

## HYDRAULIC TRAITS ARE INFLUENCED BY PHYLOGENETIC HISTORY IN THE DROUGHT-RESISTANT, INVASIVE GENUS *JUNIPERUS* (CUPRESSACEAE)<sup>1</sup>

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In the conifer genus *Juniperus* (Cupressaceae), many species are increasing rapidly in distribution, abundance, and dominance in arid and semiarid regions. To help understand the success of junipers in drier habitats, we studied hydraulic traits associated with their water stress resistance, including vulnerability to xylem cavitation, specific conductivity ( $K_s$ ), tracheid diameter, conduit reinforcement, and wood density in stems and roots, as well as specific leaf area (SLA) of 14 species from the United States and the Caribbean. A new phylogeny based on DNA sequences tested the relationships between vulnerability to cavitation and other traits using both traditional cross-species correlations and independent contrast correlations. All species were moderately to highly resistant to water-stress-induced cavitation in both roots and shoots. We found strong phylogenetic support for two clades previously based on leaf margin serration (serrate and smooth). Species in the serrate clade were 34–39% more resistant to xylem cavitation in stems and roots than were species in the smooth clade and had ~35% lower  $K_s$  and 39% lower SLA. Root and stem resistance to cavitation and SLA were all highly conserved traits. A high degree of conservation within clades suggests that hydraulic traits of *Juniperus* species strongly reflect phylogenetic history. The high resistance to cavitation observed may help explain the survival of junipers during recent extreme droughts in the southwestern United States and their expansion into arid habitats across the western and central United States.

**Key words:** juniper; *Juniperus*; phylogeny; vulnerability to cavitation; xylem cavitation; xylem embolism.

The environment and evolutionary history are important forces shaping the hydraulic properties that determine how plants respond to water shortages. During water stress, negative pressure in the xylem can induce cavitation through the aspiration of air bubbles into a functional xylem conduit from a neighboring air-filled conduit (Zimmermann, 1983). Cavitation results in conduits that can no longer transport water, eventually leading to stomatal closure (Sperry and Pockman, 1993), reduced photosynthesis (Jones and Sutherland, 1991), and potentially mortality (Tyree and Sperry, 1988). Vulnerability to water-stress-induced cavitation is an index of the maximum seasonal water stress in the field and provides insight into drought tolerance (Linton et al., 1998; Brodribb and Hill, 1999; Pockman and Sperry, 2000; Pratt et al., 2007). Physiological traits relating to drought tolerance are influenced by the evolutionary history of species and will likely influence survival in new habitats. By combining physiological data and molecular hypotheses, we investigated evolutionary history and hydraulic traits in the genus *Juniperus* (Cupressaceae), a generally drought-

tolerant and invasive group of >60 conifer species (Adams and Demeke, 1993; Adams, 2004; Little, 2006).

Many *Juniperus* species in North America are invading drier habitats and increasing in abundance where they already grow by surviving droughts that other conifers cannot. In Africa, Asia, Europe, and the Caribbean, many *Juniperus* species are considered threatened or endangered, in many cases from habitat loss or degradation, agriculture, or extraction (Adams, 2004). In the western hemisphere, however, many of the species in section *Sabina* are expanding their distributions into relatively dry, lower-elevation habitats to the point of being “weedy” (Adams and Demeke, 1993; Jackson et al., 2002). For example, in the last 60 years *J. occidentalis* populations have encroached exponentially into lower-elevation grasslands and shrublands in the northwestern United States during severe droughts (Miller et al., 1994). In the southwestern United States, while co-occurring piñon pines (*Pinus* spp.) have experienced massive die-offs as a result of severe droughts in 1996 and 2002, juniper species have suffered little mortality and are increasing in dominance (Breshears et al., 2005; Mueller et al., 2005). Because of both the recent, widespread expansion of *Juniperus* species and their ability to survive severe droughts, we studied 14 New World juniper species to better understand their success in dry environments.

*Juniperus* species are among the most resistant (least vulnerable) species in the world to water-stress-induced xylem cavitation (Maherali et al., 2004), which along with conduit wall structure is one of the most important hydraulic traits determining drought tolerance in plants (Tyree and Ewers, 1991). The more resistant the xylem is to cavitation, the more negative the water potential the plant can sustain, and the stronger the conduit wall must be (Hacke et al., 2001). Within a plant, roots are generally more vulnerable than stems to water-stress-induced cavitation (Jackson et al., 2000). Species with smaller, thicker leaves have lower specific leaf area (SLA; leaf area/leaf mass) and generally occur in more stressful environments. Previous

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studies revealed correlations between SLA and maximum hydraulic conductivity and water availability (Cavender-Bares and Holbrook, 2001; Wright et al., 2001). The ratio of sapwood area to leaf area ( $A_S/A_L$ ) has also been shown to increase with habitat aridity (Cavender-Bares and Holbrook, 2001). For *Juniperus* species then, we expected greater resistance to cavitation to be correlated with higher conduit wall strength, lower SLA, and higher  $A_S/A_L$ .

On the one hand, the ability of *Juniperus* species to survive in xeric environments today may arise from a single evolutionary event, an adaptation to a prehistoric climate in a common juniper ancestor. Alternatively, a trait such as high resistance to cavitation may have arisen multiple times in different lineages. In comparative studies, inference about adaptation is hampered by the statistical nonindependence of species (Felsenstein, 1985b). Consideration of phylogenetic relationships among species accounts for this nonindependence because closely related species may have similar phenotypes due to descent from a common ancestor. For example, in a study of six conifer species, Piñol and Sala (2000) found a trade-off between sapwood-area specific conductivity and resistance to cavitation only when two groups were considered separately, pines and non-pines. In another study, Jacobsen et al. (2007) found that sapwood-area specific conductivity, hydraulic vessel diameter, and maximum vessel length in 26 angiosperm species were correlated with resistance to cavitation in standard cross-species correlations but not when phylogenetic relationships were considered. These studies demonstrate the potential importance of evaluating trends between vulnerability to cavitation and climate or hydraulic traits in a phylogenetic framework. When analyzing traits, phylogenetic information plays a crucial role in distinguishing between evolutionary convergence (i.e., homoplasy, distantly related species that are phenotypically similar) and evolutionary conservatism (closely related species that are phenotypically similar). Comparative methods can also help identify relationships between pairs of continuously varying traits that reflect correlated evolution (e.g., Preston and Ackerly, 2003).

We used two types of comparative evolutionary analyses in this study: (1) an index to quantify levels of convergent evolution for single traits and (2) a test for correlated evolutionary change between pairs of traits. We investigated the degree of convergent or conserved evolution in single traits using the Quantitative conVergence Index (QVI; Ackerly and Donoghue, 1998). Because evolutionary correlations between pairs of traits can point to potentially adaptive associations or trade-offs, we also examined correlations among hydraulic traits and between hydraulic traits and habitat moisture availability. One particularly strong tool when comparing functional correlations of continuously varying traits among taxa is the use of phylogenetically independent contrast (PIC) correlations (Felsenstein, 1985b). Independent contrasts use reconstructions of ancestral traits to identify statistically independent comparisons among species. The differences are calculated between the trait values of sister taxa in a bifurcating phylogenetic tree. Then, the differences of each trait in the trait-trait pairs or trait-environment pairs of interest are used in correlations. For both types of comparative analyses, a phylogeny is required.

Because a DNA sequence-based phylogeny for the *Juniperus* species in this study did not previously exist, another goal of this study was to generate a phylogenetic hypothesis for the 14 *Juniperus* species under study (12 American, two Caribbean; all of which are members of section *Sabina*; Fig. 1). Within section

*Sabina*, species have been divided into informal series based on leaf-margin serration, with smooth margin species mainly in eastern and northern North America, and serrate margin species mainly in western and southern North America and Central America (Gaussen, 1968; Adams and Demeke, 1993; Adams, 2004). We chose to base the phylogeny on the ITS region of nuclear ribosomal DNA, owing to its utility in numerous phylogenetic studies (e.g., Baldwin et al., 1995; Liston et al., 1999). We also used the nuclear second intron of *LEAFY* because it has been shown to be variable and phylogenetically useful in other taxa (Oh and Potter, 2003, 2005), including other gymnosperms (e.g., Won and Renner, 2003). We created a phylogenetic hypothesis using sequence variation in both ITS and *LEAFY* to examine the phylogenetic structure of physiological traits.

Our main objective was to study vulnerability to xylem cavitation and other hydraulic traits associated with resistance to water stress in roots and stems of *Juniperus* species within an evolutionary context. We explored five main hypotheses: (1) a phylogeny based on DNA sequence variation will support a division between the informal series of serrate and smooth leaf margins. (2) Vulnerability to xylem cavitation will be higher in species in the smooth leaf margin series because such species occur in more mesic sites and in tropical areas. (3) As shown for other species, roots will be more vulnerable to cavitation than stems. (4) Vulnerability to cavitation will increase with increasing mean annual precipitation in the distributions of *Juniperus* species. (5) Higher vulnerability to cavitation will be associated with lower wood density and conduit wall reinforcement from implosion, lower  $A_S/A_L$ , higher SLA, and higher hydraulic conductivity. In a similar study, Jacobsen et al. (2007) showed that higher vulnerability to cavitation was correlated with decreasing xylem density and stem mechanical strength, with and without taking into account phylogeny. Exploring the relationship between phylogenetic history and interspecific variation in water relations may help us to explain the distributions and the successful survival of *Juniperus* species after invasion into rangelands or during extreme drought. It may also offer a more general evolutionary perspective on physiological and ecological traits associated with water transport.

## MATERIALS AND METHODS

**Taxon sampling and molecular markers**—The study species have distributions ranging from the Mojave Desert of California (most arid, *J. californica*) to the Blue Mountains of Jamaica (most mesic, *J. lucayana*; Fig. 1). With the exception of the low-growing circumboreal shrub *J. communis* (section *Juniperus*), all North American junipers belong to *Juniperus* section *Sabina* (the third section, *Caryocedrus*, is monotypic; Adams, 2004). The *Juniperus* species in section *Sabina* are believed to be of relatively recent origin (Adams and Demeke, 1993). Section *Sabina* is divided into two informal series based on denticulation of leaf margins (as seen at 40× magnification): serrate (denticulate) and smooth (entire) margins (Appendix 1; Gaussen, 1968; Adams and Demeke, 1993; Adams, 2004). In addition to the 12 North American species, we added two Caribbean species to increase the number of taxa with smooth leaf margins in the study.

We sampled 14 *Juniperus* species within section *Sabina* to create a phylogeny based on DNA sequences (Fig. 1). Two accessions were sampled for each species (see Appendix 1 for collection information), and one accession of *J. communis* was used as an outgroup because it is a member of a separate section (section *Juniperus*; Adams and Demeke, 1993). All accessions were vouchered as herbarium specimens and deposited at the Duke University Herbarium in Durham, North Carolina, USA (Appendix 1). Total genomic DNAs were extracted from fresh or silica dried material using a DNeasy Plant Mini Kit according to manufacturer's protocols (Qiagen, Valencia, California, USA).

