Unraveling the Effects of Plant Hydraulics on Stomatal Closure during Water Stress in Walnut

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The objectives of the study were to identify the relevant hydraulic parameters associated with stomatal regulation during water stress and to test the hypothesis of a stomatal control of xylem embolism in walnut (*Juglans regia* × *nigra*) trees. The hydraulic characteristics of the sap pathway were experimentally altered with different methods to alter plant transpiration (E_{plant}) and stomatal conductance (g_s). Potted trees were exposed to a soil water depletion to alter soil water potential (Ψ_{soil}), soil resistance (R_{soil}), and root hydraulic resistances (R_{root}). Soil temperature was changed to alter R_{root} alone. Embolism was created in the trunk to increase shoot resistance (R_{shoot}). Stomata closed in response to these stresses with the effect of maintaining the water pressure in the leaf rachis xylem (P_{rachis}) above −1.4 MPa and the leaf water potential (Ψ_{leaf}) above −1.6 MPa. The same dependence of E_{plant} and g_s on P_{rachis} or Ψ_{leaf} was always observed. This suggested that stomata were not responding to changes in Ψ_{soil}, R_{soil}, R_{root}, or R_{shoot} per se but rather to their impact on P_{rachis} and/or Ψ_{leaf}. Leaf rachis was the most vulnerable organ, with a threshold P_{rachis} for embolism induction of −1.4 MPa. The minimum Ψ_{leaf} values corresponded to leaf turgor loss point. This suggested that stomata are responding to leaf water status as determined by transpiration rate and plant hydraulics and that P_{rachis} might be the physiological parameter regulated by stomatal closure during water stress, which would have the effect of preventing extensive developments of cavitation during water stress.

Drought is one of the most limiting environmental stresses for plant production (Kramer and Boyer, 1995). Plants respond to drought by closing their stomata, which reduces leaf transpiration and prevents the development of excessive water deficits in their tissues. The drawback of the stomatal closure for plants is that their carbon gain is lowered and their growth is impaired. Therefore, information about plant responses to drought and their underlying mechanisms will have relevance for plant adaptation to new climatic conditions and breeding programs (Schulze et al., 1987).

Many physiological variables are known to be implicated in the stomatal regulation; some of these variables are involved in the mechanics of stomatal movements, others in the signals triggering these movements (Zeiger et al., 1987). A new set of variables has been proposed in the recent literature, all of which are related to the hydraulics of plants. Indeed, it has been suggested that stomata may respond to drought-induced changes in the hydraulic characteristics of the sap pathway, such as whole-plant hydraulic resistance (R_{plant}; Meinzer and Grantz, 1990; Cochard et al., 1996a; Lu et al., 1996), soil resistance (R_{soil}), root resistance (R_{root}; Meinzer and Grantz, 1990; Cochard et al., 2000b), or shoot resistance (R_{shoot}; Salleo et al., 1992; Sperry et al., 1993). However, during water stress, changes in the above parameters are not independent and, further, correlated to variations in soil water potential (Ψ_{soil}), whose impact on stomatal function has long been recognized (e.g. Hinckley and Bruckerhoff, 1975).

It has also been suggested that stomatal closure during water stress might be associated with the maintenance of xylem integrity (Tyree and Sperry, 1988; Jones and Sutherland, 1991). Sap is transported under negative pressures in plants, and, therefore, is susceptible to cavitation events (Pickard, 1981) that render xylem conduits non-conductive. Cavitation occurs when the negative sap pressure exceeds a threshold value defined by anatomical characteristics (Sperry and Tyree, 1988). Many species have been found to operate very close to the point of embolism. Therefore, stomata control both plant water losses and sap pressure and, thus, may actively control the risk of xylem embolism (Jones and Sutherland, 1991).

The first objective of our study was to experimentally uncouple R_{soil}, R_{root}, R_{shoot}, and Ψ_{soil} to identify the specific hydraulic parameters associated with stomatal regulation during water stress. The experi-
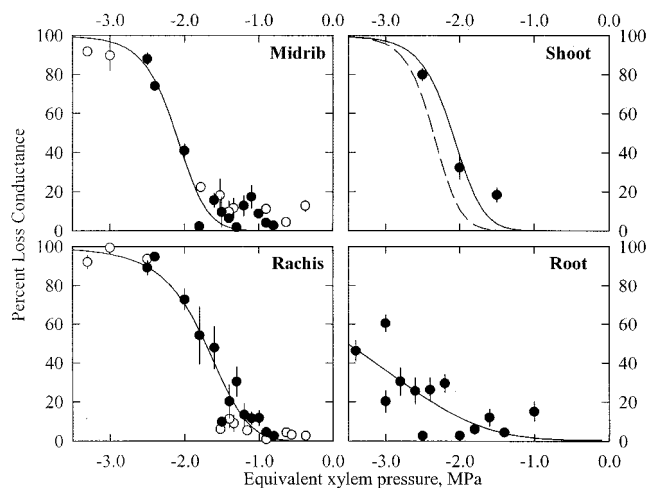

Figure 1. VCs for walnut leaf midribs and rachis, and current year shoots and roots. Close symbols refer to measures on air-pressurized segments. White symbols refer to leaf midribs and rachis collected on trees during the different experiments. Error bars are ± SE. Lines are logistic fits through the data. For shoots, the lines are logistic fits through the data published by Tyree et al. (1993) for current year (plain line) and previous year (dashed line) segments.

ments were conducted on potted walnut (*Juglans regia × nigra*) trees in a growth cabinet to minimize the effects of temperature, light intensity, air vapor deficit, and air CO_2 concentration on stomatal behavior. The second objective of our study was to test the hypothesis that stomata control embolism during water stress in walnut.

