The impact of subcanopy light environment on the hydraulic vulnerability of *Rhododendron maximum* to freeze–thaw cycles and drought

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ABSTRACT

The vulnerability of xylem to embolism development in *Rhododendron maximum* L., an evergreen diffuse-porous shrub, was investigated in relation to the frequency of winter freeze–thaw cycles in high and low light sites of the Eastern US. Though the frequency of freeze–thaw cycles during the winter was lower in North Carolina than in Virginia, the hydraulic conductivity of 3-year-old branches was reduced by up to 60% by winter embolism development in North Carolina compared to less than 30% in Virginia. Generally, small vessel diameters and volumes were associated with a significant resistance to embolism formation resulting from repeated freeze–thaws of xylem sap. In stems grown in high light sites (gaps), larger vessel volumes, and greater diameter growth of stems were associated with a significantly higher degree of freeze–thaw embolism development than in those grown in the low light sites. Thus, the growth patterns of *R. maximum* stems, under conditions of higher light availability, rendered them more susceptible to freeze–thaw-induced embolisms. Vulnerability to drought-induced embolism in stems was not affected by light environment. *Rhododendron maximum* was relatively sensitive to drought-induced embolism because 50% loss of hydraulic conductivity occurred at a water potential of −2·2 MPa. The distribution and gas exchange of *R. maximum* are constrained by the dual effects of freeze–thaw cycles and drought on vascular function.

Key-words: *Rhododendron maximum*; freeze–thaw cycles; hydraulic conductivity; leaf phenology, light environment; understorey shrub; water relations.

INTRODUCTION

Embolisms in the xylem of woody plants, which disrupt water flow and reduce hydraulic conductivity, may be induced by drought, excessive transpiration, or winter cycles of freezing and thawing of xylem water (Tyree & Sperry 1989). During freezing of the xylem, air bubbles are

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produced as air diffuses out of the xylem sap. During thaw, these bubbles may nucleate in the lumen of xylem conduits to create embolisms (Sucoff 1969). A correlation has been observed between conduit volume and the incidence of freeze–thaw-induced embolism development (Sperry & Sullivan 1992). Small-volume conifer tracheids ($< 1 \times 10^{-12}$ m^3), with diameters between 5 and 15 μm, appear resistant to embolism formation during freezing (Sperry & Sullivan 1992; Robson & Petty 1993). However, deciduous temperate trees and vines, with larger vessel volumes (e.g. 7×10^{-11} m^3 in *Betula* and $2·8 \times 10^{-9}$ m^3 in *Quercus*; Sperry & Sullivan 1992) and vessel diameters often greater than 30 μm, are vulnerable to winter embolism. For example, *Acer sacchrum* (Sperry *et al.* 1988), *Vitis* (Sperry *et al.* 1987) and *Betula* (Sperry 1993) can lose around 70–80% of their hydraulic conductivity after a winter of recurring freeze–thaw cycles. Also, the number of freeze–thaw cycles can influence the degree of embolism development over the winter (Lo Gullo & Salleo 1993; Sperry *et al.* 1994). However, in conifers, there is no correlation between the number of wintertime freeze–thaw cycles and embolism development. Rather, the occurrence of embolisms in conifers has been related to cavitation of the water column induced by transpiration during a time of limited water availability in cold soils (Sperry 1993; Sperry *et al.* 1994).

Few broad-leaved evergreen angiosperms have been investigated for susceptibility to freeze–thaw-induced embolisms. In environments subject to seasonal freeze–thaws, Sperry (1995) has predicted that evergreen broad-leaved species should be characterized by restricted vessel sizes. Wintertime gas exchange and other water flow-dependent physiological processes of evergreen plants necessitate adaptations that reduce the potential loss of xylem function due to freeze–thaw-induced embolisms. In addition, the light environment may induce xylem structural characteristics that increase vulnerability to freeze–thaw-induced embolism. In high light sites (gaps in the forest canopy) understorey plants tend to produce larger and longer stems than understorey plants in low light sites (Nilsen 1986). The larger annual stem growth in high light sites is likely to contain vessels of greater volume than wood produced by plants in low light sites. Therefore,

1264

understorey evergreen plants growing in gaps may have higher vulnerability to freeze–thaw-induced embolism than those growing under a closed forest canopy.

The most abundant understorey evergreen plant in the eastern United States is *Rhododendron maximum* L. This species is dominant in many deciduous forests from Georgia to Canada, especially along the eastern United States mountain ranges. *R. maximum* populations occur along stream boundaries or on north slopes at between 70 and 1800 m elevation, where they are likely to be subject to frequent wintertime freeze–thaws. Throughout its range, *R. maximum* grows in gaps and under closed forest canopies. There is clear evidence that *R. maximum* inhibits the growth of other forest canopy species seedlings, which reduces forest regeneration (Clinton & Vose 1996). Furthermore, the extent of *R. maximum* in the temperate forest of the eastern United States may be increasing. Due to the expanding influence of *R. maximum* on forest dynamics there is interest in understanding what factors regulate the distribution and survivorship of this species.