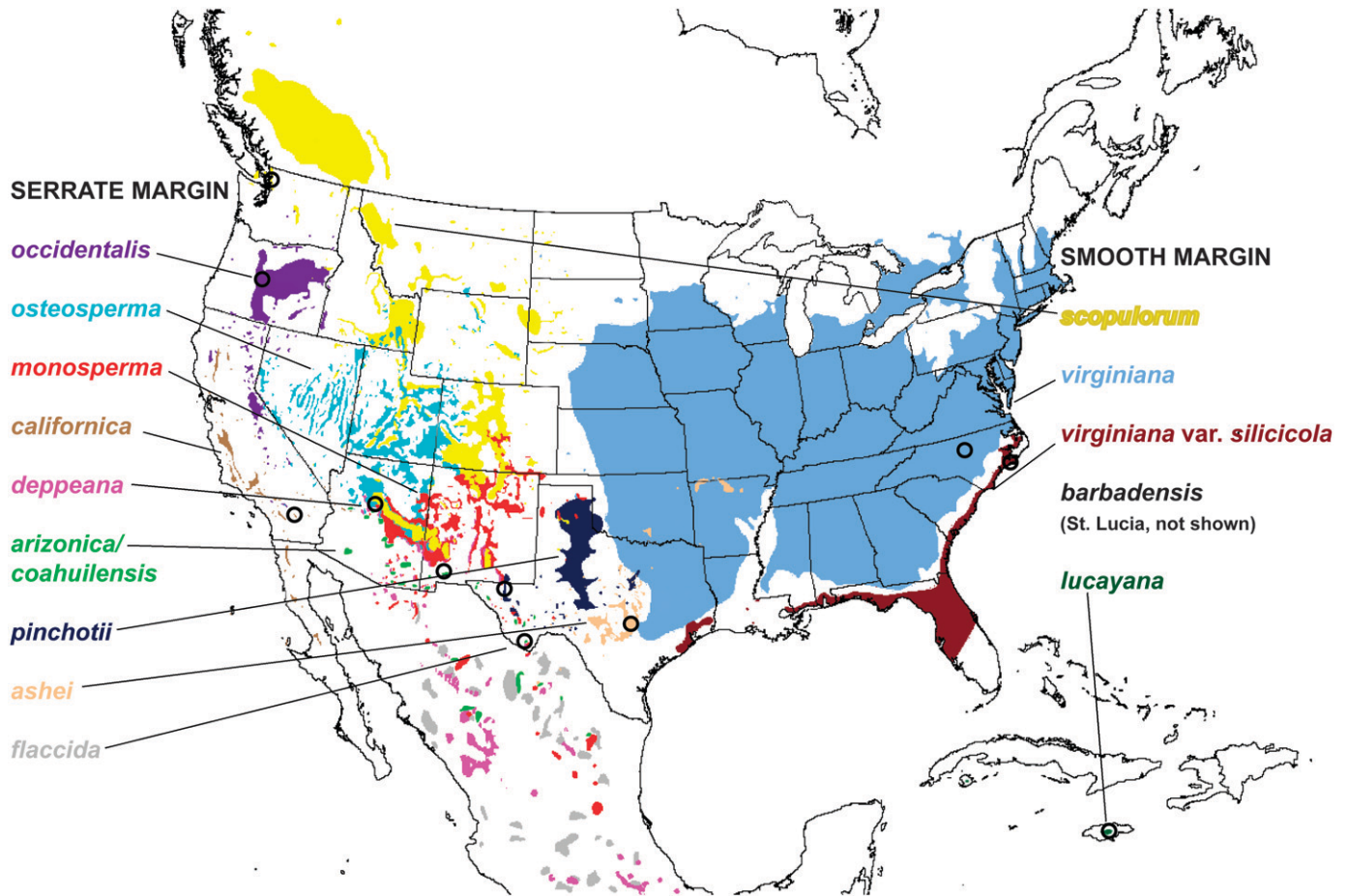


Fig. 1. Distributions for the 14 *Juniperus* species in this study (*J. barbadensis* is endemic to St. Lucia, not shown on map). Serrate leaf margin species are listed on the left and smooth leaf margin species are listed on the right. *Juniperus communis* and *J. horizontalis* are not included because their low-growing stature precludes these two species from vulnerability curve measurements. *Juniperus arizonica* was split from *J. coahuilensis* after field work was completed; *J. coahuilensis* (*sensu stricto*) was not sampled (Adams et al., 2006). Open circles indicate collection locations for vulnerability curve measurements for all taxa (except *J. barbadensis*).

Amplification of ITS (ITS 1 intron, 5.8s exon, and ITS 2 intron) was accomplished using ITS4 (White et al., 1990) and ITS5a (Stanford et al., 2000) primers. Amplification of ITS used 0.1  $\mu$ l Qiagen Taq DNA polymerase, 5  $\mu$ l Q solution, 2.5  $\mu$ l 10 $\times$  buffer, 2.5  $\mu$ l 10 mM dNTPs, and 1.25  $\mu$ l each of the two 10  $\mu$ M primers in a final volume of 25  $\mu$ l. The PCR amplification conditions were as follows: an initial 2 min at 97°C, followed by 30 cycles of 1 min at 97°C, 1 min at 48°C, and 45 s at 72°C, and a final extension cycle of 7 min at 72°C.

Amplification of the second intron of *LEAFY* was initiated by using the degenerate primers LFY1 and LFY2 described by Oh and Potter (2003). *LEAFY* is a nuclear homeotic gene that regulates the establishment of floral meristem identity and flowering time in *Arabidopsis* (Weigel, 1995; Blázquez et al., 1997). The gene is found in all plants (Frohlich and Parker, 2000; Himi et al., 2001). The gene was duplicated on the lineage leading to seed plants, so although one copy was lost in angiosperms, two copies are present in gymnosperms: *LEAFY* and *NEEDLY*. We confirmed that the allele amplified was *LEAFY* and not *NEEDLY* by comparing our sequences with *LEAFY* and *NEEDLY* gymnosperm sequences in GenBank. We then designed *Juniperus*-specific primers LFY5J (ATG TTC AGC ACG TCG CAA AGG) in the second exon and LFY4J (TTG TCG ATA TGA CCT ACA CCA G) in the third exon of *LEAFY*. Amplification of *LEAFY* used 0.2  $\mu$ l Phusion polymerase (Finnzymes, Helsinki, Finland), 10  $\mu$ l 5 $\times$  Phusion buffer, 4  $\mu$ l 10 mM dNTPs, and 2  $\mu$ l each of the two 10  $\mu$ M primers in a final volume of 50  $\mu$ l. The PCR amplification conditions were as follows: an initial 30 s at 98°C, followed by 40 cycles of 10 s at 98°C, 30 s at 55°C, and 90 s at 72°C; and a final extension cycle of 7 min at 72°C.

Amplified products were purified using the Qiagen Qiaquick purification protocol. Purified products were sequenced directly with the automated se-

quencing methodology of the ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, California, USA). Sequencing primers for ITS were ITS4 and ITS5a plus *Juniperus*-specific primers ITS2J (GCT ACA TTC TTC ATC GTG GC) and ITS3J (GCC ACG ATG AAG AAT GTA GC). Sequencing primers for *LEAFY* were ITS4J and ITS5J. Products were cleaned in Sephadex G-50 (fine) Centri-Sep spin columns (Princeton Separations P/N 901, Adelphia, New Jersey, USA), dried under vacuum, and run on either an ABI 373, 377, or 9700 Automated Sequencer at the Duke University Department of Biology Sequencing Facility. Raw sequences were assembled and edited using the program Sequencer version 3.1.1 (Gene Codes, Ann Arbor, Michigan, USA) and manually aligned using the program MacClade version 4.06 (Maddison and Maddison, 2003).

**Molecular phylogenetic analyses**—Separate and combined parsimony analyses of ITS and *LEAFY* nucleotide sequences along with any potentially informative insertion/deletion characters (indels) derived from each alignment were conducted using the program PAUP\* version 4.0b10 (Swofford, 2002). Indels were considered further and coded as binary characters when their length was uniform in all taxa sampled, whereas those of variable length where exact placement was uncertain were excluded. Settings in PAUP\* were as follows: all characters equally weighted, uninformative characters excluded, 1000 random-sequence addition replicates, saving all shortest trees under ACCTRAN optimization, tree bisection-reconnection (TBR) branch swapping, STEEPEST DESCENT off, MULTTREES on, and COLLAPSE branches if maximum length is zero (AMB-). Support for nodes resolved in the strict consensus of the most parsimonious trees for each data set was evaluated with bootstrap (BS) analyses

(Felsenstein, 1985a). Bootstrap analyses were conducted using PAUP\* with TBR branch swapping on 1000 bootstrap replicates, saving all trees. Bootstrap values of >85% support were considered strong, 70–84% moderate, 50–69% weak, and <50% lacking. Incongruence between data sets, specifically for taxa with conflicting phylogenetic placement, was evaluated using the criterion of >70% BS (Mason-Gamer and Kellogg, 1996). Initial analyses were performed using multiple accessions (2–6) per species. Then we randomly selected one accession per species to produce the tree used for comparative analyses. Overlaying of continuous characters onto the phylogeny was done using Adobe (San Jose, California, USA) Illustrator CS based on results from the program Mesquite version 1.06 (Maddison and Maddison, 2005).

**Field sites and plant material for vulnerability curves**—We collected stems and roots from 14 *Juniperus* species for vulnerability curves (Fig. 1, Table 1 for location and climate information for all sites). We collected all plant material before 1100 hours local time to minimize water stress. Stems and roots typically consisted of 3–8 growth rings. Branches were collected from the northern aspect (except for *J. lucayana*, where tree height limited branch selection) of the bottom third of the canopy. Canopy heights varied from ~2 to 5 m for the American species and from ~3 to 10 m for the Caribbean species. Branches and roots were selected to have diameters of 0.7–1.1 cm and a minimum length of 30 cm free of side branches. From each adult tree, we collected one branch and one root, for a minimum of six branches and six roots (although in some cases, only four roots were possible) per species. We collected the first roots encountered from a depth of 10–30 cm. Material was cut longer than needed (>4 cm at each end) so that embolism caused by severed conduits did not reach the segments used for vulnerability curves. Branches or root segments were immediately enclosed with damp paper towels, triple-bagged in plastic bags, and placed in a cooler to inhibit dehydration during transit to the University of New Mexico, Albuquerque, New Mexico, USA, or Duke University, Durham, North Carolina, USA. Vulnerability curves were initiated in the laboratory within 3 d of collection. For *J. flaccida* from Texas, *J. lucayana* from Jamaica, and *J. barbadensis* from St. Lucia, samples were sealed with damp paper towels and mailed overnight to Duke University for processing within 4 d of collection.

**Hydraulic conductivity and other hydraulic traits**—To construct vulnerability curves, we measured maximum hydraulic conductivity of excised segments and the percentage loss of conductivity before and after water stress treatments of increasing intensity. In the laboratory, stem or root segments were recut under water to remove any emboli formed during collection to a length of 24 cm to fit in the centrifuge rotor, and the ends were shaved with a new razor blade. From the terminal ends of the segments, 1 cm of bark was removed to prevent excess accumulation of resin and mucilage. The segments were then mounted in a tubing manifold to measure hydraulic conductivity as described in Sperry et al. (1988). Hydraulic conductivity ( $K_h$ , kg·m·MPa<sup>-1</sup>·s<sup>-1</sup>), defined as the mass flow rate of water through a segment divided by the pressure gradient

along the segment, was measured under a gravity-induced pressure head of 5–6 kPa with deionized and filtered (0.22 μm) water using an electronic balance connected to a computer. Before treatment, each segment was measured for initial  $K_h$  (hereafter referred to as  $K_{hmax}$ ). In pilot studies using *J. virginiana* from North Carolina and *J. ashei* from Texas, we found either negligible (<4%) native embolism or, in some segments, conductivity declines after flushing with deionized, degassed, and filtered (0.22 μm) water for 30 min at 40 or 50 kPa. We therefore did not flush segments in this study. Other studies have also reported that flushing decreases hydraulic conductivity in conifers (Sperry and Tyree, 1990; Pittermann et al., 2006) and angiosperms (Wang et al., 1992; Macinnis-Ng et al., 2004). Not flushing segments theoretically could result in underestimates of loss of hydraulic conductivity. However, in our pilot studies, flushing decreased hydraulic conductivity, perhaps as a result of no detectable native embolism (Pittermann et al., 2006) or of torus aspiration in conifers.