RESULTS

Vulnerability to Cavitation

Vulnerability curves (VCs) were constructed by plotting the changes in the percentage loss of xylem conductance (PLC) versus xylem pressure. The VCs for the different walnut organs are shown in Figure 1 and the parameters of the logistic fitting (Eq. 4) are given in Table I. Significant differences were found between organs. Leaf rachises were significantly the most vulnerable, roots the least vulnerable, and leaf veins and shoots intermediate. The few measurements obtained on shoot segments were in agreement with our previous study.

Pressure-Volume Analysis

Turgor pressure ($P_{leaf\ 0}$) at full turgor averaged 0.93 ± 0.06 MPa ($n = 5$, ±SE) and turgor loss point averaged −1.53 ± 0.04 MPa. The relative change in P_{leaf} ($P_{leaf}/P_{leaf\ 0}$) with decreasing leaf water potential (Ψ_{leaf}) is shown on Figure 7.

Soil Dehydration

When plants were exposed to different light levels while soil watering was stopped (procedure 1), only a moderate water stress was induced. Figure 2 shows the concurrent changes in E_{plant} and P_{rachis} for one tree. The other trees behaved the same. After 4 d, P_{rachis} measured on the plants placed in the dark (predawn P_{rachis}) was reduced only by 0.1 MPa (see y intercepts on Fig. 2). However, E_{plant}, stomatal conductance (g_s), and the P_{rachis} at maximum light intensity were considerably reduced at the end of the treatment. This was consistent with a sharp increase in R_{plant}. This increase could not be attributed to xylem cavitation because the xylem pressure never drops below the threshold for cavitation for all organs measured. The change in R_{plant} was more probably due to increase in R_{soil} and/or R_{root}.

When plants were continuously exposed to a constant and high light intensity for 1 week (procedure 2), higher levels of water stress were obtained. Figure 3 shows the results for one tree, the other trees behaving the same. E_{plant} and g_s dropped close to zero whereas P_{rachis} leveled at approximately −1.4Mpa. At this point, the degree of xylem embolism in the leaf rachis was still less than 10 PLC (not shown).

Root Chilling

Decreasing soil temperature from ambient to near zero temperature provoked a significant reduction in g_s, E_{plant}, and P_{rachis} (Fig. 4). These changes were reversed when soil temperature was returned to ambient.

Stem Pressurization

Application of a positive pneumatic pressure around the trunk of a plant induced a sharp decrease of E_{plant}, g_s, and P_{rachis} only for pressures greater than 2.5 MPa (Fig. 5). At this pressure, about 70 PLC was

Table I. *Xylem vulnerability to embolism*

Parameters of the logistic function (see equation in the text) fitted to the experimental PLC versus Ψ_{rachis} curves. Values are given ± SE. Data having a letter in common are not significantly different at $P = 0.01$.

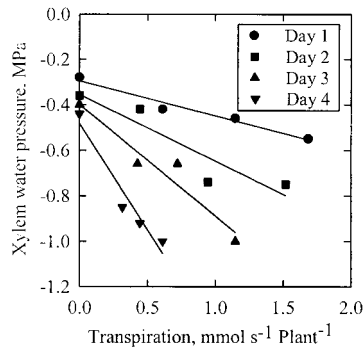|	Leaf		Shoot		Root
	Midrib	Rachis	Year n	Year $n–1$	
$P_{rachis50}$	−2.12 ± 0.06a	−1.72 ± 0.05b	−2.10 ± 0.04a	−2.36 ± 0.02c	−3.53 ± 0.39d
s	−9.77 ± 2.00ac	−5.72 ± 0.88abd	−9.82 ± 1.97bc	−12.8 ± 1.95c	−3.50 ± 1.31d

Figure 2. Typical changes of plant transpiration and xylem water pressure during the first stage of a soil water stress for one of the studied tree. The four different symbols correspond to 4 consecutive d. Four different light intensities were used on each day to vary E_{plant}. Lines are linear regressions through the data for each day. The slope of the lines represents the R_{plant}.

induced in the stem (see the VC for stems plotted on top of Fig. 5). For air pressure applications higher than 3 MPa, more than 95 PLC was induced in the stem, leaf wilting was noticed, and nearly 100 PLC was measured in the leaf rachis.

DISCUSSION

The first objective of our study was to combine different experimental approaches to unravel the effect of plant hydraulics on stomatal closure. The second objective was to analyze the effect of stomatal closure on plant hydraulics and to test the hypothesis of a stomatal control of embolism.

Unraveling the Effects of Ψ_{soil}, R_{soil}, R_{root}, and R_{shoot} in the Response of g_s to Water Stress

In this study, we focused our analysis on some of the endogenous physiological parameters likely to be altered during a water stress and that have been reported in the literature to be associated with stomatal responses. These parameters are the Ψ_{soil}, the R_{soil}, the R_{root}, and the R_{shoot}; all of these parameters are strongly correlated under natural drought condi-
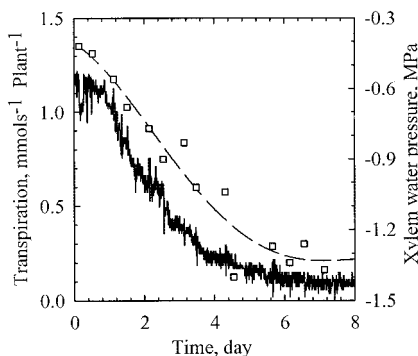

Figure 3. Typical time course of plant transpiration and xylem water pressure for a non irrigated tree exposed to a constant light intensity.
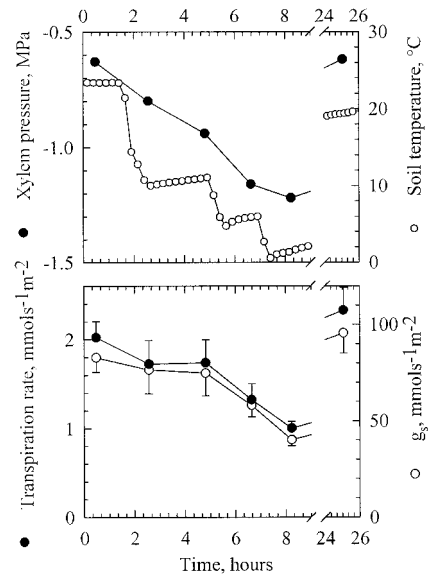

Figure 4. Typical time course of xylem water pressure and soil temperature (top panel), and plant transpiration and g_s (bottom panel) during a soil chilling experiment. Error bars are ± SE ($n = 5$).

tions. The experiments were designed to alter R_{plant} in very different ways, which probably had a primary influence on different parts of the pathway.