Two factors may be important for regulating the distribution and growth of *R. maximum*. The preferred habitat of *R. maximum* (stream sides and north slopes) suggests that water availability is important to plant survival. The frequency of freeze–thaw cycles may also be important to survival because of the climate (frequent wintertime freeze thaws) and because of the growth phenology of this species. Late bud-burst in *R. maximum* may be a consequence of wintertime embolism development in the xylem. New growth in early spring may be prevented until most vessels are refilled (Wang *et al.* 1992). The influence of both drought and freeze–thaw cycles on habitat selection and plant growth could be a result of the interaction between these climatic factors and vascular structure (Langen *et al.* 1997).

The objective of this study was to investigate the vulnerability of an evergreen diffuse-porous understorey shrub (*R. maximum*) to winter freeze–thaw- and drought-induced embolism when growing in gaps or under a closed canopy. We selected study sites in Virginia and in North Carolina where *R. maximum* is common and periods of nighttime freezing and daytime thawing occur throughout the winter. We posed the following hypotheses: (1) *R. maximum* will have vessel characteristics contributing to a low susceptibility to freeze–thaw-induced embolisms and a high vulnerability to drought-induced embolism; (2) plants growing in high light sites will have a greater susceptibility to freeze–thaw-induced embolism; (3) transpiration during thaws in midwinter will increase the potential for water stress-induced embolism formation; (4) bud-burst is delayed in the spring because of extensive wintertime embolism.

MATERIALS AND METHODS

Site characteristics

Four research sites, differing in the frequency of winter freeze–thaw cycles, were selected to study the effect of freeze–thaw cycles on hydraulic conductivity in *R. maximum*. One research area was located in the Jefferson National Forest, near Blacksburg, Virginia, and included two sites at different elevations; Poverty Creek at 685 m (37°15'37" N lat., 80°28'39" W long.) and Mountain Lake at 1198 m (37°21'50" N lat., 80°32'20" W long.). The other research area was at the Coweeta Hydrologic Laboratory, near Otto, North Carolina, where two sites at different elevations were selected; Grady Creek at 730 m (35°03'03" N lat., 83°26'18" W long.) and Stewart Trail at 1172 m (35°02'16" N lat., 83°27'44" W long.). The regional climate for the sites located in Virginia is classified as temperate and humid, with cool summers and winters, and adequate rainfall during all seasons. In North Carolina, the regional climate is classified as marine and humid, with cool summers, mild winters, and adequate rainfall during all seasons. At all sites, deciduous oak (*Quercus* sp.) and maple (*Acer* sp.) dominate the canopy and there is an abundant evergreen understorey component comprised of *R. maximum*.

Maximum and minimum daily air temperatures (monitored continually at climate recording stations at or near each site) and the freezing temperature of the stem tissue (determined monthly) were used to estimate the number of freeze–thaw cycles occurring during the winter. From previous climate records (1980–1995), an average of 54 freeze–thaw events (ranging from 28 to 76 events per year) have occurred between October and April at 685 m elevation at the Coweeta Hydrologic Laboratory. For the same period and season, an average of 66 freeze–thaw events (ranging from 49 to 77 d of freeze–thaws per year) have occurred near Poverty Creek, VA. The number of freeze–thaw events during the year in which this study was conducted was 30% lower than the yearly average but was in the normal range.

Species description

Rhododendron maximum L. (Ericaceae) is a large understorey diffuse-porous shrub distributed throughout the mountain regions of the eastern United States. At the Virginia study sites, *R. maximum* shrubs are commonly 2 m in height, while at the North Carolina sites, they are around 5 m in height. Growth is determinate and each year's shoot growth is characterized by a whorl of leaves. Internodal length ranges from 3·9 cm (under low light conditions) to about 15 cm (under high light conditions) (Nilsen 1986). At each research site, plants were selected for study from two relative light environments: gaps (high light) and under canopy (low light). The light environment was not quantified.

Field measurements of air, leaf and stem temperatures

In order to verify that tissues froze in the field, air, leaf and stem temperatures at all sites and in all light environments were continually monitored over periods of several days,

every second month during the winter (November to April). Tissue temperature was measured with copper-constantan thermocouples (36 gauge) at 20 s intervals. Air temperature was measured at 0·5 m above the ground. Leaf and stem temperatures were monitored by placing thermocouples directly against the tissues (abaxial side of the leaf) without rupturing the surface tissues. Temperatures were recorded with a datalogger (CR-21X, Campbell Scientific, Logan, UT).

