conductivity through the petiole-rhizome junction. All differences were considered significant at the 0.05 level.

RESULTS

Vessel Measurements

Maximum vessel lengths differed significantly (F_{2, 87} = 135.29, p< 0.0001) among all the *Galax* plant parts that I measured (Figure 3). Below-ground runners had the longest maximum vessel lengths with a mean of 10.83 ± 0.64 cm. Rhizomes had the shortest, 1.32 ± 0.15 cm and petioles were intermediate averaging 4.20 ± 0.31 cm.



Figure 3. Maximum vessel lengths (mean \pm standard error) in petioles, rhizomes, and runners in *Galax urceolata*. Each mean was significantly different from the others at $\alpha = 0.05$. N = 30.

Figure 4. Frequency (A) and hydraulic frequency (B) distribution of vessel diameter size classes within the petioles and runners of *Galax urceolata*. N = 5.



Vessel diameters in *Galax* were larger within runners when compared to petioles (Figure 4A). Within *Galax* petioles the vessels in the 20μ m diameter size class were the most frequent (35%) and contributed the largest amount to the hydraulic conductance of the xylem conduits (42%) (Figure 4B). Within runners vessels in the 25μ m diameter size

class were the most frequent, comprising 31% of all vessels (24% hydraulic frequency), but vessels in the 30μ m size class contributed the most to the overall hydraulic conductance at 31% (23% frequency).

According to the Hagen-Poiseuille equation, flow is proportional to the fourth power of the vessel radius. Therefore, the larger vessels within a xylem conduit may conduct a larger portion of water than smaller vessels even though there are a greater number of smaller vessels. In *Galax* runners, vessels with diameters 30μ m and larger made up only 36% of the vessels but accounted for 57% of the hydraulic frequency (Figure 4A and B). Alternatively, *Galax* petioles with vessel diameters 15μ m or smaller accounted for nearly half of all vessels (49%) but contributed only 17% to the hydraulic frequency.

Maximum Hydraulic Conductivities

Maximum hydraulic conductivities (max k_h) were compared between the two age classes to determine if the xylem conduits decreased in their ability to conduct water as they aged. Current year petioles had a mean of 0.379 ± 0.044 mg mm s⁻¹ kPa⁻¹ and oneyear old petioles had a mean of 0.406 ± 0.027 mg mm s⁻¹ kPa⁻¹. There were no significant differences in the max k_h between the current year and one-year old petioles (Figure 5). Figure 5. Maximum hydraulic conductivities (k_h) for current year and one-year old *Galax* petioles (mean ± standard error). Means not significantly different at $\alpha = 0.05$. N = 18.



Susceptibility to Drought-Induced Cavitation: Centrifuge

Although older petioles tended to cavitate at slightly higher xylem water potentials than younger petioles, there was no significant difference found in the susceptibility to water-stress induced xylem cavitation between current year petioles and one-year old petioles (Figure 6). There was an initial increase in hydraulic conductivity found in both age classes after the first centrifugal application which may have resulted from small occlusions that were blown out of the vessels upon spinning (Sperry, personal communication). Figure 6. Vulnerability curve of hydraulic conductivity during water stress for current year (N = 6) and one-year old (N = 4) Galax petioles using the centrifuge method. Percent of maximum hydraulic conductivity (mean \pm standard error) at each water potential.



Susceptibility to Drought-Induced Cavitation: Pressure Sleeve

As in the centrifuge method, older petioles tended to cavitate at a slightly higher xylem water potential than younger petioles using the pressure sleeve method (Figure 7). However, there were no significant differences between current year and one-year old petioles in susceptibility to cavitation except at -3 MPa water potential. There was no

initial jump as was seen using the centrifugal method.

Figure 7. Vulnerability curve of hydraulic conductivity during water stress for current year and one-year old Galax petioles using the pressure sleeve method. Percent of maximum hydraulic conductivity (mean \pm standard error) at each water potential.

* indicates significant difference between petiole ages at $\alpha = 0.05$. N = 5.



