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No local adaptation in leaf or stem xylem vulnerability to embolism, but consistent vulnerability segmentation in a North American oak

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Summary

Vulnerability to embolism varies between con-generic species distributed along aridity gradients, yet little is known about intraspecific variation and its drivers. Even less is known about intraspecific variation in tissues other than stems, despite results suggesting that roots, stems and leaves can differ in vulnerability. We hypothesized that intraspecific variation in vulnerability in leaves and stems is adaptive and driven by aridity.

We quantified leaf and stem vulnerability of *Quercus douglasii* using the optical technique. To assess contributions of genetic variation and phenotypic plasticity to within-species variation, we quantified the vulnerability of individuals growing in a common garden, but originating from populations along an aridity gradient, as well as individuals from the same wild populations.

Intraspecific variation in water potential at which 50% of total embolism in a tissue is observed (P_{50}) was explained mostly by differences between individuals (>66% of total variance) and tissues (16%). There was little between-population variation in leaf/stem P_{50} in the garden, which was not related to site of origin aridity. Unexpectedly, we observed a positive relationship between wild individual stem P_{50} and aridity.

Although there is no local adaptation and only minor phenotypic plasticity in leaf/stem vulnerability in *Q. douglasii*, high levels of potentially heritable variation within populations or strong environmental selection could contribute to adaptive responses under future climate change.

Key words: common gardens, drought tolerance, intraspecific variation, North American oaks, plant hydraulic traits, vulnerability segmentation, xylem vulnerability to embolism

Introduction

Drought renders plant water transport systems vulnerable to dysfunction, because increasing tension in the xylem water column caused by dehydration is associated with higher risk of air entry that causes blockages (i.e. embolism). Consequently, the capacity of xylem to withstand air entry (i.e. to resist embolism) is a key component of plant drought tolerance. The water potential at which 50% of total embolism in a tissue is observed (known as P_{50}) provides an important metric of xylem vulnerability to embolism. Several studies have highlighted the fact that large variation in vulnerability to embolism may exist between species within genera (e.g. in *Quercus* (Lobo *et al*., 2018; Skelton *et al*., 2018) and *Callitris* (Larter *et al*., 2017)). Much interspecific variation in vulnerability to embolism appears to be adaptive, with most studies reporting that species with lower vulnerability tend to occupy more arid sites (e.g. Pockman & Sperry, 2000; Maherali *et al*., 2004; Larter *et al*., 2017; Skelton *et al*., 2018; see also Brodribb *et al*., 2014). Although substantial literature focuses on interspecific variation in vulnerability (of mainly woody stems), our knowledge of intraspecific variation in vulnerability to embolism remains limited. Yet understanding the amount of variation that exists within a species, and the factors that influence this variation, has important implications for predicting the sensitivity of species to climatic shifts (Hoffmann & Sgrò, 2011; Sgrò *et al*., 2011). Both the amount of local adaptation present on the landscape and the capacity of trees to plastically and evolutionarily alter their phenotype may influence the ability of a species to cope with rapid climate change.

Previous studies indicate that intraspecific variation in vulnerability to embolism between the same organs of different individuals can be substantial (Kolb & Sperry, 1999; Volaire *et al*., 2018), equal to approximately a third of the variation in P_{50} found between species within a genus (Anderegg, 2015). However, comprehensive datasets that examine the amount and underlying drivers of intraspecific variation in vulnerability to embolism are limited to a few species and typically only include variation in stem P_{50}. Typically, these studies show that within-population (and/or provenance) variation in stem P_{50} tends to be higher than that found among populations (Wortemann *et al*., 2011; Lamy *et al*., 2014; Lobo *et al*., 2018). In some species (e.g. *Pinus pinaster*) between-population variation in stem P_{50} has been found to be extremely low, both within common gardens, and among populations occurring in sites with contrasting climates, indicating a lack of genetic variability and limited phenotypic plasticity (Lamy *et al*., 2014). Such findings have led the authors of these studies to conclude that vulnerability to embolism is a relatively "canalized" trait (i.e. genetically fixed and robust to genetic perturbation), potentially to avoid loss of critical drought tolerance (Lamy *et al*., 2011). Other studies have shown that phenotypic plasticity in vulnerability to embolism can be high in some species (e.g. *Fagus sylvatica* and *Pinus canariensis*), particularly when populations from marginal sites are considered (López *et al*., 2016; Stojnić *et al*., 2018). Populations occurring at the arid edge of the species range are

often shown to be less vulnerable to embolism than those occurring toward the center of a range (López *et al.*, 2016; Stojnić *et al.*, 2018). Existing results ranging from no variation to large variation in xylem vulnerability to embolism between populations within a species, the limited number of studies and study species currently documented, and the difficulty of determining plastic vs genetic causes of variation in vulnerability limit our ability to draw general conclusions at present.

Intra-individual variation in xylem vulnerability to embolism also is physiologically significant, particularly because different tissues have been known to vary in their capacity to withstand xylem embolism (Zimmermann, 1978, 1983; Tyree & Ewers, 1991; Johnson *et al.*, 2011). So-called "vulnerability segmentation" between tissues possibly serves to create hydraulic fuses within the plant to protect the more valuable tissues from drought damage (Zimmermann, 1983; Choat *et al.*, 2005). Support for this vulnerability segmentation hypothesis has come from observations that distal tissues in some woody trees, particularly leaves of drought-deciduous species, are more vulnerable to water deficit than stems or large branches (Cochard *et al.*, 1992; Tyree *et al.*, 1993; Choat *et al.*, 2005; Johnson *et al.*, 2011; Hochberg *et al.*, 2017). However, several different studies have shown a lack of segmentation between leaves and stems for other species, indicating that vulnerability segmentation may be species-specific (Skelton *et al.*, 2017a, 2018; Klepsch *et al.*, 2018). However, to our knowledge, no study has explored intraspecific variation in both leaf and stem vulnerability to embolism (or the degree of segmentation between tissues) at the population level, and the factors contributing to intraspecific variation in leaf xylem vulnerability to embolism and vulnerability segmentation between leaves and stems remain unclear.