Tissue freezing temperature

Once a month, between November and April, whole branches were collected, sealed in plastic bags and transported quickly to a laboratory for determination of tissue freezing temperature. Copper-constantan thermocouples were placed in contact with the leaves and stem without piercing the plant tissue. The branches were placed inside an insulated Styrofoam chamber between two heat exchangers through which 45 volumes water/55 volumes ethylene-glycol was circulated. The temperature of the coolant was adjusted using a refrigerated bath (RTE-140 with programmable temperature controller; Neslab, Newington, NH). The temperature of the chamber was lowered at a rate of 3 °C h^{-1}, from ambient to −12 °C, similar to natural rates of cooling observed in stems of *R. maximum* in the field. The temperatures of the chamber and the plant tissue were continuously recorded, at 20 s intervals, using thermocouples connected to a datalogger (Campbell CR-21X, Campbell Scientific, Logan, UT). The first freezing exotherm was indicated by a transient exothermic event marked by an increase in temperature. We utilize the first exotherm because this represents freezing of xylem water which is part of the apoplastic water fraction (Burke *et al.* 1976; Calkins & Swanson 1990).

Measurement of embolism

The development of xylem embolisms during the winter months, when the xylem sap was subjected to repeated freezing and thawing under natural conditions, was measured as a reduction from maximum hydraulic conductivity. Hydraulic conductivity was defined as the mass flow rate of solution through an excised stem segment per unit pressure gradient and was measured using the methods described in Sperry *et al.* (1988). Monthly throughout the winter, three different shrubs in each light environment (from gaps and from under canopy) had branches removed for determination of the presence of embolisms. Two- to three-year-old stem segments were excised from these large branches. These stem segments were attached to an apparatus where the initial hydraulic conductivity was determined for each segment by flowing a solution of 10 mmol dm^{-3} oxalic acid through the segment using low pressure (11 kPa). Then, after a high-pressure (150 kPa) flush was used to remove any air bubbles in embolized conduits, the maximum hydraulic conductivity of each segment was determined. The degree of embolism development and resultant loss of conductivity was calculated as the percentage that the initial hydraulic conductivity was reduced from the maximum hydraulic conductivity. Branches collected were much longer (at least 1·5 m) than the stem segments that were excised later for hydraulic conductivity measurements. Excised stem segments for conductivity measurements were between 0·3 and 0·8 cm in diameter and about 5–10 cm long. These excised stem segments were cut from larger branches under water to avoid introducing new air embolisms.

Vessel volume

Thin cross-sections of 3-year-old *R. maximum* stems from five individuals per site/light environment were prepared using a sliding microtome, then mounted and dyed with safranin. For each stem cross-section, the diameters of all vessels in a radial sector between a randomly selected ray and the next closest ray, from the vascular cambium to the pith, were measured using a light microscope fitted with a micrometer (Ewers *et al.* 1990). The diameter of each vessel was taken between the interior walls in two directions, 180° from each other, to calculate the average diameter of each vessel. Vessel diameter frequency distribution (based on measurements of over 400 vessels for each site/light environment) and average number of vessels per stem cross-sectional area were determined. The estimated percentage of total conductance contributed by each diameter class was calculated (Zimmerman 1983) as the percentage contribution of each diameter class to the sum of the fourth power of the radius of all conduits. Vessel length was measured following the techniques of Zimmerman & Jeje (1981). Intact branches of at least 1·5 m in length were collected from five plants growing in high and low light conditions at each site. The base of each stem was cut under water. A paint solution was prepared as described by Zimmerman & Jeje (1981) and perfused into the base of the stems for 3 d. Thin sections of the stems were made at 1 cm distance increments until 5 cm past the point where no paint-filled vessels could be found. Vessel length distribution was calculated as in Zimmerman & Jeje (1981). Vessel volumes were calculated based on the assumption that vessel diameter is randomly associated with vessel length. This is a conservative estimate of vessel volume distribution (Zimmerman & Potter 1982) and has been used in studies of other species (Pockman & Sperry 1997). The total xylem volume (in vessels) of 3-year-old stems was calculated by multiplying the average vessel volume, times the average number of vessels per stem cross-sectional area, times the average cross-sectional area of xylem.

Physiological measurements

One diurnal cycle of stomatal conductance and transpiration was measured every second month at each site with a steady-state porometer (Model 1600: Licor Inc., Lincoln, NE) during the winter study period on *R. maximum* leaves. Leaf water potential was measured concurrently using a

PMS pressure bomb (Model T1000: PMS instruments, Corvallis, OR). Stomatal conductance was correlated to air temperature, and a polynomial regression was developed to provide an indication of the days during the winter when there was potential stomatal activity, based on midday maximum air temperatures at each site.

Vulnerability curves

A vulnerability curve, which provides information on the relationship between declining xylem water potential and the resulting reduction in the hydraulic conductivity of stems, was developed using the dehydration technique (Sperry & Sullivan 1992; Sperry & Saliendra 1994) on 3-year-old *R. maximum* stems. The vulnerability curve was determined by dehydrating replicate shoots from five to six individuals from each site/light condition and formulating the relationship between water potential and hydraulic conductivity.