Maximum Hydraulic Conductivity: Petiole-Rhizome Junction

The hydraulic conductivity through the petiole-rhizome junction was much reduced compared to the petioles alone (Figure 8). Conductivities were only about one tenth of what they were in isolated petioles. There was no significant difference found between the max k_h of current year and one-year old petioles when water passed first through the rhizome junctions and then through the petioles.







DISCUSSION

Galax leaves normally persist for three years (McCarron 1995) but occasionally may last up to five years (A.T. and B.G. Hallowell, personal communication). Rates of gas exchange decline each year as the leaves age (McCarron 1995). Maximum rates of photosynthesis (A_{max}) drop from 4.3 µmol m⁻² s⁻¹ in current year leaves to 1.3 µmol m⁻² s⁻¹ by the next fall (McCarron 1995). This decrease in photosynthetic capacity can either be attributed to changes in stomatal conductance, or to a decline in the photosynthetic capacity of the mesophyll, or both (Björkman 1981). Stomatal conductance (g_s) of twoyear-old *Galax* leaves averages only half of what they do in the previous fall (0.02 vs. 0.04 mol m⁻² s⁻¹) (McCarron 1995). A lower g_s could reduce the rate of photosynthesis (A) or a lower A could reduce g_s by increasing internal CO₂ (c_i).

Using a hydraulic conductivity model, Sperry *et al.* (1998) demonstrated that as cavitation lowers k_h the water potential of the leaf decreases, which in turn lowers g_s and limits the rate of transpiration. I was interested in determining if the petioles of *Galax* leaves decreased in their ability to transport water as they aged. If over time the xylem vessels became non-functional and filled with occlusions, a drop in k_h would result. A decrease in the k_h of a *Galax* petiole as it aged would limit g_s , which in turn would limit its rate of photosynthesis. Alternatively, the rate of photosynthesis may decline from a reduction in the photosynthetic capacity of the mesophyll. The subsequent rise in c_i would feed back to lower g_s . In this scenario, hydraulic conductivity would remain high and the xylem conduits would actually have excess capacity to conduct water and a reduced water supply would not be the cause of the lowered photosynthesis.

Although rates of photosynthesis decline with age in *Galax*, the ability of petioles to conduct water remains constant (Figure 5). In fact, I saw a slight increase in max k_h with age although it was not statistically significant (0.379 ± 0.044 10⁻⁷ vs. 0.406 ± 0.027 10⁻⁷ kg m sec⁻¹ MPa⁻¹). This may result from pit membrane degradation as has been found in ageing twigs of *Populus tremuloides* (Sperry *et al.* 1991). The increase in pit membrane permeability of *P. tremuloides* was attributed to the removal of materials from around cellulose fibrils, possibly in the middle lamella components of the pit membranes.

Since hydraulic conductivity in petioles was found not to decrease with age, I then decided to see if older *Galax* petioles were more sensitive to drought than their younger counterparts. It has been found that the larger the pit pore the more susceptible a vessel is to cavitation (Tyree and Sperry 1989). If the pit pores in *Galax* vessels indeed increase in size with age their susceptibility to drought-induced xylem cavitation would also increase. As the petioles age and their rates of gas exchange decrease it may be beneficial for the plant to direct water toward the younger leaves during drought events. If drought-induced cavitations accumulate in the older petioles at a faster rate than in the younger ones, the Ψ_w of the older leaves would decrease faster than younger leaves during periods of water stress. In turn, both g_s and A would be lower in the older leaves and more water would pass into the younger petioles through the rhizome, maintaining a higher leaf Ψ_w and allowing a greater rate of photosynthesis.

When the centrifugal method was used, older petioles did seem to cavitate at a slightly faster rate than younger petioles (Figure 6). The differences however, were not statistically significant. I saw the same trend using the pressure-sleeve method (Figure 7) with the exception that at -3 MPa there was a transient statistically significant difference found between the two age classes in their vulnerability to water-stress-induced xylem cavitation. Overall older petioles do not appear to cavitate faster than younger petioles during periods of extreme water stress. Thus, differences in k_h or vulnerability between younger and older *Galax* petioles does not appear to explain the declines in A and g_s observed by McCarron (1995).

