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Seasonal Conductivity Patterns in the Roots and Stems
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Frank Ewers
Major professor

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SEASONAL CONDUCTIVITY PATTERNS IN THE ROOTS AND STEMS OF THE TWO CLONAL RING-POROUS TREES, *SASSAFRAS ALBIDUM* AND *RHUS TYPHINA*.

By

Laura L. Jaquish

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ABSTRACT

SEASONAL CONDUCTIVITY PATTERNS IN THE ROOTS AND STEMS OF THE TWO CLONAL RING-POROUS TREES, *SASSAFRAS ALBIDUM* AND *RHUS TYPHINA.*

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Seasonal xylem (wood) conductivity and embolism (air blockage) patterns were monitored in roots versus stems of two clonal tree species, *Sassafras albidum* and *Rhus typhina,* throughout 1996 and 1997. Stems of both species were embolized in the early spring following thaw, while the roots were already conductive. Stems became conductive by late June following leaf expansion and maturation of the new earlywood vessels. Stems reached their maximum hydraulic conductivity rates by mid-July and August, and then became totally embolized by early November, following leaf drop. The dye patterns in xylem growth rings indicated *Rhus* stems were only conductive in the currents year's growth ring. *Sassafras* stems were more variable in the number of conductive rings, but most of the conductivity was in the outer growth ring. In contrast, the roots of both species had many growth rings of conductive xylem throughout the monitoring period. No positive root pressure was detected in either species. The stems of these ring-porous species appeared to fit the expected pattern of high vulnerability to freezing-induced embolism. The maximum frost depth of 204mm ± 11 for 1996 and 149mm ± 10 for 1997 was much deeper than the sampled root depth of *Rhus* (71mm ± 6; 57mm ± 3) and but not deeper than many sampled roots of *Sassafras* (225mm ± 17; 299 ± 35). Despite occasional winter freezing of the soil water, the roots either avoided xylem embolism altogether or they were able to reverse embolism by a mechanism other than positive root pressures.

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I would like to thank Dr. Frank Ewers for serving as my advisor and mentor. I would especially like to thank him for all the invaluable advice and encouragement. Thanks to my committee members, Dr. Stephen Stephenson and Dr. Frank Telewski, for advice and editorial assistance. Special thanks to Michele Pruyn and Dr. Telewski for help with NIH Image and the image analysis equipment. I would also like to thank David Dominic with the DNR for permitting me to use state game area land throughout Mid-Michigan.

Thanks to Tim Tibbets for showing me the ropes and teaching me how to operate the ganglion of tubes and clamps also known as the Sperry Apparatus. And also for instructing me in the creation of and implementation of bubble manometers. Many thanks to the two undergraduates, Elizabeth Webster and Amy VanSluyters, who assisted me in the field and in the lab. Amy VanSluyters accompanied me on excursions to collect samples for hydraulic conductivity measurement and long hours of wrestling with the Sperry Apparatus. Elizabeth Webster assisted in slide making and image analysis.

I would especially like to thank Cal Steinorth who never complained once about accompanying me on pre-dawn bubble manometer checks in the dark with an extra dose of mosquitoes. And for help scouting out *Sassafras* clone locations along miles of Mid-Michigan dirt roads. Thanks for help sampling for hydraulic conductivity, for digging a lot of deep holes with small hand trowels, for sporting the orange uniform while braving the woods with me during various hunting seasons, and especially for supporting me emotionally while I raced to finish my thesis just days before our wedding.

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INTRODUCTION

Seasonal patterns of xylem embolism (air blockage) in temperate woody plant stems are well documented. Such stems show the greatest percent embolism in the winter (Cochard and Tyree 1990; Sperry and Sullivan 1992; Cochard et al. 1994; Sperry et al. 1994; Tognetti and Borghetti 1994; Magnani and Borghetti 1995; Hacke and Sauter 1996). However, seasonal studies of xylem embolism are lacking for roots of woody plants that experience severe winter freezing conditions.

Freezing-induced embolism occurs in stems when the xylem sap freezes and air bubbles are forced out of solution. Upon thaw, and especially when the stem xylem sap is under negative pressure (tension), the air bubbles expand, forming embolisms that block water transport. The larger the xylem conduit, the more vulnerable it is to cavitation during freeze-thaw events (Zimmermann 1983; Ewers 1985; Wang et al. 1992; Sperry and Sullivan 1992; Lo Gullo and Salleo 1993; Sperry et al. 1994; Tyree et al. 1994; Sperry 1995). Increases in percent embolism have been correlated with the number of freeze-thaw cycles (Sperry et al. 1988; Cochard and Tyree 1990; Sperry et al. 1994; Wang et a.l 1992; Sperry 1993, 1995). Faster thaw rates can result in cavitation occurring at higher (less negative) pressure (Langan et al. 1997). In stems of *Larrea tridentata* the minimum sub-freezing temperature impacts the amount of embolism. With freezing at –9°C there was no increase in embolism, whereas minimum temperatures below -19°C resulted in 100% embolism.

In general, stems of temperate woody plants are able to overcome the potentially debilitating effects of freezing-induced embolisms. The two most well known mechanisms for embolism recovery are replacement of embolized xylem by new xylem production and refilling of embolized xylem by positive root pressure. In temperate ring-porous trees the vessels of stems generally remain conductive for only one growing season. Such trees can tolerate the loss of function in previous year's growth rings because of the production of new, wide diameter, earlywood vessels in the early spring, prior to leaf maturation (Zimmerman 1983; Ellmore and Ewers 1986; Cochard and Tyree 1990; Sperry et al. 1994; Hacke and Sauter 1996). Xylem refilling by positive root pressure has been reported for many temperate deciduous diffuse-porous trees and for temperate species of Vitis, (Scholander et al. 1955; Sperry et al. 1987; Sperry et al. 1994) but not for temperate ring-porous trees. Root pressures, which are able to dissolve embolisms by raising the pressure within the xylem conduits to atmospheric pressures or above, originate from water absorption into the roots. Positive root pressures have been detected before dawn, during rainstorms, and in the spring before bud burst when transpiration levels are minimal (Sperry et al. 1987; Cochard et al. 1994; Ewers et al. 1997; Fisher et al. 1997).

Some studies suggest that roots of diffuse-porous trees are potentially more vulnerable to drought-induced embolism than stems. This is based upon vulnerability curves of roots versus stems in *Betula occidentalis* (Sperry and Saliendra 1994) and *Acer grandidentatum* (Alder et al. 1996). Because tree roots tend to have wider vessel diameters than stems (Zimmermann and Potter 1982; Gasson 1985; Pate et al. 1995; Gartner 1995; Ewers et al. 1997), roots could also be more vulnerable to freezing-induced embolism.

Results suggested that in roots of the ring-porous tree *Sassafras albidum*, the wide vessels remained conductive for many years, versus just one year for stems (Bosela and Ewers, in preparation). The present study investigated the seasonal patterns of xylem conductivity and embolism in the roots versus stems of two clonal tree species, *Sassafras albidum* and *Rhus typhina*. My hypothesis was that the roots, but not the stems, would be able to reverse winter, freezing-induced embolism.

Both species are ring-porous temperate deciduous trees that spread clonally from root buds that arise from horizontally growing parent roots. The clonal growth habit of the two species allowed for genetically identical samples to be taken monthly from clones throughout 1996 and 1997. Frost gauges were used to determine if the soil water surrounding the horizontal parent roots froze. In addition to monitoring the seasonal change in conductivity and embolism, various phenological events were recorded including bud burst, cessation of leaf growth, cessation of twig elongation, peak flowering, appearance of fruit (*Rhus* only), leaf color change, and leaf drop. The mechanism for possible recovery from winter embolism was also investigated using bubble manometers to measure root pressure. In addition, crystal violet dye was used to mark the conducting xylem elements in roots and stems throughout the study period, to see which elements were more prone to embolism.

MATERIALS AND METHODS

Plant Material

The *Sassfras albidum* (Nutt.) Nees and *Rhus typhina* L. clones sampled in this study were located within Clinton, Ingham, Gratiot, and Shiawassee counties in southern lower Michigan (Table 1). Each stem arising from the horizontal root system was considered a ramet. The smallest sampled *Sassfras* and *Rhus* clones had 119 and 161 ramets respectively. Four clones of each species were monitored in 1996-97 and eight in 1997-98 (Table 1). In 1996, 32 *Sassfras* and 34 *Rhus* ramets were sampled, versus 56 ramets of each species in 1997. Different clones of a species were separated from each other by more than 160 m in each case, so each clone was assumed to be a separate genet. All the sampled clones had many stems that arose from horizontally spreading roots.

Hydraulic Conductance

Measurements were made as described by Sperry et al. (1988) and Tyree and Ewers (1991). A single ramet from each of the clones was sampled per month throughout the monitoring period. The horizontal root systems of the sampled ramets were excavated to approximately 25 cm both proximally and distally. Roots were cut in the field and ramets were transported to the lab in water. Stem, junction, and root segments were then cut under water to approximately 14 cm in the lab and hydraulic conductance per unit pressure gradient (K_h) and percent embolism were measured using a Sperry Apparatus (Sperry et al. 1988). The cut ends of the segments were re-cut with a razor blade and connected to sections of vinyl tubing. A solution of 10mol m^{-3} citric acid (pH = 3) was used in order to discourage microbial growth. The solution was run through a

Table 1: Clone locations for hydraulic conductivity sampling for 1996-97 and 1997-98.

	Genet	County	Townshi	Section	Site
Sassafras albidum					
1996-97					
	*^1	Ingham	Alaiedon	6	S. side of Lott South Woodland -Sandhill Rd., Michigan State University
	*2	Clinton	Bath	24	0.1 mi south on Peacock Rd. off Clark Rd., Rose Lake Research Area
	*3	Clinton	Bath	27	0.15 mi west on Stoll Rd. off Upton Rd., Rose Lake Research Area
	*4	Gratiot	Elba	4	0.51 mi east on Garfield Rd.off Ransom Rd., Gratiot-Saginaw State Game Area
1997-98					
	1	Gratiot	Elba	4	0.08 mi east on Garfield Rd. off Ransom Rd., Gratiot-Saginaw State Game Area
	2	Gratiot	Elba	4	0.18 mi east on Garfield Rd. off Ransom Rd., Gratiot-Saginaw State Game Area
	3	Gratiot	Elba	4	0.4 mi east on Garfield Rd. off Ransom Rd., Gratiot-Saginaw State Game Area
	*4	Gratiot	Elba	4	0.51 mi east on Garfield Rd. off Ransom Rd., Gratiot-Saginaw State Game Area
	*^5	Ingham	Ingham	32	0.6 mi south on Potter Rd.off Dexter Tr., Dansville State Game Area
	6	Ingham	Bunkerhil	4	0.8 mi west on Ewers Rd.off Williamston Rd., Dansville State Game Area
	^7	Ingham	Bunkerhil	3	0.1 mi east on Ewers Rd.off Williamston Rd., Dansville State Game Area
	*8	Ingham	Ingham	34	1.2 mi south on Williamston Rd.off Dexter Tr., Dansville State Game Area
Rhus typhina					
1996-97					
	*^1	Ingham	Alaiedon	6	S. side of Lott South Woodland -Sandhill Rd., Michigan State University
	*2	Clinton	Bath	26	1 mi west on Stoll Rd.off Peacock Rd., Rose Lake Research Area
	3	Clinton	Bath	24	0.1 mi west on Stoll Rd. off Peacock Rd., Rose Lake Research Area
	4	Shiawassee	Woodhull	20	0.6 mi west on Bath Rd from Woodbury Rd., Rose Lake Research Area
1997-98					
	1	Clinton	Bath	24	0.1 mi west on Stoll Rd. off Peacock Rd., Rose Lake Research Area
	*2	Clinton	Bath	26	N. side of Rose Lake near headquarters, Stoll Rd., Rose Lake Research Area
	3	Clinton	Bath	26	1 mi west on Stoll Rd.off Peacock Rd., Rose Lake Research Area
	4	Clinton	Bath	23	0.8 mi north on Upton Rd.from Stoll Rd., Rose Lake Research Area
	*^5	Ingham	Ingham	29	0.5 mi south on Potter Rd.off Dexter Tr., Dansville State Game Area
	^6	Ingham	Bunkerhil	3	0.1 mi east on Ewers Rd.off Williamston Rd., Dansville State Game Area
	7	Ingham	Ingham	33	edge of parking area at the end of Seven Gables Rd,Dansville State Game Area
	*8	Ingham	Ingham	33	edge of field off the end of Seven Gables Rd, Dansville State Game Area

* indicates frost tube site locations

^ indicates common sites to both species

0.2 μm Gelman filter. A pressure head of 2.5 kPa was used for measuring the conductivity in the native state ($K_{h\ initial}$) and a pressure of 173 kPa was used to flush out any embolism. Volumetric flow rate was determined with a 0.1ml pipette and a stopwatch. This process was repeated until a maximum value ($K_{h\ max.}$) was achieved. To determine which were the conductive xylem elements, segments were perfused for 15 minutes with 0.5% crystal violet to mark conductive vessels. Dyes were used both on control, to represent the native or initial state, and on segments with the embolisms removed (final). Specific conductivity (K_s), (K_h) divided by the xylem area, was calculated for all segments measured in 1996 and 1997. The xylem area was calculated from the xylem diameter, with the pith area then subtracted.

