Starch storage capacity of sapwood is related to dehydration avoidance during drought

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PREMISE: The xylem tissue of plants performs three principal functions: transport of water, support of the plant body, and nutrient storage. Tradeoffs may arise because different structural requirements are associated with different functions or because suites of traits are under selection that relate to resource acquisition, use, and turnover. The structural and functional basis of xylem storage is not well established. We hypothesized that greater starch storage would be associated with greater sapwood parenchyma and reduced fibers, which would compromise resistance to xylem tensions during dehydration.

METHODS: We measured cavitation resistance, minimum water potential, starch content, and sapwood parenchyma and fiber area in 30 species of southern California chaparral shrubs (evergreen and deciduous).

RESULTS: We found that species storing greater starch within their xylem tended to avoid dehydration and were less cavitation resistant, and this was supported by phylogenetic independent contrasts. Greater sapwood starch was associated with greater parenchyma area and reduced fiber area. For species without living fibers, the associations with parenchyma were stronger, suggesting that living fibers may expand starch storage capacity while also contributing to the support function of the vascular tissue. Drought-deciduous species were associated with greater dehydration avoidance than evergreens.

CONCLUSIONS: Evolutionary forces have led to an association between starch storage and dehydration resistance as part of an adaptive suite of traits. We found evidence for a tradeoff between tissue mechanical traits and starch storage; moreover, the evolution of novel strategies, such as starch-storing living fibers, may mitigate the strength of this tradeoff.

KEY WORDS   anatomy; anisohydric; cavitation; chaparral; deciduous; embolism; evergreen; isohydric; NSC; xylem.

The xylem tissue of woody plants performs three principal functions: transport of water, mechanical support of the plant body, and storage of nutrients (Pratt and Jacobsen, 2017). Much previous research has focused on the two functions of water transport and mechanical support in the context of xylem tradeoffs, and there has been less research into the function of storage (however, see Plavcová et al., 2016; Chen et al., 2020). In the present study, we focused on how storage relates to other xylem functions. It is likely that xylem functions are interdependent, such that wood proficient at one function is necessarily poor at another, giving rise to tradeoffs. Tradeoffs can arise due to the structural demands required to support a particular function that is at odds with another function (Janssen et al.,

2020). Another possibility is that evolutionary forces lead to wood functions that are associated because they are part of an adaptive suite of traits that determine rates of resource acquisition, use, and turnover (Reich, 2014).

One force that strongly influences xylem function is water limitation in seasonal environments. Plants widely differ in dehydration tolerance during drought, which we define as how negative their water potentials are during dry periods. One trait that is associated with dehydration tolerance is cavitation resistance (Pockman and Sperry, 2000; Kursar et al., 2009; Parker et al., 2016). Cavitation occurs when the tension of the xylem sap reaches a point where gas is pulled into water-filled conduits, filling them with gas emboli.

Emboli-filled conduits cannot transport water; thus, when emboli are widespread, the flow of water to the leaves cannot keep up with transpiration and tissues dehydrate, triggering further "runaway cavitation" (Tyree and Sperry, 1988; Anderegg et al., 2012). Dehydration avoidance or tolerance is not necessarily related to drought resistance (survival during a drought), and this is an area of active research (Pausas et al., 2016; Jacobsen and Pratt, 2018; Dietrich et al., 2019; Janssen et al., 2020).

We studied chaparral shrub species occurring in the semiarid mediterranean-type environment in southern California, where the seasonal environment has a strong influence on carbon storage and water balance (Parker et al., 2016). The mediterranean-type climate is characterized by cool moist winters and hot dry summers and it is common to have a 6 mo period with no rainfall in the summer and fall months. Photosynthetic rates are limited in the winter because of short days and cool temperatures, highest in the spring and lowest during the summer and fall months when soil water is limited and hot, dry conditions trigger stomatal closure (Mooney et al., 1975; Parker et al., 2016). This region is also prone to droughts (Jacobsen and Pratt, 2018), during which there are protracted periods when no growth occurs and these systems turn into annual net carbon sources (Harvey and Mooney, 1964; Luo et al., 2007). During the winter and spring growing seasons, stored carbohydrates are mobilized to support vegetative and reproductive growth (Mooney and Hays, 1973; Palacio et al., 2018). As water becomes limiting in the late spring and early summer, growth stops and photosynthesis continues leading to a replenishment of stores. During a typical summer dry season, the balance between carbon uptake and carbon sinks, particularly respiration, may be positive or negative through the summer and fall dry seasons. This is due to stomatal closure during the summer, but it is also due to the hot and sunny conditions, because high temperatures increase rates of cellular respiration (Oechel and Lawrence, 1981) and photorespiration of this C_3-dominated community. During the driest months and during droughts, stored reserves are important for maintaining tissues (Mooney and Chu, 1974; Pratt et al., 2014). Climate change and climate-change-type droughts are associated with hotter and drier conditions that further limit carbon gain and put a strain on stores (Jacobsen and Pratt, 2018). Plants with higher maintenance costs and greatly restricted photosynthesis during the dry season should be most reliant on stored reserves (McDowell et al., 2008; Sala et al., 2012). These are likely to be dehydration-avoiding species with strong stomatal closure, large root systems, or leaf shedding; thus, we predicted that dehydration-avoiding species would require greater stores.

There are other traits that can affect chaparral shrub carbon and water balance, such as leaf habit and resprouting after fire. Chaparral shrub communities are dominated by mostly evergreen shrubs, but there are also co-occurring deciduous species that drop their leaves during the dry season. These different leaf habits are expected to have an effect on carbon storage and balance (Martínez-Vilalta et al., 2016; Palacio et al., 2018). We predicted that deciduous species would store more carbohydrates in stems to aid the growth of a new canopy at the onset of the winter rainy season and because being leafless makes them wholly reliant on those stores for maintenance costs (Palacio et al., 2018). We also predicted that deciduous taxa would be better at avoiding tissue dehydration than the evergreen taxa. Another driver of this community is periodic fire, and there are species that resprout and those that do not resprout after crown fires. The ability to store carbohydrates may be influenced by resprouting ability (Schwilk and Ackerly, 2005), which in turn may be associated with dehydration tolerance (Pausas et al., 2016). Thus, we compared resprouters to non-resprouters to assess whether they differed in dehydration tolerance and xylem function.