Since the k_h of the petioles did not decrease significantly with age and they do not become more susceptible to water stress over time, I focused my attention on the petiolerhizome junction. As the *Galax* rhizome grows it puts up one to three leaves per year. The leaf bases are added acropetally to the rhizome just past the last year's leaf bases. I hypothesized that as the *Galax* plant aged and new leaf bases were added to the rhizome, water flow through the older petiole-rhizome junction might become restricted and reduce k_h (Meinzer *et al.* 1992).

Again, this turned out not to be the case. No significant differences could be found in the max k_h through the petiole-rhizome junctions between current and one-year old leaves (Figure 8). Therefore, factors other than reductions in hydraulic conductivity in the xylem tissue must account for the decrease of carbon assimilation in ageing *Galax* leaves. Yoshie and Kowana (1986) found that *Pachysandra terminalis*, another evergreen, understory herb, did not generate stomatal limitations in photosynthesis over time and the loss in photosynthetic capacity was attributed to a reduction in the photosynthetic capacity of the mesophyll. The chloroplasts may simply be breaking down in some manner, reducing their ability to photosynthesize over time. In *Rhododendron maximum*, Nilsen *et al.* (1988) found that plastoglobules (chloroplastic inclusions formed from the breakdown of chloroplast membranes) accumulated over the winter. High irradiance and cold temperatures damaged the chloroplasts and reduced their photosynthetic capacity. Whether the same phenomenon is occuring in the leaves of *Galax* is currently unknown. More research is required to determine the exact physiological cause of reduced photosynthetic capacity in ageing *Galax* leaves.

During this study I found that the hydraulic architecture of *Galax* is well-suited for the environment in which it is found. The leaf litter in which *Galax* grows is prone to dessication between rain events, even though the southern Appalachians as a whole receive plentiful rainfall. *Galax* may be more resistant to drought-induced xylem cavitation than other plants found in association with it. These other plant species may tap the deeper layers of soil which do not dry out as readily as the leaf litter. *Rhododendron maximum* is a more deeply-rooted evergreen shrub found in association with *Galax*. Vessel diameters within *R. maximum* stems (mean diameter of 20 μ m) are slightly larger than the vessel diameters found within *Galax* petioles (mean diameter of 13 μ m) (Lipp and Nilsen 1997). Their resistances to drought-induced xylem cavitation, however, differ considerably. Lipp and Nilsen (1997) found *R. maximum* could have up to a 20% loss of k_h at -1 MPa and nearly 50% by -2.2 MPa. In contrast, *Galax* petioles do not begin to lose any hydraulic capability until tensions approach -2 MPa and they retain 50% k_h even at water potentials as low as -4 MPa (Figures 4 and 5). *Galax* vessels, therefore, are much more resistant to drought-induced xylem cavitation than *R. maximum*. Since susceptibility to water-stress-induced xylem cavitation is determined by pit pore size (Sperry and Tyree 1988, 1990), pit diameter in *Galax* petioles, therefore, must be smaller than those in *R. maximum*.

The resistance of *Galax* xylem to cavitation is comparable to that of some desert plants (Pockman and Sperry 2000). The vulnerability curves for *Galax* are similar to those found by Kolb and Sperry (1999) in three subspecies of sagebrush (*Artemesia tridentata*). These drought-resistant vessels make *Galax* well-suited for the intermittently dry leaf litter in which it is primarily rooted. Water potentials in the upper soil layers in the southern Appalachians have been measured as low as -2 to -2.5 MPa and may drop even lower during episodes of extreme drought (Stan Wullschleger, Oak Ridge Lab, personal communication). But even at -2.5 MPa, *Galax* would still maintain between 80% and 90% of its k_h (Figures 6 and 7).