Anatomical Study

To determine the number of conductive xylem growth rings, all segments were cut approximately 3 cm from the flow end on a band saw and re-cut with a fresh razor. The smooth end was then examined under a dissecting microscope so that the number of xylem growth rings and the number of active rings could be determined. A growth ring was not counted as conductive unless it had more than 20 conductive vessel elements in transverse view.

To determine diameters of conductive vessel elements, all segments from July, September, October, and November of 1997 were cut transversely with a sliding microtome set at a thickness of 40 μm. Sections were taken through an ethanol-xylene dehydration series and mounted in permount. Vessel lumen areas were measured using a light microscope interfaced with a high-resolution CCD video camera and multi-scan analog monitor (Dage-MTI, Inc) and a computer running NIH Image 1.5. All the vessels in a pie-shaped wedge, from the center to the outer edge of a transverse section and bordered by rays were sampled for each slide. A minimum of 100 vessel lumens were measured per stem, root, or junction. In some of the smaller roots all the vessels in the transverse section were measured. This method allows all vessels above a set minimum to be measured within a designated area. Vessel diameters less than 25μm were excluded in order to avoid including fibers within the measurements. Vessel diameters were calculated from lumen areas.

Macerations were done to determine vessel diameter for comparison with the image analysis technique. Wood from the November 1997 collection was slivered from the vascular cambium to the pith using a fresh razor. Material from all the segments was separated into three categories, root, junction, and stem. Slivered wood was placed in Jeffery's solution (10% chromic acid: 10% nitric acid) and kept at 60°C for 36 hours. The macerated shavings were washed with water and pelleted by centrifugation up to three times. The pellet was transferred into a vial of 70% ethanol. Macerated samples were stained with 0.1% methylene blue. Vessel members were sampled randomly in the fashion described by Ewers and Fisher (1989). A total of 100 vessels were sampled for roots, junctions and stems of each species. Vessels diameters were measured with an optical micrometer.

Phenology

One sample ramet from each of the 16 clones was monitored throughout the 1997 growing season for recording phenological events and results were averaged for each species. Methods were adapted from Gilbert (1961), Lechowicz (1984, 1995), McGee (1986) and Wang et al. (1992). Ramets chosen for phenological observation were similar in size to ramets sampled for hydraulic conductivity. Five marked buds per tagged ramet were monitored for bud burst beginning in early May 1997. Twenty-two marked leaves in *Sassafras* and 16 in *Rhus* were measured weekly throughout the growing season in 1997, to generate the mean date of cessation of leaf growth. The same method was used to generate the mean date of cessation of twig elongation. Eight twigs were tagged and monitored per species. Peak flowering was described as mean date at which greater than 50% of the individual flowers on an inflorescence were open. Appearance of fruit was noted only in *Rhus*. Mean date of fruit visibility was the date by which all monitored female ramets had visible fruit. Mean date of leaf color change was calculated by averaging the dates that colored leaves appeared in each clone. Leaf drop was defined as the mean date by which all leaves on monitored ramets had fallen. Data was recorded every week through September and then every other week through leaf drop.

Root Pressures

The possibility of positive root pressure was investigated using bubble manometers (Fisher et al. 1997). For both species in 1996 and 1997, xylem sap pressures were measured on both junctions and horizontal roots in the early spring following soil thaw but before bud burst. Measurements for *Sassafras* began the first week and continued through the third week of May. For *Rhus*, measurements were taken in the first week of May and June. Root pressures were measured on at least one individual per clone for both species in 1996 and 1997. Manometers were hooked up in the evening and checked the following morning before dawn. The root or junction was then re-cut with a fresh razor and the vinyl tubing reattached. The same root or junction was checked for three consecutive days after which a new root or junction was selected.

Frost Depth

The depth of soil frost was monitored for the winters of 1996-97 and 1997-98 with frost gauges (Rickard and Brown 1972; McCool and Mohnau 1984; and Saul and Potts, 1996). Five sites were monitored each winter, two of which remained the same for both years (Table 1). Each site had five gauges that were placed throughout the clone. Gauges were constructed of 1.5m lengths of 1.9cm ID PVC with rubber stoppers at each end. The interior tubes were constructed of 1.2m lengths of 1.6cm OD clear vinyl tubing, stoppered at both ends and filled to within 25.4cm of the top with distilled water dyed with 5 drops of 0.1% methylene blue. The inner vinyl tubing was connected by nylon string to an eye on the inner side of the top stopper of the outer PVC tube to facilitate removal for measurment. Depth of frost was measured on the vinyl tube from ground level down. As the ground froze, the water in the tube would freeze and the blue dye would settle out below the frost line. Gauges were inserted 1.0m into the ground. Frost depth was measured every other week from the first frost through the last frost and on any date when the air temperature was colder than had previously been recorded that season.

RESULTS

Percent Embolism

In 1996, *Sassafras* stem segments became conductive in late May and became fully embolized by November (Figure 1). Roots remained conductive from the start of

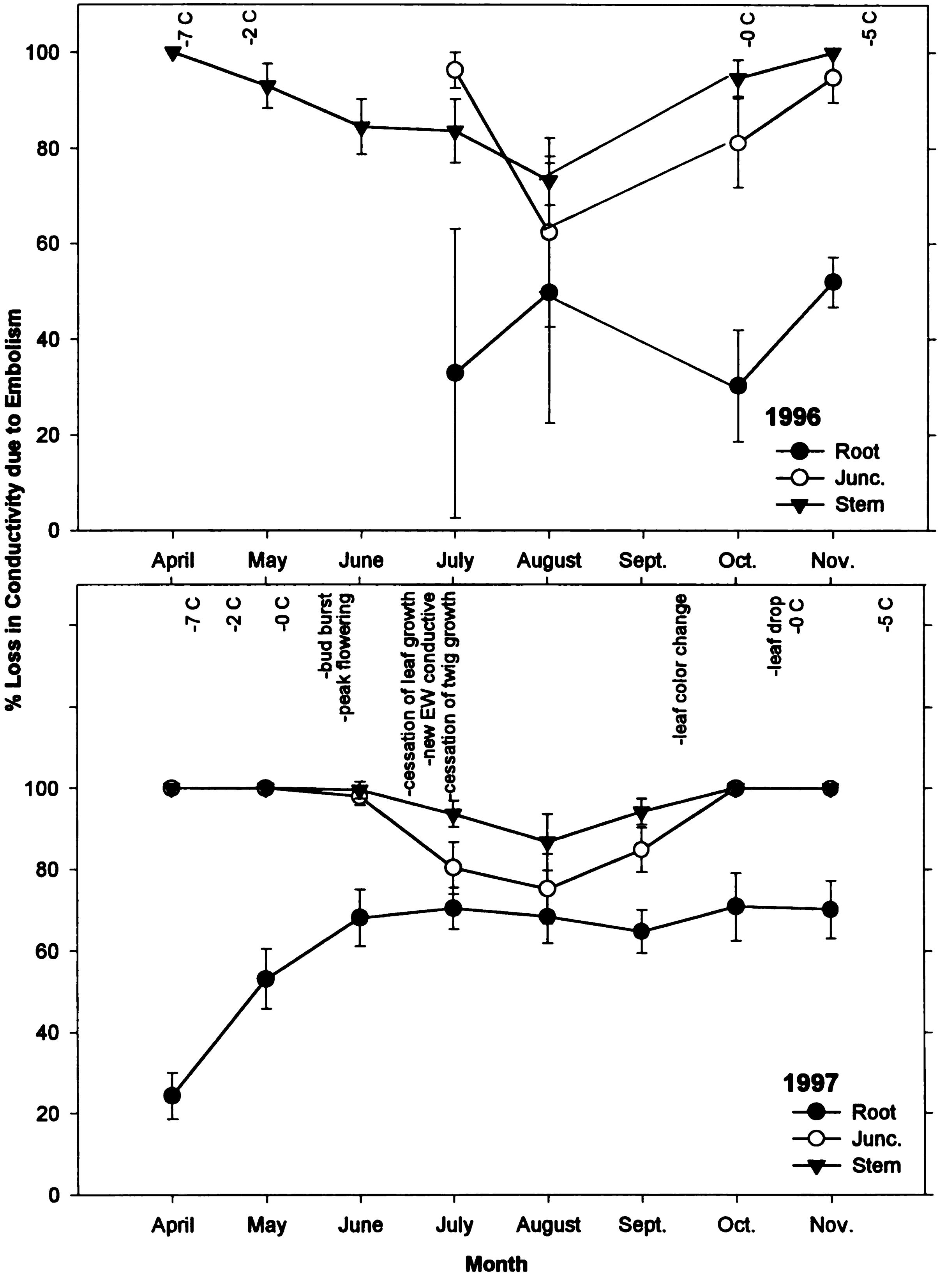

Figure 1: Percent loss in conductivity due to embolism (± SE) for *Sassafras albidum* for 1996 and 1997 (temperature data for both years from St. Johns, MI).