RESULTS

Freeze–thaw cycles

There were a greater number of daily freeze–thaw cycles in the Jefferson National Forest during the study period than at Coweeta (Fig. 1a). The higher elevation sites at both research areas were subject to a greater number of freeze–thaw days than the lower elevation sites. Freezing exotherms were observed in *R. maximum* leaves and stems frequently throughout the winter (Fig. 1b). Diurnal changes in air temperature of 15–20 °C were commonly observed at all sites/light environments, and, as the temperature of *R. maximum* leaves and stems remained close to ambient air temperature, frequent freezing and thawing of plant tissues occurred during the winter. About 80% of the total number of occurrences of nighttime tissue freezing were estimated to be subject to daytime thaws, for both regions, over the study period.

The freezing temperature of stem tissue changed throughout the winter, decreasing from around –2·5 °C in late autumn to below –4 °C at mid-winter for both research areas (Fig. 2). At Coweeta, tissue freezing temperature followed similar trends at both the high and low elevation sites. However, in the Jefferson National Forest region, the freezing temperature of *R. maximum* stems at Poverty Creek were the lowest of all sites at mid-winter.

Vessel size

Vessel diameters of *R. maximum* ranged from around 7 to 45 μm for all sites (Fig. 3a). The median vessel diameter for all sites/light environments taken together was 20 μm. Vessels less than 10 μm in diameter were distinguishable from fibres only by their more irregular shape. *Rhododendron maximum* growing in gaps had an increased occurrence of larger-diameter vessels (30–45 μm) within each growth ring than those of *R. maximum* grown under
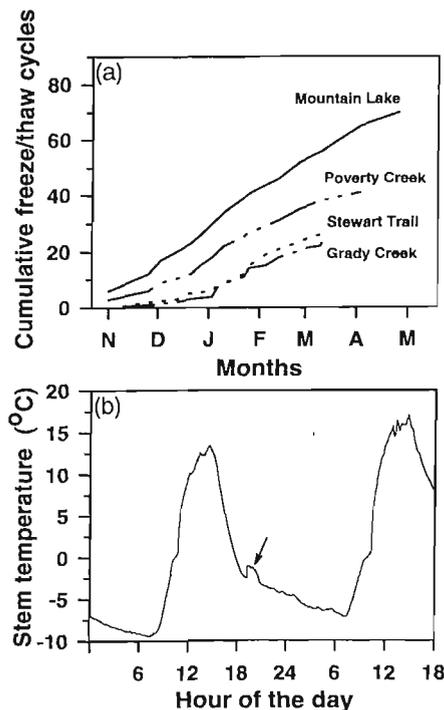

Figure 1. (a) Cumulative number of freeze–thaw cycles that occurred during the winter study period (November to April) at Mountain Lake and Poverty Creek at the Jefferson National Forest, VA and at Stewart Trail and Grady Creek at Coweeta, NC. (b) Representative diurnal course of *R. maximum* stem temperature (°C) for two winter days at Poverty Creek, VA. The arrow indicates the exotherm resulting from ice formation in a stem of *R. maximum*.

the canopy (Fig. 3a). The hydraulic mean diameter of vessels was 19·7 and 23·3 μm for the low and high light environments, respectively. In high light grown plants 40% of hydraulic conductance occurs in vessels greater than 30 μm, while in low light grown plants only 9% of hydraulic conductance occurs in these large-diameter vessels.

Over 70% of all vessels were less than 20 mm long (Fig. 3b). Plants grown in gaps at all sites had longer vessels than plants grown under the canopy. The longest vessel found in plants growing in gaps was 200 mm, while that of plants growing under the canopy was 120 mm. Vessel volumes ranged from $2·6 \times 10^{-12}$ m^3 to $8·5 \times 10^{-11}$ m^3 (Fig. 3c). The largest vessel volume for plants grown under the canopy was $3·8 \times 10^{-11}$ m^3. Vessel volumes were as large as $8·5 \times 10^{-11}$ m^3 for plants grown in gaps.