Specific hydraulic conductivity ( $K_s$ , kg·m<sup>-1</sup>·MPa<sup>-1</sup>·s<sup>-1</sup>; a measure of conducting efficiency and sapwood permeability) was calculated as  $K_{hmax}$  divided by the cross-sectional xylem area of the segment ( $A_s$ ; determined as the mean area of both ends of the segment). Active xylem area was not determined by staining, which may result in overestimated functional xylem area and underestimated  $K_s$ . Leaf-specific hydraulic conductivity ( $K_L$ , kg·m<sup>-1</sup>·MPa<sup>-1</sup>·s<sup>-1</sup>) was calculated as  $K_{hmax}$  divided by the distal leaf area supported by the segment ( $A_L$ ). Leaf area was determined from a projected leaf area to leaf dry mass ratio, or specific leaf area (SLA), for each species based on a subsample (>25% of total leaf material) of leaves per branch. The ratio of sapwood to leaf area ( $A_s/A_L$ , unitless; Tyree and Ewers, 1991) was calculated as the cross-section of conducting tissue (m<sup>2</sup>) divided by leaf area distally supported (m<sup>2</sup>). The median value of mean annual precipitation (MAP) for each species was taken from Thompson et al. (1999), or from nearby weather stations for *J. arizonica*, *J. lucayana*, and *J. barbadensis* (Table 1).

**Vulnerability to xylem cavitation**—Although both freezing and water stress can induce cavitation, we focus here on water-stress-induced cavitation in juniper stems and roots. (See Willson and Jackson, 2006, for data on freezing-induced cavitation in a subset of the study species.) The relationship between embolism, quantified as a decrease in  $K_{hmax}$ , and xylem tension and can be described in a “vulnerability curve.” We used the centrifugal force method on stem and root segments (Pockman et al., 1995). Stem and root segments were spun to negative xylem tension, inducing cavitation and reducing hydraulic conductivity. The loss of hydraulic conductivity was plotted as a percentage loss (PLC) from  $K_{hmax}$  against the corresponding xylem tension to generate a vulnerability curve using the following equation: PLC =  $(1 - K_{treated} / K_{hmax}) \times 100$ .

**Xylem conduit dimensions and wood density**—To examine the relationship between vulnerability to cavitation and conduit diameters, we measured tracheid diameters on segments used for vulnerability curves. Transverse sections

TABLE 1. Species names; series based on type of leaf margin (Gaussen, 1968; Adams and Demeke, 1993; Adams, 2004); latitude (Lat.), longitude (Long.), and elevation (Elev.) (m) of collection site; mean annual precipitation (MAP, in mm); and collection date for vulnerability curves for multiple individuals of the 14 *Juniperus* species used in this study. The median MAP of the entire distribution for most species is from Thompson et al. (1999). For the species with very limited distributions, *J. arizonica*, *J. lucayana*, and *J. barbadensis*, MAP is based on weather stations within the distribution for the period of 1971–2000. Collection sites: NF = National Forest; NG = National Grassland; NM = National Monument; NP = National Park; SP = State Park.

Species	Series	Lat. N	Long. W	Elev.	Collection site	MAP	Date
<i>J. ashei</i>	Serrate	30°22'	97°45'	182	Spicewood Springs, Austin, Texas, USA	705	Apr. 2003
<i>J. californica</i>	Serrate	34°01'	116°04'	1363	Joshua Tree NP, California, USA	355	Oct. 2002
<i>J. arizonica</i>	Serrate	32°10'	107°36'	1463	Rockhound SP, New Mexico, USA	390	Oct. 2002
<i>J. deppeana</i>	Serrate	35°10'	111°31'	2050	Walnut Canyon NM, Arizona, USA	470	June 2001
<i>J. flaccida</i>	Serrate	29°16'	103°16'	2100	Big Bend NP, Texas, USA	620	Mar. 2002
<i>J. monosperma</i>	Serrate	35°10'	111°31'	2050	Walnut Canyon NM, Arizona, USA	355	June 2001
<i>J. occidentalis</i>	Serrate	44°06'	121°08'	731	Crooked River NG, Oregon, USA	485	Oct. 2001
<i>J. osteosperma</i>	Serrate	35°10'	111°31'	2050	Walnut Canyon NM, Arizona, USA	310	June 2001
<i>J. pinchotii</i>	Serrate	31°53'	104°48'	1673	Guadalupe Mtns. NP, Texas, USA	500	Oct. 2002
<i>J. barbadensis</i>	Smooth	13°50'	61°03'	725	Petit Piton, St. Lucia	2187	Oct. 2002
<i>J. lucayana</i>	Smooth	18°04'	76°40'	1493	Blue Mountains NP, Jamaica	2685	Sep. 2002
<i>J. scopulorum</i>	Smooth	48°22'	122°31'	15	Fidalgo Island, Washington, USA	515	May 2002
<i>J. virginiana</i> var. <i>silicicola</i>	Smooth	34°42'	76°40'	12	Carrot Island, North Carolina, USA	1320	Sept. 2001
<i>J. virginiana</i>	Smooth	35°57'	79°04'	150	Duke Forest, North Carolina, USA	1005	Aug. 2001

(20–30  $\mu\text{m}$  thick) were cut from stem ( $N=6$ ) and root ( $N=4-6$ ) segments using a sliding microtome (American Optical, Buffalo, New York, USA). The sections were stained with toluidine blue, then rinsed in deionized water and mounted in glycerol on a glass slide. Images along each of 3–4 radial axes approximately 90 degrees apart were captured at a magnification of 100 $\times$  for roots and 200 $\times$  for stems for one section per stem or root with a Nikon CoolPix 990 digital camera (Nikon, Melville, New York, USA) mounted on a light microscope (Nikon E400). Along each of the 3–4 radial axes, enough images were taken to cover the outer one-quarter of each radial axis. A minimum of 1000 tracheids per section was analyzed. After these images were downloaded to a computer, we measured lumen area of all intact tracheids with image analysis software (Scion Image v.4.02 for Windows, Scion, Frederick, Maryland, USA). Because the tracheids were more square than circular or rectangular in cross section, tracheid diameter ( $d$ , in  $\mu\text{m}$ ) was determined to be equal to the side of a square with an area equal to the lumen area. To determine the functional significance of conduit size distribution, we also calculated the hydraulically weighted mean diameter ( $d_h$ , in  $\mu\text{m}$ ) for each segment as  $\Sigma d^5 / \Sigma d^4$  (Pockman and Sperry, 2000).

To examine the relationship between vulnerability to cavitation and construction costs, we measured the reinforcement of conduit walls against implosion and a related trait, wood density. For pairs of tracheids with diameters within 3  $\mu\text{m}$  of  $d_h$ , we measured maximum span across a tracheid ( $B$ ) and thickness of the double wall between the pair ( $T$ ) using Scion Image. Conduit wall reinforcement,  $(T/B)^2$ , was measured using digital images of sections of stems and roots used for determining  $d$  and  $d_h$  (Hacke et al., 2001). The  $(T/B)^2$  value was determined for a minimum of 50 tracheid pairs per stem or root. We measured a related parameter, wood density ( $D$ ; dry mass per fresh volume,  $\text{g}\cdot\text{cm}^{-3}$ ) on the same segments used for vulnerability curves ( $N=4-6$ ). Segments 2.5 cm long were cut from stems and roots without heartwood. The bark was peeled away, and fresh volume was determined as the volume of a cylinder. The segments were then oven-dried at 75°C for 72 h to obtain dry mass.

**Statistical analyses**—The tension inducing a percentage loss of conductivity (PLC) of 50% ( $P_{50}$ ) was estimated by fitting vulnerability curves using an exponential sigmoidal function:  $\text{PLC} = 100 / \{1 + \exp[a(\Psi - b)]\}$ , where  $\Psi$  is tension,  $b$  is  $P_{50}$ , and  $a$  is proportional to the slope of the vulnerability curve (Pammenter and Vander Willigen, 1998). Vulnerability curves were fit to data from stems and roots using the nonlinear mixed model procedure (NLINMIX), which did not require log transformation of the data, in the program SAS 9.1 (SAS Institute, Cary, North Carolina, USA). Significant differences in  $P_{50}$  and other traits were determined with one-way ANOVA for species or two-way ANOVA for tissue type (stem, root), species and tissue type  $\times$  species effects using the program JMP IN 5.1 (SAS Institute). Multiple comparisons were made using Tukey's honestly significant difference (HSD) using JMP IN 5.1.

**Comparative methods: single trait analyses**—One comparative method used in this study quantifies the degree of evolutionary conservatism or convergence, or phylogenetic signal, in single continuous traits. For single hydraulic traits, we determined the quantitative convergence index (QVI; Ackerly and Donoghue, 1998), which quantifies the amount of convergent evolution in each trait over the phylogeny based on linear parsimony methods, using CACTUS 1.13 software (Comparative Analysis of Continuous Traits Using Statistics; Schilck, 2001; Schilck and Ackerly, 2001). The QVI varies from 0, for highly conserved traits, such that phenotypically similar species are closely related, to 1, for highly convergent traits such that similar phenotypes are distantly related. Significant levels of conserved or convergent evolution were determined using the same program based on randomization methods to test for levels of homoplasy that are greater or less than expected due to chance. Significance testing was accomplished in CACTUS by comparing the calculated QVI to that under the null model of no relationship between character values and the phylogeny (i.e., character values are randomly shuffled across the tips of the phylogeny).

**Comparative methods: correlations between traits**—We used two methods to determine correlations between pairs of continuous traits, both with and without considering phylogenetic relationships among taxa. Phylogenetic independent contrast (PIC) correlations were used to test for correlated evolutionary change among pairs of traits (Felsenstein, 1985b). PICs are calculated as the difference between values in both traits of the sister taxa at terminal or internal nodes, such that  $n$  taxa result in  $n - 1$  contrasts. Correlations are then determined through the set of differences. Independent contrasts were standardized to meet the assumptions of parametric statistics by dividing the contrast value

by the branch lengths as determined by the number of changes along each branch. PIC correlation coefficients and significance levels were determined using CACTUS 1.13. We also calculated Pearson correlation coefficients for cross-species correlations, without considering phylogeny, using JMP IN 5.1.

## RESULTS

**Phylogenetic analyses**—When analyzed separately (Appendices S1 and S2, see Supplemental Data with online version of this article) or together (Fig. 2), both ITS and *LEAFY* produced strong support for the division of the 14 species into two clades, one with the nine western U. S. species in the serrate leaf margin clade, and the other with five species in the Caribbean and eastern United States, plus the western U. S. species *J. scopulorum*, in the smooth leaf margin clade. The ITS sequence data were 1119 bp long and yielded 86 informative characters, and the *LEAFY* sequence data were 1052 bp long and yielded 112 informative characters. Eight ITS indels (insertions or deletions of base pairs in the DNA sequence) and 12 *LEAFY* indels were coded as binary characters. Because there was low bootstrap (BS) support for the areas of disagreement between the two sets of sequence data, we combined the ITS and *LEAFY* sequences for each individual (Mason-Gamer and Kellogg, 1996). Parsimony analysis of 198 informative characters in the combined ITS and *LEAFY* data set yielded a single tree (Fig. 2). In the serrate margin clade, *J. californica* is basal to the other eight species, and *J. ashei*-*J. flaccida*-*J. deppeana* form a clade that is sister to a *J. arizonica*-*J. pinchotii*-*J. monosperma*-*J. osteosperma*-*J. occidentalis* clade (Fig. 2). In the smooth margin clade, *J. virginiana* and *J. virginiana* var. *silicicola* form a highly supported clade (97% BS), which is sister to a weakly supported *J. scopulorum*-*J. lucayana*-*J. barbadensis* clade (65% BS). Strong support (100% BS) for both the serrate and smooth leaf margin clades (Fig. 2) confirmed previous taxonomic division into informal series based on leaf margin serration (Gaussen, 1968) and random amplified polymorphic DNAs (RAPDs; Adams and Demek, 1993). We noted some topological differences between our results and the trees produced by Little (2006) in a broad-level study of the Cupressaceae clade. Specifically, Little (2006) placed *J. californica* as sister to *J. osteosperma* based on ITS and chloroplast DNA sequences; however, only five taxa are common to both studies, thus a more complete phylogenetic study of the genus, including intraspecific sampling, is needed to resolve this discrepancy.

