Constraints on water transport posed by xylem embolism: implications for drought and frost resistance in woody plants

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Contents

Presentació

D'entre els molts i interessants temes que es poden investigar, certes coincidències còsmiques que em resten completament opaques van voler que jo em dediqués a l'estudi de l'embolisme en plantes. Aquest tema té un gran desavantatge, i és bo que això quedi perfectament clar ja des d'ara: la paraula embolisme en l'accepció que s'usa en aquest treball no existeix ni en català, ni en castellà ni en anglès[1]. Això dóna idea de l'enorme rellevància del tema en general i, per extensió, de les disquisicions que se'n fan en aquesta memòria. Si bé al començament vaig percebre el problema de la inexistència de l'objecte d'estudi com un desavantatge, ara veig les coses d'una manera menys apassionada i he après a valorar-ne els avantatges. Entre els significats de la paraula embolisme que sí que existeixen hi trobem una disfunció de la circulació sanguínia que és bastant semblant al fenomen que jo he estudiat en plantes i que, en aquest cas, té unes certes implicacions pràctiques. Altres accepcions més interessants d'aquest mot d'origen grec fan referència a la inserció de dies per ajustar calendaris o de paràgrafs per ampliar textos. En castellà, la paraula també s'usa per designar confusió, barreja de moltes coses inconnexes, engany, xafarderia. Amb afany de síntesi podríem dir que el text que segueix és un gran embolisme sobre l'embolisme, afegit a aquesta presentació, i ple d'embolismes de tota mena que, tanmateix, no serveixen per ajustar calendaris.

Aclarit ja quin és l'objecte de la present memòria, podem passar a explorar-ne les motivacions. Sembla, fins i tot, un exercici saludable. Saludable i, tanmateix, difícil. És ben sabut que la major part dels fenòmens acostumen a aparèixer associats d'una manera predictible, la qual cosa té una extraordinària rellevància pràctica i ha dut als homes a distingir entre causes i efectes. De fet, aquesta regularitat aparent del cosmos és el que ha portat a l'empresa que anomenem ciència. I, tanmateix, la creença en l'existència d'aquesta regularitat, tot i haver-se demostrat molt raonable, no és pròpiament racional. Deia Einstein, que era físic, que el més sorprenent de l'Univers és que es pugui explicar. Deia Machado que deia Mairena; els dos eren poetes però l'un ho era una mica més que l'altre; que "honremos al Señor que hizo la Nada / y ha esculpido en la fe nuestra razón". En aquest context em sembla que intentar explicar els motius que em van portar a fer la tesi, sobreposant a les causes ja obscures un aire de raó conscient, és, en el millor dels casos, desenfrenat. No vull dir amb tot això que fer la tesi no fos, en algun sentit, la meva voluntat; al contrari, era el meu desig des de petit. Simplement dic que tot allò que envolta aquest desig és per a mi inescrutable. No puc, doncs, aportar llum sobre el notable fenomen pel qual un ciutadà aparentment normal esdevé un becari.

En vista del que he dit fins ara sembla fora de lloc explicar com he organitzat la present memòria i, efectivament, no ho faré. Si algú hi està interessat el remeto a la taula de continguts, apartat que he ideat amb aquest propòsit. Em limitaré a explicar com s'organitza la tesi en relació a factors

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[1] Vull dir amb això que no apareix en els principals diccionaris de les llengües respectives.

externs, al meu parer més rellevants. Pels qui encara no les heu comptades, la tesi consta de 51939 paraules. Considerant una jornada laboral normal, la qual cosa és considerar francament poc, això equival a produir unes 7 paraules per hora. Aquesta taxa és respectable, però amaga enormes diferències entre paraules. La mediana seria en aquest cas una mesura més informativa, però manquen els elements necessaris per calcular-la. La majoria de paraules no costen quasi res, algunes poden costar anys. Hi ha paraules que costen una vida, vides que ens han costat paraules. Hi ha, també, paraules que han canviat el món, paraules que duren un sospir. Lluny de tot això, podríem dir que en el present cas la inexistència de l'objecte d'estudi ha facilitat notablement la generació de mots i, a l'hora, n'ha viciat una mica els resultats. De tota manera, qualsevol preocupació pels resultats sembla desproporcionada atenent a la difusió que tindrà aquest opuscle. Considerant la irrellevància de tot plegat, haver-ne rebut alguns euros a canvi em sembla prova de la innegable magnanimitat de les autoritats que ens governen, de la infinita bondat de l'estat del benestar: pura extravagància.

Fent balanç, em quedo amb aquest diàleg dels germans Marx:

"Groucho: Va Ravelli, camini una mica més de pressa.
Chico: I perquè tanta pressa, capità? No anem enlloc.
Groucho: En aquest cas, correm i acabem d'una vegada amb això".

Donada la perfecta inutilitat d'una vida humana considerada en conjunt, millor anar-la assumint a trossets. Però, afortunadament, això no és tot. La ciència, com l'art, es basa en el plaer estètic que ens produeixen els seus resultats. Miratges de comprensió, vertígens de l'inútil. Plaer que es comparteix i s'encomana, que surt al diccionari i val la pena. Tot això tan remarcablement poc interessant ho explico per redimir-me: sóc culpable d'una curiositat estúpida, infantil, irreprimible.

CHAPTER 1

General Introduction

In the biosphere, water cannot be separated from life,
and life cannot be separated from water.
(V.I. Vernadsky, ***The Biosphere*** (1926))

Water as a resource

In both terrestrial and aquatic ecosystems, water provides the vertical mixing necessary to link the energy inputs from above with the main reservoir of chemical elements, located in a level below the surface reached by solar radiation. The ascent of sap through the stems of trees is for a forest what upwelling of deep water is for a pelagic ecosystem (Margalef 1997). In terrestrial ecosystems, the distance between photosynthetic surfaces and the reservoir of mineral nutrients ranges from 0 to just over 100 m, and the separation is imposed by life itself: where allowed by abiotic conditions, competition for light has stimulated the increase in height and the development of highly structured life forms that are capable of transporting aqueous solutions from the soil to photosynthetic surfaces.

Water relations were a key aspect in the establishment of the first terrestrial plants during the Silurian (Raven 1993). In particular, the evaporative water loss imposed by an atmosphere unsaturated with water vapour posed new problems (and offered new opportunities) to plant evolution. The cuticle was the main evolutionary innovation enabling plants to avoid internal drought (Schönherr 1982). The progressive specialization of plant tissues lead to the appearance of a conducting system composed of dead cells, and regulatory structures such as stomata, which allowed higher and at the same time more controllable rates of water transport and nutrient supply (Edwards ***et al.*** 1996). These new structures, together with mechanical innovations, allowed vascular plants to transport water to greater heights and, consequently, to grow taller than previous terrestrial plants (Raven 1986, Bateman ***et al.*** 1998). Current terrestrial vegetation is dominated almost completely by vascular plants (Tracheophyta), which include the whole divisions Pteridophyta (ferns) and Spermathophyta (gymnosperms and angiosperms).

Although it is clear that the presence of a vascular system offers important advantages for water transport, the exact mechanism that explains the ascent of sap in tall trees has remained elusive and it is still not completely understood. If we consider the nutrient requirements per unit of biomass produced, and the typical concentrations of these nutrients in soil water, it becomes apparent that the production of new biomass requires a great amount of water[1]. At the same time, in vascular plants stomata provide the pathway both for water loss and for entrance of CO_2 and, thus, transpiration and assimilation of carbon are inevitably linked. Since both the diffusion coefficient and the concentration gradient that drives diffusion are larger for water vapour than for CO_2, the ratio of molecules of water vapour transpired per molecule of CO_2 gained ranges between 100 and 500 in most C_3 and C_4 plants (Lambers ***et al.*** 1998). As a result, considerable fluxes of water are required to sustain typical growth rates. A substantial amount of energy is needed to pull several litres of water per day to a height of 100 m(2), as done in the tallest trees. If plants had to raise this water by active mechanisms, this would be a phenomenal expenditure of metabolic energy.

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[1] 200-300 kg of water per kg of dry matter according to Margalef (1992).

[2] Approximately 100 J per litre considering only the effect of gravity.

The currently accepted cohesion theory for the ascent of sap is based on the fact that terrestrial plants bridge a steep water potential gradient between liquid water in the soil and water vapour in the air, which is typically around 100 MPa. According to this theory, solar radiation provides the energy source for water evaporation in leaves, which together with the cohesive strength of liquid water makes the transport along the gradient of water potential possible (Steudle 1995, Tyree 1997). As a result of evaporation a curvature in the water menisci of apoplastic water is created in the evaporation sites. Surface tension forces lower xylem pressure in the liquid water in direct contact with the menisci, which is transmitted throughout a continuous water column to the root apices. In this way, evaporation in leaves drives water transport along an hydrostatic gradient throughout the xylem, and a water potential gradient between the soil and root cells. Hales (1727; cited in Floto 1999) was the first to describe the main elements of this theory. However, it was not until the end of the XIX century that the theory was established in detail and given a name (Dixon & Joly 1894). The contribution of van den Honert (1948), who proposed an analogy with electric circuits to study water flow along the different compartments of the soil-plant-atmosphere continuum (SPAC), supposed an important theoretical advance towards our current understanding of water transport in plants.