Loss of hydraulic conductivity

Generally, under hydrated growing conditions, xylem embolisms occurred as *R. maximum* shrubs transpired, which resulted in a 10–20% reduction in hydraulic conductivity. During the winter, in Virginia, there was up to 30% embolism development at both the low and high elevation sites for those *R. maximum* grown in gaps (Figs 4a & b). Embolism development was greatest in *R. maximum* at Coweeta, reaching over 60% of xylem embolized in shrubs
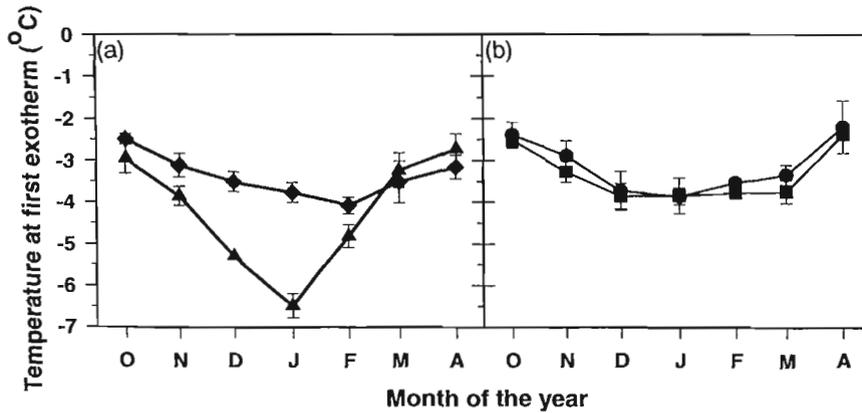
Figure 2. Stem freezing temperature (°C) measured monthly throughout the winter for *R. maximum* at Mountain Lake (diamonds), Poverty Creek (triangles), Stewart Trail (squares), and Grady Creek (circles). Symbols represent the means of measurements of at least three individual plants at each site ± SE.
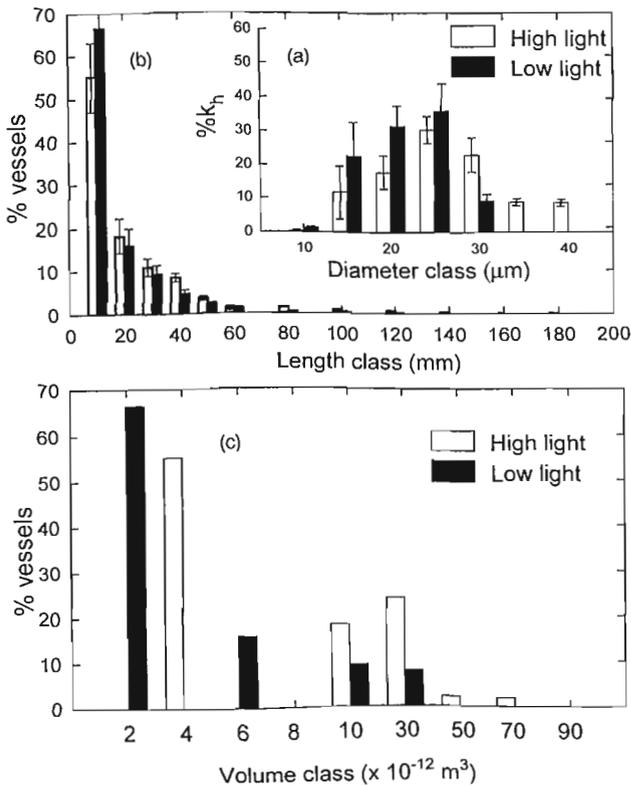

Figure 3. (a–c) Xylem vessel dimensions for 3-year-old *R. maximum* stems grown under canopy (shaded bars) and in gaps (open bars) (a) Diameter distribution in 5 μm classes reflecting the contribution of each class to total hydraulic conductance (k_h) based on the Hagen-Poiseuille relationship (Zimmerman 1983). (b) Vessel length distribution. (c) Vessel volume distribution based on vessel length and mean hydraulic diameter.

grown in gaps (Figs 4c & d). At each site, embolism development of shrubs grown under low light was mostly below 20%, except at the high elevation site in North Carolina (Stewart trail) where stomatal activity in the spring may have contributed to increased embolism development.

Potential stomatal activity (Figs 4a, b, c & d; bar chart below the reference axis), determined from a polynomial regression based on stomatal conductance and maximum midday air temperatures above 10 °C ($R^2 = 0.58$), suggests that there were infrequent brief periods during the winter when some stomatal activity was possible. There appears to be an association between recovery of hydraulic conductivity from winter embolism with the onset of warmer weather and increased stomatal activity.

Loss of hydraulic conductivity at the end of winter was strongly related to the total estimated volume of vessels present within a 3-year-old stem segment (regression line $R^2 = 0.83$, $P < 0.0005$) (Fig. 5). Plants grown in a lower light environment, under the forest canopy, suffered a smaller loss in hydraulic conductivity by the end of the season than those grown in gaps. The functional cross-sectional area of stems was greater in canopy gaps than under the canopy at all sites, and lower at the Virginia sites than at the North Carolina sites (data not shown).