**Vulnerability to xylem cavitation**—The vulnerability of roots and stems to xylem cavitation varied widely across the 14 *Juniperus* species (Fig. 3). The species most vulnerable to xylem cavitation was *J. virginiana*, the most widespread conifer in the mesic eastern United States, which reached 50% loss of conductivity at  $-5.8$  MPa in stems ( $P_{50\text{stem}}$ ) and  $-4.9$  MPa in roots ( $P_{50\text{root}}$ ). The species most resistant to water stress (least vulnerable to cavitation) was *J. californica* from the Mojave Desert in California, with an extrapolated  $P_{50\text{stem}}$  of  $-22.0$  MPa and  $P_{50\text{root}}$  of  $-14.2$  MPa (Fig. 3). In general, serrate margin species were significantly more resistant to xylem cavitation than were smooth margin species for both stems and roots (serrate mean  $P_{50\text{stem}} = -12.5 \pm 0.5$  and  $P_{50\text{root}} = -8.3 \pm 0.6$ ; smooth mean  $P_{50\text{stem}} = -9.5 \pm 0.5$  and  $P_{50\text{root}} = -5.8 \pm 0.7$  MPa;  $P < 0.001$ ). Species in the serrate margin clade were 34 and 39% (stems and roots, respectively) more resistant to xylem cavitation and had 33 and 38% (stems and roots) lower  $K_S$  and 39% lower SLA compared to species in the smooth margin clade

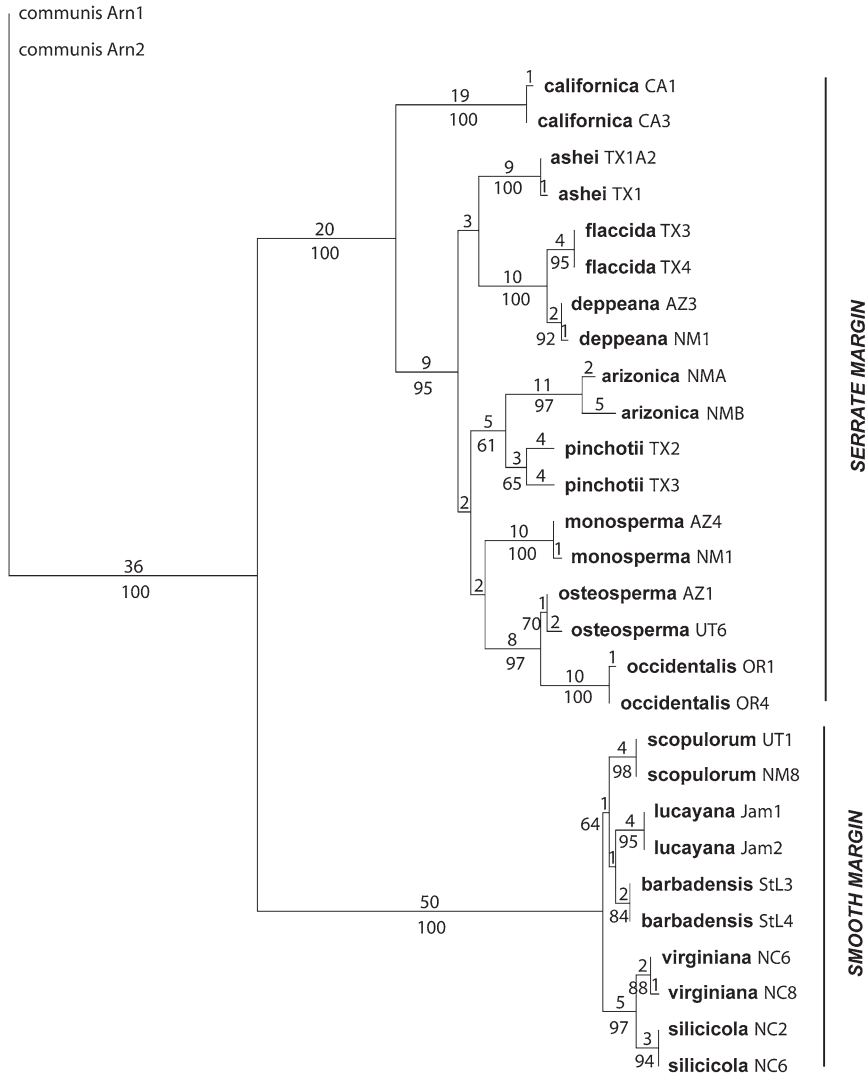


Fig. 2. Phylogram based on ITS and *LEAFY* sequence data combined for each accession created with maximum parsimony. Numbers above branches are branch lengths (number of changes) and numbers below are bootstrap support values.

(Table 2). Interestingly, species in the serrate margin clade occur in drier habitats with only half of the MAP compared to species in the smooth margin clade (Fig. 1, Table 2).

When comparing all species, roots were significantly more vulnerable than stems to xylem cavitation (Figs. 3, 4; mean  $P_{50\text{root}} = -8.1 \pm 0.3$  MPa, mean  $P_{50\text{stem}} = -10.8 \pm 0.5$  MPa;  $P < 0.001$ ). Additionally, specific conductivity ( $K_S$ ) was on average >10 times higher in roots than in stems (mean root  $K_S = 3.3 \pm 0.3$  kg·m<sup>-1</sup>·MPa<sup>-1</sup>·s<sup>-1</sup>; mean stem  $K_S = 0.3 \pm 0.1$  kg·m<sup>-1</sup>·MPa<sup>-1</sup>·s<sup>-1</sup>;  $P < 0.001$ ). Comparing each species individually, roots were significantly more vulnerable than stems for five of the 14 species: *J. californica*, *J. pinchotii*, *J. arizonica*, *J. ashei*, and *J. barbadensis* (Fig. 3). Species whose stems were generally less vulnerable to cavitation also tended to have less vulnerable roots (Figs. 3, 4). As overall vulnerability to cavitation decreased, the difference between stem and root vulnerability increased (Fig. 4). For instance, species such as *J. virginiana* and *J. virginiana* var. *silicicola* with more vulnerable xylem had similar values for  $P_{50\text{root}}$  and  $P_{50\text{stem}}$ , whereas species such as *J. pinchotii* and *J. arizonica* had much less-vulnerable stem xylem (more negative  $P_{50\text{stem}}$ ) compared to root xylem (Figs. 3, 4).

**Comparative methods: single trait analyses**—Several hydraulic traits had significant levels of evolutionary conservatism—the lack of adaptive radiation such that similarly related species have similar phenotypes—as indicated by low QVI values. (In contrast, QVI values are high for convergent evolution, when similarities have arisen independently in two or more organisms that are not closely related.) Quantitative convergence index values ranged from 0.19 for SLA to 0.92 for root  $K_S$  (Table 3). Vulnerability to xylem cavitation in both roots and stems had a substantial degree of phylogenetic conservatism (Table 3, Fig. 5). Mean conduit diameter ( $d$ ), hydraulically weighted conduit diameter ( $d_h$ ) and  $K_S$  were conserved traits for stems, but not for roots (Table 3). The most highly conserved trait was SLA (Table 3, Fig. 6).

The extent to which interspecific variation in traits is due to convergent evolution or trait conservatism can be illustrated by mapping traits onto a phylogeny. Mapping some of the most highly conserved traits onto the phylogeny shown in Fig. 2 revealed strong differences between the serrate and smooth margin clades (Figs. 5, 6). The species most resistant (least vulnerable) to xylem cavitation in both stems and roots belong

TABLE 2. Means ( $\pm 1$  SE) for stem and root hydraulic traits of species in serrate and smooth leaf margin series. Traits include vulnerability to xylem cavitation (water potential producing 50% loss in hydraulic conductivity,  $P_{50}$ ), specific conductivity ( $K_S$ ), conduit diameter ( $d$ ), hydraulically weighted conduit diameter ( $d_h$ ), wood density, conduit wall reinforcement  $[(T/B)^2]$ , leaf-specific conductivity ( $K_L$ ), sapwood to leaf area ratio ( $A_S/A_L$ ), specific leaf area (SLA), and mean annual precipitation (MAP) for 14 *Juniperus* species. Within each row, values followed by different letters are significantly different ( $P < 0.05$ ).

Trait	Units	Stem		Root	
		Serrate	Smooth	Serrate	Smooth
$P_{50}$	MPa	-12.48 $\pm$ 0.50 c	-8.27 $\pm$ 0.62 b	-9.51 $\pm$ 0.52 b	-5.81 $\pm$ 0.68 a
$K_S$	kg·m <sup>-1</sup> ·MPa <sup>-1</sup> ·s <sup>-1</sup>	0.25 $\pm$ 0.20 c	0.40 $\pm$ 0.25 c	2.79 $\pm$ 0.21 b	4.16 $\pm$ 0.27 a
$d$	µm	7.32 $\pm$ 0.37 c	8.29 $\pm$ 0.47 c	16.60 $\pm$ 0.39 b	19.17 $\pm$ 0.50 a
$d_h$	µm	8.98 $\pm$ 0.42 c	10.40 $\pm$ 0.53 c	21.05 $\pm$ 0.44 b	24.56 $\pm$ 0.58 a
Wood density	g·cm <sup>-3</sup>	0.61 $\pm$ 0.01 a	0.63 $\pm$ 0.01 a	0.49 $\pm$ 0.01 b	0.43 $\pm$ 0.01 c
$(T/B)^2$	unitless	0.28 $\pm$ 0.01 a	0.27 $\pm$ 0.02 a	0.14 $\pm$ 0.01 b	0.10 $\pm$ 0.01 b
$K_L$ ( $\times 10^{-4}$ )	kg·m <sup>-1</sup> ·MPa <sup>-1</sup> ·s <sup>-1</sup>	1.85 $\pm$ 0.19 a	2.32 $\pm$ 0.24 a	—	—
$A_S/A_L$ ( $\times 10^{-4}$ )	unitless	7.83 $\pm$ 0.52 a	6.49 $\pm$ 0.66 a	—	—
SLA	m <sup>2</sup> ·kg <sup>-1</sup>	2.07 $\pm$ 0.06 b	3.41 $\pm$ 0.09 a	—	—
MAP	mm·yr <sup>-1</sup>	470.5 $\pm$ 30.6 b	935.0 $\pm$ 44.7 a	—	—

to the serrate margin clade (the upper clade in Fig. 5). In contrast, the smooth margin clade contains the species most vulnerable to xylem cavitation in both stems and roots (the lower clade in Fig. 5). Closely related species show remarkable similarity in SLA, the most conserved trait (Fig. 6). The serrate margin clade had a mean SLA of 2.1  $\pm$  0.1 m<sup>2</sup>·kg<sup>-1</sup>, and the smooth margin clade had a mean SLA of 3.4  $\pm$  0.6 m<sup>2</sup>·kg<sup>-1</sup>. The species least vulnerable to xylem cavitation, *J. californica*, had the smallest SLA (Figs. 5, 6). The two Caribbean species, *J. lucayana* and *J. barbadensis*, were closely related and had the highest SLA values (Fig. 6).