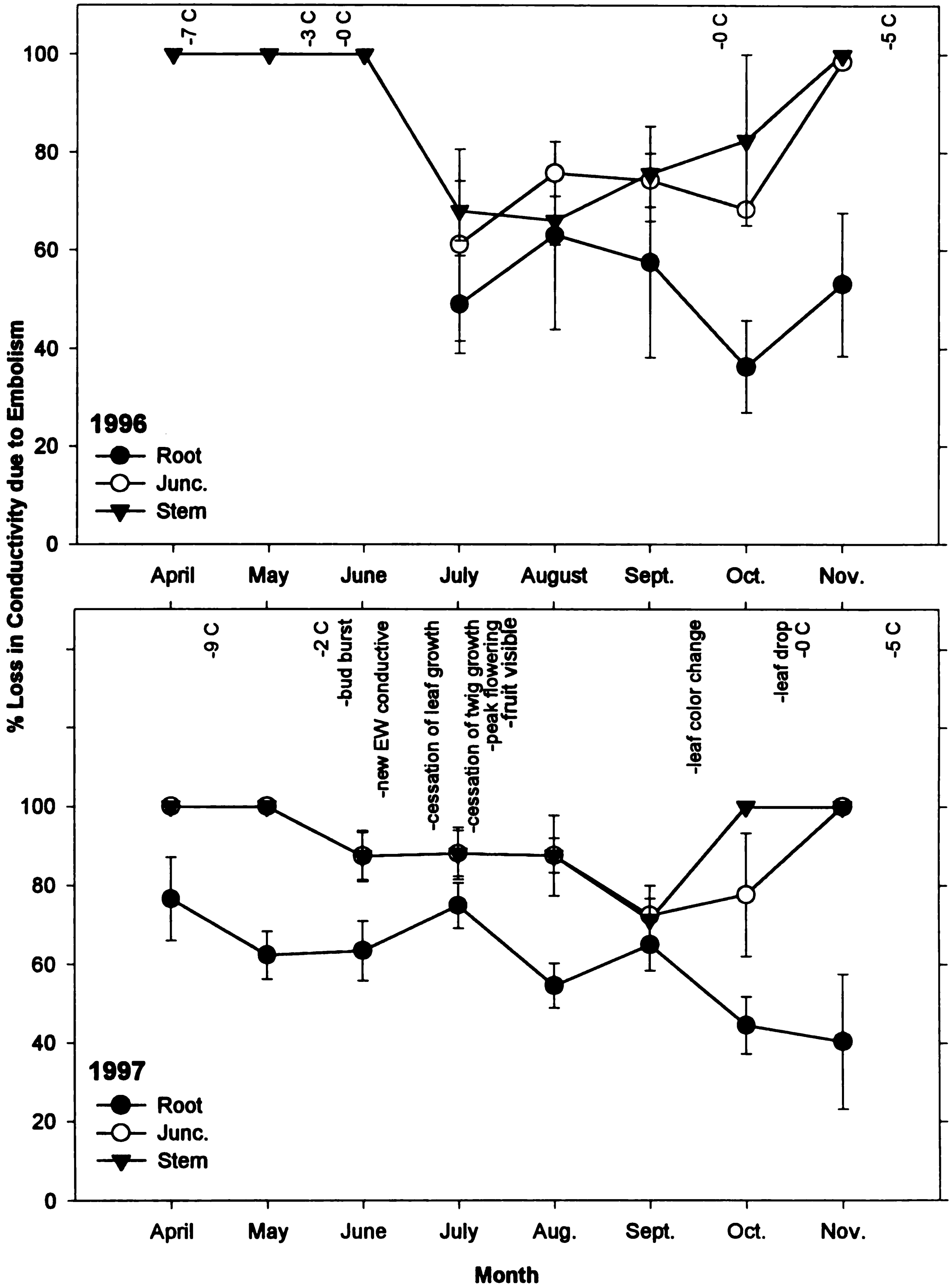

Figure 2: Percent loss in conductivity due to embolism (± SE) for *Rhus typhina* for 1996 and 1997 (temperature data for both years from East Lansing, MI).

sampling in July through November. In 1997, *Sassafras* stems and junctions remained fully embolized through May (Figure 1). Stems and junctions became conductive by late June to early July, as the new current year's earlywood (EW) became conductively active. This was well after bud burst and peak flowering. Embolism was lowest in August but began to increase in September as leaves changed color and then became fully embolized by October following leaf drop but before the first hard freeze. Roots of *Sassafras* remained conductive throughout the entire monitoring period. Root embolism increased from 25% in April to around 70% as junctions and stems became conductive in late June and early July.

In 1996, *Rhus* stem segments remained fully embolized until July and became completely embolized again by November (Figure 2). Junctions also became fully embolized by November. Roots remained conductive throughout the monitoring period. *Rhus* stems and junctions remained fully embolized through May in 1997 (Figure 2). Stems and junctions became conductive in June following bud burst, as new earlywood became conductive. Stems became fully embolized by October and junctions by November following the first hard freeze. Roots remained conductive throughout the monitoring period.

Specific Conductivity (K_s)

In both years, for both species, $K_{s\,max.}$ values were always greater in the roots than in the stems. In 1996, *Sassafras* stems and junctions had peak $K_{s\,max.}$ values in August of 4.5 ± SE 1.1 kg $s^{-1}MPa^{-1}\,mm^{-2}$ and 3.3 ± 2.0, respectively, and the root values fluctuated around an overall mean of 28.5 ± 5.1 (Figure 3). In 1997, $K_{s\,max.}$ peaked in July at 11.0 ±

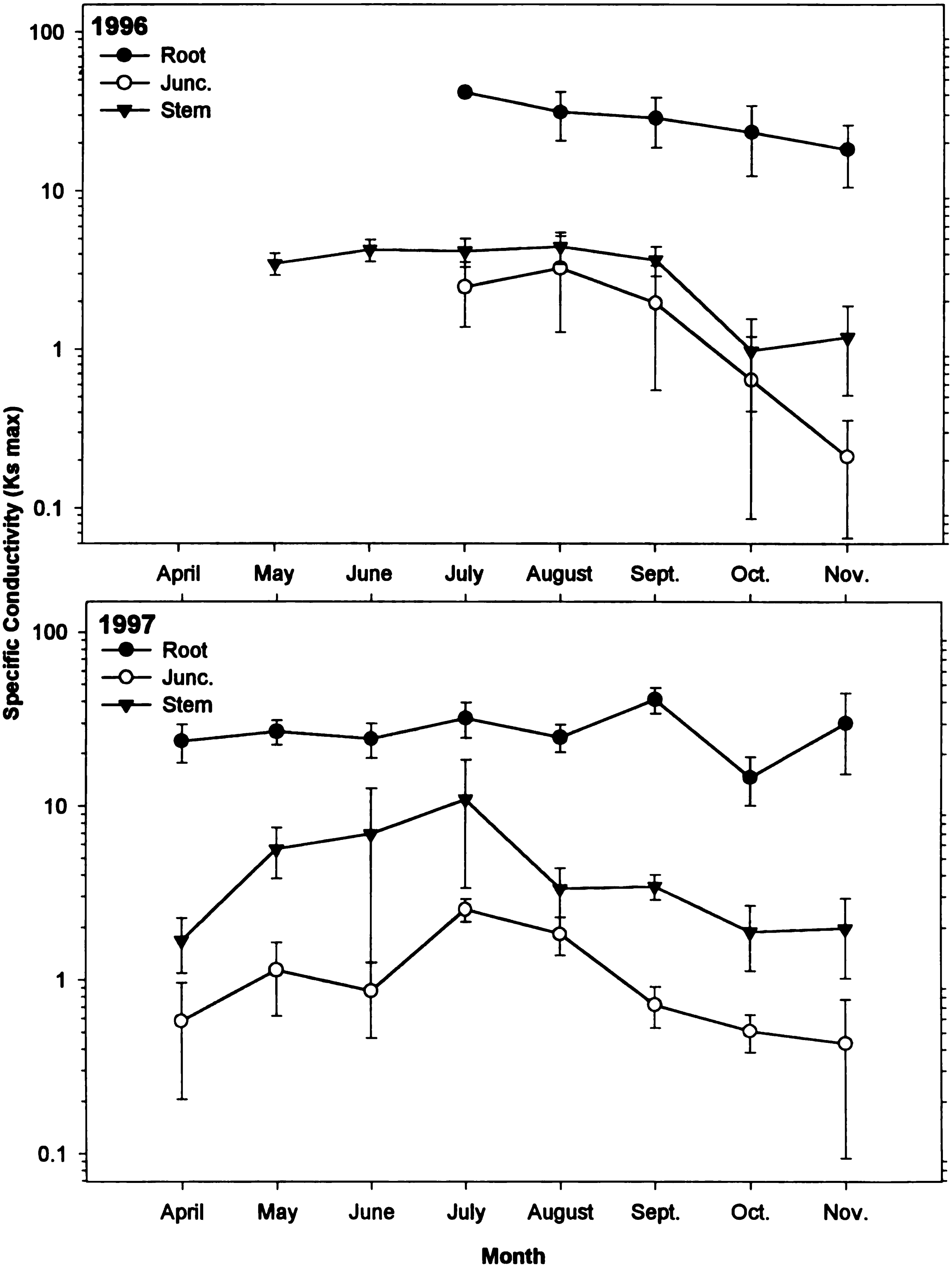

Figure 3: Seasonal maximum specific conductivity (Ks max) ± SE for *Sassafras albidum* in 1996 and 1997.

7.6 for stems and at 2.5 ± 0.4 for junctions. For the junctions, values of $K_{s\,max.}$ were associated with greater heartwood formation in the lower stem. Stems and junctions of *Sassafras* always had $K_{s\,max.}$ values above zero even when $K_{s\,initial}$ values reached zero in the early spring and fall of both years. $K_{s\,max.}$ values for the roots fluctuated very little seasonally and remained around an overall mean of 27.3 ± 2.7.

In 1996, the stems and junctions of *Rhus* had peak $K_{s\,max.}$ values in August at 5.3 ± 1.5 kg $s^{-1}MPa^{-1}\,mm^{-2}$ and 2.6 ± 0.4 respectively (Figure 4). The overall $K_{s\,max}$ mean for the roots was 6.6 ± 1.1. In 1997, initial and maximum specific conductivity values were at zero for both stems and junctions in April and May. The $K_{s\,max.}$ values peaked in July for junctions, 1.6 ± 0.4, and in late July early August for stems, 1. 8 ± 0.5. Stem $K_{s\,initial}$ values reached zero in October and junctions in November as they became fully embolized. In November 1997, 50% of stems and 50% of junctions sampled were at a $K_{s\,max.}$ value of zero. Maximum specific conductivity values of zero were associated with the formation of tyloses in the current year's growth ring. The $K_{s\,max.}$ values of the roots fluctuated around an overall mean of 6.1 ± 0.5 for 1997.

Anatomical Study

Percent Conductive Lumen Area

In the stems of both species, the percent conductive lumen area prior to embolism removal declined from July to October/ November. However, the seasonal trend that was seen in the stems was not present in the roots of either species. In *Sassafras*, the percent conductive vessel lumen area for stems in their native condition declined from a value of 31.3% ± 11.4 in July to 0% by October and November (Figure 5). The values for the