One area of active research is tradeoffs in xylem function, and some tradeoffs are well established (Hacke et al., 2001; Pratt and Jacobsen, 2017). For example, cavitation resistance generally comes at the cost of hydraulic transport efficiency; however, there is wide interspecific variability in this tradeoff (Gleason et al., 2016). Another cost is that tissues need to be reinforced with thick cell walls to resist the strains associated with dehydration (higher mechanical support and investment) and highly negative xylem pressures in cavitation-resistant species (Hacke et al., 2001; Jacobsen et al., 2005). This latter association forms the basis for our hypothesis, which is that high cavitation resistance may trade off with carbohydrate storage across species because high stores of carbohydrates require abundant parenchyma (Plavcová et al., 2016; Pratt and Jacobsen, 2017; Chen et al., 2020), and parenchyma cells have thin walls that are the least mechanically supportive at the tissue level (Willson et al., 2008; Pratt and Jacobsen, 2017; Janssen et al., 2020). High tensions can strain tracheary elements to the point of collapse (i.e., implosion) if they are not sufficiently reinforced by their own cell walls and by surrounding tissues including robust fibers (Hacke et al., 2001; Brodribb and Holbrook, 2005; Voelker et al., 2011). The amount of fibers in xylem is strongly and inversely related to the amount of parenchyma (Ziemińska et al., 2015; Pratt and Jacobsen, 2017; Chen et al., 2020; Janssen et al., 2020). Thus, we predicted that (1) tissues that store abundant carbohydrates have abundant parenchyma and (2) this leads to mechanically weaker tissues that place vessels at risk of collapse (Jacobsen et al., 2005).

To test for tradeoffs among starch storage and other traits, we examined starch storage, xylem structure, cavitation resistance of stem xylem, and seasonal water potential of species that broadly differ in dehydration tolerance, focusing on 30 shrubs from southern California chaparral communities. Starch was expressed per mass of dry tissue and per volume of tissue, because differences in tissue density can affect expressions on a per mass basis (Hoch et al., 2002). Because we were interested in testing for a tradeoff between storage and dehydration tolerance in xylem tissue, we focused our sampling on distal stems. This approach assumes that this tissue represents an important compartment of carbohydrate storage. A plant may store carbohydrates in leaves, stems, bark, roots, or specialized structures such as lignotubers (Furze et al., 2019). Sapwood of small stems has been shown to be an important location of stored carbohydrates and to commonly experience large seasonal changes in trees (Newell et al., 2002; Palacio et al., 2018; Piper, 2020). One reason for this is that sapwood in these small distal stems is close to the carbohydrate source (leaves); storing in this tissue thus minimizes transport costs. It also means that these stores are nearby to support growth of new shoots, including reproductive structures.

MATERIALS AND METHODS

We sampled 36 species of evergreen ($n = 20$) and drought-deciduous ($n = 16$) shrubs from four different field sites in southern California (Table 1; Appendices S1 and S2). However, six of the species were outliers in some of our data sets, and for most analyses we have $n = 30$ species. The outliers included *Eriogonum fasciculatum* and *Trichostema lanatum*, neither of which ever had

TABLE 1. Species sampled, location (for GPS, see Appendix S1; A = Angeles; P = Phantom; SJ = San Jacinto; S = Silverwood), leaf habit (e = evergreen, d = deciduous), the presence (Y) or absence (N) of living fibers or fiber-tracheids, resprouting (R+) or non-resprouting (R−) after disturbance, and mean (± SE) midday water potential during the dry season. Taxonomy follows Baldwin et al. (2012).

Species	Family	Site	LeafHabit	Living fibers	R+ or R−	Ψ_{md} (-MPa)
Adenostoma fasciculatum	Rosaceae	All	e	Y	R+	5.91 ± 0.31
Arctostaphylos glandulosa	Ericaceae	A	e	Y	R+	7.27 ± 0.22
Arctostaphylos glauca	Ericaceae	A	e	Y	R−	6.77 ± 0.14
Brickellia californica	Asteraceae	A	d	Y	R+	2.41 ± 0.13
Ceanothus crassifolius var. *crassifolius*	Rhamnaceae	A	e	N	R−	8.24 ± 0.08
Ceanothus leucodermis	Rhamnaceae	A	e	N	R+	3.34 ± 0.28
Fraxinus dipetala	Oleaceae	A	d	Y	R+	4.07 ± 0.21
Heteromeles arbutifolia	Rosaceae	A	e	Y	R+	4.35 ± 0.30
Quercus berberidifolia	Fagaceae	A	e	N	R+	5.19 ± 0.09
Quercus wislizeni	Fagaceae	A	e	N	R+	4.21 ± 0.12
Rhamnus ilicifolia	Rhamnaceae	A	e	N	R+	5.71 ± 0.14
Ribes malvaceum	Grossulariaceae	A	d	Y	R+	1.89 ± 0.15
Sambucus nigra ssp. *caerulea*	Adoxaceae	A	d	N	R+	1.67 ± 0.13
Trichostema lanatum	Lamiaceae	A	d	N	R−	7.27 ± 0.49
Rhus ovata	Anacardiaceae	A & SJ	e	Y	R+	3.80 ± 0.11
Ceanothus spinosus	Rhamnaceae	P	e	N	R+	5.06 ± 0.22
Encelia californica	Asteraceae	P	d	Y	R+	3.32 ± 0.06
Eriogonum cinereum	Polyganaceae	P	e	Y	R+	4.54 ± 0.11
Juglans californica	Juglandaceae	P	d	N	R+	2.06 ± 0.08
Malosma laurina	Anacardiaceae	P	e	Y	R+	2.44 ± 0.09
Ribes speciosum	Groassulariaceae	P	d	Y	R+	1.70 ± 0.15
Senegalia greggii	Fabaceae	SJ	d	Y	R+	2.89 ± 0.25
Adenostoma sparsifolium	Rosaceae	SJ	e	Y	R+	3.72 ± 0.11
Bernardia incana	Euphorbiaceae	SJ	d	Y	?	7.80 ± 0.17
Prunus fasciculata var. *fasciculata*	Rosaceae	SJ	d	Y	R+	5.11 ± 0.46
Purshia tridentata	Rosaceae	SJ	e	Y	R+	3.73 ± 0.53
Quercus cornelius-mulleri	Fagaceae	SJ	e	N	R+	3.90 ± 0.31
Ziziphus parryi	Rhamnaceae	SJ	d	N	R+	No data
Cercocarpus betuloides	Rosaceae	S	e	Y	R+	6.25 ± 0.23
Ceanothus vestitus	Rhamnaceae	S	e	N	R−	7.01 ± 0.24
Ericameria parishii	Asteraceae	S	e	Y	R−	2.16 ± 0.06
Fremontodendron californicum	Malvaceae	S	d	N	R+	1.72 ± 0.04
Keckiella ternata	Plantaginaceae	S	d	Y	R+	3.17 ± 0.41
Prunus emarginata	Rosaceae	S	d	Y	R+	2.87 ± 0.06
Frangula californica	Rhamnaceae	S	e	N	R+	2.33 ± 0.08
Rhus aromatica	Anacardiaceae	S	d	Y	R+	2.83 ± 0.15