The cohesion theory has several important implications. The main one, and the one that explains why this theory is still controversial, is the prediction that water in the xylem is at negative pressures (tensions). At ambient temperature (20ºC), liquid water at pressures below +2.3 kPa is thermodynamically unstable, and tends to evaporate. Therefore, if the cohesion theory is correct all water transport must take place under a metastable state. It was not until Scholander ***et al.*** (1965) demostrated the long-term permanence of negative pressures in the xylem with the introduction of the pressure chamber, that the cohesion theory became the current paradigm. However, the ascent of sap in plants is far from being completely clarified, and despite important studies supporting the cohesion theory (Holbrook ***et al.*** 1995, Pockman ***et al.*** 1995, Sperry ***et al.*** 1996, Tyree 1997, Stiller & Sperry 1999), recent experimental results suggest that the mechanism is more complex than previously thought. In the first place, direct measurements of xylem pressure using the xylem pressure probe introduced by Balling & Zimmermann (1990) failed to report the large tensions required by the cohesion theory (Zimmermann ***et al.*** 1994, 1995). These authors also raised doubts on the validity of the pressure chamber to adequately measure xylem pressure in transpiring plants (Zimmermann ***et al.*** 1994). Although some of the discrepances between the two methods disappear when leafy twigs measured with the pressure chamber are covered before measurement to avoid the non-equilibrium conditions associated with transpiration (Wei ***et al.*** 1999, Zimmermann ***et al.*** 2000), some inconsistencies remain (Melcher ***et al.*** 2001).

The second objection to the current interpretation of the cohesion theory comes also from the existence of large negative pressures inside the xylem. As we have already seen, liquid water at large tensions is thermodynamically unstable and, thus, as pressure decreases in xylem conduits it is eventually reached a point in which water evaporates. This phenomenon is called cavitation (Apfel 1972, Sedgewick & Trevena 1976, Plesset & Prosperetti 1977). Homogeneous cavitation of water normally occurs below –100 MPa (Green ***et al.*** 1990), a pressure well below the values predicted by the cohesion theory. However, the presence of heterogeneous nucleating sites causes the cavitation of water within the physiological range of xylem water potentials. Cavitation in xylem conduits is followed by the entering of air from the surrounding tissue to form embolisms (Tyree & Sperry 1989). These embolisms were considered irreversible unless positive or near-positive pressures were reached in the xylem, which seldom happens in terminal branches of transpiring trees (Tyree & Sperry 1989, but see Pickard 1989). Recently, a considerable amount of observations has accumulated supporting that active refilling of previously cavitated vessels can occur even at high xylem tensions (Salleo ***et al.*** 1996, Canny 1997, McCully ***et al.*** 1998, Zwieniecki & Holbrook, 1998, Tyree ***et al.***, 1999, Salleo ***et al.*** 2000, Melcher ***et al.*** 2001; but see also the methodological criticisms by Cochard ***et al.*** 2000, 2001). There is no known mechanism within the cohesion theory capable of explaining these results. It has been proposed that living xylem parenchyma surrounding cavitated vessels may play a role in vessel refilling (Holbrook & Zwieniecki 1999), and some experimental support of this view has been obtained (Zwieniecki & Holbrook 2000).

Water as a limiting factor

As all resources, water can become a limiting factor. In fact, water availability has been recognized as one of the main factors controlling the distribution of vegetation at the global scale (Kramer 1983, Woodward 1987, Grace 1997, Walter 1998). It is enough to compare a world map of the main vegetation types with the geographic distribution of precipitation to be convinced of this. The limiting role of water availability is usually ascribed exclusively to the need to avoid desiccation and, at the same time, maintain carbon assimilation. Because assimilation and transpiration are linked, there is an obligate compromise between carbon gain and the avoidance of excessive water loss. The role of water in nutrition is considered to be of secondary importance under most circumstances, because the costs of carbon acquisition are usually greater than those of nutrition in terms of both water and nutrients. As a result, the demands for water and for nutrients tend to vary independently (Bloom ***et al.*** 1985).

From the previous paragraph, it is clear that water flux through a plant should be high enough to secure carbon assimilation and nutrition. In this way, water availability poses an upper limit to productivity. At the same time, a balance between water entering roots and water loss from leaves should be maintained to prevent desiccation. Since physiological processes are extremely sensitive to water deficit (Bradford & Hsiao 1982) the conservation of water to maintain reasonably high water potentials tends to be the main concern of plants exposed to water shortage, at least over the short term.

Although some plants can tolerate desiccation in the vegetative state (Gaff 1989), these "resurrection plants" constitute less than 0.1% of all Spermatophyta, and are generally limited to a stature of less than 1 m (Raven 1986). The rest of plants have adapted to drought either by completely avoiding water shortage or by partial tolerance.

There are several mechanisms that allow plants to avoid drought: (1) most desert annuals have adapted to germinate only after substantial rains and complete their life cycle before the soil dries again. (2) Where the environment is more predictable, the seasonal reduction of leaf area during the driest months has a similar effect. This happens in summer deciduous species, and has the additional advantage of being compatible with perennial life forms. (3) Phreatophytes escape drought by producing extremely deep roots that tap the water table. In general, drought-avoiding plants have high rates of photosynthesis and growth. However, these advantages are offset by the costs associated with this strategy (short life, production of new leaves or deep roots), and these species are replaced by drought-tolerant evergreens as water availability increases (Mooney & Dunn 1970).

Plants accommodate the supply of water from the soil with the evaporative demand imposed by the atmosphere. As a result, the different levels at which a plant can show tolerance to drought correspond to the main compartments of the SPAC, including: (1) water loss from leaves, (2) water absorption by roots plus radial transport to root xylem, and (3) water transport in the xylem. These compartments are described in the following paragraphs. Throughout the pathway, but particularly in roots and leaves, osmotic adjustment and the modification of cell-wall elasticity allow plant cells to maintain turgor at low water potentials (Tyree & Jarvis 1982).

Since diffusion from mesophyll cells to the air offers the greater resistance to water movement through the SPAC, canopy structure and stomata are key elements in the regulation of water flow through plants. Low maximum stomatal conductance and a high sensitivity to moisture conditions are typical characteristics of drought-tolerant plants. The response of stomata to a variety of environmental variables has been the object of extensive research (reviewed in Whitehead 1998). It is established that stomatal closure is linked to transpiration rate (Mott & Parkhurst 1991, Monteith 1995) and xylem hydraulic conductivity (Hubbard ***et al.*** 2001), in both cases probably via local changes in water potential within the leaf. There is also strong evidence supporting that stomata can respond to soil moisture by chemical signals (ABA) originated in the root system (Davies & Zhang 1991).

In most vascular plants water uptake occurs primarily, if not exclusively, through roots. The influence of roots in drought-tolerance is, thus, obvious. Within a given soil, the extension, shape and structure of the root system determines water availability (Kramer & Boyer 1995). Plants with extensive root systems, of which phreatophytes are the extreme case, are in this way able to maintain gas exchange even when the surface horizons of soil are completely dry. Once water enters a root, the radial pathway to root xylem normally imposes the major resistance to water flow (Weatherley 1982). Recent results suggest that the different pathways in radial water transport combine to regulate water uptake according to the demands from the shoot and that this regulation can play an important role in resistance to drought (Steudle 2000).

The ability of xylem to transport water from roots to leaves is determined by its hydraulic conductivity. Although the xylem is designed to offer the least resistance to water flow and has hydraulic conductivities millions of times greater than parenchymatous tissues (Pallardy 1989), its contribution to total resistance from soil to leaves can be substantial in tall plants (20-60% in woody species according to Sperry (1995); see also modelling results in Sperry ***et al.*** 1998). Since the conductivity of a circular conduit is related to the diameter raised to the fourth power, the dependence of hydraulic conductivity on conduit size is critical (Zimmermann 1983). In agreement with lower rates of water transport, conducting elements tend to be smaller in xeric areas (Baas 1986). Hydraulic conductivity is not a constant property, but tends to decrease at low water potentials as a result of xylem embolism. High levels of xylem embolism are dangerous because they can lead to a complete loss of conductivity ("runaway embolism", Tyree & Sperry 1988) and, eventually, to branch (or plant) dieback (***e.g.,*** Rood ***et al.*** 2000). Plants differ widely in their vulnerability to drought-induced embolism and this variation is associated with the range of water potentials experienced in the field. Species from arid environments are normally more resistant to xylem embolism than plants from mesic or humid areas (Hacke ***et al.*** 2000; Pockman & Sperry 2000; Sperry 2000). Plants also store some water in the wood and cortex, which can temporally supply the water for transpiration (Holbrook 1995). Although the contribution of stored water is quantitatively small in most plants, it can be important under some circumstances (Waring ***et al.*** 1979, Borchert 1994, Goldstein ***et al.*** 1998) and in succulents it is the key adaptation to drought (Nilsen ***et al.*** 1990).