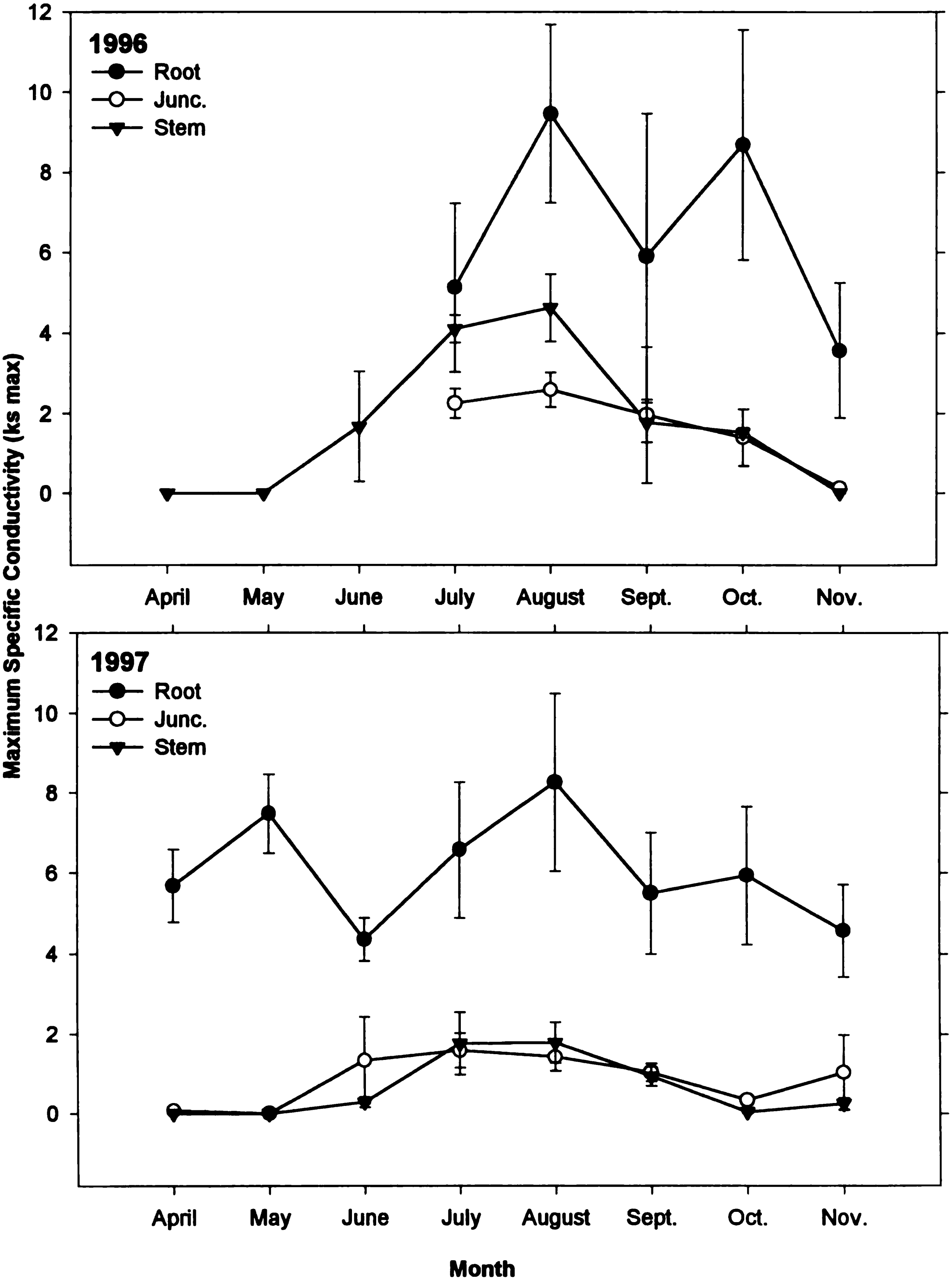

Figure 4: Seasonal maximum specific conductivity (Ks max) ± SE for *Rhus typhina* in 1996 and 1997.

roots in their native condition did not change significantly and remained around an overall mean of 42.1% ± 1.9. In *Rhus* the percent conductive lumen area for the stems in their native condition decreased from a value of 32.3% ± 6.6 in July to 0% in October and November (Figure 6). *Rhus* roots did not show a significant change in percent conductive lumen area seasonally and fluctuated around an overall mean of 39.6 ± 3.2. The junctions, not shown in Figures 5 and 6, displayed a pattern very similar to the stems in both species.

Number of Conductive Rings

In both species, the number of conductive growth rings was far greater in roots than in stems. In *Sassafras*, the number of conductive xylem growth rings in stems (initial) prior to embolism removal varied seasonally (Figure 7). From April, the number of conductive xylem growth rings increased from zero to 2.1 ± 0.9 rings in August (the August sample contained a stem which had 7 out of 7 xylem growth rings conductive) and then decreased to zero conductive rings again by October. Roots (initial) fluctuated around an overall mean of 5.1 ± 0.3 rings, and did not demonstrate a clear seasonal pattern. *Sassafras* roots (initial) had a significantly greater number of conductive rings than stems (initial) (paired t-test, $P<0.000001$).

Sassafras stems (final) following embolism removal had a significantly greater number of conductive rings than native stems (initial) (paired t-test, $P< 0.000005$). The number of conductive xylem growth rings in stems (final) fluctuated around an overall mean of 3.6 ± 0.2 rings throughout the monitoring period but were always above zero. The average ratio of conductive rings to total rings was 0.9 ± 0.03, which indicated that

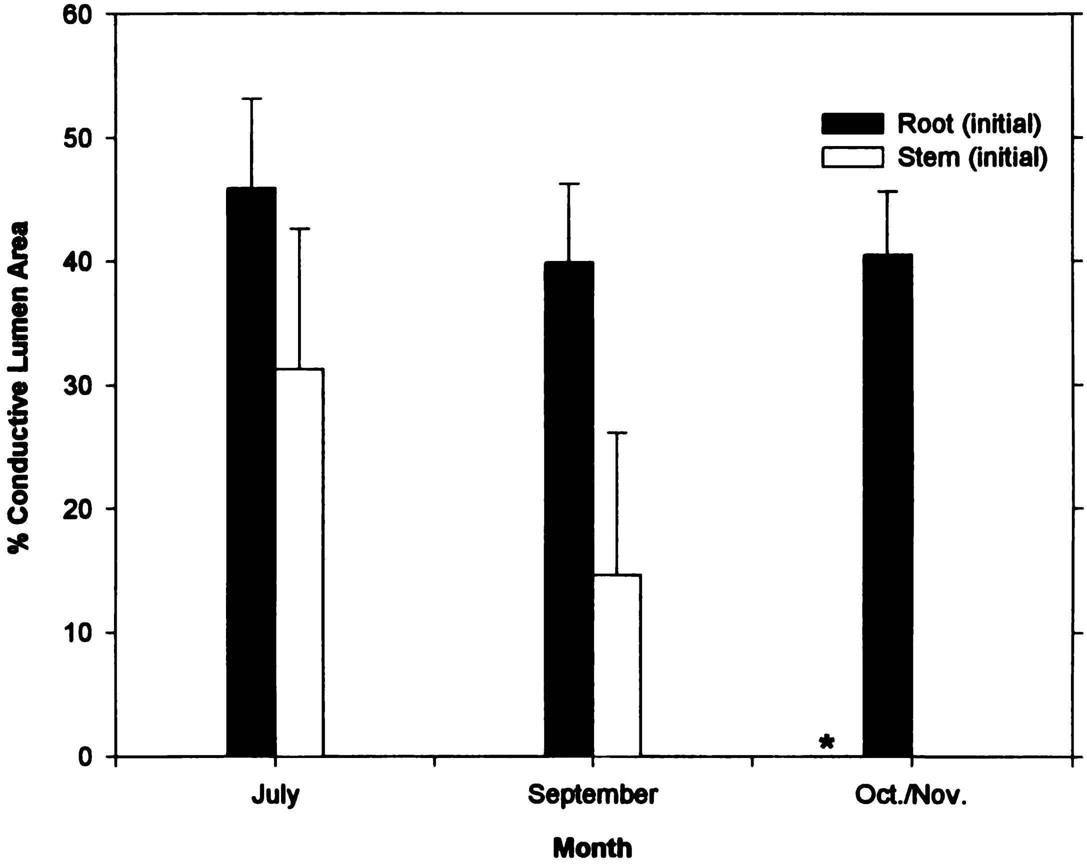

Figure 5: Percent conductive lumen area (± SE) for *Sassafras albidum* for 1997, before embolism removal (initial) (n = 16 for roots and 8 for stems; * stem value at zero).

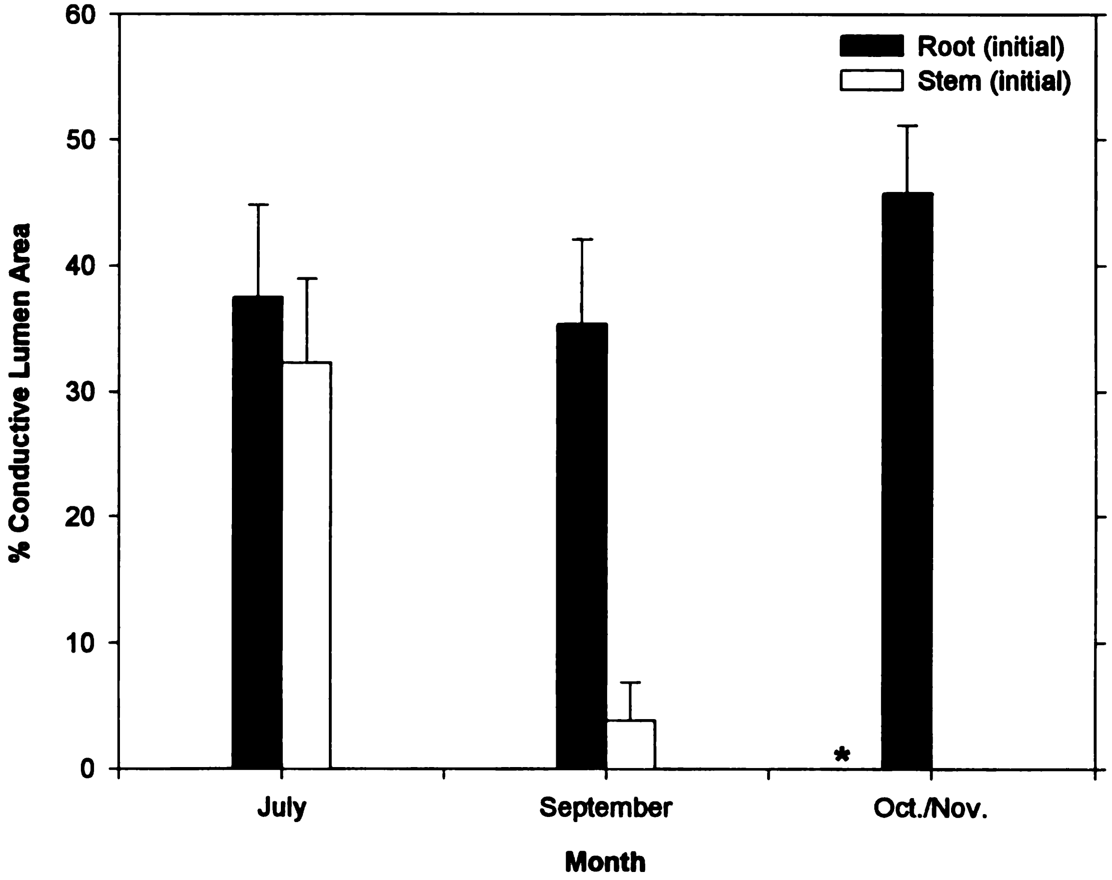

Figure 6: Percent conductive lumen area (± SE) for *Rhus typhina* for 1997, before embolism removal (initial) (n = 16 for roots and 8 for stems; *stem value at zero).