In the present paper we investigated the intraspecific variation in stem and leaf xylem P_{50} in *Quercus douglasii*, a long-lived, drought-adapted and ecologically dominant oak species from California, USA. We asked two related questions: (1) What is the level of intraspecific variation in vulnerability to embolism in leaves and stems of *Q. douglasii*? (2) What are the main environmental drivers of intraspecific variation? We hypothesized that intraspecific variation in vulnerability to embolism in *Q. douglasii* would be caused by adaptation to aridity such that *Q. douglasii* populations from drier sites would be less vulnerable to embolism than those from more mesic sites, even when grown together in the same environment, and that differences would be exacerbated under field conditions due to additional contributions of adaptive plasticity (so-called co-gradient variation; Eckhart *et al.*, 2004).

Materials and Methods

Intraspecific variation in xylem vulnerability to embolism

Quercus douglasii Hook & Arn. is a long-lived, deciduous, noticeably ring-porous, woody tree species endemic to California, USA. To assess

intraspecific variation in vulnerability to embolism in *Q. douglasii* we quantified leaf and stem xylem vulnerability to embolism of multiple individuals from seven *in situ* populations, as well as individuals from the same populations that were growing in a common garden. The location and climatic data associated with each study site is shown in Fig. 1 and described in Table 1. Climate variables were obtained from the California state-wide Basin Characterization Model (Flint *et al*., 2013) and were based upon historical data records for the period between 1951 and 1980. Individual populations were selected to capture variation in climate variables and geographical locality and the climates of the populations that we sampled for the garden cover *c*. 95% of the entire range of *Q. douglasii*.
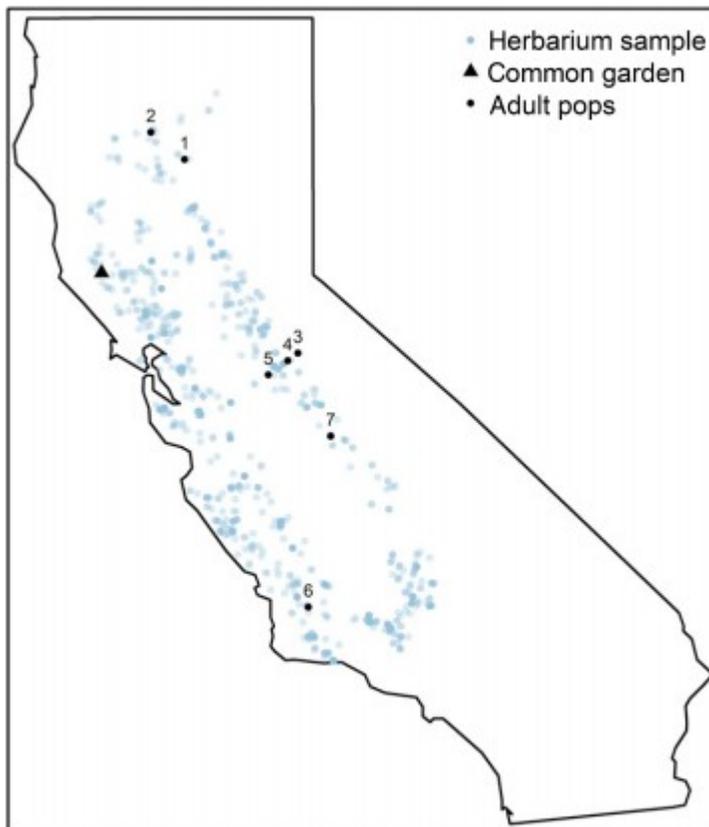
Fig. 1 Distribution of *Quercus douglasii* (blue points) within California, showing the location of the seven wild populations (black numbers), and the common garden population.

Table 1 Environmental variables for wild populations and garden sites of *Quercus douglasii*

Population	AI	CWD (mm)	Precip. (mm)	AET (mm)	T_{max} (°C)	Elevation (m asl)
1	0.74	672	849	456	22.08	658
2	1.04	690	1222	481	23.59	230
3	0.85	734	1014	393	21.17	903
4	0.66	783	837	441	22.18	638
5	0.35	982	455	291	23.49	104
6	0.41	986	560	355	23.05	610
7	0.34	993	472	354	24.52	348
Common garden	0.84	733	978	421	22.78	256

Data are for sites shown in Fig. 1. Populations are numbered according to site climatic water deficit (CWD). AI, aridity index; Precip, annual precipitation; AET, actual evapotranspiration; T_{max}, mean monthly maximum temperature.

Common garden

In order to assess the contribution of genetic variation to leaf and stem xylem vulnerability to embolism we took advantage of an existing replicated provenance trial at the Hopland Research and Extension Center (California, USA; Table 1; Fig. 1). The common garden was established in the 1990s when acorns were collected from 26 different *Q. douglasii* populations across California and planted in randomized block design (J. McBride, pers. comm.; see also McBride *et al.*, 1997). Thus, the garden trees were at least 25 yr old.

We subsampled seven of the 26 populations, stratified across the regional aridity gradient. For each of the seven populations we randomly sampled eight individuals for stem vulnerability to embolism and at least three individuals for leaf vulnerability to embolism. We sampled sunlit, south-facing branches to reduce the potential effects of microsite and/or intra-individual (canopy-level) variation (see Leaf and stem xylem vulnerability to embolism section below for more details on sampling protocol). One to two individuals per each population were collected from the garden at each sampling point between April and July 2018 to control for temporal variation. However, date of collection had no significant effect on xylem vulnerability.

Wild sites

We also sampled individuals growing in the original source populations from the same seven populations sampled in the common garden to assess the contribution of phenotypic plasticity to intraspecific variation. In each of the seven wild sites we sampled eight individuals for leaf and stem xylem vulnerability to embolism (see the 'Sample collection and optical setup'

section below) between April and July 2018, roughly controlling for time since leaf out (i.e. all sites were collected after leaf expansion ceased, earlier leafing sites were collected first). All measurements were conducted on sunlit, upper canopy, south-facing branches from adult trees.