any starch in their sapwood; thus, they were excluded from analyses. This was observed with an enzymatic starch assay (described below) and by staining with I_2KI at different times of the year. For *Ziziphus parryi*, we were not able to get a reliable seasonal water potential when it was leafless, so it was excluded from those analyses. Lastly, for the three species in the family Asteraceae that store fructans (Table 1), they were all outliers with extreme high values, as described below.

All sites were climatically similar (mediterranean-type; Appendix S1), with cool moist winters and hot dry summers; moreover, the chaparral indicator species *Adenostoma fasciculatum* (chamise) occurred at all sites; thus, they all represented a type of "chamise chaparral" in the context of vegetation classification (Sawyer and Keeler-Wolf, 1995). Sites were selected because they contained a diverse mix of co-occurring species (mixed chaparral) with both evergreen and deciduous leaf habits. None of the sites had experienced a burn in ≥20 yr. After fire, some species resprout from a root crown (R+), while others are killed and either recruit from a fire-cued dormant seed bank or disperse in from unburned areas (R−; Table 1). The criteria we used to define resprouting ability are outlined in Pausas et al. (2016).

Water potentials were measured on plants at field sites in the fall of 2009 and again in 2010 using a pressure chamber (model 2000; Plant Moisture Stress, Albany, Oregon, USA). September and October were chosen because this is the time of year when soil water is at the seasonal low and plant tissues are at peak water deficits. These measurements allowed us to assess the degree to which species avoided or tolerated tissue water deficits. The annual precipitation during the 2008–2009 rainfall season was moderately below average at all field sites and in southern California (Appendix S1). The 2009–2010 precipitation year was slightly above average (Appendix S1). Water potentials were measured predawn and at midday on 6–12 individuals per species at each site, which included two to four subsamples per individual. The minimum seasonal water potential (Ψ_{min}) used in analyses was the midday water potential measured for each species.

Cavitation resistance was measured on stems collected during the fall months of 2009. We selected branches that were fully illuminated (south facing) and that were ~6 mm in diameter. Stems were removed from plants in the field by cutting long branches under water. Stems were double-bagged along with a moist paper towel and transported to a laboratory at California State University,

Bakersfield, on the same day and were measured within 3 d. Stems were kept bagged and at 4°C during storage. Stem segments were removed from larger branches, and measured segments were ~6 mm diameter and 14 cm long. All cuts were made under water. Stem segments were flushed under pressure (100 kPa) with a 20 mM KCl solution that was filtered (0.1 μm pore) and degassed to remove emboli in the xylem to measure maximum conductivity in the absence of emboli. Stem ages were mostly 3–6 yr old; it was not possible to only sample the current year's growth because of very short annual grow increments. Conductivity was measured using a tubing system connected to a pressure head of the KCl solution at the upstream end and an analytical balance at the downstream end. Vulnerability to drought-induced cavitation was estimated using a standard centrifuge method that has undergone recent scrutiny and has been shown to be valid in the sense that data generated from it match the level of embolism from chaparral shrubs growing in the field (Sperry et al., 2012; Tobin et al., 2013; Pratt et al., 2015). When measuring samples after centrifuge treatments, pressure heads were kept low (<4kPa) to avoid displacing emboli with solution. Such measurements allowed us to calculate losses in conductivity due to embolism and express it as a percentage in relation to maximum conductivity. Cavitation resistance was expressed as the pressure potential corresponding to 50% ($P50$), 75% ($P75$), and 90% ($P90$) loss of conductivity.

Carbohydrate content of sapwood was measured on stem segments collected in the fall of 2009. The carbohydrate content of distal stems in shrubs from mediterranean-type environments is seasonally dynamic. During winter and spring, stores generally fall as they are mobilized to support vegetative (Palacio et al., 2018) and reproductive (Mooney and Hays, 1973) growth. In the late spring and summer, growth becomes water limited, but photosynthesis can continue to replenish carbohydrate stores over the summer and into the fall for evergreens and until leaf drop for deciduous species. Depending on the availability of water and temperatures, carbohydrates should accumulate to peak values in late spring and through the fall. With drought or extreme heat, carbon balance could be negative in the later summer and fall and stores may have a moderate drop at this time (Mooney and Hays, 1973). During a typical fall period, this should represent an estimate of the near maximum value of stored carbohydrates for these species (Palacio et al., 2018). The rainfall during the period that we sampled was slightly below average for the region and thus approximates a "typical" year (Appendix S1). One site (Silverwood) had lower rainfall compared to the average than the others, so values may have been lower at this site, but any such difference was not significant (Appendix S1). Plants may store starch in leaves, roots, and other regions, but our focus was on stems, and specifically sapwood, to examine the possibility of a tradeoff (Furze et al., 2019). To that end, the bark and pith were removed.

Samples for carbohydrate analysis were collected from stems that were chosen in a similar fashion to those sampled for cavitation resistance, although these cuts were not made under water. Harvested stem segments were ~5 cm long and were immediately sealed in a plastic bag and kept on ice. Stems were transported to a laboratory on the same day that they were collected and were immediately put in an oven at 100°C for 1 h and then kept in the oven for ≥3 d at 60°C. For long-term storage, dried stems were kept in desiccators.