Diurnal measurements of stomatal conductance and transpiration in winter revealed that, even with some loss of stem hydraulic conductivity, there was some leaf gas exchange occurring (Fig. 6). Typical values for stomatal conductance of a *R. maximum* leaf in full sun at midday, during much of the winter, were between 4 and 18 mmol m^{-2} s^{-1} when air temperatures were above freezing. Stomata became particularly active when air and leaf temperatures exceeded 10 °C during the day (Fig. 6a). In early spring, stomatal conductance increased to around 65 mmol m^{-2} s^{-1} at midday (Fig. 6b). Typical midday water potentials ranged from −0.6 to −1.2 MPa, becoming high again (< −0.05 MPa) overnight (Fig. 6c).

Vulnerability curve

As leaf water potential declined, loss of hydraulic conductivity increased curvilinearly (Fig. 7). There was no discernable difference in this relationship among sites (data not shown). At water potentials above −1 MPa, there was a
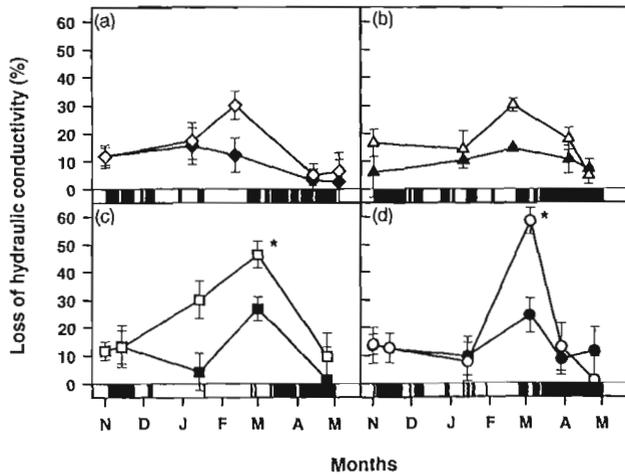
Figure 4. Loss of hydraulic conductivity (%) in 3-year-old *R. maximum* stems during the winter months, for plants grown under canopy (shaded) and in gaps (open) at (a) Mountain Lake, (b) Poverty Creek, (c) Stewart Trail and (d) Grady Creek. Data points represent the means of measurements made on at least three plants from each light environment at each site. Below the zero reference line of each graph, a bar chart indicates periods of potential stomatal activity based on midday air temperatures over 10 °C at each site. Asterisks indicate significant differences (*P* < 0·05), as determined by non-parameteric rank sum tests, between the loss of hydraulic conductivity for plants grown in gaps and under the canopy, for that particular date and site.

0–20% loss of stem hydraulic conductivity. This degree of loss in conductivity is common during periods of transpiration. As water potential declined below −1 MPa during dehydration, the number of embolisms increased. The leaf water potential at 50% loss of hydraulic conductivity was around −2·2 MPa.

DISCUSSION

Vulnerability to embolism development

Rhododendron maximum vessel diameters and volumes are small, falling within a range comparable to the ranges for small diffuse-porous desert species (Pockman & Sperry 1997) and conifer tracheids (Sperry & Sullivan 1992; Sperry *et al.* 1994). In a variety of other species tested, xylem conduits greater than 30 μm diameter are most commonly embolized during repeated freeze–thaws (Sperry & Sullivan 1992; Lo Gullo & Salleo 1993). In 3-year-old stems of *R. maximum*, vessel diameters of this size or larger were infrequent and found primarily in those plants grown in relatively high light environments.

The degree of wintertime embolism development in young *R. maximum* stems was significantly related to the total volume of the vessels within a stem internode and was unrelated to the frequency of freeze–thaw cycles. Under low light conditions, there was less than a 25% loss of hydraulic conductivity due to embolism development in *R. maximum* at all research sites.This is within the range of embolism development that would be expected to occur

during a normal diurnal cycle as a result of transpiration (as determined from the vulnerability curve with leaf water potential at or higher than −1·0 MPa). Thus, the first part of hypothesis 1 is accepted because the vessel anatomy of *R. maximum* serves to minimize freeze–thaw-induced embolism in an environment with frequent freeze–thaw cycles each winter.

However, xylem embolism development was much greater in the higher light environments, particularly in the North Carolina sites (up to 60% loss of hydraulic conductivity). Higher wintertime embolism in high light sites was related to increased xylem vessel length, increased frequency of larger-diameter vessels, larger stem diameters and increased frequency of larger-volume vessels. Therefore, the second hypothesis is accepted because plants grown in high light sites had a higher susceptibility to freeze–thaw-induced embolism and experienced higher levels of embolism at the end of the winter at all sites compared to plants grown in low light.