Leaf and stem xylem vulnerability to embolism

Sample collection and optical setup

Large branches of individuals of each population were collected in the early morning from healthy-looking individuals. To avoid any potential artefact associated with open vessels we ensured that the cut branches were longer than the species' maximum recorded vessel length (124 cm; Skelton *et al.*, 2018). Upon excision, xylem was relaxed by re-cutting under water. After the cut ends were wrapped in parafilm, branches were immediately placed in at least two plastic bags with damp paper towels to prevent further water loss and transported back to the laboratory at the University of California, Berkeley for processing. There we used an optical method to capture embolism in both leaves and branches by using flatbed scanners in a dark, temperature-controlled room. Branches from different individuals were used to capture embolism events within the leaves according to the methods described by Brodribb *et al.* (2016b) and within small branches (< 0.5 cm in diameter) according to the methods described by Brodribb *et al.* (2017). We scanned all images at a resolution of 4200 dpi. Full details, including an overview of the technique, image processing, as well as scripts to guide image capture and analysis, are available at http://www.opensourceov.org. Also, extensive validation of the techniques can be found in several recent publications (Brodribb *et al.*, 2016a,b, 2017; Skelton *et al.*, 2017a, 2018).

Leaf and stem image collection

For leaves we secured a healthy, intact leaf between two microscope slides on a flatbed scanner (Epson perfection V800 or V850 Scanner; Epson America, Long Beach, CA, USA) using duct tape. We scanned each leaf in transmission mode (as opposed to reflective mode, to allow light to pass through the leaf xylem) at least once every 4 min for a period of a few days (usually < 4 d). For stems, we carefully removed a small section of bark to expose the xylem, placed it face down on the scanner and secured it in place using duct tape. Stems were scanned in reflective mode, which allowed us to observe embolism within the outer few layers of xylem in each stem. The small sizes of the branches that we observed reduced the possibility that our method might have missed significant radial variation in embolism within branches (i.e. between rings), although this possibility cannot be entirely excluded. Examples of leaves and stem tissue used in our analysis can be viewed in the Supporting Information (Videos S1, S2).

Leaf and stem xylem water potential

As branches were being scanned for leaf or stem embolism, we simultaneously monitored their stem and leaf xylem water potential. For

stem xylem water potential, we attached a stem psychrometer (ICT International, Armidale, Australia) on each branch at > 60 cm from the cut end of the main branch. Stem psychrometers were connected to the xylem, sealed with high-vacuum grease (Dow Corning Corp., Midland, MI, USA), and secured with Parafilm (Bemis NA, Neenah, WI, USA) to prevent moisture loss. Stem xylem water potential was recorded every 10 min for the duration of the scanning process. We verified the accuracy of the stem psychrometer readings for a subset of individuals by periodically measuring leaf xylem water potential using a Scholander-type pressure chamber (PMS Instruments, Corvallis, OR, USA). For leaf xylem water potential, we excised leaves neighboring the scanned leaves, immediately wrapped them in moist paper towel and aluminium foil, and placed them in plastic bags to prevent further water loss. Because branches were largely equilibrated as a result of being kept in the dark, variation among neighboring leaves was slight (always < 0.1 MPa).

Image processing

Upon completion, image sequences were analyzed to identify embolism events, seen as changes in the reflection of the stem xylem or changes in the transmission of light through the leaf xylem. Image subtraction of subsequent images conducted in IMAGEJ (National Institutes of Health, Bethesda, MD, USA) was used to reveal rapid changes in light transmission or contrast produced by each embolism event. Slow movements of the stems or leaves caused by drying could easily be distinguished from embolism events and were filtered from the analysis. Embolism events were thresholded, allowing automated counting of each event using the analyse-stack function in IMAGEJ. From the thresholded stack of embolism events we could extract a time-resolved count of embolism events (using the time stamp of each image). We then converted the raw embolism counts to a percentage of total pixels embolized, producing a dataset of time-resolved percent embolism.

Vulnerability curves

The time-resolved percent embolism data were combined with the water potential timeline to estimate the leaf or stem xylem water potential associated with each embolism event. Due to technical issues we were unable to extract relevant water potential measurements or embolism data from a few individuals, which meant that we were unable to construct complete vulnerability curves for those individuals. Vulnerability to embolism was recorded as the relationship between percent embolism and water potential (Ψ), and modeled using a sigmoid function:

$$\text{Percent embolism} = 100 - 100/(1 + e^{a(\Psi - b)})$$

(*a*, sensitivity to decreasing water potential (proportional to the slope of the equation); *b*, water potential associated with 50% embolism). From the fitted model for each individual, we were able to extract the air-entry water

potential (P_{12}, MPa), defined as the leaf or stem xylem water potential associated with ~ 12% embolism for each branch, the water potential associated with 50% loss of hydraulic conductance (P_{50}, MPa), and the water potential associated with 88% loss of hydraulic conductance (P_{88}, MPa). Previous studies have suggested that the point of air-entry (i.e. P_{12}) represents a point of incipient damage to plant functionality (Skelton *et al.*, 2017b, 2018), P_{50} represents the water potential associated with significant damage, and P_{88} the water potential often associated with canopy dieback in angiosperms (Urli *et al.*, 2013).

Statistical analysis

A Type III ANOVA was used to examine statistical effects of tissues, sites and populations on vulnerability to embolism. Tissues, sites and population were set as fixed factors. To test for any difference between garden and wild populations (i.e. phenotypic plasticity) we included an interaction between site and population. Before performing the ANOVA analysis, the normality of the data were assessed using QQ plots and the Shapiro–Wilk test of normality. For each combination of the three different factors we found no substantial deviations from normality. Tukey's honest significant differences test was used to test the *post hoc* effects of factors found to be significant. A Type III ANOVA was used to assess the statistical effects of sites and populations on vulnerability to embolism of leaves and stems, and vulnerability segmentation between leaves and stems. Site and populations were set as fixed factors. All ANOVA analyses were run in R/CAR (Fox & Weisberg, 2011). To further quantify the amount of intraspecific variation in vulnerability to embolism in *Q. douglasii* we calculated the coefficient of variation (CV) of P_{50} values within and between populations. We also used linear models to examine the relationship between vulnerability to embolism (response variable) and a range of environmental driver variables associated with climate.