Mechanical ball mills were used to grind samples to powder, and carbohydrates were extracted in sodium acetate buffer (pH 4.8) and analyzed as described by Pratt et al. (2014) using a method modified

from Cruz and Moreno (2001). In brief, we used an enzymatic approach whereby starch was converted to glucose and then was analyzed as glucose spectrophotometrically (Genesys 10UV; Thermo Electron, Madison, Wisconsin, USA) using commercially available kits (Hexokinase assay kit; Sigma-Aldrich, St. Louis, Missouri, USA). The total amount of starch was assessed by first measuring glucose content of extracts and then measuring glucose again after converting starch to glucose using amyloglucosidase (product no. 10115, Sigma-Aldrich). The total amount of starch was determined by subtracting the post-enzyme treatment glucose from the pre-enzyme glucose. Values were expressed as a percentage of xylem dry mass that was starch or as a percentage of xylem volume. Species in the family Asteraceae store fructans instead of starch. To analyze fructans, inulinase enzyme was used instead of amyloglucosidase to hydrolyze fructans (Megazyme, Chicago, Illinois, USA). Phosphoglucose isomerase (Sigma-Aldrich) was then used to isomerize fructose to glucose before the final step of analyzing glucose as described above. Precision was ensured by measuring a reference sample (control of ground ornamental pear wood) every time an assay was conducted, and if a particular assay fell >20% outside of the range, we reran the entire set of samples. To ensure the accuracy of assays, we ran a four-point standard curve for glucose and starch every time we conducted an assay.

The percentage of different cell types in cross section (vessels, parenchyma, fibers, and tracheids, if present) were measured for the same stems used to determine cavitation resistance ($n = 3–6$ stems/species). Thin sections were made using a sledge microtome and mounted in glycerol. Samples were examined at 200× magnification with a microscope (Axio Imager.D2; Carl Zeiss Microimaging GmbH, Gottingen, Germany) connected to a digital camera (AxioCam MRc; Carl Zeiss Microimaging). Each section was imaged in four locations, the area in cross section of the parenchyma and vessels were measured, and the remaining fiber area was determined by subtraction. For species with tracheids, tracheid area was analyzed when they could be clearly identified in cross section. For some species, tracheids were present but it is difficult to separate from fibers in cross section and, for these species, tracheid and fiber area were analyzed together. Measurements of parenchyma area included both axial and ray parenchyma. Many species contained living fibers (often septate) or fiber tracheids (we did not distinguish between these two) that stored varying amounts of starch (Carlquist, 1988). This was determined by staining cross sections with I_2KI and looking for relatively small thick-walled cells with starch granules or dense cytosol. Living fibers were included in the fiber cross-sectional area.

A phylogenetic tree was inferred using partial gene sequences from a plastid matK (930 bps) and nuclear small-subunit 18S rDNA (1660 bps) genes (Appendix S2). A DNA extraction kit was used (DNeasy plant kit; Qiagen, Valencia, California, USA) with a modified CTAB protocol. PCR amplification and DNA sequencing used universal primers (as suggested by P. Soltis, D. Soltis, and L. Majure, University of Florida, personal communication). Purified PCR products were sequenced at the University of Florida's DNA Sequencing Core Facility. Concatenated sequences were aligned using CLUSTAL X (default). The phylogenetic tree and branch lengths were inferred using maximum likelihood from the model with the lowest AIC (GTR+I+G) implemented in MEGA5.2 (Tamura et al., 2011). TML parameters were selected using iModelTest (Posada, 2008). The phylogenetic tree was used to calculate phylogenetic independent contrasts (PICs; Paradis et al., 2004). Analyses were run

using these branch lengths and also using branch lengths set to one (Ackerly, 2000).

Relationships between traits, including phylogenetic independent contrasts, were assessed using correlation analyses (Minitab 15; State College, Pennsylvania, USA). Differences between deciduous and evergreen taxa and resprouting type were compared using general linear models that included site, leaf habit (nested in species), resprouting type (nested within species), and species (random factor). All raw data for analyses are included in the appendices.

RESULTS

Species and site differences

Species broadly differed in minimum seasonal midday water potential (Ψ_{min}; −1.67 to −8.25 MPa), indicating that the level of dehydration their tissues experienced dramatically differed during the dry season in southern California. Other broad differences were found for cavitation resistance ($P75$ ranged from −1.5 to −15.0 MPa), starch content of the xylem (2.43–12.89% starch by dry mass and 0.016 to 0.087 starch per volume), the percentage of xylem parenchyma area (7.11–31.28%), and the percentage of fiber area (42–84%). These broad differences in water potential and xylem traits were observed across the whole data set and within each field site where plants were sampled (Appendix S3). Minimum seasonal water potential was strongly correlated to cavitation resistance ($R = 0.728$, $P < 0.001$) as found in previous studies (Jacobsen et al., 2007b; Pratt et al., 2007; Markesteijn et al., 2011); moreover, parenchyma and fiber area were strongly negatively correlated ($R = −0.699$, $P < 0.001$; Appendix S4).

Evergreen and deciduous species differed with respect to dehydration avoidance and tolerance traits. In particular, $P50$ was more negative for evergreens than for deciduous species, indicating greater cavitation resistance; however, $P75$ was not different between the two leaf habits (Table 2). The Ψ_{min} was more negative for evergreens than for deciduous species (Table 2). The xylem starch content was greater among deciduous than among evergreen species (marginally so on a per volume basis; Table 2), while the percentage xylem parenchyma area and fiber area were not different between the two (Table 2). Species that do not resprout after fire (R−) were more cavitation resistant and experienced greater Ψ_{min} than the resprouting species (R+; Table 3). They did not differ in starch content, parenchyma area, or fiber area of the xylem (Table 3).

Associations between dehydration tolerance and xylem traits

We found an association between Ψ_{min} and starch stored in the xylem (Fig. 1A). None of the species that had the lowest Ψ_{min} had high stores of starch. The opposite was not true, and at high Ψ_{min} (less negative) species had a broad range of starch content (Fig. 1A); however, the species with the greatest starch content of the xylem were those that had the least negative Ψ_{min}. This analysis was supported by PICs (Fig. 1C). These patterns held whether starch was expressed on a per mass or per volume basis (Fig. 1A; Appendix S5). This was due to the very strong correlation between starch expressed per mass or per volume (Appendix S6). Including the three sampled species in the family Asteraceae (Table 1) did not change the outcome of analyses, but all three were extreme outliers for carbohydrate content. These species store fructans instead of starch and had values much higher than the starch-storing species (21%, 26%, and 47%); thus, they were excluded from reported analyses that included carbohydrates.