The effects of the different experiments conducted on the same plant material can be analyzed according to their main impact on Ψ_{soil}, R_{soil}, R_{root}, and R_{shoot}. Soil dehydration provoked mainly a drop in Ψ_{soil} and an increase of R_{soil}. The resistance of the interface
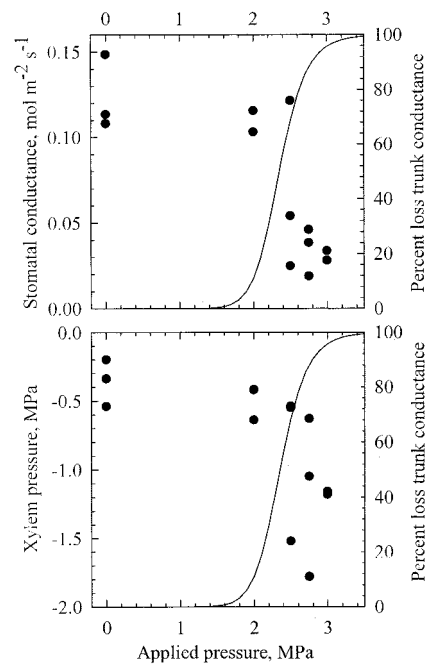

Figure 5. Changes in g_s (top panel) and xylem water pressure (bottom panel) of trees exposed to increasing pneumatic pressures around their trunk (x axis). The curve represents the change in embolism in the trunk versus the applied pressure.
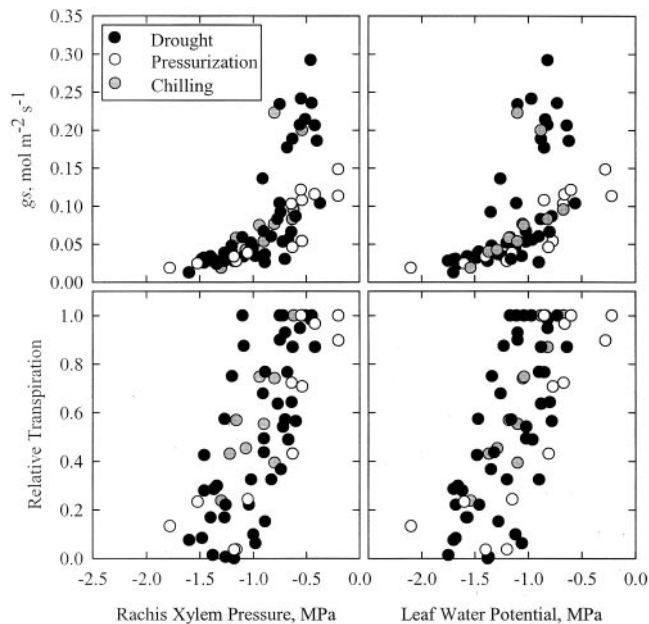
Figure 6. Dependence of g_s (top panels) and transpiration (E_{plant}) on xylem water pressure (left panels) and Ψ_{leaf}. The different symbols represent the different experiments conducted in this study. Transpiration was normalized by the transpiration of each tree before treatment (E_{plant}/E_{max}).

between the soil and the root probably also increased (Nobel and Cui, 1992). R_{root} was modified to the extent that the radial flow into the root xylem was altered. R_{shoot} was probably not altered, because the level of xylem embolism remained low during these experiments. However, if the ionic composition of the sap changed dramatically as a result of the drought, then R_{shoot} may have varied (Zwieniecki et al., 2001). Root chilling altered mainly R_{root} and R_{soil}. The soil being watered to maximum capacity in this experiment, R_{soil} was very low compared with R_{root}, and its variations were not quantitatively significant. Ψ_{soil} remained constant because the temperature dependence of Ψ_{soil} is very low (Muromtsev, 1981). Stem pressurization provoked only an increase in R_{shoot} when the pressure exceeded the point of embolism induction (about 2.0 MPa; Tyree et al., 1993; Fig. 5). If the air was propagated along the xylem flow path significantly beyond the injection point, R_{root} and R_{leaf} may also have being altered. Therefore, combining the results of all these experiments, it is possible to determine whether g_s and E_{plant} were specifically responding to main changes in Ψ_{soil}, R_{soil}, R_{root}, and/or R_{shoot} or not. Because air humidity, air temperature, and light intensity were maintained constant in our experiments, leaf to air vapor deficits and leaf boundary layer conductances were also constant. Therefore, the g_s and E_{plant} patterns corresponded in our study. The relationship between g_s and hydraulic parameters are likely to depend on these environmental conditions, contrary to the relationships with E_{plant} (Monteith, 1995).