Normally, tolerance at one of the levels (***e.g.,*** roots) allows avoidance at another (***e.g.,*** leaves). For that reason, but also because of the trade-offs and interrelationships between the different characters related to drought-resistance (see below), plants are better classified according to its general strategy to cope with water shortage. This strategy normally includes several characters associated in predictable ways and leads to the general concept of plant hydraulic architecture (Zimmermann 1983). Leonardo da Vinci was probably the first to note that wood area is approximately conserved as we move from the base of a tree to its terminal branches. The generalization of this idea lead to the "pipe model" theory (Shinozaki ***et al.*** 1964) and suggests that the relationship between wood or, more precisely, sapwood and the supported leaf area is an important component of plant design (Huber 1928, Zimmermann 1983). In recent literature, the inverse of this relationship (***i.e.,*** leaf area per unit of sapwood area, A_L:A_{SW}) has been commonly used in comparative studies to give an idea of the total water transport supported per cross-sectional area of xylem. It has been repeatedly found that the A_L:A_{SW} ratio is lower in species and populations from drier sites (Mencuccini & Grace 1995, Comstock 2000, DeLucia ***et al.*** 2000). For the same reason, the relationship between the area available for water uptake (***i.e.,*** surface area or dry weight of roots) and leaf area (or the weight of aboveground parts) has also received considerable attention. This ratio tends to increase in arid areas (Kramer 1983, but see Kummerow 1981), reaching values of ***ca.*** 7 kg kg^{-1} in some desert ecosystems (Archibold 1995).

The combination of high evaporative demand and low water availability is not the only scenario under which water stress can occur. Low temperatures may also lead to an stress functionally very similar to water shortage, and usually referred to as frost-drought (Tranquilini 1982). Freezing of water along the SPAC, typically in the soil and/or xylem, limits or completely interrupts water transport and, as a result, causes acute plant desiccation. The formation of extracellular ice crystals also causes cell dehydration because of the very negative water potential of ice and the resulting gradient towards the extracellular spaces. Adaptations to frost-drought include changes in the properties of the cell wall and in the osmotic potential (Rajashekar & Lafta 1996), and resistance to freezing-induced xylem embolism (Langan ***et al.*** 1997, Pockman & Sperry 1997). Since vulnerability to cavitation caused by freezing is related to the size of xylem conduits (Sperry & Sullivan 1992, Davis ***et al.*** 1999b, see below), small conduits should be typical of plants from cold regions. This prediction is confirmed by the decrease in vessel member length and diameter with increasing latitude or altitude in mesic floras (Baas 1986).

Xylem embolism and its ecological implications

Although transpirational losses and soil water availability are major determinants of plant water economy, there is an increasing body of evidence supporting that the efficiency of water transport in the xylem and internal storage play also an important role. The continuation of gas exchange is a requisite for all plants that maintain physiological activity under drought conditions. In consequence, the complete blockage of the conducting system due to xylem embolism is something to be avoided (Tyree & Sperry 1989, Sperry 1995). Some authors have recognized resistance to xylem cavitation as perhaps the most important parameter that determines drought resistance (Tyree & Ewers 1991). Despite previous experimental (Kelso ***et al.*** 1963, Milburn & Johnson 1966) and theoretical studies (Oertli 1971, Pickard 1981) on xylem embolism in relation to sap ascent, it was not until the brilliant synthesis by Zimmermann (1983) that the systematic study of the mechanism and implications of xylem embolism began.

There is now much theoretical and experimental evidence (Sperry & Tyree 1988, Cochard ***et al.*** 1992, Sperry & Saliendra 1994, Jarbeau ***et al.*** 1995) supporting that drought-induced xylem embolism is caused by air seeding as proposed by Renner (1915) and, more recently, by Zimmermann (1983). According to this hypothesis, cavitation occurs when air from outside a functional conduit is aspirated through the pores of the conduit wall, normally the pit membrane that separates adjacent xylem conduits. This aspirated air forms small bubbles inside the functional conduit that provide the nucleation sites for cavitation. The pressure difference required to push air into a water filled conduit is inversely proportional to the radius of the pore and, thus, pore size is the key factor determining vulnerability to drought-induced cavitation. Pit membranes function, in this way, as valves preventing the spread of embolisms throughout the conducting system. In conifers, the mechanism is slightly more complicated, being the displacement of the torus in bordered pits what provides the required sealing.

Freezing-induced xylem embolism occurs through a completely different process. Since the solubility of air is lower in ice than in liquid water, freezing forces air out of the xylem sap solution forming bubbles. On thawing, these bubbles can either re-dissolve or nucleate cavitation, depending on the balance between the surface tension forces and xylem pressure (Lybeck 1959; Hammel 1967, Sucoff 1969; Zimmermann 1983). Larger conduits will result in larger air bubbles after freezing and, thus, they will tend to be more vulnerable to freezing-induced embolism. However, some experimental results show an effect of minimum freezing temperature rather than freezing itself on embolism formation (Lo Gullo & Salleo 1993, Pockman & Sperry 1997). This is difficult to fit into the previous mechanism, because if bubbles formed after a freeze-thaw cycle are the only cause of embolism, the minimum temperature below the freezing point should be unimportant.

The hydraulic conductivity of most woody species changes seasonally as a result of xylem embolism (Sperry ***et al.*** 1994, Magnani & Borghetti 1995, Pockman & Sperry 2000). At the same time, it has been shown that, as suggested by Tyree & Sperry (1988), plants tend to operate at water potentials close to the values causing catastrophic xylem dysfunction (Sperry 1995, Davis ***et al.*** 1999a, Kolb & Sperry 1999a, Hacke ***et al.*** 2000, Pockman & Sperry 2000). These narrow safety margins raise the question of why there is a selective disadvantage in having an overly resistant xylem. One possibility is that there are direct benefits from cavitation. Declining hydraulic conductivity has been shown to decrease stomatal conductance and, thus, amplify the stomatal regulation of transpiration during drought (Saliendra ***et al.*** 1995, Oren ***et al.*** 1999, Hubbard ***et al.*** 2001). As a result, xylem embolism may be considered an additional control mechanism which, together with stomatal activity, helps the plant to adjust water uptake to maintain an acceptable water balance over the longest time. Two questions are particularly relevant. First, this advantage could easily disappear if there is competition for water between species or individuals. Additionally, it requires that xylem cavitation is at least partially reversible. Reversibility is the main reason why xylem cavitation has been traditionally considered a “negative” dysfunction while stomatal closure has been regarded as a “positive” control mechanism. As we have seen in a previous section, evidence is accumulating supporting that cavitations and embolisms are reversible even at negative xylem water potentials. However, this is far from being completely clarified, and a recent study has shown that cavitation and refilling cycles can have a long-term impact in some species by weakening the cavitation resistance of xylem (cavitation fatigue, Hacke ***et al.*** 2001b).

A second reason to expect narrow safety margins may be that there are indirect costs associated with having a resistant xylem. Regarding this, the different mechanisms of drought- and freezing-induced xylem embolism have an important implication. While there is a direct link between conduit size and freezing-induced embolism, there is no obvious reason to expect such a relationship with drought-induced embolism (Tyree ***et al.*** 1994). If wider conduits were always more vulnerable to embolism, there would be a strong trade-off between efficiency and safety of the conducting system, with important ecological and evolutionary implications (Tyree ***et al.*** 1994). A drought-related trade-off across taxa has not been consistently reported in the literature (***e.g.,*** Cochard 1992; Sperry & Sullivan 1992; Sperry ***et al.*** 1994), and although some meta-studies show a weak negative correlation between efficiency and security at the global scale (Tyree ***et al.*** 1994; Pockman & Sperry 2000), it still remains an open question in the study of plant water transport (Kolb & Sperry 1999a). A recent study by Hacke ***et al.*** (2001a) shows a strong linkage between wood density and resistance to cavitation, which suggests that the relationship between hydraulic and mechanical considerations is probably more important than previously thought.

The possible relationships between resistance to xylem embolism and conducting efficiency or wood density have implications at several scales, from tissues to communities. Plant tissues differ in the conditions under which they operate. Within a transpiring plant, water potential decreases from roots to leaves, a decrease that can be of several MPa. Therefore, it would be reasonable to expect also a gradient of hydraulic properties within plants. Vulnerability to drought-induced embolism, the size of xylem conduits, and hydraulic conductivity has been shown to decline from roots to twigs (Zimmermann 1983, Sperry & Saliendra 1994, Sperry & Ikeda 1997). These ideas lead Zimmermann (1983) to the concept of plant segmentation, by virtue of which the hydraulic construction of plants would allow them to sacrifice peripheral parts (***e.g.,*** terminal branches) to protect more valuable organs (***e.g.,*** the main stem). This hypothesis has obtained considerable experimental support (Tyree ***et al.*** 1993, Joyce & Steiner 1995) and was extended to the root system by Alder ***et al.*** (1996). Other implications of anatomical gradients within plants are just becoming apparent: tappered conduits from the trunk to petioles can buffer hydraulic conductance from path length-effects, thus allowing plants to grow in height with minimum hydraulic costs (West ***et al.*** 1999, Becker ***et al.*** 2000).

Because of the differences in water potential, the relevant question in comparing roots and stems is whether roots live closer to the critical water potentials causing dangerous levels of xylem embolism. Some studies (***e.g.,*** Hacke ***et al.*** 2000) indicate that safety margins are indeed much narrower in roots, and several arguments have been proposed to explain these results, ranging from the easier refilling of roots due to higher water potentials to the lower construction costs of root xylem (Sperry & Saliendra 1994, Alder ***et al.*** 1996, Kolb & Sperry 1999b, Hacke ***et al.*** 2000). In this way, root xylem seems to be the limiting factor for the maintenance of gas exchange during drought for many woody species (Kolb & Sperry 1999b). A picture is emerging in which roots act as hydraulic valves regulating water uptake in plants, with potential large-scale implications (Jackson ***et al.*** 2000). However, little work has been done on root xylem to date (Sperry 2000).