**Comparative methods: correlations between traits**—In most cases, we found relationships between stem or root  $K_S$  and other traits that were significant using both cross-species and PIC analyses (Table 5). Stem  $K_S$  was negatively correlated with  $A_S/A_L$  and positively correlated with SLA and MAP in the cross-species analysis (Table 5). These relationships weakened somewhat in the PIC analysis. Root  $K_S$  was negatively correlated with  $A_S/A_L$  and positively correlated with SLA and MAP in the PIC analysis, but not in the cross-species analysis (Table 5).

TABLE 3. Summary of convergent evolution statistics for vulnerability to xylem cavitation (water potential producing 50% loss in hydraulic conductivity,  $P_{50}$ ), specific conductivity ( $K_S$ ), conduit diameter ( $d$ ), hydraulically weighted conduit diameter ( $d_h$ ), wood density, conduit wall reinforcement  $[(T/B)^2]$ , leaf-specific conductivity ( $K_L$ ), sapwood to leaf area ratio ( $A_S/A_L$ ), and specific leaf area (SLA) for 14 *Juniperus* species. Low quantitative convergent index (QVI) values indicate that a trait is highly conserved, and high QVI values indicate that a trait illustrates convergent evolution. Values in boldface are statistically significant. \* $P < 0.05$ ; \*\* $P < 0.005$ .

Trait	QVI	
	Stem	Root
$P_{50}$	<b>0.53*</b>	<b>0.39**</b>
$K_S$	<b>0.54*</b>	0.92
$d$	<b>0.45*</b>	0.79
$d_h$	<b>0.48*</b>	0.81
Wood density	0.78	0.72
$(T/B)^2$	0.79	0.66
$K_L$	0.70	—
$A_S/A_L$	0.89	—
SLA	<b>0.19**</b>	—

In contrast, we found relationships between  $P_{50\text{stem}}$  or  $P_{50\text{root}}$  and other traits that were significantly correlated when using standard cross-species analysis, but not when using the PIC analysis (Table 4). When we compared pairs of traits, there were significant cross-species correlations between increasing SLA and increasing vulnerability to xylem cavitation in both stems and roots (Fig. 7A, F). SLA was not correlated with  $P_{50\text{stem}}$  or  $P_{50\text{root}}$ , however, according to PIC correlations (insets in Fig. 7A, F). In stems, conduit wall reinforcement,  $(T/B)^2$ , and wood density were not correlated with  $P_{50\text{stem}}$  in the cross-species analysis, but they were correlated in the PIC analysis such that more vulnerable stem xylem had lower  $(T/B)^2$  and density (Fig. 7B, C). The opposite pattern occurred in roots, with significant correlations in the cross-species analysis but not in the PIC analysis (Fig. 7G, H). Vulnerability to xylem cavitation was not correlated with  $K_S$  in either the cross-species or PIC analysis for stems and roots (Fig. 7D, I).

DISCUSSION

Traits observed in nature often reflect both the adaptation of species to their present habitats and the legacy of traits found in species' ancestors. Overall, all 14 *Juniperus* species in our study are more resistant to xylem cavitation compared to taxa in previous studies (e.g., Maherali et al., 2004). *Juniperus californica* had an extrapolated  $P_{50\text{stem}}$  of -22.0 MPa, one of the most resistant species ever reported (along with other members of Cupressaceae; e.g., *Actinostrobus acuminatus*, Brodrigg and Hill, 1999). Such resistance to cavitation could be particularly important because the recent expansion of junipers during the past two centuries has occurred during a period of increasing aridity (Miller and Wigand, 1994). The high resistance to water-stress-induced cavitation in *Juniperus* might help explain the successful survival of junipers during drought and after their expansion into drier environments.

The 14 *Juniperus* species in this study separated into two groups on the basis of their phylogeny and hydraulic traits, coinciding with taxonomic divisions based on leaf margin serration (Figs. 2, 5, 6). The most striking feature of the phylogeny produced in this study is the strong support for the taxonomic divisions into informal series based on leaf margin serration: serrate or smooth margins (Fig. 2; Gaussen, 1968; Adams and Demeke, 1993; Adams, 2004). Our results were similar to those

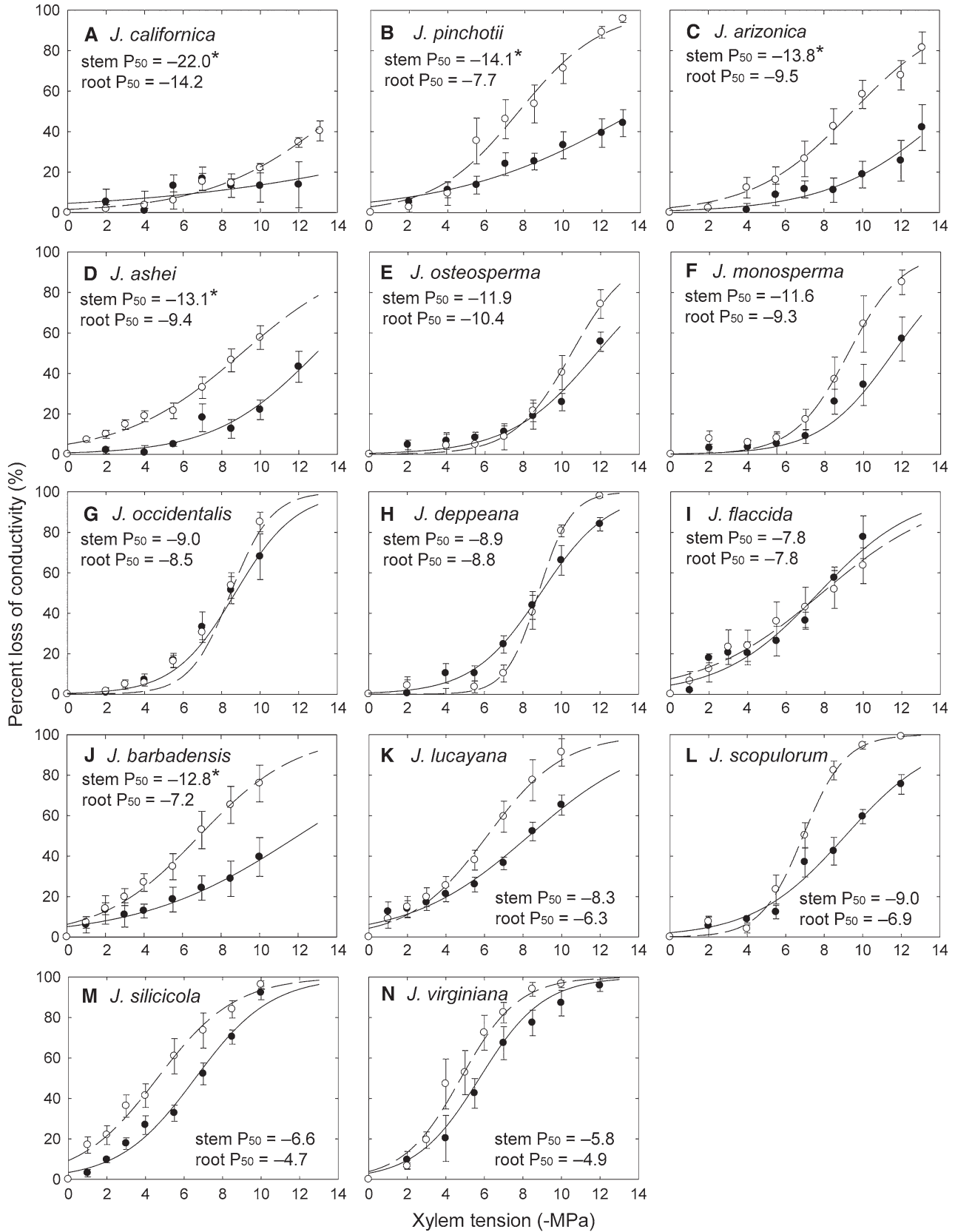


Fig. 3. Curves of the vulnerability of xylem to cavitation for stems (filled circles and solid lines;  $N = 6$  per species) and roots (open circles and dashed lines;  $N = 4-6$  per species) for 14 *Juniperus* species. Although means ( $\pm 1$  SE) are presented, curves were fit using all data. The xylem tension causing 50% loss in hydraulic conductivity ( $P_{50}$ ; same as variable  $b$ ) for each species is shown in each panel. Means for variable  $a$ , related to the slope of the vulnerability

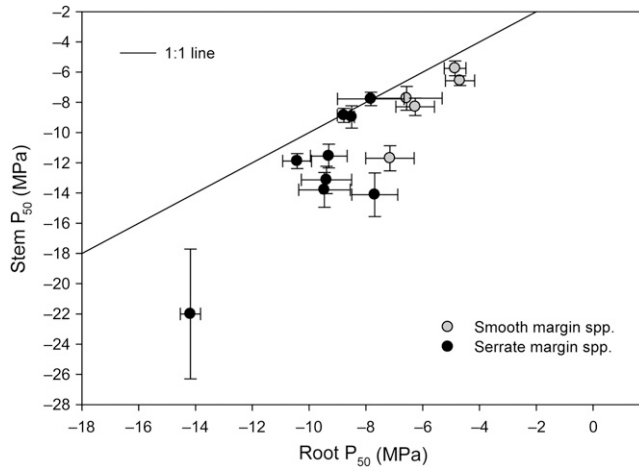


Fig. 4. The xylem tension causing 50% loss in hydraulic conductivity ( $P_{50}$ ) for stems plotted against the same parameter for roots for 14 *Juniperus* species. Species in the smooth leaf margin group are shown as gray circles, and species in the serrate leaf margin group are shown as black circles. Stem  $P_{50}$  and root  $P_{50}$  were correlated, both when all 14 species as shown are included ( $r = 0.87$ ,  $P < 0.001$ ) and also when *J. californica* is excluded (the point in the lower left of the figure;  $r = 0.72$ ,  $P = 0.005$ ).

of Adams et al. (2006) for southwestern U. S. junipers in that *J. osteosperma* and *J. occidentalis* formed a clade, and *J. californica* was distinct among the serrate species. The smooth margin species in this study, *J. virginiana*, from the eastern United States, *J. virginiana* var. *silicicola* from the coastal, southeastern United States, and *J. scopulorum* from the Rocky Mountains of the western United States, formed a clade with *J. lucayana* from Jamaica (also found in Cuba and the Bahamas) and *J. barbadensis* from St. Lucia (Fig. 2). This supports the suggestion that Caribbean junipers appear to have arisen from an ancestor of *J. virginiana* or *J. scopulorum* from the Appalachian-southeastern U. S. region rather than from the junipers in southern Mexico and Guatemala, which belong to the serrate margin series (Adams, 1995). When averaging species within series, the serrate margin *Juniperus* species, common in the more arid western United States, were more resistant to xylem cavitation but had lower  $K_s$  than the smooth margin *Juniperus* species (Table 2, Fig. 5). In general, conduit diameters ( $d$  and  $d_h$ ) were narrower in the serrate margin species than in the smooth margin species, but the differences in  $K_s$ ,  $d$ , and  $d_h$  were only significant in roots and not in stems (Table 2). Specific leaf area (SLA) and mean MAP for the serrate series were lower than for the smooth series (Table 2, Fig. 6). This pattern of traits associated with clade membership leads us to suggest that physiological traits related to hydraulic architecture yield a strong phylogenetic signal in *Juniperus*.