Our results showed that the different experiments all significantly reduced E_{plant} and g_s. Therefore, the response of g_s to Ψ_{soil}, R_{root}, R_{soil}, and R_{shoot} was neither specific nor exclusive. An alternative analysis of the problem is not to consider Ψ_{soil}, R_{root}, R_{soil}, and R_{shoot} individually but rather to examine their combined effect on P_{rachis} or Ψ_{leaf}. The relationship between P_{rachis}, Ψ_{soil}, R_{root}, R_{soil}, E_{plant}, and g_s under steady-state conditions is well described by the Ohm's law analogy (Tyree and Ewers, 1991):

$$P_{rachis} = \Psi_{soil} - (R_{soil} + R_{root} + R_{shoot}) \cdot SF_{plant} \cdot g_s \cdot D \quad (1)$$

where SF_{plant} is the plant leaf area and D the air vapor pressure deficit, two parameters that remained constant during our experiments. The gravity term and the xylem sap osmotic potential are assumed negligible in equation 1. A similar relationship is obtained with Ψ_{leaf} if we further include the leaf blade hydraulic resistance. The dependency of g_s or E_{plant} on P_{rachis} and Ψ_{leaf} is shown on Figure 6 for all of the experiments. The responses were similar whatever the experiments. This would suggest that in these experiments, g_s was not correlated to changes in Ψ_{soil}, R_{soil}, R_{root}, or R_{shoot} per se but rather to P_{rachis} and/or Ψ_{leaf}. Another illustration on the combining effects of R_{soil} R_{root}, and R_{shoot} on E_{plant} is shown on Figure 8. An identical relationship was obtained between E_{plant} and C_{plant} (defined as $[R_{soil} + R_{root} + R_{shoot}]^{-1}$). Data for plants exposed to a high soil water depletion (procedure 2) are not included on Figure 8. These results are in agreement with the finding of Saliendra et al. (1995), Sperry (2000), and Hubbard et al. (2001). Our data do not enable us to test whether g_s was more specifically responding to changes in Ψ_{leaf} or P_{rachis} because both values were highly correlated and differed only by a few 0.1 MPa. Experiments where P_{rachis} and Ψ_{leaf} are uncoupled are awaited.

Evidence for a Stomatal Control of Xylem Embolism in Walnut

From the above discussion, it could be concluded that the effect of the stomatal closure in our study was to maintain P_{rachis} above a threshold value around -1.4 MPa and Ψ_{leaf} above approximately -1.6 MPa. To further understand this behavior, we have to identify a major physiological trait that would threaten plant integrity at lower P_{rachis} and/or Ψ_{leaf} values. The answer to this question is obviously very complex, because many traits are probably involved and correlations between them probably exist. We will argue that, in our study, xylem cavitation was a trait correlated with the stomatal closure.

A physiological trait associated with a stomatal closure during water stress should meet at least the following three main conditions. First, its impairment should represent a serious threat to plant functioning. This results from the consideration that the

cost associated with stomatal closure is high (reduced carbon gain, reduced growth, reduced reproductive success, etc.) so the gain associated with the regulation should overcome the loss. Cavitation is a serious threat for plants because it impairs the xylem conductive capacity and may eventually lead to leaf desiccation and branch mortality (Rood et al., 2000). Indeed, leaf desiccation was not observed in our study as long as the xylem integrity was maintained. Leaf desiccation was noticed only when high levels of embolism were measured in the leaf petioles. The gain associated with stomatal closure was thus the maintenance of leaf vitality, which largely overcomes the drawbacks cited above.

The second condition is that the impairment of the trait should be water deficit dependent because the effect of stomatal closure is precisely to prevent excessive leaf dehydration. The mechanism of water stress-induced cavitation has been well documented (Sperry and Tyree, 1988; Cochard et al., 1992; Jarbeau et al., 1995). Air is sucked into the xylem lumens through pores in the pit wall when pressures in the sap exceed the maximum capillary pressures that can sustain the pores. Therefore, the likelihood of cavitation occurrence is directly determined by the degree of water deficit in the xylem, more precisely by P_{rachis}. The maintenance of leaf turgor above cell plasmolysis is another physiological trait that might also satisfy these first two conditions.

The third condition is that the impairment of the trait should have the same water deficit dependence as stomata. Stomata were completely closed in walnut trees when P_{rachis} reached about approximately −1.4 MPa and Ψ_{leaf} about approximately −1.6 MPa. The impairment of the trait associated with stomatal closure should therefore occur at comparable P_{rachis} or Ψ_{leaf} values. On Figure 7 (top panel) is represented the dependencies of E_{plant} and rachis embolism on P_{rachis}. The leaf rachis was the most vulnerable organ along the sap pathway in the xylem and was also exposed to the lowest xylem pressure values. Leaf rachis is therefore the Achilles' heel of the walnut tree sap pathway. Segmentation in xylem vulnerability to cavitation has been demonstrated for several other species (Cochard, 1992; Sperry and Saliendra, 1994). A lot of variation exists between species, and occasionally the roots appear to be the most cavitation sensitive organs in the plant (Sperry and Ikeda, 1997). From Figure 7, it is clear that the dependencies of leaf rachis xylem embolism and transpiration on water deficit were very similar. Stomata were completely closed at the incipience of xylem embolism in the leaf rachis. Figure 7 (bottom panel) shows the concurrent variations of E_{plant} and leaf turgor pressure (P_{leaf}) with bulk Ψ_{leaf}. It is also clear from this graph that stomata were completely closed at the incipience of leaf cell plasmolysis (turgor loss point). The maintenance of xylem integrity and leaf turgor
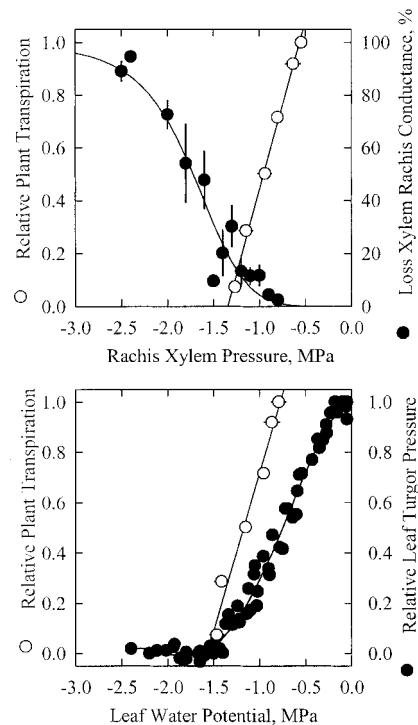

Figure 7. Dependence of plant transpiration and xylem rachis embolism on rachis xylem pressure (top panel) and dependence of plant transpiration and leaf turgor pressure on Ψ_{leaf} (bottom panel). Error bars are ± SE. The VC was replotted from Figure 1, and data for transpiration rate were averaged from Figure 7.

were closely associated with stomatal closure during water stress in walnut.