The possible existence of universal trade-offs between plant traits has also important implications among species. It is becoming clear that the relationships among fundamental functional traits are constrained in a similar manner among taxonomically diverse species from a wide range of biomes, resulting in a global convergence in plant functioning (Reich ***et al.*** 1997, Stratton ***et al.*** 2000, Meinzer ***et al.*** 2001). Recent models based on relatively simple transport constraints through “fractal-like” vascular networks (Enquist ***et al.*** 1998, West ***et al.*** 1999, Enquist & Niklas 2001) or on efficient transportation networks (Banavar ***et al.*** 1999) suggest that many macroecological features of communities may be due to few general constrictions and trade-offs operating at the level of the individual, and point to the importance of size and allometric scaling in the organization of plant communities. The clarification of the underlying relationships between the traits involved is thus of great importance.

Climate change

Besides the undeniable theoretical interest, the study of the different strategies that plants use to cope with water shortage and the underlying mechanisms on which they are based has also implications on applied grounds. Climate change is modifying the balance between atmospheric demand and water availability on a global scale, and this modification is predicted to increase in the near future (IPCC 2001). In many areas, such as the Mediterranean, this indirect effect of global warming will have a major regulatory role (Shaver ***et al.*** 2000). The prediction of the effects of climate change on biological communities is of obvious practical interest, and has attracted enormous scientific effort. Species are predicted to respond to climate change via adaptation ***in situ*** and/or modification of their physiology, phenology or distribution, with the resulting changes in species interactions and community structure and composition. Some of these changes have been already observed (Hughes 2000).

There are different approaches to study the effects of climate change or, more generally, global change on plant species, including[3]: (1) the description of the ecophysiological characteristics and/or other factors that determine the actual distribution of species (***e.g.,*** Peñuelas ***et al.*** 1998). (2) The study of the response to past or present situations that are representative of expected future conditions. These include past climates (***e.g.,*** Peñuelas & Azcón-Bieto 1992), extreme climatic events such as droughts (***e.g.,*** Peñuelas ***et al.*** 2000), or anomalous concentrations of carbon dioxide (***e.g.,*** Tognetti ***et al.*** 1998). (3) Controlled experiments, preferably under field conditions (***e.g.,*** Borghetti ***et al.*** 1998). (4) Modelling (***e.g.,*** Osborne ***et al.*** 2000). Since the responses to climate change are likely to be very complex, an integrated effort using the

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[3] All the examples are from studies conducted in the Mediterranean basin.

data from (1) to (3) in the parameterisation and validation of biologically realistic models would probably be the most powerful approach.

Objectives

The aim of this work is to explore some of the open questions in the study of xylem embolism and its ecological implications that have been highlighted in the previous pages. These questions range from considerations on the mechanism of embolism repair in plants to ecophysiological characterizations aimed at predicting the possible impacts of climate change on the species studied. All the chapters but one deal with water stress in Mediterranean ecosystems. The general objectives of the thesis are the following:

(1) to study the **mechanism of freezing-induced xylem embolism** in ***Larrea tridentata***. [Chapter 2]

(2) to compare **xylem structure and function between roots and stems**, specially with regard to their possible role in limiting water transport. [Chapters 2, 3 and 4]

(3) to test the existence of a **trade-off between conducting efficiency and security in the xylem** in species from a given community, and explore its possible causes and consequences. [Chapters 3 and 4]

(4) to study the **safety margins** at which the different species of a given community operate. [Chapters 2, 3 and 4]

(5) to characterize the **strategies to cope with drought stress** of several Mediterranean species from different communities. [Chapters 3, 4, 5 and 6]

(6) to predict the likely **effects of climate change** on the species studied. [All the chapters to a greater or lesser extent, but particularly Chapter 7]

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CHAPTER 2

The vulnerability to freezing-induced xylem cavitation of *Larrea tridentata* in the Chihuahuan desert

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> ***One*** Larrea ***(a perennial shrub) was found within the inner 300 feet [from the Zero point where the first atomic bomb ever exploded on July 16, 1945, at the Trinity site, NM]. It was so large that one observer claimed it "must have germinated after almost the first rain after the bomb was set off".***
>
> (F.M. Szasz, ***The Day the Sun Rose Twice*** (1984);
> the text between brackets is mine)

Resum

En aquest treball s'estudia la vulnerabilitat a l'embolisme induït per glaçada en una població de ***Larrea tridentata*** al desert de Chihuahua (Nou Mèxic, USA). Al camp vam mesurar les temperatures de la fusta, l'aire i el sòl, els potencials hídrics al sòl i a les fulles, i l'embolisme nadiu present a rels i branques. Aquestes mesures les vam completar amb estudis de l'anatomia del xilema i experiments al laboratori en els quals una cambra aïllada connectada a un bany tèrmic s'utilitzava per congelar mostres d'arrels i branques en condicions controlades. Els nostres resultats els vam comparar amb els obtinguts per Pockman & Sperry (1997) en una població de ***L. tridentata*** al desert de Sonora. Encara que la població del desert de Chihuahua va resultar una mica més resistent a l'embolisme induït per congelació, l'impacte de les glaçades hi va ser també major perquè estava sotmesa a temperatures molt més baixes. Aquest resultat concorda amb l'observació d'una elevada mortalitat de branques a la població estudiada (***ca.*** 50%), i suggereix que les baixes temperatures contribueixen a limitar la distribució actual de ***L. tridentata*** a la zona central de Nou Mèxic. Dins de plantes, les branques estaven més limitades per les glaçades que les arrels, al contrari del que passa amb l'embolisme induït per sequera. Els experiments al laboratori van corroborar les troballes de Pockman & Sperry (1997) quant a l'existència d'una relació entre la temperatura mínima de congelació i el % d'embolisme. La correlació que vam observar entre l'àrea de l'exoterma de baixes temperatures produïda durant els tractaments i el % d'embolisme resultant suggereix que la congelació de l'aigua a l'interior de les cèl.lules del parènquima estava associada amb l'ocurrència de l'embolisme. Aquesta troballa concorda amb evidències recents que apunten cap a un paper actiu de les cèl·lules vives en el transport d'aigua a llargues distàncies dins la planta.

Abstract

The vulnerability of ***Larrea tridentata*** to freezing-induced xylem embolism was studied in a Chihuahuan desert population (central New Mexico, USA). In the field we measured wood, air and soil temperatures, soil and leaf water potentials, and native embolism in roots and branches. These measurements were combined with anatomical studies and laboratory experiments in which an insulated chamber attached to a temperature bath was used to freeze stem and root samples under controlled conditions. We compared our results with the measurements by Pockman & Sperry (1997) in a Sonoran desert population of ***L. tridentata***. The comparison showed that although the Chihuahuan population was slightly more resistant to freezing-induced xylem embolism, the impact of freezing was also greater in this population because of the much lower temperatures. This result agrees with the observation of ***ca.*** 50% stem mortality in the population studied, and suggests that low temperatures contribute to limit the present distribution of ***L. tridentata*** in central New Mexico. Within plants, stems were more limited by freezing than roots, as opposed to what happens with drought-induced embolism. Our laboratory experiments corroborated the findings by Pockman & Sperry (1997) regarding the existence of a relationship between minimum freezing temperature and % embolism. The correlation observed between the area of the low temperature exotherms produced during the freezing treatments and the resulting % embolism strongly suggested that the freezing of water inside parenchyma cells was associated with the occurrence of xylem embolism. This finding adds to the existing evidence supporting an active role of living cells in long-distance water transport.

Introduction

Low temperatures and, in particular, freezing injury, have been reported as the main factors limiting plant distribution in many habitats (Burke ***et al.*** 1976, Sakai & Larcher 1987, Woodward 1987). While supercooling (Rada ***et al.*** 1985) and other mechanisms (***e.g.,*** Beck ***et al.*** 1984) have been identified that prevent the freezing of water in plant cells, freezing in xylem conduits (apoplast) generally occurs within a few degrees below 0. Among the consequences of apoplastic freezing are cell dehydration and collapse (Rajashekar & Lafta 1996), and the disruption of long-distance water transport as a result of xylem embolism (Zimmermann 1983, Tyree & Sperry 1989). The vulnerability to freezing-induced xylem embolism and its ecological significance have been studied in a number of species, including conifers (Sperry & Sullivan 1992, Sperry ***et al.*** 1994), diffuse-porous (Sperry & Sullivan 1992, Hacke & Sauter 1996, Langan ***et al.*** 1997) and ring-porous species (Cochard & Tyree 1990, Sperry & Sullivan 1992, Hacke & Sauter 1996).