By examining single traits across a phylogeny, we established that several hydraulic traits gave strong phylogenetic signals in *Juniperus*. Root  $P_{50}$  (QVI = 0.39) was more highly conserved than stem  $P_{50}$  (QVI = 0.53, Table 3, Fig. 5). The

most highly conserved trait was SLA (QVI = 0.19, Table 3, Fig. 6). SLA has been shown to decline along gradients of decreasing moisture and/or nutrient availability (Cunningham et al., 1999). In accordance, SLA was highest in the two tropical *Juniperus* species, next highest in the two eastern U. S. species, intermediate in the southwestern U. S. species, and lowest in the Mojave Desert species (Fig. 6). High phenotypic and ecological similarity between closely related species implies that some traits have changed little since a divergence from a common ancestor or that parallel evolutionary changes have occurred since divergence. The lack of change in evolutionary lineages could be due to (1) lack of genetic variation upon which selection can act, (2) insufficient time since an evolutionary divergence, or (3) stabilizing selection that maintains ancestral traits (Ackerly, 2003; Caruso et al., 2005). At least some *Juniperus* species have likely arisen recently because Bermuda's soil was formed only during the first interglacial period of the Pleistocene (Cox, 1959). Although these reasons cannot be distinguished here, we propose that because phylogenetic branch lengths were relatively short, speciation may be relatively recent, which could explain why *Juniperus* species show a high degree of conservatism in vulnerability to cavitation and other hydraulic traits.

Interestingly, species more resistant to xylem cavitation, such as many in the serrate series, tended to have greater differences between stem and root  $P_{50}$  values than less resistant species (Fig. 4). Notably, several species had no significant differences in stem and root vulnerability (Figs. 3, 4). Similarly, Linton et al. (1998) found no difference in vulnerability to cavitation in stems and roots of *J. osteosperma*. In general, however, roots were more vulnerable than stems when all species are considered (mean  $P_{50\text{stem}} = -10.6 \pm 0.5$  MPa, mean  $P_{50\text{root}} = -8.0 \pm 0.3$  MPa,  $P < 0.001$ ; Fig. 3). Roots are often more vulnerable to cavitation than stems in most angiosperms and conifers (Linton et al., 1998; Kavanagh et al., 1999; Jackson et al., 2000; Maherli et al., 2004, 2006; McElrone et al., 2004). A possible reason for why root xylem is more vulnerable than stem xylem, according to the vulnerability segmentation hypothesis, is because xylem tension is highest in peripheral organs such as leaves and minor twigs, so embolism occurs first in peripheral organs (Zimmermann, 1983). Cavitation in the roots that are more easily replaced may protect stem xylem from further water loss and possible cavitation. Roots are also likely partly buffered from the most extreme water potentials faced by stems and leaves. In addition, greater vulnerability in roots may be less harmful than in stems because roots are typically lower in construction cost, as indicated by wood density and  $(T/B)^2$  (Table 2), and they may have the opportunity for refilling of emboli by root pressure or other mechanisms (Sperry, 1995).

When levels of convergent evolution are low and conservatism are high, there are often discrepancies between cross-species and evolutionary correlations (Ackerly and Donoghue, 1998). A deep divergence, coupled with reduced divergence within the descendant lineages can explain correlations among traits using traditional cross-species correlations that diminish

curve, were not significantly different in stems, and there were few significant differences in roots. Mean  $a$  ( $\pm 1$  SE) for stems and roots in the following species order were (A)  $0.18 \pm 0.05$  and  $0.32 \pm 0.06$ , (B)  $0.24 \pm 0.03$  and  $0.64 \pm 0.08$ , (C)  $1.12 \pm 0.68$  and  $0.44 \pm 0.02$ , (D)  $0.42 \pm 0.04$  and  $0.35 \pm 0.05$ , (E)  $0.47 \pm 0.08$  and  $0.70 \pm 0.09$ , (F)  $0.75 \pm 0.17$  and  $0.90 \pm 0.19$ , (G)  $0.68 \pm 0.07$  and  $1.03 \pm 0.13$ , (H)  $0.65 \pm 0.09$  and  $1.28 \pm 0.18$ , (I)  $0.41 \pm 0.06$  and  $0.40 \pm 0.03$ , (J)  $0.26 \pm 0.03$  and  $0.40 \pm 0.03$ , (K)  $0.34 \pm 0.03$  and  $0.55 \pm 0.11$ , (L)  $0.49 \pm 0.06$  and  $1.01 \pm 0.16$ , (M)  $0.54 \pm 0.03$  and  $0.59 \pm 0.09$ , (N)  $0.87 \pm 0.19$  and  $0.93 \pm 0.19$ . \*Significant differences between stem and root  $P_{50}$  ( $P < 0.05$ ).

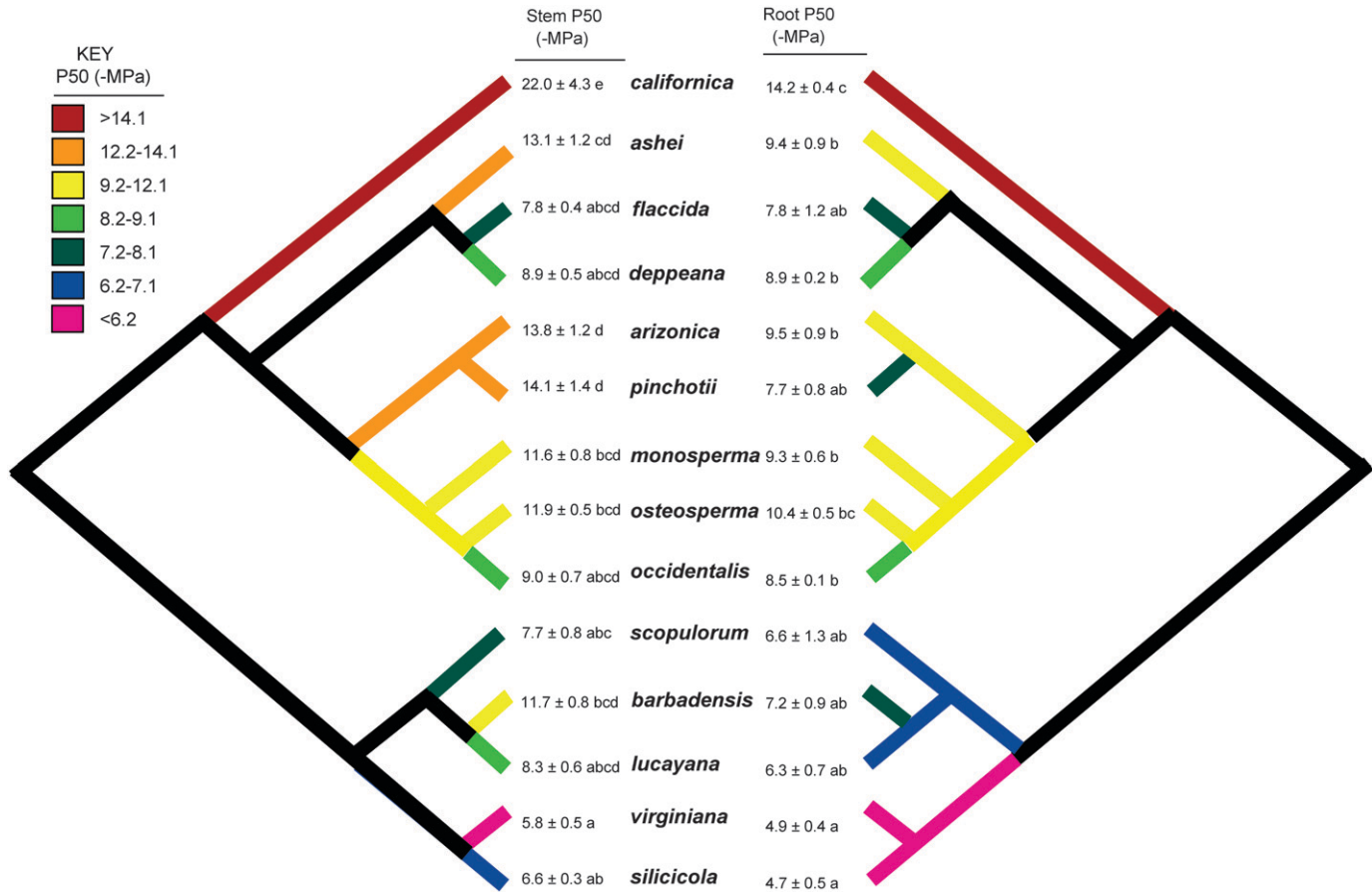


Fig. 5. Phylogenetic tree constructed with maximum parsimony methods showing the evolution of vulnerability to water-stress-induced xylem cavitation in stems (left) and roots (right) of 14 *Juniperus* species. Convergence statistics for  $P_{50}$  are shown in Table 3. Continuous  $P_{50}$  data were grouped into seven discrete color-coded categories to aid presentation. Ambiguous state assignments along branches leading to hypothetical ancestors are in black.

with PIC correlations. In contrast, traits with a higher degree of homoplasy (e.g., convergent evolution) tend to have less discrepancy between cross-species and PIC correlations. Not sur-

prisingly then, stem and root  $P_{50}$  were both significantly correlated with SLA in the cross-species correlation but not in the PIC correlation (Fig. 7), since stem and root  $P_{50}$  and SLA

TABLE 4. Magnitude and statistical significance of Pearson correlations ( $r$ ) or phylogenetically independent contrast (PIC) correlations for relationships between stem or root vulnerability to xylem cavitation ( $P_{50}$ ) and other traits for 14 *Juniperus* species. Other traits include specific conductivity ( $K_S$ ), conduit diameter ( $d$ ), hydraulically weighted conduit diameter ( $d_h$ ), wood density, conduit wall reinforcement  $[(T/B)^2]$ , leaf-specific conductivity ( $K_L$ ), sapwood to leaf area ratio ( $A_S/A_L$ ), specific leaf area (SLA), and mean annual precipitation (MAP) for 14 *Juniperus* species. Values in boldface are statistically significant. † $P < 0.10$ ; \* $P < 0.05$ ; \*\* $P < 0.005$ .

Trait or contrast	Pearson correlations		PIC correlations	
	Stem $P_{50}$	Root $P_{50}$	Stem $P_{50}$	Root $P_{50}$
Root $P_{50}$	<b>0.87**</b>	—	<b>0.71*</b>	—
Root $K_S$	—	0.35	—	0.10
Root $d$	—	<b>0.47†</b>	—	0.04
Root $d_h$	—	<b>0.60*</b>	—	0.15
Root wood density	—	<b>-0.49†</b>	—	-0.29
Root $(T/B)^2$	—	<b>-0.58*</b>	—	-0.27
Stem $K_S$	0.40	—	0.08	—
Stem $K_L$	0.35	—	-0.08	—
Stem $d$	<b>0.48†</b>	—	0.09	—
Stem $d_h$	0.41	—	0.08	—
Stem wood density	-0.31	—	<b>-0.54*</b>	—
Stem $(T/B)^2$	-0.42	—	<b>-0.49†</b>	—
$A_S/A_L$	-0.04	-0.23	-0.02	-0.18
SLA	<b>0.63*</b>	<b>0.75*</b>	0.26	0.24
MAP	0.31	<b>0.52†</b>	-0.26	0.02

are all highly conserved traits in *Juniperus* (Figs. 5, 6). For stem  $P_{50}$ , the significant PIC correlation suggests that evolutionary increases in vulnerability to cavitation in stems are associated with evolutionary decreases in  $(T/B)^2$  and wood density (Fig. 7). For root  $P_{50}$ , it appears that large differences in trait values between species in the serrate and smooth series caused the PIC correlations to be insignificant. It should be acknowledged that PIC analyses can be sensitive to phylogenetic uncertainty, branch lengths, and nonrandom species sampling (Ackerly, 2000), although other recent studies also found inconsistencies between cross-species and PIC correlations of cavitation resistance and wood density or  $(T/B)^2$  (Jacobsen et al., 2007; Pratt et al., 2007). A lack of correlated evolution between cavitation resistance and  $(T/B)^2$  may point to other anatomical traits, such as fibers in the angiosperm species *Ceanothus crassifolius* or *Cercocarpus betuloides* as suggested by Jacobsen et al. (2007), that contribute to resistance to implosion.