The data from Figure 7 suggest that stomatal closure was rather preemptive in avoiding cavitation. This behavior might be explained by the potential for "catastrophic xylem failure" (Tyree and Sperry, 1988). There is a feedback between xylem conductance and xylem pressure during cavitation. Cavitation decreases xylem conductance, which in turn decreases xylem pressure and thus provokes more cavitation. Tyree and Sperry (1988) and Jones and Sutherland (1991) have computed that catastrophic xylem failure occurs at the expense of some xylem conductance and at a critical transpiration rate (E_{crit}) only slightly greater than the actual maximum E. The hypothesis of a stomatal control of catastrophic xylem failure was evaluated with an hydraulic model of a walnut tree explicitly taking into account the feedback between xylem pressure and xylem conductance. Our simulations confirmed the results of Sperry et al. (1998) and Comstock and Sperry (2000). Transpiration was maximized (E_{crit}) at the expense of all conductance in the distal leaf rachis segment. E_{crit} was therefore much higher than the actual E_{plant} (Fig. 8, plain line). Using the same model, we have computed E_{plant} provoking 1% (E_{1PLC}) and 10% (E_{10PLC}) loss of rachis conductance (Fig. 8, dotted and dashed
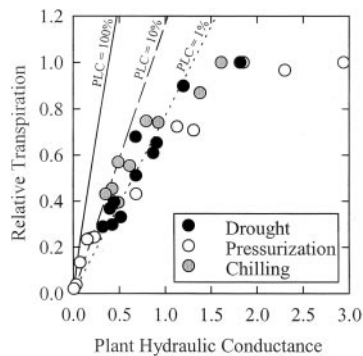
Figure 8. Plant transpiration versus whole plant hydraulic conductance. Different symbols represent different experiments. The plain, dashed, and dotted lines represent the critical transpiration rate provoking 100%, 10%, and 1% loss conductance in the leaf rachis, respectively.

lines, respectively). The onset of tree water loss regulation occurred when E_{plant} reached E_{1PLC} and E_{plant} tracked E_{10PLC} when plant conductance was further reduced. This model suggests that the risk of catastrophic xylem failure was not associated with the stomatal regulation in walnut. g_s was not maximized at the expense of all xylem conductance. Rather, xylem conductance was maximized at the expense of all g_s. To experimentally validate these computations, we have tried, without success, to feed stressed plants with fusiccocine, a drug supposed to promote stomatal opening. The use of mutants lacking efficient stomatal regulation is probably a better way to test such hypotheses (Cochard et al., 1996b).

Mechanisms Triggering Stomatal Closure

The mechanisms by which stomata could sense changes in P_{rachis} to adjust g_s and avoid cavitation remain hypothetical. It seems improbable that P_{rachis} itself was the triggering parameter, so we must identify a parameter correlated to P_{rachis} during water stress. Our stem pressurization experiments dismiss the implication of parameters associated with soil or root water stress (such as Ψ_{soil} or abscisic acid production by roots [Tardieu et al., 1992]). Because the onset of cavitation events in leaf blades was correlated with the onset of stomatal closure in laurel, it has been suggested that stomata were responding to hydraulic signals generated by cavitation (Salleo et al., 2000; Nardini et al., 2001). However, in our study, stomatal closure occurred before the onset of cavitation in leaf blades and midribs (Fig. 6) and only after 70% loss of conductance in the trunk (Fig. 5). The possibility that stomata were responding to Ψ_{leaf} cannot be rejected because of the high correlation between P_{rachis} and Ψ_{leaf} in our study. Ψ_{leaf} could have a direct impact on guard cells turgor (Meidner and Edwards, 1996) or an indirect impact on their sensitivity to abscisic acid (Tardieu and Davis, 1993). A

stomatal response to changes in leaf water status has long been suggested (Cowan, 1977) and has recently been supported by root pressure chamber experiments on woody plants (Fuchs and Livingston, 1996; Comstock and Mencuccini, 1998). These experiments demonstrate that stomatal closure caused by soil drought or decreased air humidity can be partially or wholly reversed by root pressurization.

CONCLUSIONS

In conclusion, combining different experimental procedures, we have established that stomata were not responding to changes in Ψ_{soil}, R_{soil}, R_{root}, or R_{shoot} per se but rather to their impact on P_{rachis} or Ψ_{leaf}. Cavitation avoidance was a likely physiological function associated with stomatal regulation during water stress in these experiments. However, the mechanism triggering the stomatal closure probably resides in the leaf mesophyll itself as stomatal closure was also correlated to bulk leaf turgor. The significance of cavitation avoidance seems crucial to understand plant water relations during water stress, probably more than the occurrence of cavitation itself. The possibility still exists that the striking relations that were observed between cavitation and stomatal function were only correlations and that the main physiological trait involved in the regulation was elsewhere. However, it seems to us that cavitation avoidance fits the three conditions we have identified for such a trait better that any other trait so far.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Experiments were conducted on 3-year-old walnut (*Juglans nigra* × *regia* cv NG38 grafted on *Juglans regia* rootstocks) trees grown in 35-L pots containing one-third peat and two-thirds natural clay soil from the Limagne region (France). The plants, grown in a nursery, were transferred to growth cabinets where air temperature (25°C) and air humidity (60% relative humidity) were constant. The light intensity at foliage level was adjusted between 0 and 480 μmol m^{-2} s^{-1}. A total of 17 trees were used in the different experiments. Because the experiments were time consuming, we chose to combine multiple experiments with a rather limited number of repetitions.