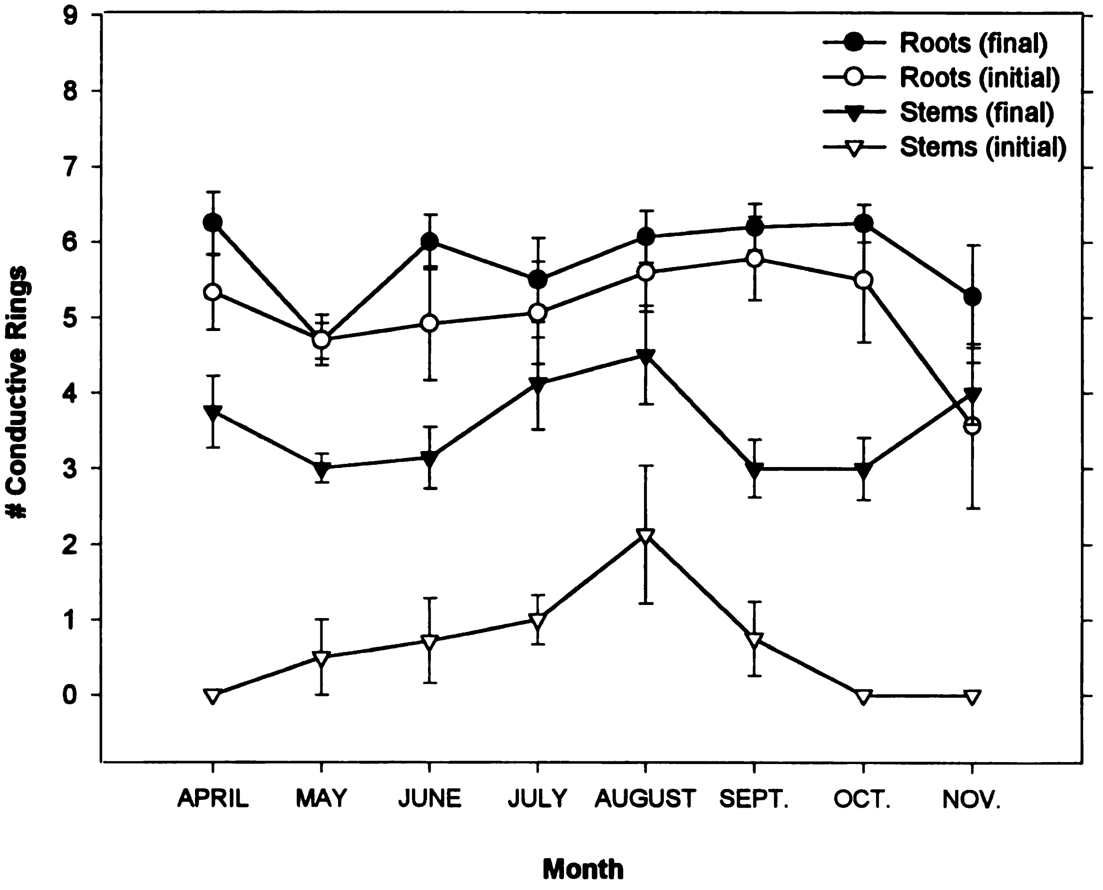

Figure 7: Seasonal change in the number of conductive rings (± SE) of *Sassafras albidum* for the 1997 growing season, before (initial) and after (final) removal of embolism (n = 16 for roots and 8 for stems; mean root age = 5.70 ± 0.27; mean stem age = 4.87 ± 0.28).

most embolisms could be removed in this species. Junctions followed the same seasonal trend as stems. In roots, removal of embolisms also resulted in a significantly greater number of conductive xylem growth rings (paired t-test, $P<0.01$). Roots of *Sassafras* had a significantly greater number of conductive rings than stems (paired t-test, $P<0.0005$).

Stems (initial) of *Rhus* had no conductive rings until June in 1997 (Figure 8). For June, July and August, stems (initial) had 1.0 ± 0.0 conductive rings and then dropped back down to zero again by October. In the roots (initial) of *Rhus*, the number of conductive rings did not show a seasonal pattern. The number of conductive rings was significantly greater in the roots (initial) than in the stems (initial) (paired t-test, $P<0.000005$).

Following laboratory removal of embolisms in April and May, 25% and 50%, respectively, of *Rhus* stems (final) showed conductive earlywood in the current year's xylem growth ring and some conductive latewood from the previous year's growth ring. Like the stems (initial), stems (final) had 1.0 ± 0.0 conductive rings for the months of June, July and August. By October, 75% of the stems (final) had one conductive xylem growth ring following embolism removal. In November, *Rhus* stems (final) had zero conductive xylem growth rings. Stems of *Rhus* never had more than one conductive xylem growth ring, which was always the current year's growth ring. Embolisms in previous years' xylem growth rings were usually irreversible by the technique used. The difference in number of conductive rings between stems (initial) and stems (final) of *Rhus* was not significant. Junctions followed the same seasonal trend as stems. Flushing did not have a significant effect in the roots, as there was no significant difference between

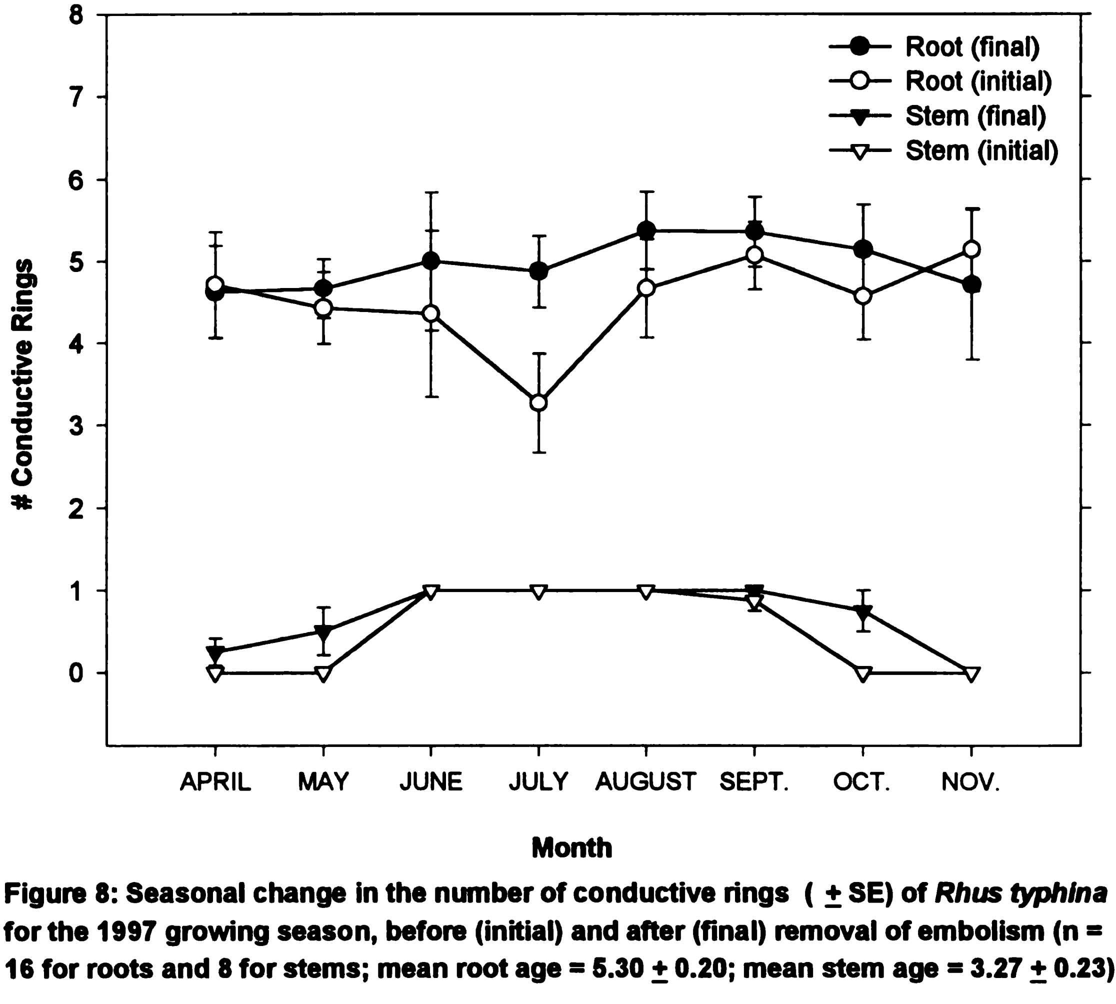

Figure 8: Seasonal change in the number of conductive rings (± SE) of *Rhus typhina* for the 1997 growing season, before (initial) and after (final) removal of embolism (n = 16 for roots and 8 for stems; mean root age = 5.30 ± 0.20; mean stem age = 3.27 ± 0.23).

roots (initial) and roots (final).

Comparing the two species, roots (final) of *Sassafras* had a significantly greater number of conductive rings than roots (final) of *Rhus* (unpaired t-test, P<0.005), but roots (initial) were not significantly different between species. Stems (final) of *Sassafras* had a significantly greater number of conductive rings than stems (final) of *Rhus* (unpaired t-test, P<0.00000005). Stems (initial) of both *Sassafras* and *Rhus* were not significantly different as they showed the same seasonal pattern.

Vessel Diameters

In *Sassfras*, the mean conductive vessel diameters of the stems and roots did not show a clear seasonal pattern in the difference between initial and final conditions (Figure 9). For stems of *Sassfras*, a significant difference between initial and final conditions was present in July (paired t-test, P<0.0005) and in October/November (paired t-test, P<0.0000005) there was a significant difference between conditions because there were no conductive vessels in stems (initial). The difference between initial and final conditions in stems sampled in September could not be tested because several individuals were already 100% embolized. There was no significant difference in the mean conductive vessel diameters of the initial versus the final conditions in the roots for the months of July and September (Figure 9), but there was a small difference between conditions in October/November (paired t-test, P<0.05). The mean conductive vessel diameters in *Sassafras* roots were significantly greater than in the stems (paired t-test, P<0.01).

In *Sassafras*, clear seasonal trends between initial and final conditions for maximum conductive vessel diameters were also not present (Figure 10). In the stems, a

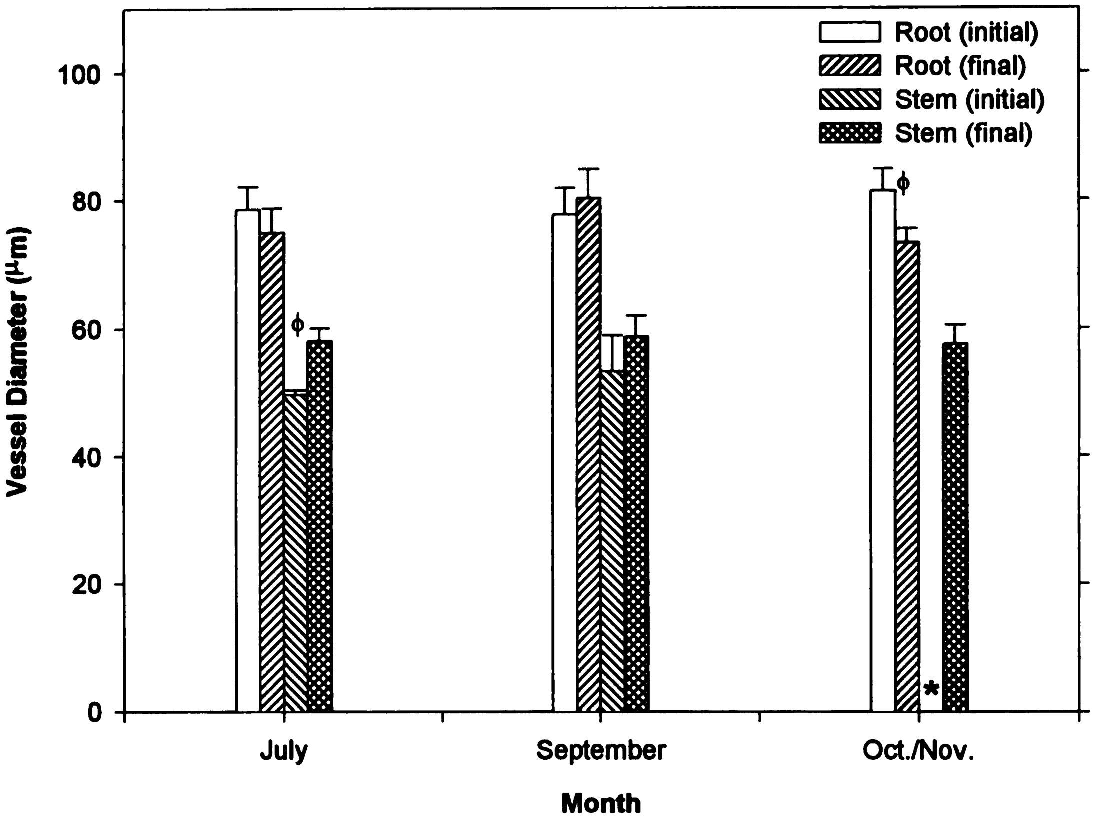

Figure 9: ***Sassafras albidum*** **mean conductive vessel diameters (± SE) for July, September, and October/November 1997, before (initial) and after (final) removal of embolism (n = 16 for roots and 8 for stems; * stem value at zero; ϕ indicates significance between initial and final at .05 level).**