Results

Intraspecific variation in leaf and stem xylem vulnerability to embolism of *Q. douglasii*

Tissue type differed in vulnerability to embolism ($P < 0.00002$ for P_{50}), contributing to 16% of the combined variation in leaf and stem xylem vulnerability to embolism (Tables 2, 3a, S1; Fig. 2; see also the section on 'Vulnerability segmentation between leaves and stems' below). Mean P_{50} of populations in the common garden differed by ≤ 1.3 MPa for leaves (Table 2; Fig. 2a) and 1.02 MPa for stems (Table 2; Fig. 2b). In comparison to the common garden populations the range in mean P_{50} of wild populations was lower for leaves (0.36 MPa; Fig. 2a; Table 2), but similar for stems (1.01 MPa; Fig. 2b; Table 2).

Table 2 Vulnerability to embolism (P_{50}) of leaves and stems of seven different *Quercus douglasii* populations in a common garden and *in situ*

Population	Mean $P_{50} \pm$ SE (MPa)			
	Common garden		Wild	
	Leaves	Stems	Leaves	Stems
1	−4.19 ± 0.30 (3)	−4.41 ± 0.31 (4)	−3.91 ± 0.18 (3)	−4.98 ± 0.17 (4)
2	−3.68 ± 0.35 (2)	−4.60 ± 0.26 (5)	−3.81 ± 0.45 (3)	−4.45 ± 0.35 (6)
3	−4.62 ± 0.50 (3)	−5.08 ± 0.44 (5)	−3.74 ± 0.37 (4)	−4.19 ± 0.41 (4)
4	−3.31 ± 0.13 (3)	−4.93 ± 0.27 (5)	−3.71 ± 0.25 (4)	−4.52 ± 0.27 (3)
5	−3.79 ± 0.27 (3)	−4.43 ± 0.39 (5)	−3.89 ± 0.06 (2)	−4.03 ± 0.24 (8)
6	−3.87 ± 0.08 (3)	−4.76 ± 0.30 (5)	−3.56 ± 0.28 (4)	−3.97 ± 0.05 (6)
7	−4.27 ± 0.25 (2)	−4.05 ± 0.51 (4)	−3.92 ± 0.33 (4)	−4.26 ± 0.31 (6)

Corresponds to data shown in Supporting Information Fig. S1. Sample size in brackets.

Table 3 Results from the full three-way ANOVA testing the effect of tissue, site and population on vulnerability to embolism of *Quercus douglasii* (a), and the two-way ANOVAs to assess the impact of site and population on variation in vulnerability to embolism in leaves and stems of *Q. douglasii* (b)

	Factor	SS	df	F	P	% var		Tissue	Factor	SS	df	F	P	% var
(a)	Tissue	9.01	1	**20.35**	**0.00002**	15.6	(b)	Stems	Site	1.18	1	2.31	0.13	3.2
	Site	1.2	1	2.72	0.1	2.1			Population	2.91	6	0.95	0.47	8.0
	Population	2.05	6	0.77	0.6	3.6			Site × Pop	3.65	6	1.19	0.32	10.1
	Tissue × site	0.06	1	0.13	0.72	0.1			Residuals	28.59	56			78.7
	Tissue × Pop	2.71	6	1.02	0.42	4.7		Leaves	Site	0.30	1	0.95	0.34	2.2
	Site × Pop	3.0	6	1.13	0.35	5.2			Population	2.26	6	1.2	0.33	17.0
	Tissue × Site × Pop	1.91	6	0.72	0.63	3.3			Site × Pop	1.69	6	0.9	0.51	12.7
	Residuals	37.64	85			65.4			Residuals	9.05	28			68.0

Corresponds to data shown in Fig. 2. SS, sum of squares; df, degrees of freedom; F, F-ratio; P, probability; % var, proportion of the total variance explained by each factor (expressed as a percentage). Bold values indicate statistically significant results.
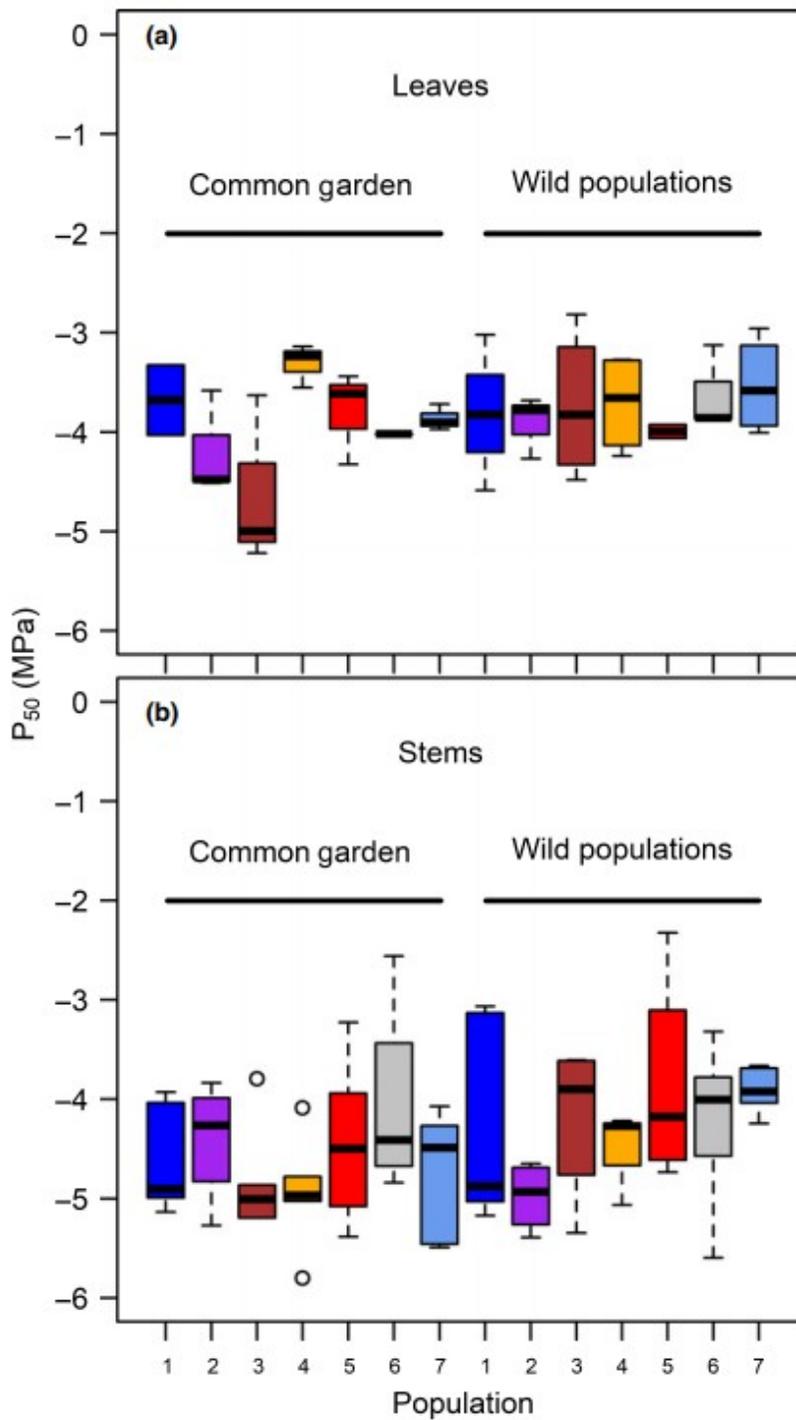
Fig. 2 Boxplots of mean xylem water potential at which 50% of total embolism in a tissue is observed (P_{50}) of leaves (a) and stems (b) of *Quercus douglasii* populations growing in a common garden and *in situ*. Horizontal bars represent the minimum and maximum (outer bars connected by dashed lines), the median (inner, thick lines), and the first quartile and third quartile in the dataset of each population (colored sections).