Freezing-induced xylem embolism occurs because the solubility of air is lower in ice than in liquid water. As a result, freezing forces air out of the xylem sap solution forming bubbles (Lybeck 1959, Sucoff 1969, Zimmermann 1983). On thawing, these bubbles can either re-dissolve or nucleate the cavitation of water inside the conduits. Whether a bubble dissolves or not depends on the balance between the surface tension forces and xylem pressure (Ψ_x). Cavitation will occur whenever $\Psi_x \leq -2T/R$, where T is the surface tension of the xylem sap and R the radius of curvature of the bubble (Sperry & Sullivan 1992, Davis ***et al.*** 1999). The cavitation of water inside a conduit is followed by the entering of air from the surrounding tissue until it becomes air-filled or embolized (Tyree & Sperry 1989). An embolized conduit no longer contributes to water transport unless it is refilled by liquid water. In principle, refilling can only occur if xylem pressure is close to the atmospheric pressure. More precisely, if $\Psi_x \geq -2T/R$, where R is now the radius of the larger gas void in the cavitated conduit. However, active refilling has been recently observed even at high xylem tensions (Salleo ***et al.*** 1996, Zwieniecki & Holbrook 1998, Tyree ***et al.*** 1999) and possible mechanisms have been proposed (Salleo ***et al.*** 1996, Holbrook & Zwieniecki 1999, Zwieniecki & Holbrook 2000).

Because vulnerability to freezing induced cavitation is tightly linked to conduit dimensions, we might expect that, as happens with drought-induced embolism (Hacke ***et al.*** 2000), roots should be also more vulnerable to freezing-induced embolism than aboveground tissues. Roots tend to have much wider conduits than branches (Zimmermann 1983, Ewers ***et al.*** 1997) and unlike what happens with drought, the relationship between conduit size and vulnerability to embolism caused by freezing is both understood and well established (Tyree ***et al.*** 1994, Davis ***et al.*** 1999). Although the soil is a good insulator, minimum soil temperatures can easily fall below zero in the first centimeters of soil, even in temperate areas. In a recent study, Jaquish & Ewers (2001) showed that seasonal levels of embolism remained much lower in surface roots than in stems for two ring-porous species. However, our knowledge about the hydraulic limitations posed by belowground tissues, particularly in relation to freezing, is still very limited.

Pockman & Sperry (1997) studied the vulnerability to freezing-induced xylem embolism in creosote bush (***Larrea tridentata*** Cov.), and related it to its distribution. ***L. tridentata*** is an evergreen, drought-tolerant shrub that lives throughout the warm deserts of North America (MacMahon 2000). In their study, Pockman & Sperry found that the temperatures that caused a complete xylem embolism in the lab (−16-(−20)ºC) corresponded closely to the minimum recorded temperatures in the northern limit of the range of ***L. tridentata***. With one exception: the relationship did not hold for the Chihuahuan desert, the coldest of the three warm deserts of southwestern USA, where ***L. tridentata*** occurs in spite of lower mean annual and extreme minimum temperatures. The authors suggested that this different behavior in the Chihuahuan desert may be related to the existing differences in ploidy between the populations of the three deserts. Another interesting result of the study was that, below the temperature that began to cause significant losses of hydraulic conductivity (***ca.*** –11ºC), % embolism increased linearly with minimum temperature. This result is difficult to explain because if, as normally assumed, bubbles formed after a freeze-thaw cycle are the only causes of embolism, the minimum temperature below the freezing point should be unimportant.

In this study we explore the vulnerability to freezing-induced xylem embolism in a ***L. tridentata*** population from the Chihuahuan desert. We combined field measurements of xylem embolism in a natural population in central New Mexico with laboratory measurements of xylem embolism of roots and branches under carefully controlled freezing conditions. The objectives were: (1) to test whether this population was more resistant to freezing-induced embolism than the one studied by Pockman & Sperry (1997); (2) to explain the relationship between embolism and minimum temperature found by Pockman & Sperry (1997); and (3) to assess the possible role of the vulnerability of the root system in the response to freezing temperatures.

Materials and methods

Study site

All the field monitoring and most measurements were conducted at the Five Points area of the Sevilleta National Wildlife Refuge, since 1989 an NSF supported Long Term Ecological Research site in central New Mexico, USA (34º 22’ N, 106º 4’ W, elevation: 1610 m). The long-term (1961-1990) mean temperature is 13.3ºC, and annual rainfall averages 242 mm. The number of days with sub-zero temperatures per year ranged from 103 to 160 between 1989 and 2000 (Sevilleta LTER data). The region is a transition zone for a number of biomes, including the Great Plains grassland, the Chihuahuan desert, and the Colorado Basin shrub-steppe. ***L. tridentata*** expanded its distribution in this area in the early XX century, probably as a result of a northward invasion (Buffington & Herbel 1965; but see Betancourt ***et al.*** 2001). With the exception of several isolated populations, the area represents the northern limit of ***L. tridentata*** and contains some of the populations of ***L. tridentata*** living under coldest conditions (Pockman & Sperry 1997). Plants of similar size (ranging between 0.9 and 1.5 m in height) from a single population were selected for use in all experiments. The study period lasted from October 2000 to May 2001.

Field monitoring

Air, soil, stem, and root temperatures were monitored at the Five Points population using a datalogger (Model CR-10X, Campbell Scientific, Logan, UT, USA). From late October to early December 2000 we measured the temperatures of stems ***ca.*** 8 cm in diameter from nine different individuals using copper-constantan thermocouples (AWG No. 36). Additional thermocouples were placed in a piece of dry wood, as a reference for temperatures observed in living stems, and in the soil (***ca.*** 10 cm deep) under one of the monitored plants. Air temperature was measured with a thermistor sheltered from direct solar radiation. In early December 2000, additional thermocouples were placed in the same area. From then to the end of the study, the monitoring included 10 different plants. In four of them two thermocouples were placed in the same stem, one proximal (***ca.*** 8 cm in diameter), and the other distal (***ca.*** 5 cm in diameter). In the other plants we maintained the proximal thermocouples installed in October. Two soil temperature profiles were also installed under studied plants, with thermocouples at 10 20, and 30 cm depth. In each profile, thermocouples were also installed in two roots (7-10.5 cm diameter), at depths between 10 and 20 cm. The air thermistor was substituted by four air thermocouples shielded from direct solar radiation, two installed within the canopy of studied plants, and the other two in the open spaces between them (***ca.*** 1.25 m in height). Another thermocouple was also added in a second piece of dry wood.

From late October to early December temperatures were recorded every 2 hours until air temperature fell below 0ºC. At air temperatures below 0ºC all the temperatures were measured every 10 s and the 5-min average was stored. From early December on we increased the storage frequency to 2-min averages to capture exotherms and endotherms associated with freezing and thawing. Wood thermocouples were installed in small holes into the outer layer of xylem as described in Pockman & Sperry (1997). We used epoxy glue to hold the thermal junctions in place.

Freezing exotherms were revealed by sharp increases in wood temperature without a parallel change in air or dry wood temperatures. The starting points were identified by means of a semi-automatic procedure, but it was impossible to analyze them in the same detail as in the laboratory experiments (see below) because of the lower frequency of storage and the much higher temporal variability of air temperature in the field.

Water potentials

We used a Scholander pressure chamber (PMS Instruments, Corvallis, OR, USA) to measure leaf water potentials (Turner

1987). Predawn (04:30-05:30 solar hours) and midday water potentials (11:30-12:30 solar hours) were measured through the study period in two terminal stems of each of the ten monitored plants. Water potentials were measured also in the lab before each freezing treatment in all the experiments. A single profile of soil psychrometers was installed in the study area in early February 2001 to monitor soil water potential at different depths. The sensors were placed at 15, 30, 50 and 80 cm.

Sampling for native xylem embolism and freezing experiments

We defined xylem embolism as the percent loss in hydraulic conductivity (in relation to the maximum) due to gas blockage of xylem vessels. Native xylem embolism refers specifically to the percent loss of conductivity under field conditions (Sperry, Donnelly & Tyree 1988).

Native xylem embolism of the stems was measured through the study period in the Five Points population. At each measurement date, 10 different individuals within a radius of 100 m from the monitored plants were sampled. We cut one stem per plant if only native embolism was to be measured, and three per plant if freezing experiments were to be carried out (Langan ***et al.*** 1997; see below). Initial measurements showed that the variability in native embolism within plants was approximately half than among plants (n=4 plants and n'=5 stems per plant). Stems were of similar size and at least 0.8 m in length. This length was enough that few, if any, of the conduits embolized during collection extended to the measured segments (the mean of maximum vessel length was 0.35 m, n=3; air-injection method, Ewers & Fisher 1989). Measured plants were never sampled again in the following measurement dates. Branches were triple-bagged with a humid towel and carried to the laboratory to be measured within 3-4 hours of collection.

Root native embolism was measured only once, in connection with a freezing experiment. Five plants were excavated and 3 roots per plant were cut at the distal end at least at 0.5 m from the crown. The entire root crown of each plant with the roots attached was triple bagged with a humid towel and carried to the University of New Mexico. Once in the laboratory the proximal ends of the roots (as close as possible to the crown) were cut underwater. Considering that the mean of maximum vessel length for roots was 0.46 m (n=3, air-injection method, Ewers & Fisher 1989) it is likely that some of the conduits embolized during collection reached the measurement segment in the shortest roots. However, it is unlikely that any of them completely crossed the segment and the ones that reached it were probably very few. Indeed, no effect of the total length of the sampled roots was found on percent embolism, although root length varied from 0.5 to 1 m. Measurements were carried out within 24 hours of collection.