Ψ_{leaf} and Plant Transpiration

Ψ_{leaf}s were measured with a Scholander-type pressure chamber on leaflets randomly sampled on the trees. The xylem water pressure at the base of the leaflet blades (P_{rachis}) was measured on leaflets enclosed for at least 2 h in an air proof aluminum bag (Turner and Long, 1980). To minimize the reduction in tree leaf area due to sampling, only one replicate of P_{rachis} and Ψ_{leaf} were performed each time.

Plant water loss (E_{plant}) was estimated with two independent techniques. Sap flow through the trunk was monitored by the heat balance technique of Valancogne and Nasr (1989). E_{plant} was also measured gravimetrically with an electronic balance (model ID1, resolution 1g, Mettler, Viroflay, Switzerland). In addition, leaf transpiration and leaf g_s were measured with a steady-state porometer (model 1600, LI-COR, Linclon, NE). The measurements were performed on five leaflets randomly chosen in each trees.

Xylem Embolism

The degree of xylem embolism due to the presence of air in the vessels was assessed following the procedure introduced by Sperry et al. (1988) and recently substantiated (Cochard et al., 2000a). We used the new XYL'EM apparatus (Xylem Embolism Meter, Instrutec, Montigny les Cormeilles, France), based on a high-resolution liquid mass flowmeter, to determine the PLC in the leaf rachis. For each PLC determination, one leaf was cut from the trees under tap water. Six segments 15 to 20 mm long were excised, still under water, from the leaf rachis, and four segments were detached from the midribs of four leaflets. The segments were attached to the tubing of the XYL'EM apparatus and their initial hydraulic conductance (K_{init}) determined with a hydrostatic pressure gradient of approximately 3 kPa. The samples were then flushed with water pressurized to 0.1 MPa and the maximum conductance (K_{max}) determined as above. The PLC was calculated as follows:

$$PLC = 100 \times (1 - K_{init}/K_{max}) \qquad (2)$$

R_{plant}

The R_{plant} was computed as the ratio between the water potential drop along the sap pathway and the whole-plant transpiration:

$$R_{plant} = (\Psi_{soil} - P_{rachis})/E_{plant} \qquad (3)$$

It should be noted that, as we have defined it, R_{plant} does not include the leaf blade hydraulic resistance. Ψ_{soil} was estimated by the P_{rachis} value measured on the plants placed in the dark (Améglio et al., 1999).

VCs

The vulnerability of the xylem conduits to air embolism was determined following the pressure chamber dehydration technique of Cochard et al. (1992). The technique consisted in inserting a plant segment in a pressure chamber with only the cut end protruding. The pressure in the chamber was then adjusted to a target value and maintained constant until sap exudation ceased (usually after 15–30 min). The pressure was then released and xylem segments were excised under water and their PLC value determined as above. The plot of the PLC value versus the applied air pressure represents the VCs of the plant segment. VCs were first obtained for leaflet midribs, leaf rachises, and fine roots. A few measurements were also made on current year stem segments to confirm our previous results (Tyree et al., 1993).

For midrib and rachis VCs, entire leaves were pressurized. Four to six rachis segments were measured on each leaf. The basal 20 cm of the leaf rachis was discarded to avoid artifacts caused by air entry into cut vessels. On the same leaves, midribs were excised from the four apical leaflets. Root VCs were obtained on lateral roots excised from entire root systems gently washed with tap water. A total of 13 leaves and 13 roots from three different trees were used to construct the VCs. The following logistic function was fitted to the different curves (Cochard et al., 1999).

$$PLC(P_{rachis}) = 100/(1 + (P_{rachis}/P_{rachis50})^s) \qquad (4)$$

where $P_{rachis50}$ is the water pressure inducing 50 PLC and s is a slope parameter.

Pressure-Volume Analysis

Pressure-volume curves were obtained for five individual leaves using the bench top dehydration method (Ritchie and Hinckley, 1975). Leaves were harvested, enclosed in a plastic bag, and let to rehydrate overnight with their petiole immersed in distilled water. Bulk tissue estimates of turgor pressure (P_{leaf}) and turgor loss point were made using the method of Schulte and Hinckley (1985).

Soil Dehydration

The stomatal response to a soil water deficit was studied on six different trees following two complementary procedures. The water deficit was induced by withholding irrigation. For the first procedure, the light intensity in the chamber was increased by steps during the day. Four light intensities (0, 170, 250, and 450 μmol m^{-2} s^{-1}) were applied for 2 h each, to obtain different transpiration rates so the slope of E versus P_{rachis} could be determined for getting R_{plant}. After 2 h, plant transpiration was stable, enabling determination of Ψ_{leaf}, P_{rachis}, and g_s. During the night, the light level was maintained at 170 μmol m^{-2} s^{-1} to progressively lower the soil water content. The procedure was repeated for 4 consecutive d for each of the three trees used in this experiment. In a second procedure, plants were continuously exposed to 450 μmol m^{-2} s^{-1} during 7 consecutive d. Ψ_{leaf}, P_{rachis}, and g_s were determined twice a day. Leaves were periodically sampled for embolism assessment. The second procedure was repeated on three different trees.

Stem Pressurization

The aim of this experiment was to analyze the stomatal response to an increase in trunk hydraulic resistance. We used the air injection technique (Cochard et al., 1992; Salleo et al., 1992) to induce air embolism in the trunk and thus increase the trunk resistance. A 20-cm-long pressure chamber was fastened to the trunk of three different trees exposed to a constant light intensity (450 μmol m^{-2} s^{-1}). The pressure chamber was similar to the chamber described by Salleo et al. (1992), except that it was divided in two parts and could be clamped on intact trees (Améglio et al., 1994). To facilitate air entry into the xylem conduits, six 1.3-mm-diameter holes were drilled radially through the trunk in-

side the chamber. The holes were aligned vertically to minimize the damage to the xylem conduits. In a preliminary experiment, we verified that fastening the pressure chamber to the tree had no effect on tree transpiration and Ψ_{leaf}. The trunk segment in the chamber was exposed to increasingly higher air pressures (up to 3.5 MPa). Each target pressure was maintained constant for 15 min. Two hours after each pressurization, two leaflets were sampled, one to measured P_{rachis}, and one to measure Ψ_{leaf} with a pressure chamber, and g_s was measured on five leaflets. Leaves were also periodically sampled for embolism assessment.