Mean P_{50} of garden and wild sites did not differ significantly for leaves (Fig. 2a; Table 3b) or stems (Fig. 2b; Table 3b). In the garden the between-population CV of leaf P_{50} was higher (10.9%) than among the wild populations (3.5%). Between-population CV of stem P_{50} was similar among the garden and wild populations (7.6% and 7.9%, respectively) (Table 4; Fig. 2). However, neither site (i.e. garden vs wild) nor population were significant factors in determining differences in P_{50} and there was no significant effect of an interaction between tissue, site and population (Table 3).

Table 4 Mean within-population coefficient of variation (CV) of the water potential associated with 50% embolism (P_{50}) of leaves and stems of *Quercus douglasii* for populations occurring in a common garden and *in situ*

Clade	Group	Site(s)	CV (%) – leaves		CV (%) – stems	
			Between	Within	Between	Within
Q. douglasii	Populations	Common garden	10.9 (7)	10.8 ± 1.9 (7)	7.6 (7)	16.7 ± 1.8 (7)
		Wild sites	3.5 (7)	13.8 ± 2.5 (7)	7.9 (7)	13.5 ± 2.5 (7)
	Populations	All		12.3 ± 1.6 (7)		15.1 ± 1.9 (7)
Quercus[1]	Species		25.69 (11)		27.35 (11)	

Also shown is the between-population CV of mean P_{50} of seven populations occurring in the common garden and *in situ*, and the CV among species of *Quercus*. The number in brackets indicates the sample size in each group.
[1]Data from Skelton *et al.* (2018).

Most of the variation in vulnerability to embolism of leaves (68% of total variance) and stems (79% of total variance) occurred between individuals (Table 3; Fig. S1). Extreme individuals within a single population were found to vary in vulnerability to embolism by ≤ 1.66 MPa in leaves and 2.1 MPa in stems (Fig. S1). The mean maximum range in vulnerability to embolism between extreme individuals within populations was 1.09 ± 0.22 MPa in leaves and 1.36 ± 0.27 MPa in stems. Within-population CV of leaf and stem P_{50} was similar among the garden and wild populations (Table 4; Fig. 2a,b). In addition, the mean within-population CV of vulnerability to embolism was similar for leaves and stems (15.1% and 12.3%, respectively; Table 4; $t = -0.87$, df = 11.97, $P = 0.40$).

Similar ANOVA results as obtained for P_{50} were obtained for P_{12} (Table S2). However, site was shown to be a significant factor for P_{88} (Table S3), with garden individuals found to have significantly lower P_{88} than wild individuals.

Relationship between P_{50} and environmental conditions

We found no significant relationships between leaf xylem vulnerability to embolism of individuals growing in a common garden and metrics of aridity of the site of origin (Fig. 3a; Table S4). There also were no significant relationships between leaf xylem vulnerability to embolism of individuals growing in the wild and metrics of site aridity (Fig. 3a; Table S4). There was a significant, but weak positive relationship between stem xylem vulnerability to embolism of individuals growing in the wild and CWD (Fig. 4b). In addition, we found a significant, but weak negative relationship between stem P_{50} of individuals from wild sites and actual evapotranspiration (Table S4). No relationship was found between stem vulnerability to embolism of garden