Our results are consistent with a growing body of evidence suggesting a lack of a trade-off between safety from xylem cavitation and efficiency of xylem transport. Zimmermann (1983) hypothesized that there was a “safety vs. efficiency” trade-off in xylem such that wider conduits were more efficient in water transport yet more vulnerable to xylem cavitation. Although evidence for such a correlation between  $P_{50}$  and  $K_S$  has been shown to exist in studies of single communities (e.g., Pockman and Sperry, 2000; Martínez-Vilalta et al., 2002), studies comparing numerous species show little or no evidence for the trade-off (Tyree et al., 1994; Hacke and Sperry, 2001; Maherali et al., 2004) or no trade-off when phylogenetic relationships are taken into account (Maherali et al., 2006; Jacobsen et al., 2007). Still other studies have shown that the trade-off exists at the level of the individual plant; i.e., that roots are more vulnerable than stems yet roots have higher  $K_S$  (Hacke and Sperry, 2001; Willson and Jackson, 2006). We found no relationship between  $P_{50}$  and  $K_S$  within stems or roots using either standard cross-species or PIC correlations (Fig. 7). According to the Hagen–Poiseuille relationship, flow through a conduit is related to its diameter to the fourth power (Tyree and Sperry, 1989). In conifers, hydraulic conductivity is largely a function

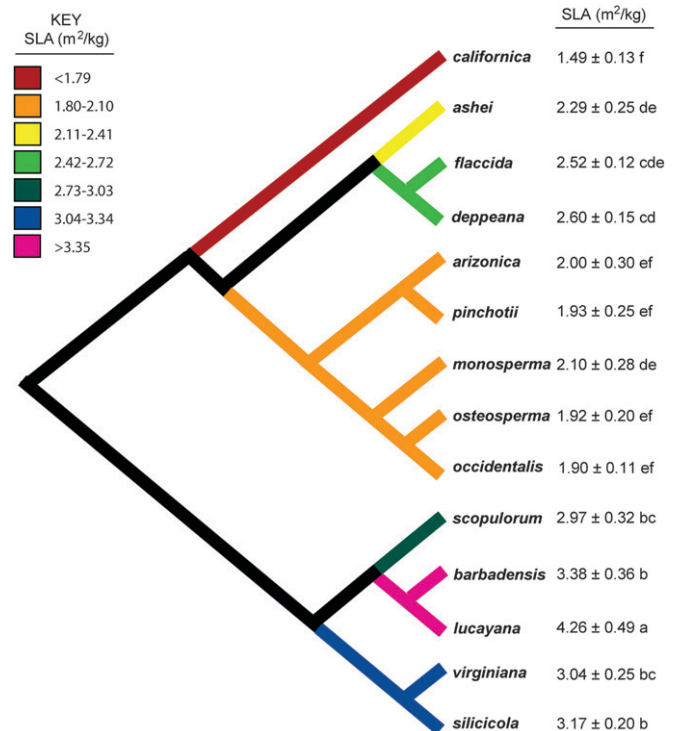
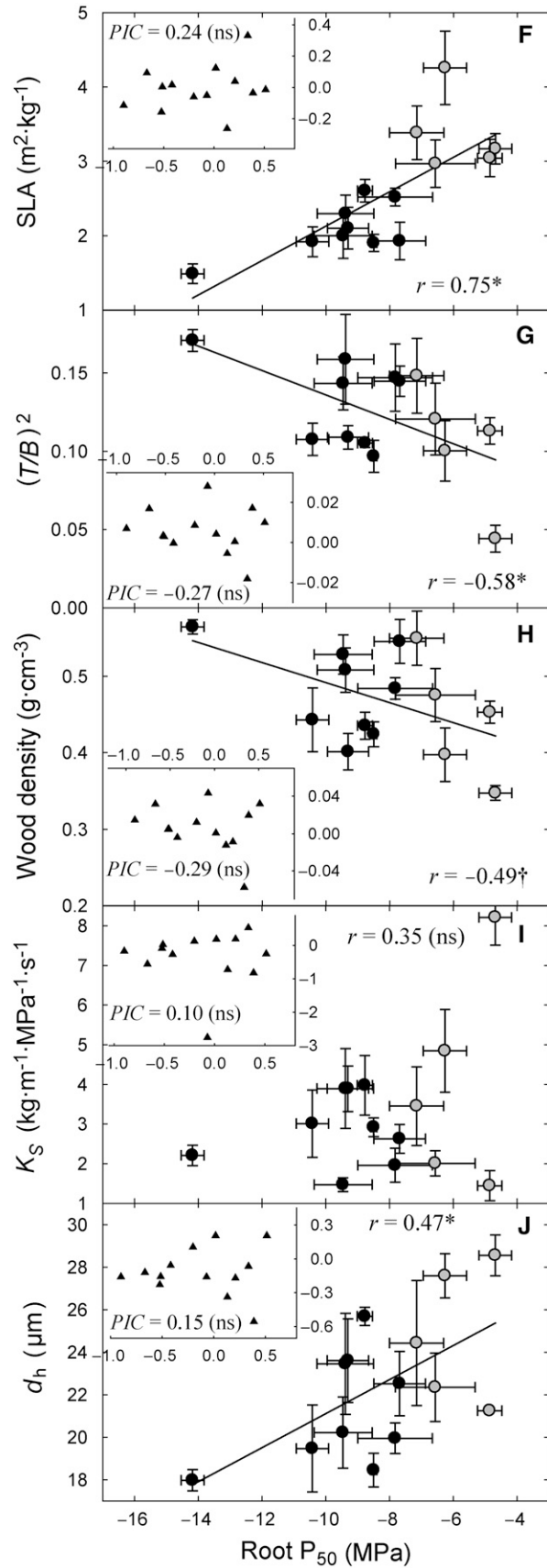
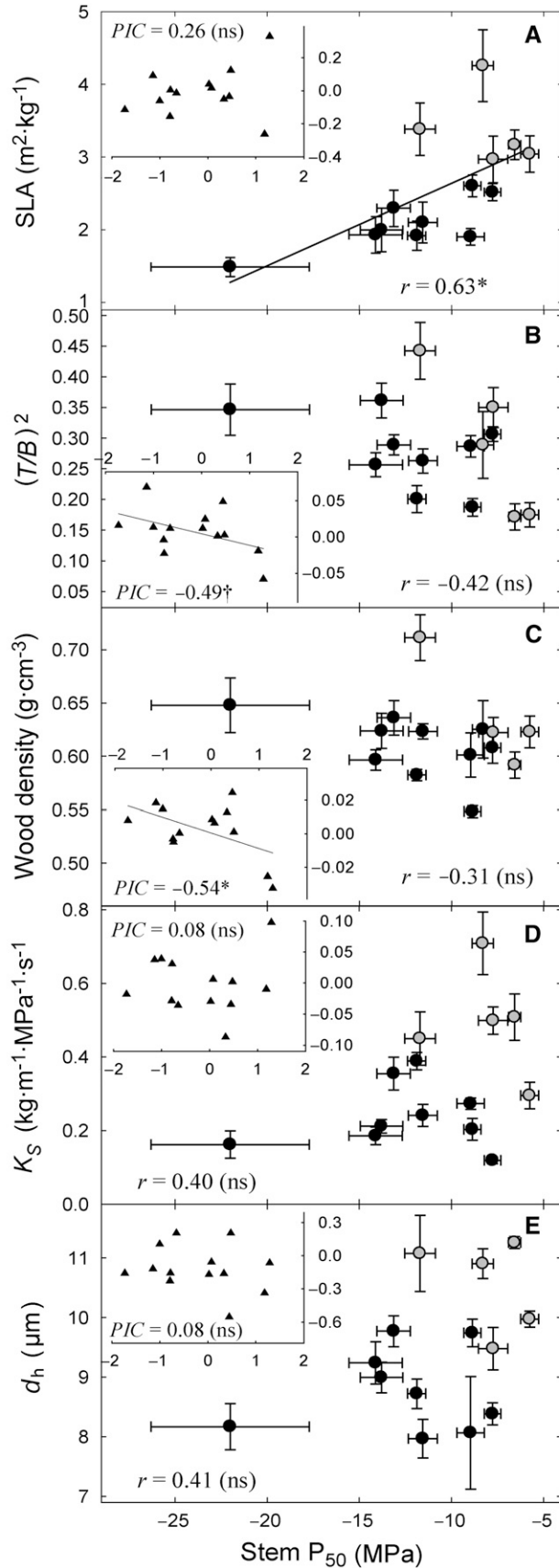


Fig. 6. Phylogenetic tree constructed with maximum parsimony methods showing the evolution of specific leaf area (SLA;  $m^2 \cdot kg^{-1}$ ) for 14 *Juniperus* species. Convergence statistics for SLA are in Table 3. Continuous SLA data were grouped into seven discrete color-coded categories to aid presentation. Ambiguous state assignments along branches leading to hypothetical ancestors are in black.

of conduit diameter and length, whereas vulnerability likely depends on strength or flexibility of the torus-margo complex in the pits between adjacent tracheids (Sperry and Tyree, 1990; Hacke et al., 2004), so efficiency and safety from cavitation are not necessarily coupled.

TABLE 5. Magnitude and statistical significance of Pearson correlations ( $r$ ) or phylogenetically independent contrast (PIC) correlations for relationships between stem or root specific conductivity ( $K_S$ ) and other traits for 14 *Juniperus* species. Other traits include vulnerability to xylem cavitation ( $P_{50}$ ), conduit diameter ( $d$ ), hydraulically weighted conduit diameter ( $d_h$ ), wood density, conduit wall reinforcement [ $(T/B)^2$ ], leaf-specific conductivity ( $K_L$ ), sapwood to leaf area ratio ( $A_S/A_L$ ), specific leaf area (SLA), and mean annual precipitation (MAP) for 14 *Juniperus* species. Values in boldface are statistically significant. † $P < 0.10$ ; \* $P < 0.05$ ; \*\* $P < 0.005$ .