Measurement of xylem embolism

One segment (***ca.*** 7 mm in diameter) was cut underwater from each stem or root, re-cut with a new razor blade, and attached to the tubing manifold for measurement. Segment length was approximately 150 mm for stems and 200 mm for roots. Hydraulic conductivity (K_h) was calculated as the water flow rate through a wood segment divided by the pressure gradient across it (Sperry, Donnelly & Tyree 1988). The flow rate under known pressure differences (***ca.*** 5.5 kPa for stems and 5 kPa for roots) was determined by repeated measurements of the flow of filtered (0.02 µm), deionized water onto an electronic balance attached to a computer. Flow rates were measured before and after flushing the segments at ***ca.*** 100 kPa for 30 min to remove all embolisms, and the percent loss of conductivity due to xylem embolism (PLC) was then calculated as $100(1 – K_{h,after}/K_{h,before})$. Specific conductivity (K_S) was calculated as the hydraulic conductivity per unit cross sectional area of the measured segment (without bark). The same protocol was used to measure native embolism and for the measurement of embolism after laboratory freezing experiments.

Laboratory freezing experiments

Freezing experiments were carried out under controlled conditions in a way similar to Pockman & Sperry (1997). Samples were collected four times from early November to mid December. From the three stems or roots sampled per individual, one was used as a control, and the other two were subjected sequentially to different freezing treatments. All the experiments were performed the same day of collection (stems) or the day after (roots). The water potential was measured in all stems just before treatment. A segment of ***ca.*** 7 mm in diameter was marked in each stem/root, and a copper-constantan thermocouple (AWG No. 36) was installed into the outer layer of xylem (Pockman & Sperry 1997) 5-10 cm below the segment. Thermocouples were held in place with adhesive tape. The temperatures of wood, air in two different locations and a piece of dry wood were measured every 5 s and averaged every 30 s (Datalogger Model CR-7, Campbell Scientific, Logan, UT, USA).

Temperature control was achieved with a temperature chamber (Langan ***et al.*** 1997) attached to a temperature bath (VWR Extra Low Temperature Circulator, Model 1197, VWR Scientific Products, Niles, IL, USA). The temperature chamber consisted of an inverted, insulated, black plastic garbage can (0.17 m^3), lined with ***ca.*** 35 m of coiled copper tubing. The fluid in the circulating bath was continuously passed through the copper tubing to control chamber temperature. Testing with an empty chamber allowed us to compensate for the increasing differential between bath and chamber temperature as bath temperature decreased.

In each experiment, batches of 10 stems or 5 roots were placed inside the temperature chamber and exposed to the desired treatment. Minimum (air) temperatures were: 3.5, -4, -11, -14, -17 or -22°C for stems; and -2 or -7°C for roots. All treatments started and finished at 10°C. Cooling and warming rates were 1 °C min^{-1} for above 0°C temperatures and 0.1 °C min^{-1} below 0°C. Target minimum temperatures were maintained for 90 min before chamber temperature began to increase. Exotherms were identified in the manner described for field data. Typically two exotherms were apparent, which we termed high temperature and low temperature exotherms (HTEs and LTEs; Figure 1). To calculate their exact duration and area we plotted the temperature difference between each stem and the corresponding dry wood sample (***cf***. differential thermal analysis; Burke ***et al.*** 1976) and identified the starting and end points of the exotherms. The area between a line linking these two points and the temperature curve was considered to be the area of the exotherm (Figure 1b).

The percent K_h and the K_S values measured in each stem/root after the treatment were expressed relative to the values measured on the control from the same plant. These relative variables were used in the following analyses to compare the different treatments.

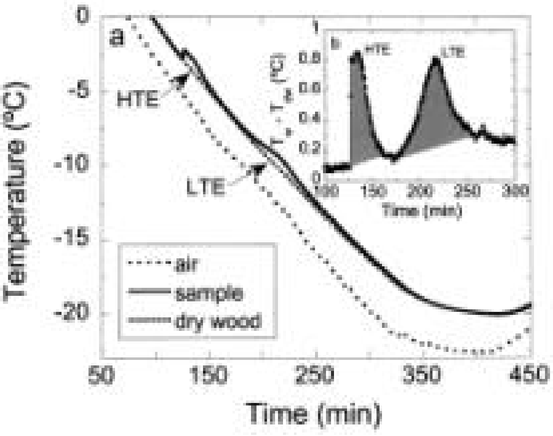

Figure 1. (a) Example of laboratory measurements of air, stem and dry wood temperatures during a typical freezing experiment. The high temperature and low temperature exotherms (HTE and LTE respectively) are marked by arrows. (b) Difference between stem (T_w) and dry wood temperature (T_{dw}) during the same experiment. The filled polygons correspond to the exotherm areas that were used in the analyses.

Xylem anatomy

Vessel diameters were measured on 10 stems and eight roots from the Five Points population. Transverse sections were cut from segments collected for native embolism measurements using a rotary microtome (Model 820, American Optical Co., Buffalo, NY, USA). The sections were stained with toluidine blue (0.05%) to improve contrast, mounted in glycerol, and viewed with a compound microscope (Nikon Eclipse, Nikon Co., Tokyo, Japan) at 100x (stems) or 50x (roots). Two representative regions from the outermost rings, situated 90° apart, were photographed from each section with a digital camera (Nikon Coolpix 990, Nikon Co., Tokyo, Japan). The images (black and white) were transferred to a computer and analyzed with Scion Image (v. β4.02 for Windows, Scion Co., Frederick, MD, USA). Within each image all open vessels with a diameter larger than 7.4 µm (stems) or 8.8 µm (roots) were sampled. These values were selected in each case to maximize the agreement between the visually identified vessels and the ones selected by the computer. For each selected conduit the program determined the total cross sectional area and perimeter. At least 300 conduits were measured from each section.

Vessel-anatomy measurements were also carried out on 10 stems from the same population studied by Pockman & Sperry (1997), at the Cienaga Creek Nature Reserve, Arizona (32º 01’ N, 110º 37’ W). The purpose of these measurements was to test whether there were differences in the obtained diameter distributions because of the different methods used in the two studies. Two variables were used to characterize the xylem anatomy of each population: the mean vessel diameter (d, in µm) and the mean hydraulic diameter (d_h, in µm). The hydraulic diameter was calculated considering that hydraulic conductivity is proportional to vessel diameter raised to the fourth power. The following expression was used to calculate the hydraulic mean diameter for each segment: $\sqrt[4]{\sum d_i^4 / N}$ (Tyree ***et al.*** 1994).

Data analysis

Two-way ANOVAs with repeated measurements were used to compare the freezing temperatures at which exotherms were observed at proximal and distal parts of the stems (only the exotherms that were detected at both parts of the stems were considered). The field measurements of PLC from consecutive sampling dates were compared by means of t-tests. The laboratory experiments were analyzed in two ways: PLC and K_S measurements from each treatment were compared to their corresponding controls using t-tests, and differences between the relative values of percent K_h and K_S among treatments were evaluated using one-way ANOVA. The attributes of the exotherms were compared using one-way ANOVA when comparing tissues or treatments (only for stems), or one-way ANOVA with repeated measurements when comparing HTEs and LTEs. The vessel diameters of different tissues and populations were compared by means of t-tests. All variables were normalized when not normally distributed (arcsine transformation in the case of PLC and logarithmic for the rest).

Results

Temperature measurements in the field

During the study period (182 days) minimum air temperature (T_{air}) was below 0ºC in 129 days, and fell below –10ºC in 13 days (Figure 2). For the days with below-zero temperatures, maximum T_{air} was below-zero only three times, and the mean difference between maximum and minimum daily temperature was 18.3ºC. The minimum recorded air temperature was –16.0ºC, reached on 28 December 2000. As expected, thermal oscillations were smaller in the soil than in the air, and these oscillations decreased with depth below the surface (Figure 2). The number of days with below-zero soil temperatures was 52 at 10 cm, 12 at 20 cm, and none at 30 cm. The minimum temperature measured in the soil was –3.27ºC (at 10 cm).

Freezing exotherms were only detectable in the field when the storage frequency was increased to 2-min averages (from early December onwards). For that period, exotherms were observed in stems between –1.46 and –10.17ºC (mean=-5.42ºC, N=400). Water froze at approximately the same time (within our 2-min resolution) on the proximal and distal part of the stem (p=0.003). Since temperatures were slightly lower at the proximal parts, freezing occurred there at an average of 0.73ºC lower temperatures. On average, sap freezing was detected once every three days in the monitored stems, and was never observed in roots.

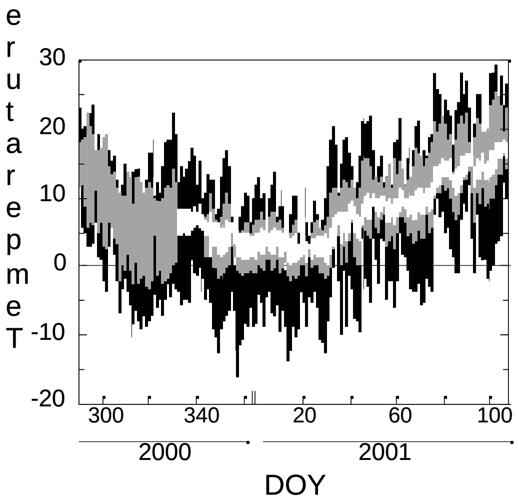

Figure 2. Daily maximum and minimum air (black) and soil temperatures at a depth of 10 (gray) and 30 cm (white) during the study period at the Five Points site.