The ability of *Galax* to maintain k_h at such low soil water potentials allows this species to photosynthesize at almost any time of the year as long as it is warm enough and there is sufficient light. Since *Galax* grows in the understory of deciduous forests, it is light-limited throughout much of the year when the canopy is closed. *Galax* photosynthesis peaks during the spring and fall when light levels are at their highest on the forest floor and day time temperatures are above freezing (McCarron 1995). The ability for *Galax* to conduct water whenever conditions are appropriate for gas exchange may be critical for the survival of this species. However, a high resistance to drought-induced cavitation, achieved by having small diameter vessels and small pit pores, as elaborated earlier, limits g_s (Sperry *et al.* 1998). In turn this will set limits on the amount of carbon assimilation *Galax* leaves are capable of in a single growing season. Chabot and Hicks (1982) suggest leaf life-spans are determined by the construction and maintenance costs related to the leaf's photosynthetic ability to pay back these costs. The evergreen habit of *Galax* is a result of the high construction costs of thick, waxy leaves in a light-limited environment. Coupled with a rather low k_h and the subsequent low A, more than a single growing season is required to offset the high construction costs of *Galax* leaves.

Some other interesting information about the ecology of *Galax* was revealed by the vessel measurements. Maximum vessel lengths within the petioles, rhizomes, and runners differed significantly (Figure 3). Maximum vessel lengths averaged only 1.3 cm in *Galax* rhizomes, which is approximately as long as a rhizome grows in a single growing season. Rhizomes typically only grow long enough to allow for the addition of the new year's leaf bases. However, petioles and runners grow several centimeters in a single growing season. Petioles had a mean maximum vessel length of approximately 4 cm and have determinant growth. They grow to their final size in just a few weeks after emerging in the spring. Runners averaged the longest maximum vessel lengths with a mean of nearly 11 cm. These rhizomatal offshoots act to disperse asexual propagules, or ramets. The runner grows apically and upon reaching a certain length (often after more than one growing season), forms a new rhizome. This new *Galax* ramet must form far enough away from the parent plant to ensure that competition for water, nutrients, and light are limited. A single *Galax* genet may contain several hundred *Galax* ramets.

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Vessels within below-ground runners have larger diameters than those found within the petioles of *Galax* (Figure 4). The k_h of runners, therefore, is probably greater than that of the petioles. In turn, petioles should be more resistant than runners to drought-induced xylem cavitation. During periods of extensive drought ramets may become hydraulically isolated from each other if runners accumulate embolisms more easily then petioles. This ensures that an individual *Galax* ramet will conserve water for itself, as opposed to connected ramets, during severe water stress. However, the possibility remains that ramets located in pockets of greater soil moisture may supply a connected ramet in a drier area with water. Further research on the water movement between *Galax* ramets is warranted.

There is also a severe constriction to water flow found at the petiole-rhizome junctions. Mean max k_h at the junction are only a fifth of what they are in the petiole itself (Figure 8). During periods of extreme water-stress petioles would fill with embolisms more quickly than the rhizome, hydraulically isolating it from the petioles. In contrast, if the drought was so severe that the leaves perished, the rhizome would be able to survive and produce new leaves the next growing season.

A considerable amount of attention is now being given to embolism dissolution during active transpiration (Zimmerman *et al.* 1993, 1994; Milburn 1996; Salleo *et al.* 1996; Canny 1995, 1997; Zwieniecki and Holbrook 2000; Zwieniecki *et al* 2000). Davis *et al.* (1999) found that, in general, vessels greater than $30\mu m$ were extremely sensitive to cavitation caused by freeze-thaw events. In *Galax* petioles less than 3% of all vessels are greater than $30\mu m$ (Figure 4A). Therefore, not only is *Galax* resistant to water-stresss induced xylem cavitation, it should also be highly resistant to freeze-thaw induced xylem cavitation. During the spring and fall, when *Galax* A is at its peak, overnight temperatures often dip below freezing. Therefore, *Galax* xylem elements must recover from any freeze-thaw or drought-induced xylem cavitation from the previous day and night in order to begin photosynthesizing at maximum rates. *Galax* would then appear to be an ideal plant with which to study drought-induced and freeze-thaw induced cavitation and embolism dissolution.

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