individuals and any of the metrics of aridity of the site of origin (Fig. 4b; Table S4).
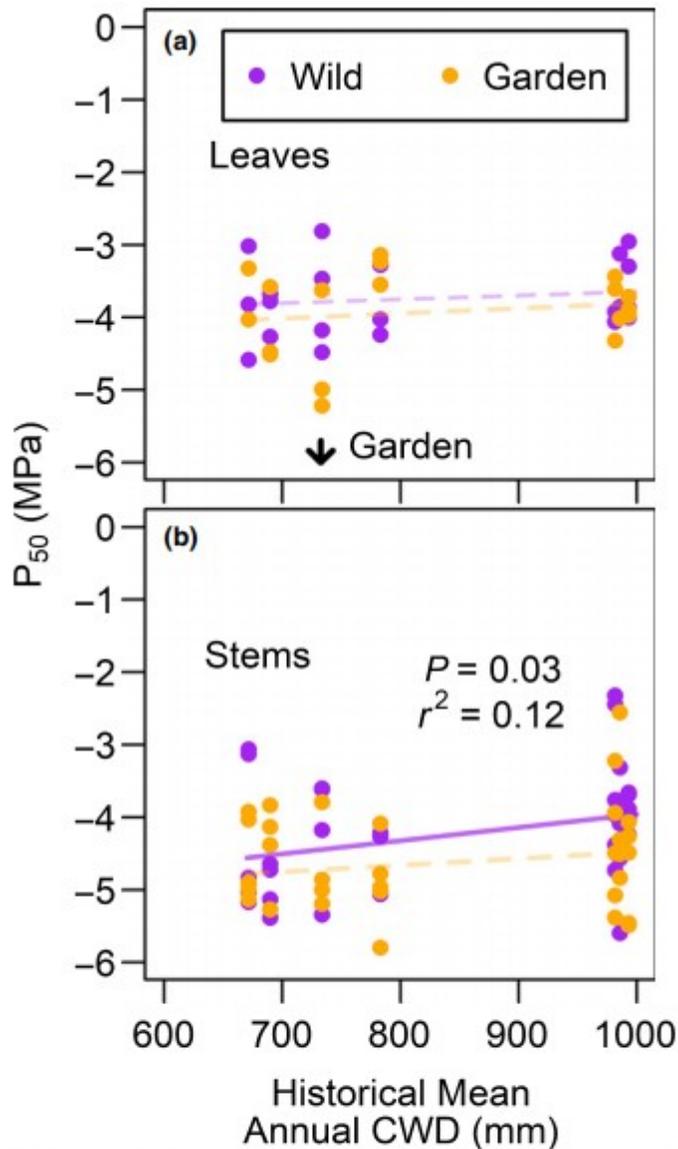
Fig. 3 Relationships between climatic water deficit (CWD) – a measure of site aridity – and xylem water potential at which 50% of total embolism in a tissue is observed (P_{50}) of leaves (a) and stems (b) of individuals of *Quercus douglasii* growing in the wild populations (purple) and the common garden (orange). P_{50} of individuals growing in the garden are plotted against the CWD of the source population and the CWD of the garden site is indicated with a black arrow. Solid lines indicate statistically significant relationships between variables.
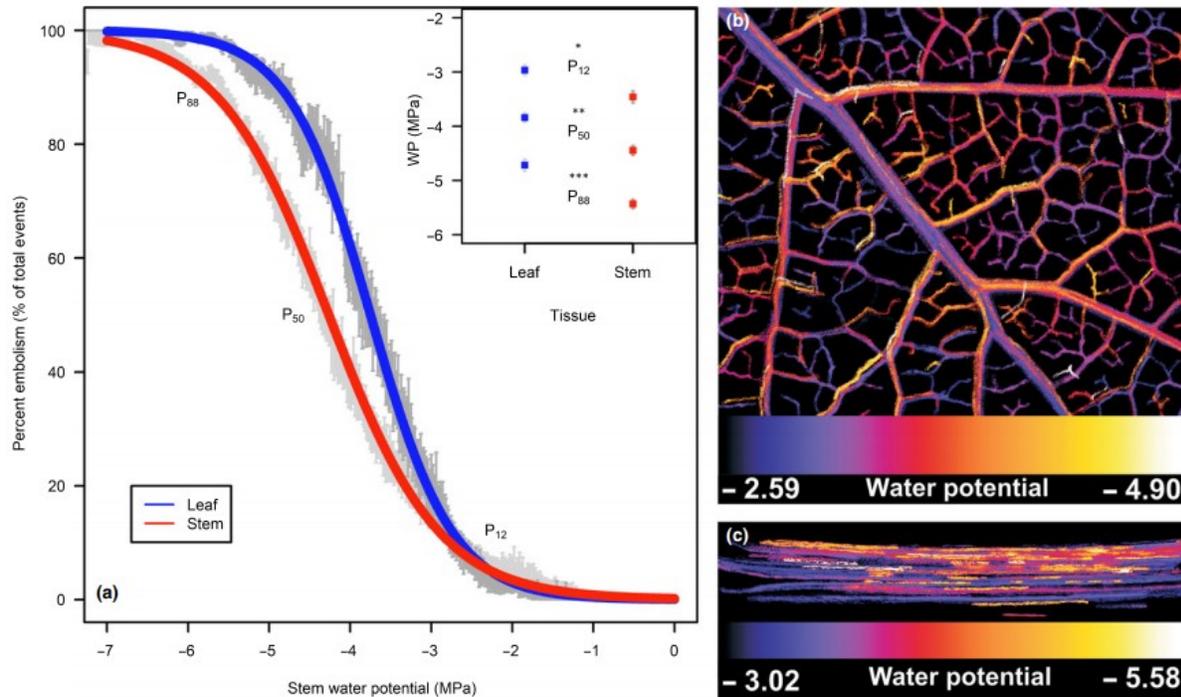
Fig. 4 Mean xylem vulnerability to embolism curve for leaves (green) and stems (brown) of *Quercus douglasii* individuals from both a common garden and wild populations, showing the vulnerability segmentation between the tissues (a). Also shown are images of total observed embolism in leaf (b) and stem (c) xylem of a segmented individual. Gray vertical bars in (a) are SE of percent embolism for each water potential value. Different colours in (b) and (c) indicate the water potential at which embolism events were observed (see colour bar for reference). The asterisks indicate significance: *, $P < 0.05$; **, $P < 0.005$; ***, $P < 0.001$.