Water potentials and native embolism

Water potentials remained considerably low (less than –2.5 MPa at predawn and less than –3.5 MPa at midday) through the study period (Figure 3a). These low values were observed despite surface soil looked quite wet during most of the period and its water potential was indeed high when it was measured from early February on (Figure 3b). In fact, the measured levels of native embolism were very high (PLC always >70%), and did not show a sharp decrease until the advent of warm temperatures at the end of the study period (Figure 3c). PLC values were already high in early November, just after the first two freezing episodes (T_{min}=-3.35ºC), which suggests that current-winter freezing was not the cause of this high native embolism (see below). Only one significant increase in PLC was detected after this first measurement, and was associated with the first temperatures below –10ºC. A second increase was observed between December and early February, which was marginally significant (p=0.058). The decrease in PLC observed in late November coincided with considerably low temperatures (daily minimums always < -7ºC and down to –8.60ºC), and was not associated with any precipitation event. In the only native embolism measurement in roots (mid December), PLC was 63.4 ±7.2, much lower than the values measured in stems.

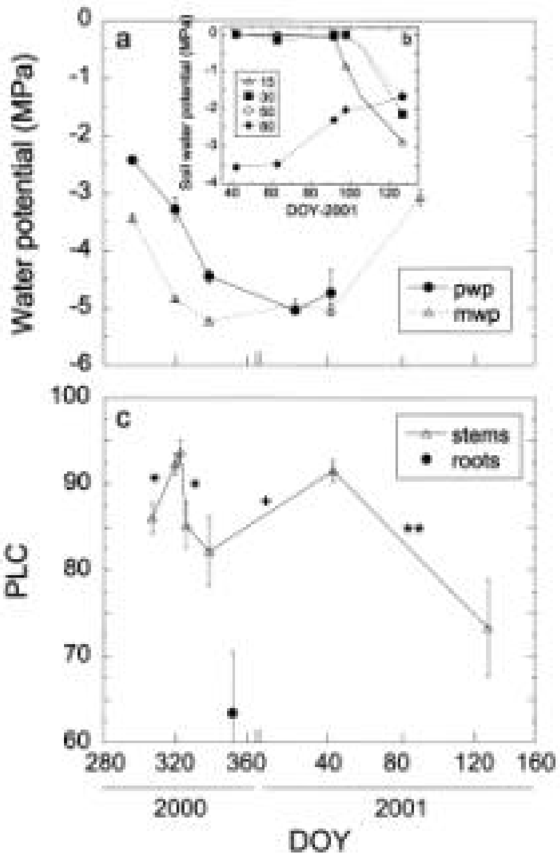

Figure 3. Seasonal pattern of predawn (pwp) and midday (mwp) leaf water potentials (a), soil water potentials at different depths (in cm) (b), and percent loss of conductivity (PLC) due to xylem embolism (c). Error bars are standard errors. Asterisks and the plus sign mark significant differences between two consecutive embolism measurements ($^{+}$: p<0.1, *: p<0.05, **: p<0.01; t-test, n=10).

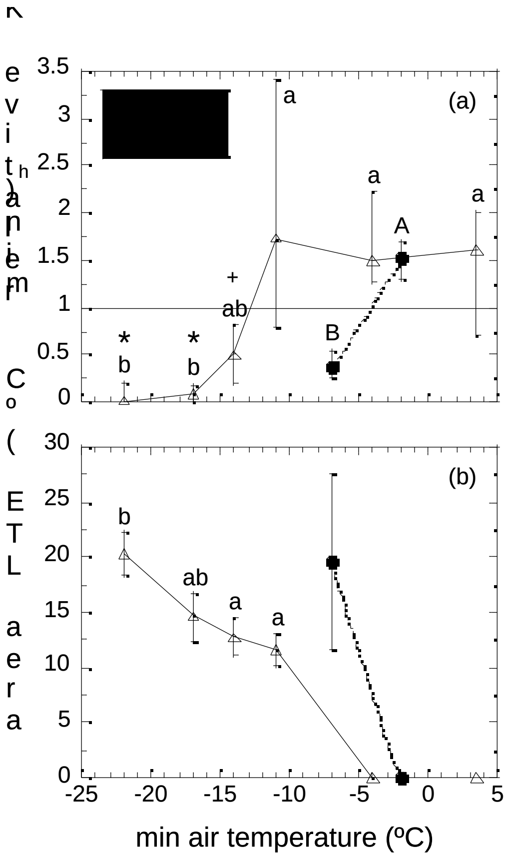

Figure 4. Results of the freezing experiments. (a) Percent conductivity in relation to the controls (relative K_h) after each treatment (controls=1), for stems and roots from the Five Points site. As the variable was not normally distributed, the points represent the median and the error bars the quartiles. Asterisks and the plus sign indicate the presence of significant differences in relation to the controls ($^{+}$: p<0.1, *: p<0.05; t-tests). (b) Mean area of the low temperature exotherms (LTEs) observed after each treatment. Error bars are standard errors. In both (a) and (b) different letters (lowercase for stems and uppercase for roots) denote significant differences between treatments (one-way ANOVA).

Freezing experiments

In the laboratory, two types of freezing exotherms were observed in most samples (Figure 1). The first (HTE) started at temperatures between –1.45 and –7.28ºC in stems (N=48) and between –3.79 and –5.26ºC in roots (N=5). The second (LTE) occurred at –5.94-(–9.53)ºC in stems (N=38) and at –4.51-(–5.44)ºC in roots (N=4). HTEs occurred at lower temperatures in roots (p=0.037), whereas for LTEs the reverse was true (p 0.001). No significant differences between tissues were observed in the area of LTEs or HTEs. LTEs were characterized by having higher duration, less amplitude and higher area than HTEs (p 0.001 in all cases) (Figure 1b). While there was no difference among sub-zero treatments in the area of the HTE, the area of the second exotherm increased as a function of the minimum temperature from –4ºC, where no LTE was observed, to –22ºC (Figure 4b).

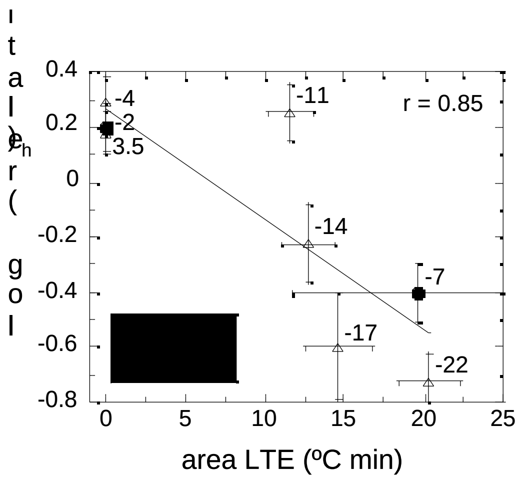

Figure 5. Relationship between the mean area of the low temperature exotherm (LTE) and the logarithm of the percent

conductivity in relation to the controls (relative K_h) observed after each treatment. Logarithms are used to normalize relative K_h. The numbers indicate the minimum temperatures (in ºC) corresponding to each treatment. The linear fit combining both stems and roots is shown (p=0.008). Error bars are standard errors in the case of the area of LTEs and the quartiles for relative K_h.

No increase in PLC was detected for temperatures down to –11ºC. The effect was marginally significant at –14ºC (p=0.090). Stems frozen to –17 or –22ºC had significantly higher embolism than the control (Figure 4a). This result is qualitatively similar to the pattern reported in Pockman & Sperry (1997), although their stems were more vulnerable to freezing-induced embolism. Roots were more vulnerable to cavitation than stems, with a significant increase in PLC at –7ºC (Figure 4a). There was a significant correlation between the average area of the LTE and the relative K_h of each treatment (p=0.008) (Figure 5). Figure 5 may suggest a threshold effect more than the linear relationship which we have assumed. However, since it would be based on a single point (T=-11ºC) and we lack any additional evidence supporting a threshold effect, we preferred to use the simplest model that fitted the data reasonably well. In any case, our main point, namely that the area of the LTE and embolism were related, would remain unaffected. The freezing treatments had no effect on maximum K_S (p=0.277 for stems, and p=0.192 for roots).

Xylem anatomy

Although the differences were quantitatively small, actual vessel diameters and hydraulic diameters were higher in roots than in stems (Table 1 and Figures 6a and 6b). Between populations, vessel diameters tended to be slightly higher in the stems of Sonoran individuals. The difference was only significant for mean diameters (Table 1). Our anatomy measurements for the Arizona population were similar though significantly different (p=0.030) to the ones given by Pockman & Sperry (1997) (21.5 ±0.6 vs. 19.7 ±0.5 μm). This discrepancy may be due to the fact that we only measured the vessels in outermost rings while Pockman & Sperry measured entire sectors.