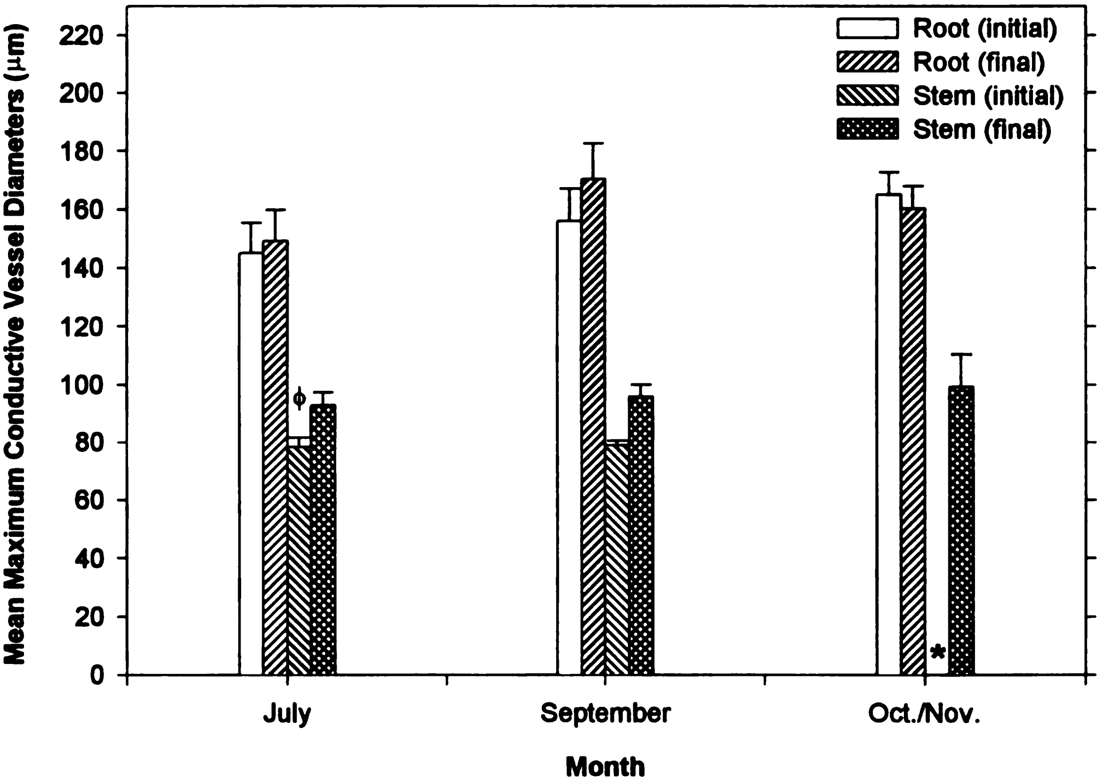

Figure 10: Maximum conductive vessel diameters (± SE) for *Sassafras albidum* for 1997, before (initial) and after (final) removal of embolism (n = 16 for roots and 8 for stems; * no stem value at zero; ϕ indicates significance between initial and final at .05 level).

significant difference was present between initial and final conditions in July and October/November. There were no significant differences between initial and final conditions of roots during any of the sampling periods. The roots, in both the initial and final conditions, had significantly greater maximum vessel diameters than the stems (paired t-test, $P<0.05$).

In *Rhus*, there was no significant difference in mean conductive vessel diameter between stems in initial and final conditions in July or in October/November when both initial and final conditions had zero conductive vessels (Figure 11). The difference between initial and final conditions in stems sampled in September could not be tested because several individuals were already 100% embolized. There were no significant differences in mean conductive vessel diameters between initial and final conditions in the roots. There were no significant differences in maximum vessel diameters between initial and final conditions for roots or stems of *Rhus* during July, September, or October/November (Figure 12). In October/November, both initial and final conditions for stems had no conductive vessels.

Comparing stems of the two species, the mean and maximum conductive vessel diameters were not significantly different in either the initial or the final conditions. In contrast, mean and maximum conductive vessel diameters of *Sassafras* roots, in both the initial and final conditions, were significantly greater than in *Rhus* roots (unpaired t-test, $P=<0.005$).

Macerations were done to quantify the proportion of vessel diameters below $25\mu m$ excluded from the image analysis results. In *Sassafras albidum* roots, 4% of

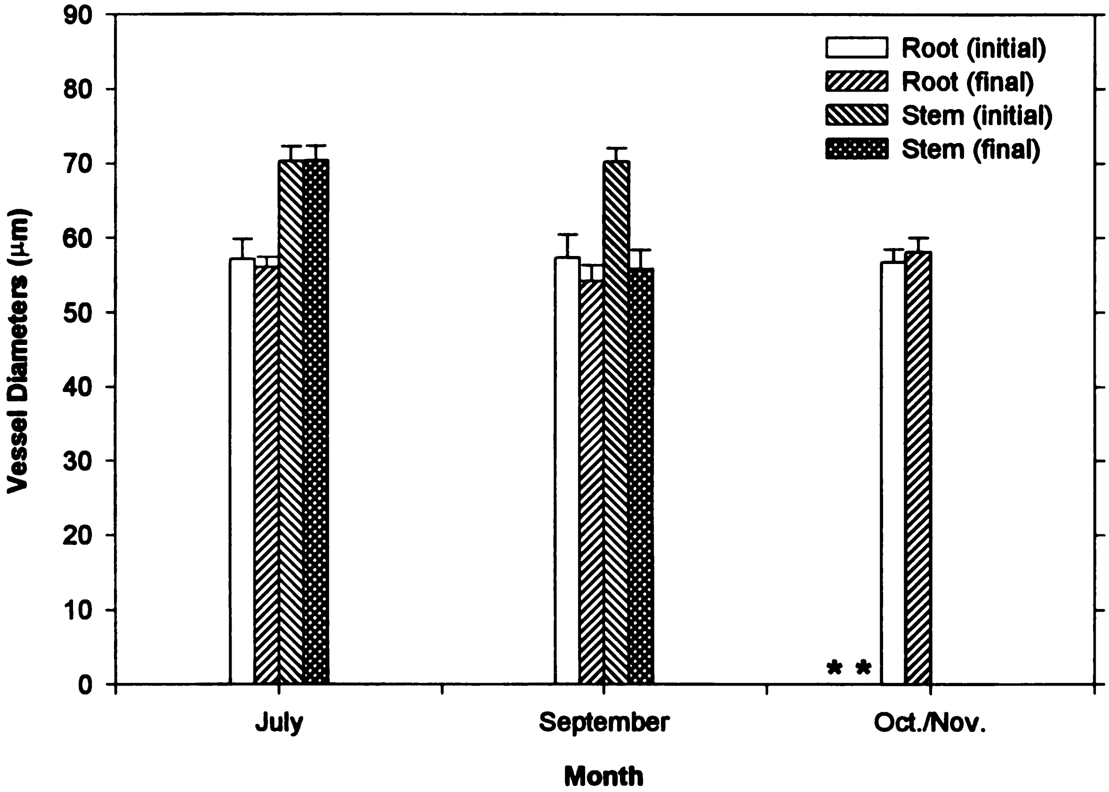

Figure 11: ***Rhus typhina*** **mean conductive vessel diameters (± SE) for July, September, and October/November for 1997, before (initial) and after (final) removal of embolism (n = 16 for roots and 8 stems; *stem value at zero).**

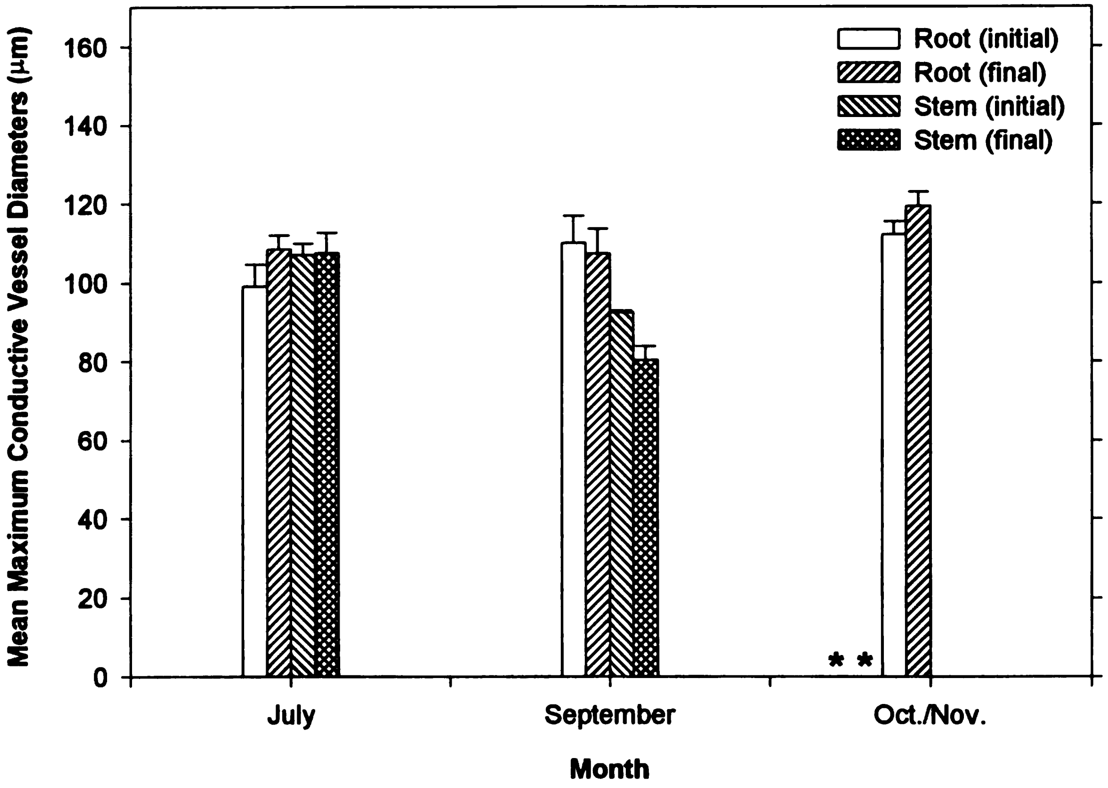

Figure 12: Maximum conductive vessel diameters (± SE) for *Rhus typhina* for 1997,before (initial) and after (final) removal of embolism (n = 16 for roots and 8 for stems; * stem value at zero).

vessels had diameters below 25µm. In the stems, 2% of vessel diameters measured were below 25µm. In *Rhus typhina,* the roots had 5% and the stems had 6% below 25µm. The proportion of the theoretical conductance excluded by disregarding vessels with diameters less than 25µm was less than .01 % for roots and stems of both species.

Root Pressures

Bubble manometers were attached to both species in early spring of 1996 and 1997 before bud burst or significant leaf expansion had occurred. Manometers were attached to both the horizontally spreading roots (12 *Rhus* and 3 *Sassafras*) near the junction and to stems (11 *Sassafras*) cut off just above the root stem junction. No positive pressures were detected in either species in either year.