Vulnerability segmentation between stems and leaves

Post hoc analysis of the difference between stem and leaf vulnerability to embolism showed that leaves were consistently more vulnerable to embolism than stems (Fig. 4; $P < 0.00002$). The P_{50} of leaves (-3.88 ± 0.09 MPa) was generally 0.5 MPa less negative than stem P_{50} (-4.47 ± 0.10 MPa). Individuals in the common garden were as segmented (mean difference in $P_{50} = -0.68 \pm 0.27$ MPa) as individuals in the wild populations (mean difference in $P_{50} = -0.57 \pm 0.11$ MPa; Tables 5, S5). The degree of segmentation between stems and leaves tended to increase with a greater extent of observed embolism (Fig. 4; Table S6), such that the extent of segmentation between stems and leaves measured at P_{88} (mean segmentation = -0.84 MPa) was greater than at P_{12} (mean segmentation = -0.31 MPa) (Fig. 4; Table S6; $t = 2.54$; df = 11.8; $P = 0.026$). No relationship was found between vulnerability segmentation and any of the metrics of aridity in our dataset (Table S7).

Table 5 Difference (mean ± SE) in the water potential associated with 50% embolism between stems and leaves (i.e. degree of vulnerability segmentation) for populations of *Quercus douglasii* in the common garden and wild populations

Population	Vulnerability segmentation (MPa)	
	Common garden	Wild
1	−0.67 ± 0.12 (2)	−0.93 ± 0.29 (3)
2	−0.74 ± 0.14 (2)	−0.73 ± 0.84 (3)
3	−0.92 ± 0.48 (3)	−0.45 ± 0.22 (4)
4	−1.87 ± 0.35 (3)	−0.90 ± 0.88 (2)
5	−0.71 ± 0.47 (3)	−0.35 ± 0.45 (2)
6	−0.41 ± 0.09 (3)	−0.44 ± 0.22 (4)
7	0.57 ± 1.39 (2)	−0.22 ± 0.29 (4)
Overall	−0.68 ± 0.27	−0.57 ± 0.11

Sample sizes for each population are given in brackets.

Discussion

We found evidence of substantial intraspecific variation in xylem water potential at which 50% of total embolism in a tissue is observed (P_{50}) in *Quercus douglasii*, with mean values for different extreme populations ranging from −3.3 to −5.1 MPa. Over two-thirds of the total observed variance in xylem vulnerability to embolism within the species occurred between individuals regardless of population, whereas 16% was explained by differences between leaf and stem xylem. Less than six percent of the total observed variance in xylem vulnerability to embolism was explained by differences between populations or site. Greater within- than between-population variation in stem or leaf xylem vulnerability to embolism, a lack of a difference in leaf and stem P_{50} between populations in the common garden, and no relationship between P_{50} of garden individuals and metrics of aridity of their sites of origin provide compelling evidence that there is no local adaptation to aridity for both leaf and stem xylem. Although we found a significant relationship between stem P_{50} of wild individuals and two metrics of site aridity the slope was the opposite of what we predicted based on our original hypothesis. Thus, leaf and stem xylem provide no evidence to support the hypothesis that adaptive and plastic intraspecific variation in xylem vulnerability to embolism in *Q. douglasii* is driven by aridity.

Lack of local adaptation to aridity in stem and leaf vulnerability to embolism

We observed moderate to low variation in stem vulnerability to embolism between populations in both the common garden and in the wild, even between populations occurring in locations that differed substantially in aridity. The observed variation in stem vulnerability to embolism between seven *Q. douglasii* populations growing in the garden was like that reported

for seventeen populations of *Fagus sylvatica* (Wortemann *et al*., 2011), but greater than that reported for four provenances of European sessile oak (*Quercus petraea*) (Lobo *et al*., 2018) and much greater than that reported for six populations of *Pinus pinaster* (Lamy *et al*., 2014).

To our knowledge no previous study has assessed intraspecific variation in leaf vulnerability to embolism. Our results show, for the first time, that the amount of variation in leaf xylem vulnerability to embolism between *Q. douglasii* populations occurring in a common garden is comparable to the amount of variation in stem xylem vulnerability to embolism. However, leaf xylem vulnerability to embolism varied by half as much as stem xylem vulnerability to embolism between *Q. douglasii* populations occurring along an aridity gradient. By contrast to the low variation between populations, the mean co-efficient of variation in leaf and stem xylem vulnerability to embolism within populations was moderate to high (> 10%), equating to *c*. 50% of the coefficient of variation between species in North American *Quercus* (Table 3; Skelton *et al*., 2018). The observed within-population variation in stem xylem vulnerability to embolism was like that reported for *Q. petraea* and *F. sylvatica*, but higher than that reported for *P. pinaster* (Wortemann *et al*., 2011; Lamy *et al*., 2014; Lobo *et al*., 2018).

Despite observing moderate variation in leaf and stem vulnerability to embolism between individuals, we were unable to detect differences between populations sourced from sites along an aridity gradient in California but growing in a common garden. These results are contrary to our *a priori* hypothesis that aridity might drive intraspecific variation of xylem vulnerability to embolism. We formulated this hypothesis based on previous studies showing that interspecific variation in xylem vulnerability to embolism in oaks is driven by aridity (e.g. Lobo *et al*., 2018; Skelton *et al*., 2018). Instead, low variation in leaf and stem vulnerability to embolism between garden populations indicates that there is a lack of local adaptation in these key drought tolerance traits. Consequently, our results show that intraspecific variation in vulnerability to embolism is influenced by different factors to those driving interspecific variation.

Mechanisms that can reduce local adaptation within a species include high gene flow between populations, canalization of a trait driven by uniform selection pressure and/or lack of genetic variation, substantial genetic drift (and weak selection pressure), and/or lack of reliable environmental signals (Kawecki & Ebert, 2004). Given that *Q. douglasii* occurs in a Mediterranean-type climate region characterized by very little rainfall in the summer months that has been present since the mid- to late Miocene (Raven & Axelrod, 1978; Major, 1988), where even the wetter sites regularly experience intra-annual water stress (Osuna *et al*., 2015, and data shown in Table 1), we suggest that populations are likely to be exposed to reliable contemporary environmental signals. In addition, interannual variation in rainfall within sites occasionally produces severe drought events known to

cause adult mortality (Brown *et al*., 2018), suggesting that selection pressure for drought tolerance traits is likely to be strong.