We found that cavitation resistance and starch storage of the xylem were correlated (Fig. 1B; Appendix S5). No species that had high levels of xylem starch also had high resistance to cavitation, and the species with low resistance to cavitation included the species that had the highest levels of xylem starch (Fig. 1B; Appendix S5); moreover, the species with the greatest starch contents had low cavitation resistance. The trends were broadly similar for both evergreen and deciduous species. This analysis was supported by PICs suggesting that there is an evolutionary basis to this relationship (Fig. 1D; Appendix S5). We analyzed cavitation resistance using two other xylem pressures besides $P75$ ($P50$ and $P90$), and the association was highly significant regardless of the pressure used ($R = −0.523$, $P = 0.002$ for $P50$; $R = −0.648$, $P < 0.001$ for $P90$).

We tested for associations between the percentage of parenchyma and fiber area in the xylem. The parenchyma area was not significantly correlated with xylem starch content (Fig. 2A). Analyzing PICs for this correlation resulted in a weak, but significant, positive correlation between these traits (Fig. 2A, inset panel). When analyzed for evergreen and deciduous species separately, this correlation was marginally significant for deciduous, but not for evergreen taxa (Fig. 2A; Appendix S7). Cavitation resistance was negatively correlated with parenchyma area analyzed across both leaf habits, and this was supported by PICs (Fig. 2C) but was not significant for either evergreen or deciduous species separately analyzed (Fig. 2C). Finally, Ψ_{min} and parenchyma area were not significantly correlated, they were not correlated using PICs, and separate analyses for the two leaf habits were not significantly correlated (Fig. 2E). Showing the opposite pattern to parenchyma, the area of fibers in cross section was generally negatively correlated to starch content and positively correlated to cavitation resistance and Ψ_{min}. These negative relationships were also generally stronger than the equivalent comparisons for parenchyma area, particularly when fibers were correlated to starch and Ψ_{min} (Fig. 2B, D, F; Appendix S7).

Most of the sampled species had living fibers and, for some, these fibers contained abundant starch (Table 1). These fibers were counted

TABLE 2. Cavitation resistance (the pressure potential at 50% [$P50$] and 75% [$P75$] loss of conductivity), starch content (percent dry mass and mass per volume of tissue), minimum midday water potential (Ψ_{min}), parenchyma (par.) area (%), and fiber area (%) in stem xylem tissue for evergreen and deciduous taxa.

Leaf habit	P50 (MPa)	SE	P75 (MPa)	SE	Starch (%)	SE	Starch (g/cm³)	SE	Ψ_{min} (MPa)	SE	Par. (%)	SE	Fiber (%)	SE
Evergreen	−3.87	0.49	−5.89	0.68	4.20	0.42	0.026	0.003	−4.80	0.39	18.22	1.41	66.11	1.99
Deciduous	−2.60	0.39	−4.54	0.92	6.20	0.96	0.038	0.004	−3.39	0.50	16.99	1.54	60.81	2.70
F	4.16		2.95		4.32		3.59		5.53		0.2		2.65	
df	1, 28		1, 28		1, 29		1, 29		1, 27		1, 28		1, 28	
P-value	0.050		0.096		0.046		0.068		0.026		0.658		0.119	

TABLE 3. Cavitation resistance (the pressure potential at 50% [*P*50] and 75% [*P*75] loss of conductivity), starch content (percent dry mass and mass per volume of xylem), minimum midday water potential (Ψ_{min}), parenchyma (par.) area (%), and fiber area (%) in stem xylem tissue of post-fire resprouters (R+) and those that do not resprout (R−) after fire.

Resprout	*P*50 (MPa)	SE	*P*75 (MPa)	SE	Starch (%)	SE	Starch (g/cm³)	SE	Ψ_{min} (MPa)	SE	Par. (%)	SE	Fiber (%)	SE
R−	−5.82	1.19	−8.89	1.41	3.66	0.69	0.024	0.010	−6.29	1.06	16.14	3.92	71.29	3.39
R+	−2.78	0.27	−4.35	0.43	5.26	0.55	0.032	0.003	−3.71	0.28	18.00	1.08	63.05	1.69
F	14.60		14.46		0.88		0.60		10.80		0.37		3.15	
df	1, 33		1, 33		1, 28		1, 28		1, 32		1, 33		1, 33	
P-value	<0.001		<0.001		0.355		0.443		0.002		0.544		0.085	

as part of the fiber area. Species with abundant starch-storing fibers could confound analyses examining a relationship between parenchyma and starch. To assess this possibility, we analyzed the relationship between parenchyma area and starch for the 12 species that did not have starch-storing fibers and that also stored starch (i.e., not fructans). When this was done, there was a significant positive relationship between log of xylem starch content and parenchyma