Frost Depth

The depth of soil freezing was variable between sites and between years (Figure 13 and 14). The deepest freezing at a site in 1996 was 315mm, versus 242mm in 1997. For 1996 the mean maximum depth of frost was 204mm versus 149mm for 1997 (Table 2). Sampled *Sassafras* roots were deeper on average than the mean depth of frost for both years at 225mm and 299mm respectively (Figures 13 and 14). However, many sampled ramets of *Sassafras* were shallower than the mean depth of frost and could have froze (Figure 15). Sampled roots of *Rhus* were much shallower (71mm in 1996 and 57mm in 1997) than the mean depth of frost recorded for both years, thus in both years, all sampled roots of *Rhus* were in soil that had frozen (Figures 13 and 14).

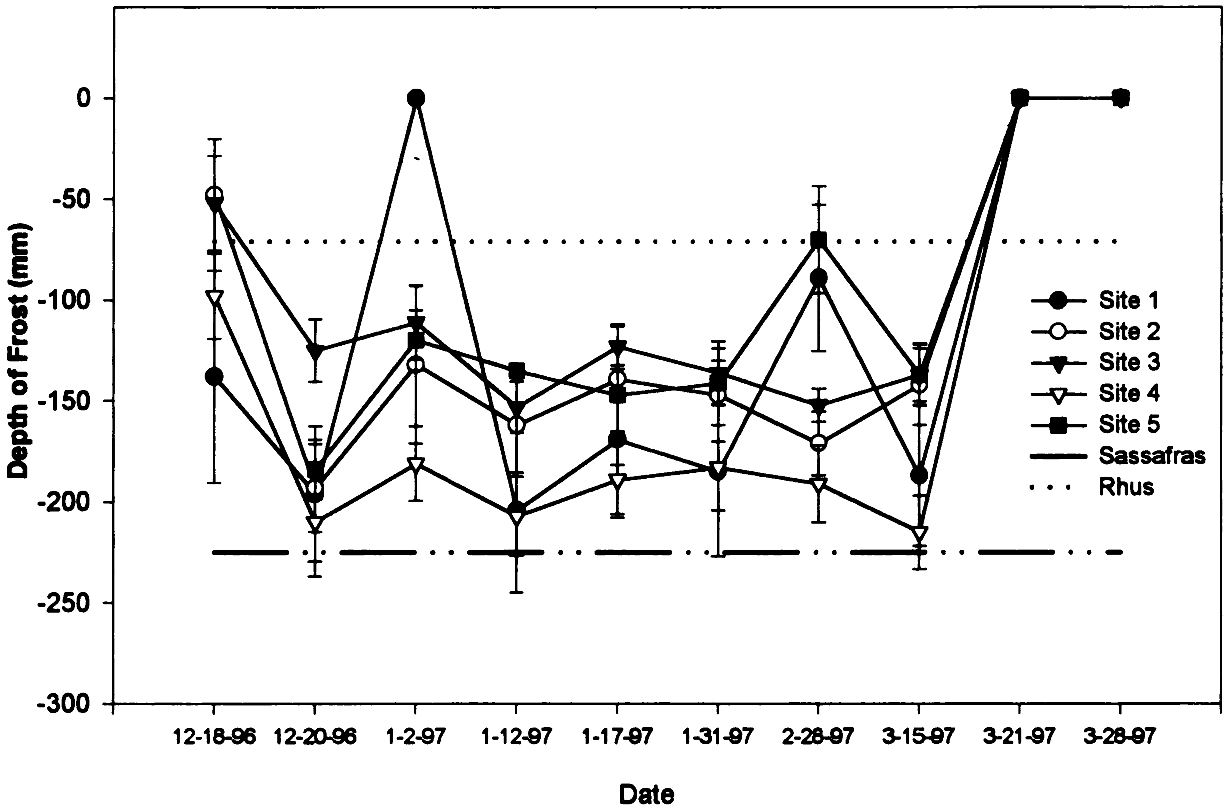

Figure 13: Mean frost depth (± SE) for each of five sites in 1996-97 (dotted lines indicate mean depth of sampled roots of *Sassafras albidum* and *Rhus typhina*).

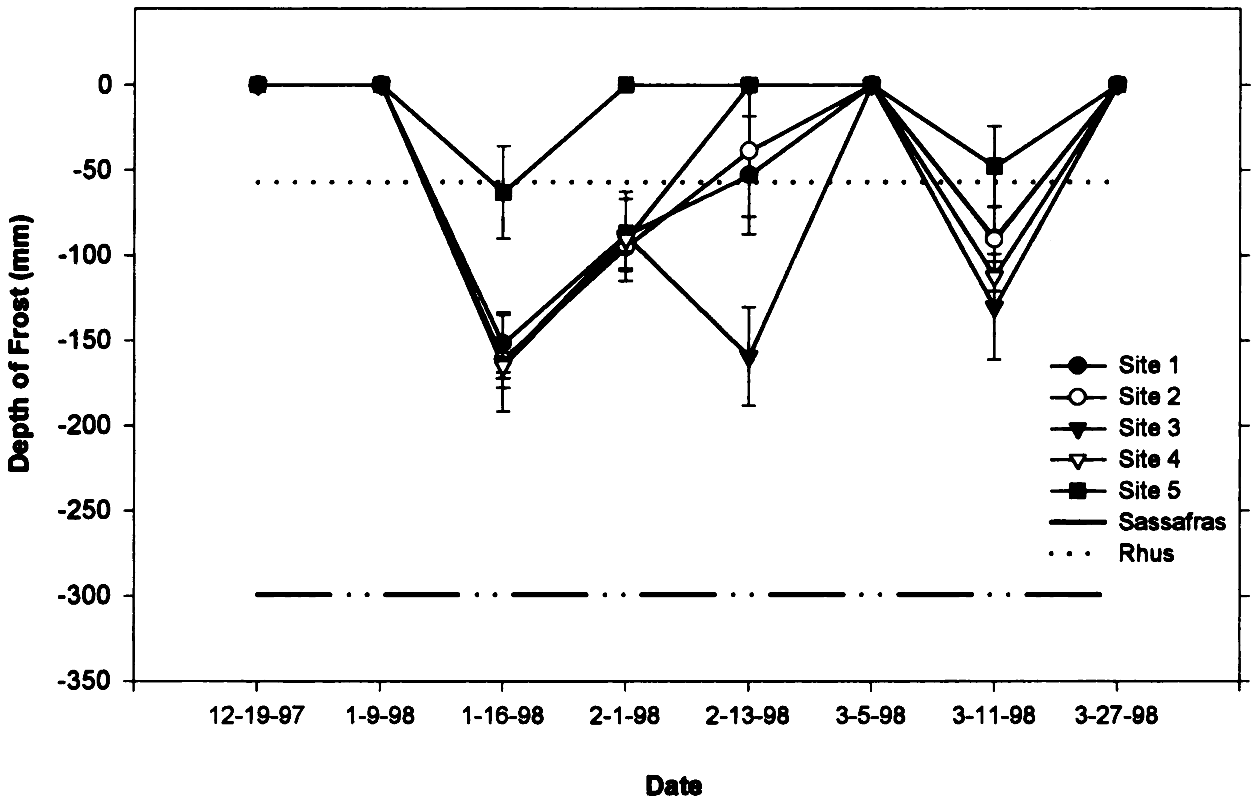

Figure 14: Mean frost depth (± SE) for each of five sites for 1997-98 (dotted lines indicate mean depth of sampled roots of *Sassafras albidum* and Rhus typhina).

Table 2: Comparison of mean maximum frost depth ± SE (n=number of frost gauges) to mean sampled root depth ± SE (n=number of root samples) for 1996 and 1997.

	1996		1997	
	X ± SE (range)	**n**	**X ± SE (range)**	**n**
frost depth	204mm ± 10.8 (125-315)	25	149mm ± 10.4 (51-242)	25
root depth:				
Sassafras albidum	225mm ± 16.9 (140-370)	31	299mm ± 35.1(100-690)	100
Rhus typhina	71mm ± 6.3 (40-120)	28	57mm ± 3.4 (30-110)	100

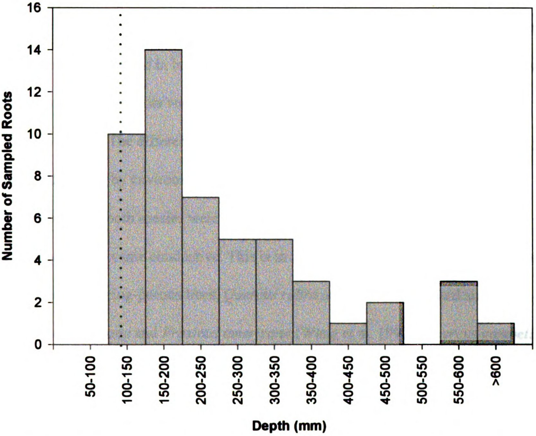

Figure 15: The number of sampled *Sassafras albidum* roots at different soil depths in 1997 (dotted line indicates maximum frost depth for winter 1996).

DISCUSSION

The seasonal patterns in hydraulic conductivity and embolism in the stems of *Sassafras* and *Rhus* are consistent with previous studies of temperate deciduous ring-porous tree stems, but the patterns in the roots are novel because such studies on roots versus stems have not been reported. Roots of both species were conductive throughout the entire monitoring period in both 1996 and 1997, with many conductive growth rings. Percent embolism in the roots was lower than in the junctions and stems of both *Sassafras* and *Rhus*. The difference in percent embolism of the roots between years may have been influenced by environmental conditions.

The stems of both species were completely embolized in the early spring before the new earlywood became conductive. This is in agreement with previous studies on the temperate deciduous ring-porous trees, *Quercus rubra* and *Q. alba* (Cochard and Tyree 1990), *Ulmus americana* and *Fraxinus americana* (Wang et al. 1992), *Quercus gambelii* (Sperry and Sullivan 1992; Sperry et al. 1994), and *Fraxinus excelsior* (Hacke and Sauter 1996). All of these ring-porous species reach high rates of embolism by late winter. In the present study *Sassafras* and *Rhus* stems were 100% embolized by November in both 1996 and 1997. This was before the minimum temperatures had dropped to -5°C in 1996 and 0°C in 1997.

Embolism in the stems of *Sassafras* was reversible by flushing even in growth rings that were several years old and apparently no longer conductive. In contrast, by November, embolism in the stems of *Rhus* could not be restored following embolism removal by flushing, suggesting permanent blockage had occurred following embolism. Blockage by tyloses has been studied in the stems of *Quercus rubra* and *Q. alba* (Cochard and Tyree 1990) and *Ulmus americana* (Ellmore and Ewers 1986). In these species tyloses do not block embolized earlywood vessels completely until the next growing season. However, in stems of black locust, *Robinia pseudoacacia*, tylose growth blocked the earlywood of the outer growth ring by December (Reichenbach, H. von. (Anonymous) 1846; Fujita et al. 1978; Zimmermann 1979).

Percent embolism values for *Sassafras* and *Rhus* were high even during the summer, and were never at 0%, perhaps because of the method used to measure maximum hydraulic conductivity ($K_{h\,max.}$) (Sperry et al. 1988). In *Sassafras* vessels from previous years' xylem growth rings, earlywood and latewood, are apparently not blocked by tyloses. Thus they contribute to the high maximum K_h values following removal of embolisms. It is unclear whether these vessels in nature may ever become conductive again.

In *Sassafras* stems, the new earlywood was visible before June in 1997, but based upon crystal violet dyes used in the isolated segments, was non-conductive until late June. Prior to the new earlywood of the stems becoming functional, embolism could be removed in nearly all xylem growth rings. In *Rhus* stems, the new earlywood was formed by late April in 1997, but remained non-conductive until June. Prior to June, the new earlywood and a few latewood vessels of the previous year's xylem growth ring did stain following removal of embolisms by flushing. Both species were consistent with the idea that cambial activity begins well before leafing out in temperate deciduous ring-porous trees (Wang et al. 1992).