The vascular system of *R. maximum* is relatively sensitive to drought-induced embolism. Greater than 50% loss of hydraulic conductance occurred at a water potential of 2·2 MPa. This result is the same as the water potential (−2·2 MPa) that induces the maximum frequency of acoustic clicks (another measurement of cavitation) in the xylem of *Rhododendron ponticum* (Crombie *et al.* 1985). This water potential value is comparable to that of the most sensitive species measured by several authors (Jarbeau *et al.* 1995; Pockman *et al.* 1995). Therefore, the second part of hypothesis 1 also is accepted because *R. maximum* stems are relatively sensitive to drought-induced embolism.
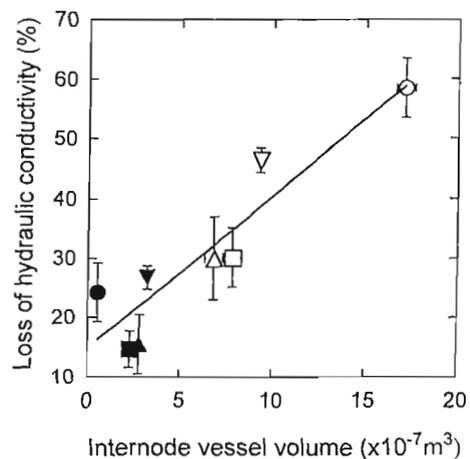

Figure 5. Relationship between the maximum percentage loss of hydraulic conductivity measured near the end of the winter season and the estimated total vessel volume within an internode of 3-year-old *R. maximum* stems at Mountain Lake (diamonds), Poverty Creek (triangles), Stewart Trail (squares) and Grady Creek (circles). Open symbols represent stems sampled from gaps, and closed symbols represent stems sampled from under the canopy. Each symbol represents the means of three to six individuals ± SE. Some standard error bars are smaller than the symbol. Linear regression line has an $R^2 = 0.83$.
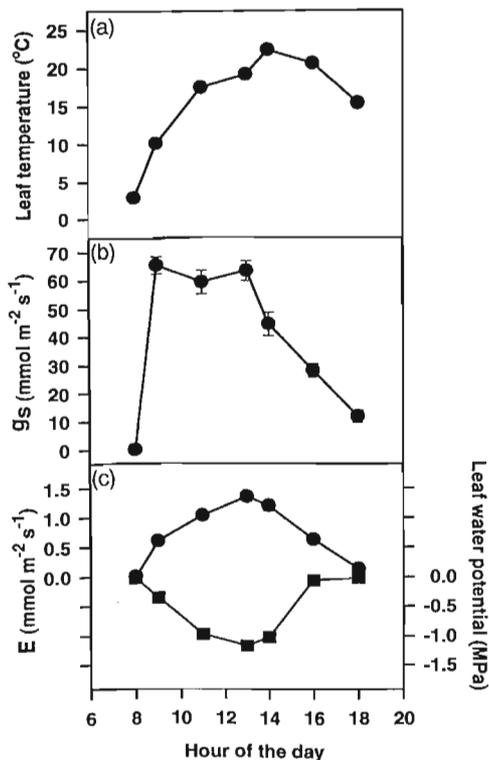
Figure 6. Diurnal track of *R. maximum* (a) leaf temperature, (b) stomatal conductance, g_s (mmol m^{-2} s^{-1}), and (c) transpiration, E (mmol m^{-2} s^{-1}) (circles), and leaf water potential (MPa) (squares) in mid-March at Grady Creek, Coweeta. Symbols represent the means of five measurements on three different plants taken at each sampling time during the day ± SE.

in the nocturnal reabsorption of embolisms for shrubs and small trees (Pickard 1989). Generally, if the rate of thawing is high and transpiration is low, then positive pressures in the xylem would cause bubbles to be redissolved (Tyree & Yang 1992). However, should negative pressures develop in the xylem from transpiration during a thaw then bubble expansion can occur, creating more embolisms (Robson & Petty 1993). In conifers, recovery of hydraulic conductivity often occurs without detectable root pressures (Sperry 1993). In *R. maximum*, negative pressures during brief mid-day periods of wintertime stomatal activity may result in embolisms, but redissolution of embolisms may occur as a result of positive pressures which appear to develop in the xylem overnight. We observed, from the exudation of sap from newly cut branches, that positive pressures can occur in the xylem in the early morning hours.

Embolisms also may be refilled by the action of 'Münch' water (Milburn 1996). This water is constantly being discharged from phloem elements as solutes are transported in vascular rays to support the construction of new xylem. The impact of this water source is primarily in the outer vascular layers. Vessels of *R. maximum* are active at least 10 years into the wood, and thus it is unlikely that Münch water could refill embolisms that occur deep in the wood of *R. maximum*. In addition, Münch water is generated when solutes are flowing to new xylem construction layers. Wood of *R. maximum* does not develop until late spring (Nilsen 1986). Thus, refilling of xylem vessels in the early spring is not likely to be the result of Münch water.