area, but not for PICs of these traits (Fig. 3A). We could not separate deciduous and evergreen species for this analysis because there were only two deciduous species that did not contain living fibers. Cavitation resistance was significantly and negatively correlated with parenchyma area for these traits and this was supported by PICs (Fig. 3B). Finally, there was also a significant negative correlation between Ψ_{min} and parenchyma area that was also supported by PICs (Fig. 3C).
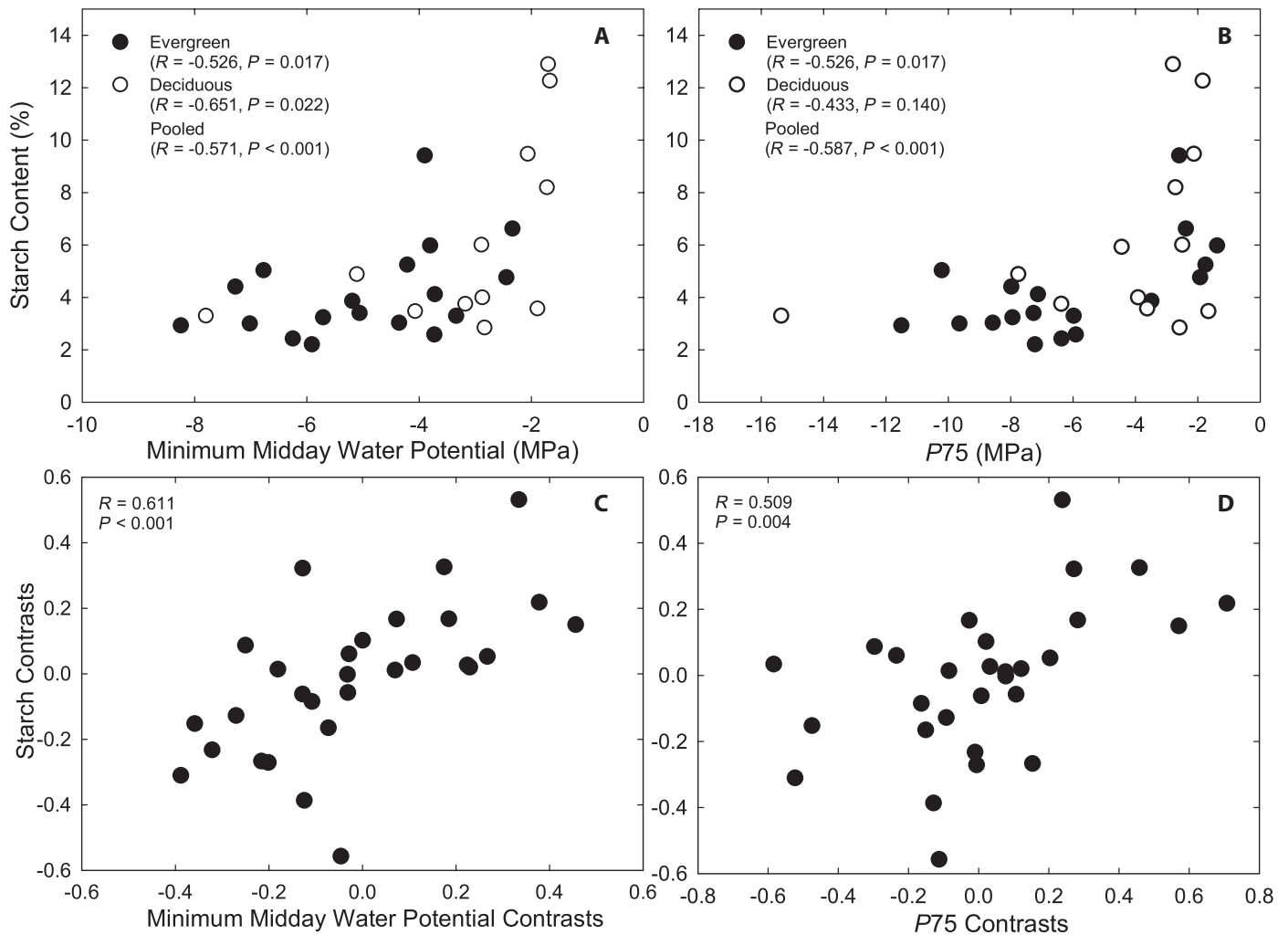
FIGURE 1. Scatterplots of (A) minimum midday water potential and starch content of sapwood and (B) cavitation resistance (estimated as xylem pressure potential at 75% loss of conductivity, or *P*75) and starch content of sapwood tissue for 30 evergreen (*n* = 18) and deciduous (*n* = 12) shrub species. Each point is a mean of four to eight replicates. (C, D) Phylogenetic independent contrasts for each trait. *R*-values and *P*-values are from Pearson correlation analyses of log-transformed data.
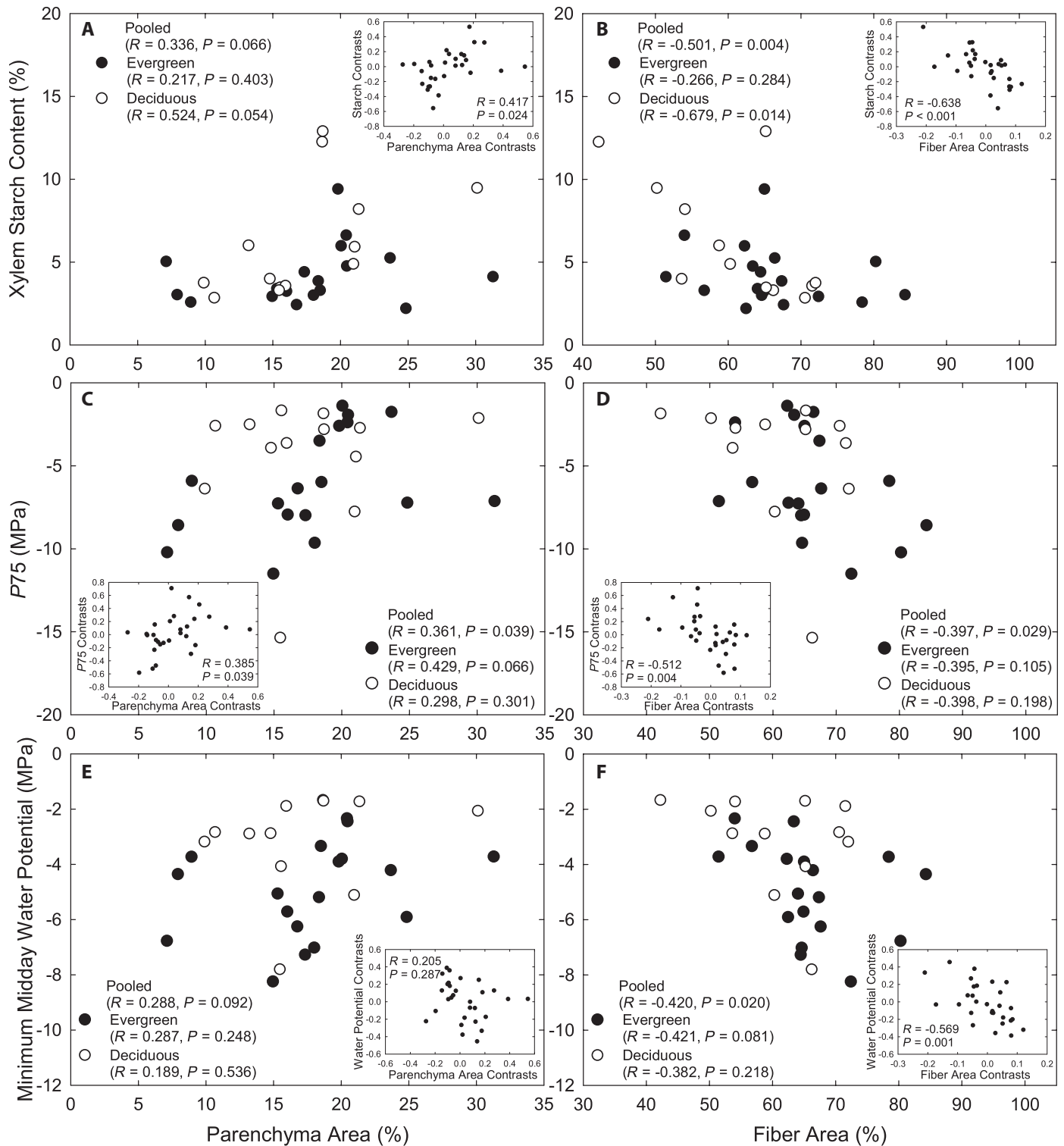
FIGURE 2. Scatterplots of (A, B) xylem starch content, (C, D) cavitation resistance (estimated as xylem pressure potential at 75% loss of conductivity, or $P75$), and (E, F) water potential plotted against the percentage of xylem parenchyma and fibers in cross section of 30 evergreen ($n = 18$) and deciduous ($n = 12$) shrub species. Each point is a mean of four to eight replicates. Insets show scatterplots of phylogenetic independent contrasts. R-values and P-values are from Pearson correlation analyses.
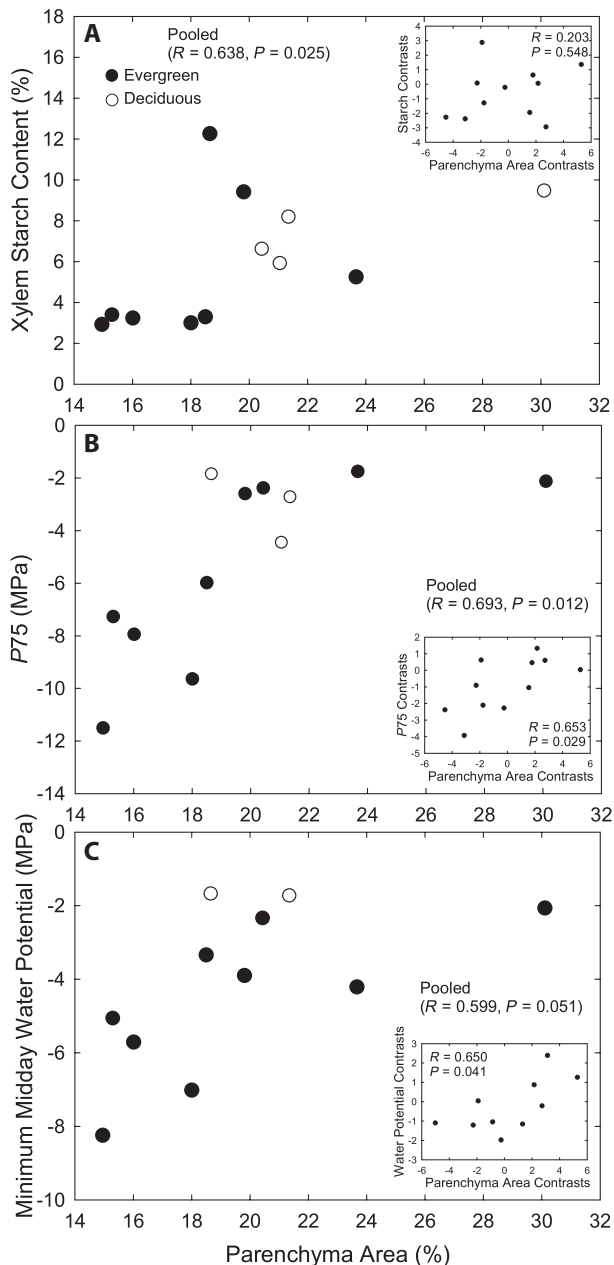
FIGURE 3. Scatterplots of (A) xylem starch content, (B) cavitation resistance (estimated as xylem pressure potential at 75% loss of conductivity, or P75), and (C) water potential plotted against the percentage of xylem parenchyma of evergreen (*n* = 9) and deciduous (*n* = 2 or 3) shrub species. These plots include only the species that do not have living fibers that store starch. Each point is a mean of four to eight replicates. Insets show scatterplots of phylogenetic independent contrasts. *R*-values and *P*-values are from Pearson correlation analyses.