Root Chilling

This experiment aimed at altering root and soil hydraulic resistance by changing soil temperature (Brodribb and Hill, 2000; Cochard et al., 2000b). A well-watered tree was placed in the growth chamber with 450 μmol m^{-2} s^{-1} light intensity. Ψ_{leaf}, P_{rachis}, and g_s were measured when E_{plant} became stable. Solutions with different temperature were obtained by mixing tap water with ice. The solutions were successively poured on the soil to lower its temperature (T_{soil}) in a step-wise manner from ambient to approximately 0.5°C. T_{soil} was measured with a thermocouple inserted near the center of the pot. After approximately 75 min, Ψ_{leaf}, P_{rachis}, and g_s were measured, and a cooler solution was poured on the soil. T_{soil} was then returned to 20°C by pouring water at ambient temperature on the soil. The whole procedure was repeated three times on two different trees.

Modeling

The relationship between plant hydraulic parameters and water loss regulation was analyzed with a model similar to the ones developed by Tyree and Sperry (1988) and Sperry et al. (1998). The soil-plant continuum is represented by hydraulic resistances in series: R_{soil}, R_{root}, R_{stem}, R_{trunk}, R_{branch}, R_{rachis}, and R_{leaf}. The partitioning of resistances within a tree was measured with an high pressure flowmeter (H. Cochard, unpublished data; Tyree et al., 1994). The relationships between the different xylem resistances and the xylem pressure were derived from the logistic fits of their VCs. R_{soil} was supposed negligible and not Ψ_{soil} dependent. Therefore, our model pertains only for the understanding of stomatal behavior during our root chilling and stem pressurization experiments. Sperry et al. (1998) and Comstock and Sperry (2000) have pointed out that the accuracy of such models depends on how finely the continuum is discretized. In our model, each xylem resistance was divided into 10 tiers. A test with 1,000 tiers yielded only 1.7% higher E_{crit} values. E_{crit} was determined to the nearest 0.001 mmol s^{-1} by progressively increasing E_{plant} until 100 PLC was obtained in one tier. The model was run with increasing R_{root} and R_{trunk} values to simulate the experimental observations. The model, called "RER," was developed on an Excel (Microsoft, Redmond, WA) spreadsheet.

LITERATURE CITED

Améglio T, Cruiziat P, Bodet C (1994) A pressure chamber to induce and control embolism in situ: consequence on the sap flow. *In* Y Kouchkovsky, F Larcher, eds, Plant Sciences. Société Franchouillarde de Physiologie Végétale, Paris, p 281

Améglio T, Archer P, Cohen M, Valancogne C, Daudet FA, Dayau S, Cruiziat P (1999) Significance and limits in the use of predawn leaf water potential for tree irrigation. Plant Soil **207:** 155–167

Brodribb TJ, Hill RS (2000) Increases in water potential gradient reduce xylem conductivity in whole plants: evidence from a low-pressure conductivity method. Plant Physiol **123:** 1021–1028

Cochard H (1992) Vulnerability of several conifers to air embolism. Tree Physiol **11:** 73–83

Cochard H, Bodet C, Améglio T, Cruiziat P (2000a) Cryo-scanning electron microscopy observations of vessel content during transpiration in walnut petioles: facts or artifacts? Plant Physiol **124:** 1191–1202

Cochard H, Bréda N, Granier A (1996a) Whole tree hydraulic conductance and water loss regulation in *Quercus* during drought: evidence for stomatal control of embolism? Ann Sci Forest **53:** 197–206

Cochard H, Cruiziat P, Tyree MT (1992) Use of positive pressures to establish vulnerability curves: further support for the air-seeding hypothesis and implications for pressure-volume analysis. Plant Physiol **100:** 205–209

Cochard H, Lemoine D, Dreyer E (1999) The effects of acclimation to sunlight on the xylem vulnerability to embolism in *Fagus sylvatica* L. Plant Cell Environ **22:** 101–108

Cochard H, Martin R, Gross P, Bogeat-Triboulot MB (2000b) Temperature effects on hydraulic conductance and water relations of *Quercus robur* L. J Exp Bot **51:** 1255–1259

Cochard H, Ridolfi M, Dreyer E (1996b) Responses to water stress in an ABA-unresponsive hybrid poplar (*Populus koreana* × *trichocarpa* cv. Peace): II. Hydraulic properties and xylem embolism. New Phytol **134:** 455–461

Comstock JP, Sperry JS (2000) Theoretical considerations of optimal conduit length for water transport in vascular plants. New Phytol **148:** 195–218

Comstock J, Mencuccini M (1998) Control of stomatal conductance by leaf water potential in *Hymenoclea salsola* (T and G), a desert subshrub. Plant Cell Environ **21:** 1029–1038

Cowan IR (1977) Stomatal behavior and environment. Adv Bot Res **4:** 117–228

Fuchs EE, Livingston NJ (1996) Hydraulic control of stomatal conductance in Douglas fir (*Pseudotsuga menziesii*

(Mirb) Franco) and alder (*Alnus rubra* (Bong)) seedlings. Plant Cell Environ **19**: 1091–1098

Hinckley TM, Bruckerhoff DM (1975) The effects of drought on water relations and stem shrinkage of *Quercus alba*. Can J Bot **53**: 62–72

Hubbard RM, Ryan MG, Stiller v, Sperry JS (2001) Stomatal conductance and photosynthesis vary linearly with plant hydraulic conductance in ponderosa pine. Plant Cell Environ **24**: 113–121

Jarbeau JA, Ewers FW, Davis SD (1995) The mechanism of water-stress-induced embolism in two species of chaparral shrubs. Plant Cell Environ **18**: 189–196