Although uniform selection pressure could be acting in geographically disparate populations (possibly because populations are adapting to local extremes rather than the average; Gutschick & BassiriRad, 2003), we sampled across sites covering a substantial range in aridity, equal to the range over which other species display local adaptation (Wortemann *et al*., 2011; Lamy *et al*., 2014; Lobo *et al*., 2018) and over which we see variation in vulnerability between congeneric oak species (Skelton *et al*., 2018, see also Larter *et al*., 2017). Thus, we suggest that low local adaptation in *Q. douglasii* leaf and stem xylem vulnerability to embolism is caused either by high gene flow between populations or canalization of vulnerability to embolism, like species such as *F. sylvatica* and *P. pinaster* (Lamy *et al*., 2011; Wortemann *et al*., 2011).

Minor phenotypic plasticity in stem vulnerability to embolism

Although there was no local adaptation in leaf or stem vulnerability to embolism, site was a significant factor in explaining variation in P_{88} (an index of absolute drought tolerance of individuals). In addition, we detected significant relationships between stem P_{50} of wild individuals and metrics of growing site aridity. Two possible hypotheses for these observed trends are: (1) that purifying selection acts on seedlings and serves to alter the vulnerability of wild populations compared to garden populations, and (2) that there is an environmental component to xylem vulnerability (e.g. P_{88}) in *Q. douglasii*. In terms of the first hypothesis, it is possible that wild sites have experienced loss of more vulnerable individuals during drier years, although it is somewhat difficult to explain why populations from wetter sites might have experienced greater mortality (Fig. 2; Table S1). Unexpectedly, stem vulnerability to embolism also was lower in more mesic sites, similar to results obtained for *P. pinaster* (Lamy *et al*., 2014). Thus, although we detected minor phenotypic plasticity in stem vulnerability to embolism in *Q. douglasii*, this plasticity may not be driven by aridity. It is possible that there are species-specific factors other than aridity that influence stem xylem phenotype in *Quercus* (see, also, Lobo *et al*., 2018). The similarity of leaf xylem vulnerability to embolism between wild populations occurring along an aridity gradient demonstrates that phenotypic plasticity in leaf xylem vulnerability to embolism is highly limited in *Q. douglasii*.

Consistent vulnerability segmentation between leaves and stems in *Q. douglasii*

Tissue type was a major determinant of vulnerability to embolism in *Q. douglasii*, with leaves being consistently more vulnerable to embolism than stems. Consequently, our findings indicate that there is vulnerability segmentation between leaf and stem xylem in *Q. douglasii*, indicating that future studies on this species should consider leaves and stems to be separate traits. In addition, this finding suggests that future studies

assessing whole plant vulnerability should also consider variation in tissues other than stems, such as leaves and roots.

In terms of a possible functional significance of vulnerability segmentation, a difference of -0.5 MPa at P_{50} and -0.8 MPa at P_{88} between leaves and stems may be highly significant for survival of stem tissues during severe drought events, because loss of hydraulic conductance in the leaf associated with low water potentials will retain water in the stems (Zimmermann, 1983). Loss of leaf hydraulic conductance and sustained evaporative demand can also induce further declines in leaf water potential creating a negative feedback effect that results in complete embolism in the leaves (a phenomenon referred to as run-away embolism). Although complete embolism within leaves is catastrophic to leaf functionality it may function in some species to reduce the rate of water loss (but see Wolfe *et al*. 2016).

Vulnerability segmentation between leaf xylem and stem xylem could result from either an intrinsic difference between the two tissue types (Zimmermann, 1983; Choat *et al*., 2005) or separate environmental cues acting on the tissues. However, little is known about the structural and genetic basis of vulnerability segmentation, limiting our capacity to resolve the underlying factors that might influence it. Zimmermann (1983) hypothesized that there may be an anatomical basis to vulnerability segmentation based on the thickness of the intervessel pit membranes. Support for this hypothesis has come from studies comparing roots and stems showing that greater porosity of the pit membranes in root xylem makes roots significantly more vulnerable than stems (e.g. in *Acer grandidentatum* (Alder *et al*., 1996); see also Hacke *et al*., 2000). Klepsch *et al*. (2018) also showed that in *Betula* tissues with thicker intervessel pit membranes were less vulnerable to embolism. Consequently, leaves in *Q. douglasii* may possess more porous and thinner intervessel pit membranes than stems that make them more vulnerable to embolism. Alternatively, differential capacity to withstand embolism formation also may be related to conduit cell wall structure and thickness (Choat *et al*., 2005) or the connectivity of the xylem to pith or other air-filled conduits. For example, the xylem conduits in the leaf of *Quercus douglasii* may possess lower ratios of protoxylem to metaxylem (i.e. be less secondarily thickened) (Choat *et al*., 2005). Identifying the anatomical or developmental basis of vulnerability segmentation between leaves and stems should be a future research priority.

Conclusion

Intraspecific variation in vulnerability to embolism in *Q. douglasii* is substantial enough to be relevant for understanding patterns of drought tolerance and future climate change impacts on the species. Much of the variation in xylem vulnerability to embolism found within *Quercus douglasii* is explained by tissue type, with leaves being more vulnerable to embolism than stems. Our results conclusively demonstrate an absence of local

adaptation and limited phenotypic plasticity in leaf and stem xylem vulnerability to embolism in *Quercus douglasii*. Instead, leaf and stem xylem vulnerability to embolism appear to be highly canalized traits, suggesting that avoiding embolism is a critical component of tolerance of extreme droughts in this species. On the other hand, we found strong evidence of vulnerability segmentation between leaves and stems, highlighting a need to better understand the factors that influence vulnerability of different tissues as well as a need for future studies to consider leaves and stems as potentially separate traits.

In terms of potential climate change impacts, the lack of local adaptation suggests that managed relocation of genotypes from drier sites to more mesic sites may have limited utility for mitigating the impacts of future climate change on *Q. douglasii*. High natural variation in vulnerability to embolism within populations indicates that any attempts to identify drought tolerant phenotypes of *Q. douglasii* will have to screen many individuals to identify less vulnerable individuals. However, high levels of natural variation within populations also indicate that populations exposed to drought may persist without going extinct through the survival of less vulnerable individuals.

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