DISCUSSION

Parenchyma content tradeoffs in xylem tissue

We hypothesized a tradeoff between structure and function such that storing abundant starch necessitates greater parenchyma and this destabilizes vessels, leading to greater vulnerability to implosion under high tensions (more negative water potential; Jacobsen et al., 2005). We predicted that carbohydrates stored in stem xylem tissue would be associated with dehydration tolerance measured as Ψ_{min} and cavitation resistance, and this was supported. We suggest that there is a tradeoff between carbohydrate storage and resistance of vessels to resist implosion in angiosperm xylem. While we cannot test this directly with our experimental design, there is excellent evidence that tracheary elements that are not reinforced in a matrix of supporting tissues, such as in leaves, will deform and collapse under sufficient xylem tensions (Cochard et al., 2004; Brodribb and Holbrook, 2005; Zhang et al., 2014), and deformation can happen in woody tissues (Schweingruber et al., 2007). In the present study, the overall correlations between parenchyma and fiber area and starch content, dehydration tolerance, and cavitation resistance were consistent with our predictions. Some of the relationships were weak, suggesting that other important factors were at work (Morris et al., 2018); moreover, in nearly every case, relationships were stronger when compared using PICs, indicating that the phylogeny of the species is important to consider for these traits. Other factors that could mitigate vessel implosion are the thickness of vessel walls (Pratt et al., 2007; Willson et al., 2008) or the arrangement of supporting fibers (Jacobsen et al., 2005). This would be possible in angiosperm xylem because of the division of labor between supporting fiber cells and transporting vessels that contributes to a diversity of functional possibilities (Sperry et al., 2006; Carlquist, 2014).

Another factor that could stabilize vessels and contribute to starch content is living fibers (Carlquist, 1988; Plavcová et al., 2016). Most of our sampled species had living fibers containing starch that could be easily observed when stained with I$_2$KI. Analyses that included only the species that lacked living fibers showed the predicted relationships with parenchyma area, which is consistent with another study (Plavcová et al., 2016). It appears that living fibers function to both store and support, as opposed to the typical division of labor between cell types in the xylem. It is possible that living fibers that store starch have evolved to expand carbohydrate storage capacity while maintaining structural integrity of wood that is exposed to high tensions. The functional significance of these cells in starch storage and xylem evolution is an exciting area for future research (Yamada et al., 2011).