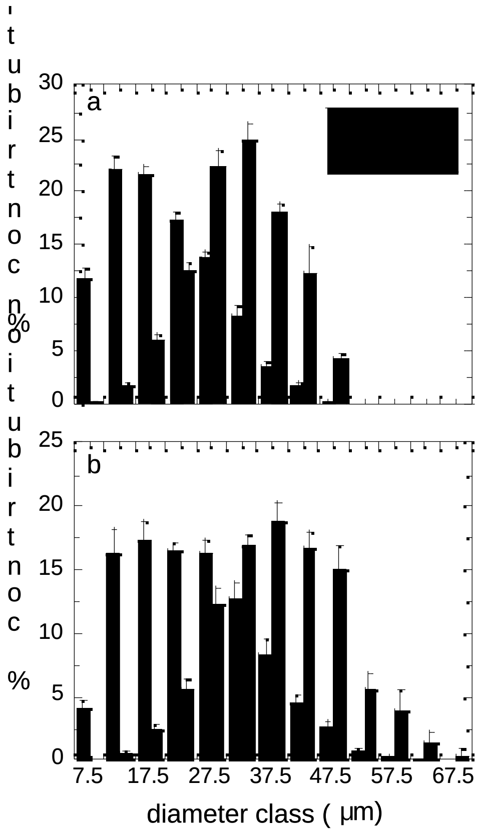

Figure 6. Vessel diameter distributions (in 5 μm size classes) of stems (a) and roots (b) from the Five Points population. Both the percent contribution to the total number of vessels and to the overall hydraulic conductivity are represented. Error bars are standard

Discussion

Comparison of the Chihuahuan and Sonoran populations

Table 1. Anatomy results. d: diameter; d_h: hydraulic diameter. Numbers in brackets are standard errors. Significant differences between stems and roots or between populations (stems) are denoted by an asterisk (t-test).

Site	Tissue	n	Mean d (mm)	Max d (mm)	Mean d_h (mm)
Sevilleta, NM	Stem	10	19.9 (0.4)	49.0	24.5 (0.5)
Sevilleta, NM	Root	8	24.7 (0.3)***	67.3	30.0 (0.9)***
Cienaga Creek, AZ	Stem	10	21.5 (0.6)*	55.3	25.6 (0.7)$^{n.s.}$

Pockman & Sperry (1997) hypothesized that New Mexican populations should be more resistant to embolism than Arizona ones. Our laboratory experiments were in agreement with that prediction, although the difference was quantitatively small. Whereas in the Sonoran desert population the decrease in PLC was already apparent at temperatures of ***ca.*** –12ºC, in our case the decrease started between –14 and –17ºC (Figure 4a). Our laboratory results were qualitatively corroborated by the fact that increases in PLC in the field were only observed after temperatures around –11ºC or lower. If we consider that in the studied area temperatures below –11ºC occur on average 15 times per year and that yearly minimums range between –14 and –20ºC (Sevilleta LTER data), it is apparent that ***L. tridentata*** is more limited by minimum temperatures in the Sevilleta than in the Sonoran population studied by Pockman & Sperry, where temperatures fell below –5ºC only once during the 1994-1995 winter. The difference in vulnerability to freezing-induced xylem embolism between both populations is not large enough to compensate for the existing climatic differences.

According to the previous considerations it is not surprising that we found very high levels of native embolism (>80% in stems for most of the winter; Figure 3c). In principle, both water stress and freezing are possible causes of this embolism. However, Pockman & Sperry (1997) measured predawn water potentials as low as –9 MPa during the summer without any increase in native embolism. Also, from ecophysiological studies ***L. tridentata*** is known to remain photosynthetically active at very low xylem water potentials (***ca.*** –8 MPa) (Odening ***et al.*** 1974). All of these data suggest that this species is very resistant to drought-induced embolism. This evidence, combined with the increases in native embolism that we observed in this study, supports the interpretation that the high levels of embolism that we have observed are mainly the result of the cumulative effect of repeated freeze/thaw events through the lifetime of the stems.

Regarding the effects of the high embolism levels, it seems that the studied population is, indeed, very close to the limit to cope with freezing stress. In mid November, where the highest levels of embolism were measured, 30% of the stems presented a PLC>95%, and in December the amount of dead standing biomass was as much as 50% of the stems in the studied population (M. Mangirón, unpublished result). However, since we were not following the demography of the stems we do not know which fraction of this mortality (if any) occurred during the winter. Qualitatively fewer dead stems were observed in the Sonoran desert population studied by Pockman & Sperry (W.T. Pockman, personal observation). Shoot mortality as a result of freezing stress has been reported in the chaparral shrub ***Rhus laurina*** (Boorse ***et al.*** 1998). The decreasing temperatures observed with increasing latitude in central New Mexico, and the associated increased accumulation of xylem embolism predicted by our results, are likely to contribute to limiting the present distribution of ***L. tridentata***.

Vulnerability of different plant tissues

In agreement with their wider vessels (Figure 6), roots were more vulnerable to freezing-induced cavitation than stems (Figure 4a). Despite that and the fact that soil temperatures reached considerably low values (Figure 2), exotherms were never observed in the field-monitored roots and their native embolism was lower than in stems. These results are in agreement with other studies (Jaquish & Ewers 2001) suggesting that roots are less limited by freezing-induced embolism than stems. In fact, it is likely that the 63.4 PLC that we measured in roots was mostly caused by water stress alone because: (1) soil temperatures at 10 cm have dropped below –5ºC (aprox. the mean exotherm temperature for roots) only once in the last 10 years, the absolute minimum being –5.3ºC (data from the Deep Well weather station, at similar altitude and 5 Km from the study site; Sevilleta LTER data); and (2) predawn water potentials remained very low during the period studied (Figure 3a). Although we did not study the vulnerability to drought-induced embolism of ***L. tridentata*** roots, the measured water potentials will be enough to cause severe embolism in the roots of most woody species (Hacke ***et al.*** 2000; Chapter 3). The low predawn water potentials (Figure 3a) together with the observed pattern of soil water potentials at different depths (Figure 3b) suggest that for some reason surface roots were hydraulically disconnected from the soil. Our results show that this was not caused by lack of hydraulic conductivity in root xylem.

Dynamics of freezing-induced xylem embolism

The existence of two non-overlapping exotherms in a wood sample is usually interpreted in the following way (Burke ***et al.*** 1976, Gusta ***et al.*** 1983): when temperature reaches the freezing point of sap inside xylem conduits the sap freezes and there is a first exotherm (HTE). As temperature decreases below that point cellular water migrates to the vessels and intercellular spaces, where it freezes. The properties of the cell wall protect cellular water from freezing and, together with the water potential of ice, regulate the rate of cell dehydration (Rajashekar & Lafta 1996). Eventually, it is reached a point in which intracellular freezing occurs in xylem parenchyma, and a second exotherm (LTE) is observed. If we accept this scenario, the increase in the area of the LTE with decreasing temperature that we have observed can easily be interpreted as the result of the freezing of more parenchyma cells as temperature reaches lower values. The differences in shape that we have found between the two exotherms (HTE sharper than LTE) will then be caused by the faster progression of freezing in the apoplast than in the symplast, which further corroborates our interpretation.

The effect of minimum temperature on embolism formation has long been recognized for conifers (Hammel 1967), but only recently has been reported in some species of angiosperms: ***Quercus ilex*** (Lo Gullo & Salleo 1993), ***Rhus laurina*** and ***R. ovata*** (S.D. Davis & F.W. Ewers, unpublished result), and ***L. tridentata*** (Pockman & Sperry 1997 and this study). Pockman & Sperry (1997) suggested that freezing injury to xylem parenchyma could contribute to this effect. The relationship between the area of the LTE (and presumably the amount of frozen cells) and embolism that we have found (Figure 5) provides strong evidence in favor of this interpretation.

The question remains whether the role of parenchyma cells on water transport is purely passive and the effect of intracellular freezing is indirect, as proposed by Pockman & Sperry (1997), or they contribute actively to the integrity of the conducting system as some recent work suggests (Holbrook & Zwieniecki 1999, Zwieniecki & Holbrook 2000). The lack of embolism formation in stems frozen at temperatures down to –11ºC despite the presence of HTEs in all samples supports the second alternative. If vessels can be hydraulically isolated upon thawing because of gas entrapment in pits, as proposed to explain refilling at low water potentials (Holbrook & Zwieniecki 1999), the local generation of positive or near-positive hydrostatic pressures would be enough to force the gas bubbles into solution (Hammel 1967). Cryo-scanning electron microscopy studies of the dynamics of xylem cavitation during freezing and thawing of several species of trees suggest that there is no long-range movement of water through vessels while cavitation is in progress (Utsumi ***et al.*** 1998, 1999). Water coming from living parenchyma cells adjacent to the vessels is a likely source of positive pressures, although the mechanism is not well understood (Holbrook & Zwieniecki 1999). In this way, xylem pressures upon thawing could be higher than predicted if the continuity between conduits was immediately restored, which would explain the absence of embolism formation at moderately low sub-freezing temperatures. In agreement with that hypothesis, Améglio ***et al.*** (2001) measured winter stem xylem pressures in ***Juglans regia*** and found that freeze-thaw cycles caused pressures to increase almost sixfold over the value accounted for by osmotic changes alone.

Conclusion

Our results show that ***L. tridentata*** populations from the Chihuahuan desert are strongly limited by freezing. Although resistance to freezing-induced xylem embolism is higher than in the Sonoran population studied by Pockman & Sperry (1997), the impact of freezing is also greater because of the much lower temperatures. Within plants, stems seem to be more limited by freezing than roots. Our laboratory experiments strongly suggest that the freezing of water inside parenchyma cells is associated with the occurrence of xylem embolism. These results are consistent with other studies that point to an active role of living cells in long-distance water transport (Holbrook & Zwieniecki 1999), and provide a reasonable explanation for the temporal dynamics of embolism and refilling observed in the field. This finding opens new perspectives in the study of the impact of frost acclimation on the vulnerability to xylem embolism. However, more detailed studies are needed to clarify the role of living cells in freezing-induced cavitation and water transport in general.

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