Studies such as those of Lechowicz (1984) and Wang et al. (1992) on the timing of foliar phenology in temperate deciduous trees assert that there is a functional linkage between foliar phenology and the loss of hydraulic conductivity due to freezing-induced embolism. A strong correlation exists between timing of spring bud burst and fall leaf drop and high rates of embolism in the winter. Thus ring-porous trees, which have the largest diameter xylem conduits and are the most vulnerable to freezing-induced embolism, leaf out significantly later than diffuse-porous trees at the same location (Wang et al., 1992). The present study is consistent with dates of bud burst previously recorded for *Sassafras* and *Rhus*. These studies indicate that both species do leaf out later than diffuse-porous trees at the same location (McGee 1986; Lechowicz 1995). Perhaps by leafing out later, ring-porous trees are more likely to avoid late spring frosts. The advantage which is incurred from later bud burst is very important for ring-porous trees, which generally rely on the current year's xylem growth ring for supplying water to the leaves. The later bud burst is associated with later initiation of cambial activity. The new xylem in ring-porous trees is thus generally not mature until danger of frost is past. The advantage of earlier leaf drop in the autumn seems less clear, because most ring-porous temperate deciduous trees become fully embolized following winter freeze. However, perhaps this early leaf drop allows better salvaging of resources from senescent leaves before the first solid freeze.

Specific conductivity (K_s), which is the hydraulic conductivity per unit pressure gradient divided by sapwood cross-sectional area, is a measure of the porosity of the stem segment (Ewers 1985; Tyree and Ewers 1991). Values for K_s reported in this study were calculated using total xylem cross sectional area, minus the pith, rather than sapwood area. I used total xylem area since the sapwood area of the stems was often very narrow and difficult to measure accurately. Thus, the $K_{s\ max.}$ values reported are lower than they would have been if they had been calculated using sapwood area.

For both species, unlike for stems and junctions, for roots there was very little variation between the overall $K_{s\ max.}$ values in 1996 versus 1997. Specific conductivity values for stems and junctions of ring-porous trees vary more from year to year compared to roots. This is because the K_s values for stems and junctions are based on the width of the current year's xylem growth ring, which is influenced by precipitation levels and extremes in temperature (Fritts 1976). Years which have lower than average precipitation rates or lower than average temperatures, result in growth rings which are narrower than those of average years (Fritts 1976).

Stems of ring-porous trees have distinct earlywood and latewood regions. Earlywood vessels are produced in the early spring at the beginning of the growth ring, and tend to have wider diameter vessels than latewood vessels, which are narrower in diameter and found towards the end of the growth ring. Latewood vessels, which have lower K_s values due to narrower diameters, are generally believed to have longer conductive spans than wider earlywood vessels (Ellmore and Ewers 1986; Salleo and LoGullo 1986; Gartner 1995). In Salleo and LoGullo's (1986) study, narrower diameter conduits experienced less cavitation than adjacent wider diameter conduits and thus had a longer conductive span. Similarly, Ellmore and Ewers (1986) reported that the latewood vessels of *Ulmus americana* remain conductive for up to six years, versus one season for the earlywood vessels. This pattern of latewood vessels remaining conductive for many years while earlywood vessels are conductive for only one year has also been observed in other ring-porous tree genera such as *Castanea* (Zimmermann 1983), *Quercus* (Cochard and Tyree 1990), and *Fraxinus* (Ewers and Cruiziat 1991).

That the latewood of the xylem growth rings of *Rhus*, and generally *Sassafras* as well, does not remain conductive for more than one growing season, as observed in other ring-porous species, is remarkable. The latewood of *Sassafras* and *Rhus* became embolized by November in both 1996 and 1997. In *Rhus* all but 25% of earlywood vessels in sampled stems became irreversibly embolized by October, while embolism in most latewood vessels was still reversible by flushing. All earlywood and nearly all latewood vessels in the stems and junctions of *Rhus* were irreversibly embolized by November in both 1996 and 1997. Embolism could only be removed in less than 20 vessels/stem in the latewood of *Rhus* and thus were not counted as a conductive ring. However it is interesting to note that such vessels, although small in number, generally did not become permanently blocked until the late spring of the following year as the new earlywood became conductive.

It is an open question as to why even narrow vessels of *Sassafras* and *Rhus* stems have such a short conductive span. The mean and maximum conductive vessel diameter data for roots and stems of both species suggest that wider vessels were not necessarily more prone to embolism than narrow vessels. This is contrary to other studies, which suggest that the narrow diameter latewood vessels have longer conductive spans than the wider diameter earlywood vessels (Zimmermann 1983; Ellmore and Ewers 1986; Cochard and Tyree 1990). Perhaps the freezing process damages their pit membranes in winter.

The pattern of higher K_s values for roots versus stems of *Sassafras* and *Rhus* are consistent with patterns seen in the three Australian shrubs, *Banksia prionotes, Banksia ilicifolia,* and *Adenanthos cygnorum* (Pate et al. 1995). The greater difference between $K_{s\ max.}$ values of roots versus stems of *Sassafras*, as compared to *Rhus*, may be due in part to the greater difference in conductive vessel diameters of roots versus stems of *Sassafras.* Although there was not much intraspecific variation for overall $K_{s\ max.}$ means of roots between years, there was considerable intergeneric variation between the roots of the two species. This may be explained, at least partially by comparing conductive vessel diameters of the roots of the two species. *Sassafras* had significantly larger mean and maximum conductive vessel diameters than *Rhus.* Specific conductivity is known to increase as the mean conduit diameter increases (Tyree and Ewers 1991). The number of vessels per cross-sectional area may also influence the intergeneric difference in $K_{s\ max.}$ values. The different rates of K_s between roots versus the stems of the two species reflect the variation in mean and maximum vessel diameter and the number of conductive rings.

Sassafras stem segments with embolisms removed had a significantly greater number of conductive rings than stems prior to embolism removal. This indicated that many of the embolisms in the stems were reversible. Embolism in the stems of *Sassafras* could be reversed in the early spring and late fall even when stems (initial) were 100% embolized, suggesting the vessels remain free of blockage by gums, resins, or tyolses for several years even though they may be non-conductive. The stems of *Rhus* never had greater than one conductive growth ring even following embolism removal. The current year's growth became irreversibly embolized by November. Stems did not become conductive in the spring until new earlywood became functional.

Stems of both species with embolisms removed by flushing showed a similar pattern in the number of conductive rings. The number of conductive rings increased from zero in early spring, increasing to exactly one conductive ring for *Rhus* and one ring on average for *Sassafras*. The number of conductive rings dropped back down to zero again by November in both species. The number of conductive rings in stems in the native state of *Sassafras* were more variable than in *Rhus*, and ranged from all xylem growth rings conductive to the more common condition with only the current year's growth ring conductive. Thus both *Sassafras* and *Rhus* stems appear to rely on the formation of new xylem growth rings in order to overcome winter embolism. This mechanism for recovery from winter embolism is consistent with previous studies of ring-porous stems.

Roots of both species had more conductive xylem growth rings than stems. Following embolism removal, all the growth rings in roots were conductive, and even prior to embolism removal, most if not all of the growth rings were conductive. The growth rings in the roots remain conductive for many years and do not show a dramatic seasonal pattern. Older growth rings in the roots do not become completely embolized as they do in the stems. Embolism in the roots may increase later in the winter as depth of frost increases and soil temperatures drop. Unfortunately, the difficulty of obtaining "natural" samples from the frozen ground prevented sampling throughout the winter.

The percent conductive lumen area in the stems of both species did suggest a seasonal pattern. The percent conductive lumen area was greatest in July and was at zero for both species by October/November. The decrease in percent conductive lumen area from July to September for both species was due to increased overall embolism, with some sampled individuals 100% embolized by September. The overall decrease in %

conductive lumen area seasonally may have been a result of water stress-induced embolism, or embolism from other causes such as pathogens, but I did not determine this.

Most studies on susceptibility of xylem conduits to water-stress-induced-embolism have found no interspecific relation between susceptibility and conduit diameter (Alder et al. 1996; Sperry and Saliendra 1994; Sperry et al. 1995). However several studies found that within a plant the wider diameter vessels were more susceptible to water-stress-induced embolism than narrow diameter vessels (Hargrave at al. 1994; Sperry and Tyree 1988; Salleo and LoGullo 1986, 1989; LoGullo and Salleo 1991). The susceptibility of the wider diameter vessels was most likely due to the connection between conduit diameter and the number and size of pit pores within an individual (Sperry and Tyree 1988; Jarbeau et al. 1995). In addition, the age of vessels (i.e. vessels in different growth rings and vessels produced at different times within one growth ring) may also affect their vulnerability to embolism (Sperry, Perry, and Sullivan 1991). It is the degradation of the pit membranes, which causes air-seeding resulting in embolism. This may have contributed to the decrease in percent conductive lumen area from July to September in 1997 in both *Sassafras* and *Rhus*.

Percent conductive lumen area in the roots was always higher than in the stems. The percent conductive lumen area for roots of *Sassafras* and *Rhus* did not vary considerably from July through November in 1997. When put into perspective with the number of conductive growth rings in the roots, it appears that conductive lumens in the roots, although never above 50%, were scattered throughout all the growth rings rather than localized in the current year's growth ring as they are in the stems.

Frost depth in soil is highly variable. A strong relationship exists between frost depth and snow cover, deeper snow results in shallower frost penetration (Saul and Potts 1986; Isard and Schaetzl 1995). Other factors that are recognized in their influence on depth of frost are canopy cover by evergreen trees (Hardy and Albert 1995), elevation (Saul and Potts 1986), and litter depth (MacKinney 1929; Sartz 1973). Due to the difficulty in predicting frost depth because of the many factors that influence it, frost gauges were used to measure frost depth around monitored *Sassafras* and *Rhus* clones in the winters of 1996-97 and 1997-98.

The soil surrounding the parent roots of all sampled roots of *Rhus* and a significant portion of sampled *Sassafras* roots froze, but whether the root xylem themselves froze is unknown. Perhaps the roots of both species employ some mechanism for avoiding freezing. It is also possible that more embolism would occur at sufficiently low subfreezing soil temperatures, as in the stems of *Larrea tridentata* (Langan et al. 1997). Further research might investigate the xylem root temperatures throughout the winter in order to determine whether the xylem sap, like the soil surrounding the roots freezes.

The roots of both species never became 100% embolized during the 1996 and 1997 monitoring periods. Since sampling was not carried out between November and April of both years, due to the difficulty of sampling in frozen ground, it is unknown whether the roots remained conductive or became fully embolized. No positive root pressures were detected in the roots of either species during the time monitored by bubble manometers in either year. It is possible that positive root pressures occurred prior to the time when root pressures were monitored or that they did not occur at all. If freezing of the roots did occur, as frost depth indicated they may have, then roots apparently reversed embolism by a mechanism other than positive root pressures. Salleo et. al. (1996) observed reversal of embolism in one-year-old stems of *Laurus nobilis* with no positive root pressures associated. The authors suggest that the reversal of embolism occurs by a phloem-mediated embolism reversal mechanism caused by an increase in phloem loading of solutes triggered by the plant growth regulator, Indol-3-Acetic Acid (IAA). Although this mechanism has been suggested to reverse embolism diurnally rather than seasonally, the possibility of embolism reversal in the roots of both species and the stems of *Sassafras*, a relative of *Laurus*, should be investigated.

In roots of plants in general, perhaps neither positive root pressures nor phloem loading are needed to reverse embolisms. When xylem pressures are at atmospheric or only very slightly negative, given sufficient time, embolisms will dissolve according to Henry's Law (Tyree and Yang 1992; Ewers et al. 1997). Such pressure conditions may occur when the soil is saturated with water, such as in the early spring in Michigan or following heavy rainfall throughout the year.

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