Jones HG, Sutherland RA (1991) Stomatal control of xylem embolism. Plant Cell Environ **14**: 607–612

Kramer PJ, Boyer JS (1995) Water relations of plants and soils. Academic Press, San Diego

Lu P, Biron P, Granier A, Cochard H (1996) Water relations of adult Norway spruce (*Picea abies* L. Karst) under soil drought in the Vosges mountains: whole-tree hydraulic conductance, xylem embolism and water loss regulation Ann Sci Forest **53**: 113–121

Meidner H, Edwards M (1996) Osmotic and turgor pressures of guard cells. Plant Cell Environ **19**: 503

Meinzer FC, Grantz DA (1990) Stomatal and hydraulic conductance in growing sugarcane: stomatal adjustment to water transport capacity. Plant Cell Environ **13**: 383–388

Monteith JL (1995) A reinterpretation of stomatal responses to humidity. Plant Cell Environ **18**: 357–364

Muromtsev NA (1981) Effects of temperature on the potential of soil water and the availability to plants. Soviet Meteorol Hydrol **5**: 68–72

Nardini A, Tyree Mt, Salleo S (2001) Xylem cavitation in the leaf of *Prunus laurocerasus* and its impact on leaf hydraulics. Plant Physiol **125**: 1700–1709

Nobel PS, Cui MY (1992) Hydraulic conductances of the soil, the root soil air gap, and the root: changes for desert succulents in drying soil. J Exp Bot **43**: 319–326

Pickard WF (1981) The ascent of sap in plants. Prog Biophys Mol Biol **37**: 181–229

Ritchie GA, Hinckley TM (1975) The pressure chamber as an instrument for ecological research. Adv Ecol Res **9**: 165–254

Rood SB, Patino S, Coombs K, Tyree MT (2000) Branch sacrifice: cavitation-associated drought adaptation of riparian cottonwoods. Trees **14**: 248–257

Saliendra NZ, Sperry JS, Comstock JP (1995) Influence of leaf water status on stomatal response to humidity, hydraulic conductance, and soil drought in *Betula occidentalis*. Planta **196**: 357–366

Salleo S, Hinckley TM, Kikuta SB, Lo Gullo MA, Weilgony P, Yoon TM, Richter H (1992) A method for inducing xylem emboli in situ: experiments with a field-grown tree. Plant Cell Environ **15**: 491–497

Salleo S, Nardini A, Pitt F, Lo Gullo MA (2000) Xylem cavitation and hydraulic control of stomatal conductance in laurel (*Laurus nobilis* L.). Plant Cell Environ **23**: 71–79

Schulte PJ, Hinckley TM (1985) A comparison of pressure-volume curve data analysis techniques. J Exp Bot **36**: 1590–1602

Schulze ED, Turner NC, Gollan T, Shackel KA (1987) Stomatal responses to air humidity and to soil drought. *In* Z Zeiger, GD Farquhar, IR Cowan, eds, Stomatal Function. Stanford University Press, CA, pp 311–321

Sperry JS (2000) Hydraulic constraints on plant gas exchange. Agric Forest Meteorol **104**: 13–23

Sperry JS, Adler FR, Campbell GS, Comstock JP (1998) Limitation of plant water use by rhizosphere and xylem conductance. Results from a model. Plant Cell Environ **21**: 347–359

Sperry JS, Alder NN, Eastlack SE (1993) The effect of reduced hydraulic conductance on stomatal conductance and xylem cavitation. J Exp Bot **44**: 1075–1082

Sperry JS, Donnelly JR, Tyree MT (1988) A method for measuring hydraulic conductivity and embolism in xylem. Plant Cell Environ **11**: 35–40

Sperry JS, Ikeda T (1997) Xylem cavitation in roots and stems of Douglas-fir and white fir. Tree Physiol **17**: 275–280

Sperry JS, Saliendra NZ (1994) Intra and inter-plant variation in xylem cavitation in *Betula occidentalis*. Plant Cell Environ **17**: 1233–1241

Sperry JS, Tyree MT (1988) Mechanism of water stress-induced xylem embolism. Plant Physiol **88**: 581–587

Tardieu F, Davies WJ (1993) Integration of hydraulic and chemical signalling in the control of stomatal conductance and water status of droughted plants. Plant Cell Environ **16**: 341–349

Tardieu F, Zhang J, Davies WJ (1992) What information is conveyed by an ABA signal from maize roots in drying field soil. Plant Cell Environ **15**: 185–191

Turner NC, Long MJ (1980) Errors arising from rapid water loss in the measurement of leaf water potential by the pressure chamber technique. Aust J Plant Physiol **7**: 527–537

Tyree MT, Cochard H, Cruiziat P, Sinclair B, Améglio T (1993) Drought-induced leaf shedding in walnut: evidence for vulnerability segmentation. Plant Cell Environ **16**: 879–882

Tyree MT, Ewers FW (1991) The hydraulic architecture of trees and other woody plants. New Phytol **119**: 345–360

Tyree MT, Sperry JS (1988) Do woody plants operate near the point of catastrophic xylem dysfunction caused by dynamic water stress? Answers from a model. Plant Physiol **88**: 574–580

Tyree MT, Yang S, Cruiziat P, Sinclair B (1994) Novel methods of measuring hydraulic conductivity of tree root systems and interpretation using AMAIZED. Plant Physiol **104**: 189–199

Valancogne C, Nasr Z (1989) A heat balance method for measuring sap flow in small trees. *In* M Borghetti, J Grace, A Raschi, eds, Water Transport in Plants under Climatic Stress. Cambridge University Press, Cambridge, UK, pp 166–173

Zeiger E, Farquhar GD, Cowan IR (1987) Stomatal Function. Stanford University Press, CA

Zwieniecki MA, Melcher PJ, Holbrook NM (2001) Hydrogel control of xylem hydraulic resistance in plants. Science **291**: 1059–1062