Small-diameter stems of *R. maximum* grown in low light environments may have the capacity to recover conductivity much more quickly than wider-diameter stems during times of thaw. Under experimental conditions, the time required to recover hydraulic conductivity in branches has been shown to be much shorter for small-diameter branches than large-diameter branches (Yang & Tyree 1992). As a result, there is some uncertainty as to whether the degree of xylem embolism, determined from periodic
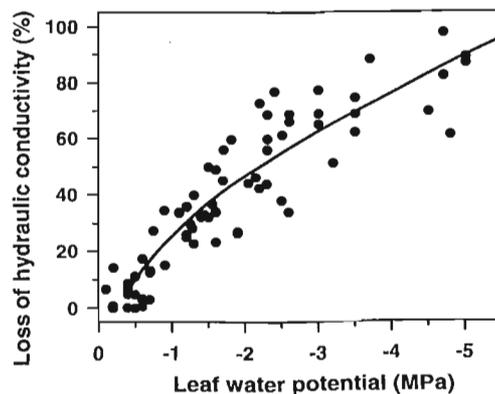
Observed winter midday xylem potentials during the study period were higher than –1·0 MPa. However, in previous years Nilsen (1987) found that winter midday leaf water potentials commonly averaged –1·5 MPa in Virginia. Transpiration during infrequent periods of warmer weather, while soils are still very cold, may increase the possibility of drought-induced xylem cavitation and embolism formation in *R. maximum*. In this study, infrequent stomatal activity did not appear to be associated with any changes in embolism formation at the Virginia sites. However, in North Carolina, early spring transpiration may have contributed in some degree to embolism formation. Thus, hypothesis 3 was rejected because wintertime transpiration did not account for the observed loss of hydraulic conductivity.

Hydraulic conductivity recovered from embolisms rapidly in the spring at all sites. Rapid recovery from cavitation was also found for *R. ponticum* (Crombie *et al.* 1985). The recovery of hydraulic conductivity from winter embolisms occurs by a variety of processes in other species. For example, in grape vines with wide vessels Sperry *et al.* (1988) found that, after winter, refilling of air-embolized vessels occurred by root pressure with the gradual dissolving of air bubbles in the xylem as the sap rose in the spring. Root pressures (commonly 0·1–0·2 MPa) may be involved

Figure 7. Vulnerability curve for *R. maximum* indicating the percentage loss of hydraulic conductivity as leaf water potential (MPa) declined as a result of dehydration. Each data point represents an individual branch sample and all data from all sites are combined. The regression line has an $R^2 = 0.89$.

measurements, reflects a cumulative amount of embolism development over the winter or the degree of embolisms occurring since the last thaw, particularly in very small-diameter branches. Also, the height of the shrub can influence the degree of capillary refilling which might occur (Sperry *et al.* 1987; Borghetti *et al.* 1991). In conifers 2 m tall, it was shown that capillary action alone could account for the refilling of embolized conduits (Borghetti *et al.* 1991). In Virginia, where *R. maximum* is commonly 2 m tall or less, refilling of embolized vessels may occur in this manner.

Ecological significance

The date when temperate deciduous trees become leafy may be strongly correlated to the degree of embolism development by the end of winter (Wang *et al.* 1992). In general, those species with a low degree of embolized xylem are more likely to become leafy sooner than those species with a high degree of embolism or those species that require new vessel growth to establish water conduction (Wang *et al.* 1992). Like many conifers, *R. maximum* appears to refill xylem relatively early in the season (Borghetti *et al.* 1991; Sperry 1993; Sperry *et al.* 1994) but, unlike other evergreen species (conifers), new leaf initiation in *R. maximum* is delayed. By mid-March at all sites studied, the environmental conditions were conducive to high stomatal conductivity, and *R. maximum* stems appeared to be fully rehydrated as indicated by over 90% restored hydraulic conductivity. However, *R. maximum* typically induces new leaf production in late May (Nilsen 1986; Lipscomb & Nilsen 1990) after the overstorey trees (typically *Acer* and *Quercus* sp.) have produced foliage. Therefore, hypothesis 4 is not supported because the degree of winter embolism development cannot explain the apparent delay of more than 2 months before *R. maximum* becomes leafy.

The ecological distribution and habitat requirements of some temperate forest species may be explained by variations in vulnerability to embolism formation. Adaptations to minimize or repair freeze–thaw-induced embolisms may be particularly important for the growth and survival of subcanopy plants growing in environments of frequent winter freeze–thaws. However, trade-offs may occur between adaptations to freeze–thaw cycles and maintenance of high water potential. Small conduits are less vulnerable to freeze–thaw embolism development but less efficient for water conduction. If a braod-leaf species had high conductance and small-diameter vessels, stem water potentials would probably be relatively low. *R. maximum* is a broad-leaf species with small-diameter vessels and is relatively sensitive to drought-induced embolism. Therefore, in order for *R. maximum* to maintain a relatively high water potential (above –2·0 MPa), stomatal conductance cannot be high. Due to this constraint to stomatal opening, the optimal environment for *R. maximum* is in low light sites of the forest understorey, where water availability is high, and water stress at any time of the year is low.

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