Trait or contrast	Pearson correlations		PIC correlations	
	Stem $K_S$	Root $K_S$	Stem $K_S$	Root $K_S$
Root $P_{50}$	—	0.35	—	0.10
Root $K_S$	<b>0.52</b> †	—	<b>0.64</b> **	—
Root $d$	—	<b>0.73</b> **	—	<b>0.81</b> **
Root $d_h$	—	<b>0.79</b> **	—	<b>0.89</b> **
Root wood density	—	<b>-0.66</b> *	—	<b>-0.65</b> **
Root $(T/B)^2$	—	<b>-0.70</b> *	—	<b>-0.78</b> **
Stem $P_{50}$	0.40	—	0.08	—
Stem $K_S$	—	<b>0.52</b> †	—	<b>0.64</b> **
Stem $K_L$	<b>0.51</b> †	—	0.10	—
Stem $d$	<b>0.66</b> *	—	<b>0.49</b> *	—
Stem $d_h$	<b>0.71</b> **	—	0.24	—
Stem wood density	0.21	—	<b>-0.44</b> †	—
Stem $(T/B)^2$	0.03	—	-0.31	—
$A_S/A_L$	<b>-0.59</b> *	-0.31	<b>-0.38</b> †	<b>-0.24</b> †
SLA	<b>0.79</b> **	0.41	<b>0.60</b> *	<b>0.35</b> †
MAP	<b>0.75</b> **	0.42	<b>0.34</b> †	<b>0.35</b> †



Surprisingly, despite the assumption that high resistance to cavitation may be costly in mesic environments, we found relatively high resistance to cavitation even in the two tropical species, *J. lucayana* and *J. barbadensis* (Fig. 3). Soil conditions can exert a strong influence over water availability (Carlquist, 1975), which might explain why some species showed more resistance to xylem cavitation than might be expected due to climate alone. For example, *J. ashei* has a range with the highest MAP values of the serrate series, yet is one of the most resistant species (Table 1, Fig. 3). Additionally, *J. barbadensis* experiences the second highest MAP of all 14 species, yet is more resistant than all of the smooth series species and many of the serrate series species (Table 1, Fig. 3). *Juniperus ashei* occurs on the Edwards Plateau in central Texas, typically on limestone bedrock with very little soil, often <10 cm depth (Jackson et al., 1999). Similarly, *J. barbadensis* is endemic to the summit of the Petit Piton on the Caribbean island of St. Lucia, where it occurs in rocky, poorly developed soil alongside succulent species and cacti. The fact that *Juniperus* species typically occur on limestone or rocky substrates or otherwise arid microsites, even in mesic environments, may help explain the relatively poor correlations of  $P_{50}$  with MAP for all species (Table 4).

Because reduced hydraulic efficiency is not consistently observed as a cost of resistant xylem, and all species are not equally resistant to xylem cavitation, there are presumably other trade-offs that are associated with xylem resistance to cavitation. Water in xylem is under tension, making conduit walls subject to bending stresses between a functional, water-filled conduit at negative pressure and an adjacent embolized, air filled conduit at atmospheric pressure (Hacke et al., 2001). Conduit collapse caused by high tension during water stress has been demonstrated in needles in *Pinus* (Cochard et al., 2004). Bending stress is related to the thickness of the double wall ( $T$ ) between the conduits and the maximum conduit span ( $B$ ) (Hacke et al., 2001). Resistance to cavitation in conifers and angiosperms is highly correlated with the ratio of the double wall thickness to span,  $(T/B)^2$  (Hacke et al., 2001). Because wood with thick walls in relation to conduit diameter [high  $(T/B)^2$ ] will also likely have high wood density, wood density is also related to  $P_{50}$  (Hacke et al., 2001). In our 14 *Juniperus* species, we found significant relationships between increasing resistance to cavitation and both increasing  $(T/B)^2$  and wood density (Fig. 7). In roots, the cross-species correlations were significant but the PIC correlations were not, and the opposite pattern occurred in stems (Fig. 7). Jacobsen et al. (2007) found in stems of 26 chaparral species that  $P_{50}$  and  $(T/B)^2$  were correlated with standard cross-species correlations, but not with PIC correlations. It appears that  $P_{50}$  and  $(T/B)^2$  may be functionally linked but may not display correlated evolutionary change. Jacobsen et al. (2007) suggested that selection may not act as long as  $(T/B)^2$  remains above the threshold necessary to resist implosion. The cost of having higher wall thickness in relation to

span, while affording greater resistance to xylem cavitation, results in higher wood density and therefore greater metabolic costs and reduced growth rate (Enquist et al., 1999; Hacke et al., 2001).

In addition to a trade-off between vulnerability to cavitation and wood reinforcement, we found strong evidence for a significant trade-off in greater wood reinforcement (i.e., higher  $(T/B)^2$  and wood density) at the expense of reduced hydraulic conductivity in roots. In our study, lower root  $K_S$  was strongly associated with higher wood density and  $(T/B)^2$ , and both Pearson and PIC correlations were nearly identical (Table 5). A correlation was not evident for stems. Similarly, Pittermann et al. (2006) found a trade-off between greater mechanical reinforcement and reduced hydraulic efficiency in a study of 18 conifer species, including two *Juniperus* species. In their study, increases in  $(T/B)^2$  (and therefore wood density) were associated with reductions in conifer tracheid diameter rather than increases in wall thickness. The decreased tracheid diameter is therefore responsible for the reduced hydraulic conductivity. As in our study, Pratt et al. (2007) found in nine Rhamnaceae species that  $(T/B)^2$  in angiosperm vessels was significantly inversely correlated to  $K_S$  in roots but not in stems. Also like our study, their correlations were strikingly similar in both standard cross-species correlations as well as PIC correlations. Tracheids function in both water transport and mechanical support. We may have seen a strong tradeoff in *Juniperus* roots but not in stems because roots have a role more limited to water transport, whereas stems have a greater mechanical support role to resist forces of wind, snow, and gravity.

Despite any associated costs, the high resistance to cavitation demonstrated here likely enables juniper species to survive drought conditions. Although the *Juniperus* species studied here were highly resistant compared to other tree species, the phylogenetic divergence of serrate and smooth leaf margins also divides the group physiologically and ecologically (Figs. 2, 5, 6). The division of our 14 *Juniperus* species into taxonomic series based on leaf margin serration (serrate or smooth) was well supported by the phylogeny based on ITS and *LEAFY* sequence variations (Fig. 2; Appendices S1, S2 [see Supplemental Data with online version of this article]). The two series were also distinguished in their ecophysiological traits, with the serrate group more resistant to xylem cavitation (Fig. 5). Vulnerability to cavitation in stems and roots, as well as SLA, showed evolutionary conservatism over the phylogeny (Figs. 5, 6). The divergence event between the two series has likely influenced the subsequent success of each group. The high resistance to cavitation seen in *Juniperus* may help explain their successful survival during the recent extreme droughts in the southwestern United States and why they have been able to expand into arid habitats across the western and central United States over the last century (e.g., Breshears et al., 2005; Mueller et al., 2005).

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Fig. 7. Relationships between traits and cavitation resistance in stems and roots. (A) Specific leaf area (SLA), (B) conduit wall reinforcement from implosion [ $(T/B)^2$ ], (C) wood density, (D) specific conductivity ( $K_S$ ), and (E) hydraulically weighted conduit diameter ( $d_h$ ) expressed as functions of xylem tension causing 50% loss in hydraulic conductivity ( $P_{50}$ ) for stems of 14 *Juniperus* species. (F) Specific leaf area (SLA), (G) conduit wall reinforcement from implosion [ $(T/B)^2$ ], (H) wood density, (I) specific conductivity ( $K_S$ ), and (J) hydraulically weighted conduit diameter ( $d_h$ ) expressed as functions of xylem tension causing 50% loss in hydraulic conductivity ( $P_{50}$ ) for roots of 14 *Juniperus* species. Pearson correlation coefficients ( $r$ ) are shown in each panel. Species in the smooth leaf margin group are shown as gray circles, and species in the serrate leaf margin group are shown as black circles. Plots of phylogenetically independent contrasts (PIC) for each pair of traits and the corresponding PIC correlation coefficients are shown in the insets. † $P < 0.10$ , \* $P < 0.05$ , \*\* $P < 0.005$ .

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APPENDIX 1. Collection data for species analyzed for both ITS and *LEAFY* DNA sequences for phylogenies shown in Fig. 2 and Appendices S1 and S2 (see Supplemental Data with online version of this article). Vouchers for the 14 study species were deposited at the Duke University Herbarium, Durham, North Carolina, USA. Collection sites: NF = National Forest; NG = National Grassland; NM = National Monument; NP = National Park; SP = State Park.

**Taxon**—Collection site; id; Voucher Specimen, GenBank accession numbers: ITS, *LEAFY*.

*Juniperus arizonica* R.P. Adams—Rockhound SP, New Mexico, USA; NMA; C. J. Willson #7; EU277686, EU277715. *J. arizonica* R.P. Adams—Rockhound SP, New Mexico, USA; NMB; C. J. Willson #8; EU277687, EU277716. *J. ashei* Buch.—Spicewood Springs Park, Austin, Texas, USA; TXCOT1; C. J. Willson #1; EU277681, EU277710. *J. ashei* Buch.—Junction, Austin, Texas, USA; TX1A2; C. J. Willson #2; EU277680, EU277709. *J. barbadensis* L.—Petit Piton, St. Lucia; StL3; C. J. Willson #3; EU277704, EU277733. *J. barbadensis* L.—Petit Piton, St. Lucia; StL4; C. J. Willson #4; EU277705, EU277734. *J. californica* Carr.—Joshua Tree NP, California, USA; CA1; C. J. Willson #5; EU277678, EU277707. *J. californica* Carr.—Joshua Tree NP, California, USA; CA3; C. J. Willson #6; EU277679, EU277708. *J. communis* L.—Arnold Arboretum, Boston, Massachusetts, USA; 50-77; Arnold1; EU277677, EU277706. *J. deppeana* Steud.—Walnut Canyon NM, Arizona, USA; AZ3; C. J. Willson #9; EU277684,

EU277713. *J. deppeana* Steud.—Cibola NF, New Mexico, USA; 470; C. J. Willson #10; EU277685, EU277714. *J. flaccida* Schlect.—Big Bend NP, Texas, USA; TXBB3; C. J. Willson #11; EU277682, EU277711. *J. flaccida* Schlect.—Big Bend NP, Texas, USA; TXBB4; C. J. Willson #12; EU277683, EU277712. *J. lucayana* Brit.—Blue Mountains NP, Jamaica; Jam1; C. J. Willson #13; EU277702, EU277731. *J. lucayana* Brit.—Blue Mountains NP, Jamaica; Jam2; C. J. Willson #14; EU277703, EU277732. *J. monosperma* (Engelm.) Sarg.—Walnut Canyon NM, Arizona, USA; AZ4; C. J. Willson #15; EU277690, EU277719. *J. monosperma* (Engelm.) Sarg.—Cibola NF, New Mexico, USA; NM; C. J. Willson #16; EU277691, EU277720. *J. occidentalis* Hook.—Crooked River NG, Oregon, USA; OR1; C. J. Willson #17; EU277694, EU277723. *J. occidentalis* Hook.—Crooked River NG, Oregon, USA; OR4; C. J. Willson #18; EU277695, EU277724. *J. osteosperma* (Torr.) Little—Walnut Canyon NM, Arizona, USA; AZ1; C. J. Willson #19; EU277692, EU277721. *J. osteosperma* (Torr.) Little—Green

Canyon, Logan, Utah, USA; UT6; *J. Leffler* #1; EU277693, EU277722. *J. pinchotii* Sud.—Guadalupe Mtns. NP, Texas, USA; TX2; *C. J. Willson* #20; EU277688, EU277717. *J. pinchotii* Sud.—Guadalupe Mtns. NP, Texas, USA; TX3; *C. J. Willson* #21; EU277689, EU277718. *J. scopulorum* Sarg.—Cibola NF, New Mexico, USA; NM8; *C. J. Willson* #22; EU277697, EU277726. *J. scopulorum* Sarg.—Green Canyon, Logan, Utah, USA; UT1; *J. Leffler* #2; EU277696, EU277725. *J. virginiana* var. *silicicola*

(Small) E. Murray—Carrot Island, North Carolina, USA; NC2; *C. J. Willson* #23; EU277700, EU277729. *J. virginiana* var. *silicicola* (Small) E. Murray—Carrot Island, North Carolina, USA; NC6; *C. J. Willson* #24; EU277701, EU277730. *J. virginiana* L.—Duke Forest, North Carolina, USA; NC6; *C. J. Willson* #25; EU277698, EU277727. *J. virginiana* L.—Duke Forest, North Carolina, USA; NC8; *C. J. Willson* #26; EU277699, EU277728.

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