A recent study did not find an association between non-structural carbohydrates and embolism resistance in temperate trees (Chen et al., 2020). In that study, carbohydrates were sampled during the growing season, when carbohydrates would have been low, whereas we sampled long after seasonal growth, when they should have been near peak. They also used a different method and expressed carbohydrates as starch and soluble sugars (non-structural carbohydrates) instead of solely as starch (as done here).

The role of carbohydrate storage in plant resource use strategies

An association between starch storage and dehydration tolerance could be driven by the evolution of an adaptive suite of traits associated with resource use. Species differ in the degree to which their tissues dehydrate. At the dehydration-avoiding end of the spectrum are species that have a suite of traits, including stomatal closure, leaf shedding, water storage, and often deep rooting (West et al., 2012). The xylem of species with this strategy is not highly resistant to dehydration-induced cavitation, and this is supported by the strong correlation between Ψ_{min} and cavitation resistance found here and elsewhere

(Pockman and Sperry, 2000; Pratt et al., 2007). Other traits associated with the dehydration avoidance strategy include larger-diameter vessels, lower wood density, reduced tissue strength, and higher leaf area/sapwood area of branches (Ackerly, 2004; Jacobsen et al., 2007a; Pivovaroff et al., 2015). We add greater starch storage to this list.

There are several reasons why starch storage may be involved in dehydration avoidance/tolerance. Species that tolerate greater tissue dehydration can conduct photosynthesis over a broader range of soil water deficits, which may mitigate the need for maintaining high starch stores (McDowell et al., 2008), an idea that has spawned much research (Quero et al., 2011; Garcia-Forner et al., 2016). While our data are broadly consistent with this idea, a robust test of it requires a more extensive consideration of carbon dynamics at the level of the whole plant, different than what was examined in this study (Ryan, 2011; Furze et al., 2019).

Another factor is that carbohydrates are known to be important in xylem tissues for refilling embolized vessels (Bucci et al., 2003; Salleo et al., 2004; Secchi and Zwieniecki, 2011; Wang et al., 2018). This does not occur when the pressure potential of xylem sap is highly negative (Hacke and Sperry, 2003); thus, dehydration tolerant species cannot refill emboli when they are dehydrated. Refilling would be a possible response for dehydration-avoiding species, and such species would need stored carbohydrates to support this. This prediction is consistent with our observed relationships between starch storage and $P75$ and Ψ_{min}. A final factor is that starch may be hydrolyzed into simpler sugars in dehydration-tolerant species in order to osmoregulate when tensions are high, which would lead to lower starch in the tissues of the species with the most negative Ψ_{min} (Martínez-Vilalta et al., 2016).

Leaf habit and resprouting

Chaparral systems are dominated by evergreen species. It has been hypothesized that this dominance is based on a more favorable carbon balance for evergreen species than for deciduous species in a mediterranean-type climate (Mooney, 1989). We found support for some aspects of this model. For example, our data suggest that evergreen and deciduous taxa were different, with deciduous taxa tending to be dehydration avoiders to a greater degree than the evergreen taxa. The deciduous taxa also stored more xylem starch than the evergreen taxa (Hoch et al., 2003; Martínez-Vilalta et al., 2016; Palacio et al., 2018; Piper, 2020). Greater carbon stores should allow them to survive protracted periods when photosynthesis is inactive, which is consistent with the hypothesis that carbohydrate storage is an adaptive trait for dehydration avoidance. The role of storage and carbon balance between evergreen and deciduous trees is an area of active research (Hoch et al., 2003; Martínez-Vilalta et al., 2016; Furze et al., 2019). There are interesting patterns in seasonal carbohydrate dynamics that are not explained by differences in growth and leaf habit (Piper, 2020). Our results suggest that some of the variation may be related to dehydration avoidance/tolerance strategies and the minimum seasonal water potential species experience. Our data collection was not designed to fully test this, and this will require a wider range of data collection including seasonal patterns of non-structural carbohydrates coupled with measures of plant water status.

Although the deciduous taxa had greater starch stores, they did not have greater parenchyma area, suggesting that their maximum storage capacity does not differ between the two leaf habits. The deciduous taxa presumably have higher rates of photosynthetic carbon gain when conditions are favorable so that they can rapidly acquire carbon during a truncated photosynthetic season (Mooney and Dunn, 1970; Field and Mooney, 1986; Mooney, 1989; Parker et al., 2016).

Fires are a recurrent disturbance in chaparral systems, and most woody chaparral species in southern California resprout after fire from a root crown (R+), while others do not resprout and must regenerate from a fire-cued seed bank or disperse into a site from adjacent unburned areas (R−). We found that R− species were more dehydration tolerant (i.e., experienced greater Ψ_{min} and were more cavitation resistant) than the R+ species, which is consistent with many previous studies (reviewed in Pausas et al., 2016). We did not find differences between R− and R+ in starch content or parenchyma area of xylem. If R+ species store more starch as part of an adaptive strategy for coping with fire, it would likely manifest as differences in belowground carbohydrate storage because a crown fire kills the shoots; thus, any starch stored in stems would not support resprouting.

Carbohydrate storage in xylem

Numerous recent studies have examined the associations between xylem storage, anatomical traits, and cavitation resistance (Pratt et al., 2007; Ziemińska et al., 2015; Morris et al., 2018; Plavcová et al., 2016; Pratt and Jacobsen, 2017; Chen et al., 2020). As more data accumulate, examining these patterns across different environments and different growth forms will soon be possible. Such studies will be valuable, because the xylem of woody plants holds important clues as to why some species survive drought and others succumb. Evidence of an important role for plant carbon storage as a component of drought resistance remains an objective of active research (Sala et al., 2010; Anderegg et al., 2012; O'Brien et al., 2014; Pratt et al., 2014; Sevanto et al., 2014; Trifilò et al., 2019; Janssen et al., 2020), and the results presented here suggest a role for carbon relations during drought.

AUTHOR CONTRIBUTIONS

R.B.P. conceived of the experiments, collected data, analyzed data, managed data, provided resources, and wrote the first draft. A.L.J. collected data, analyzed data, managed data, provided resources, and edited the manuscript. M.F.T., C.A.T., M.E.D., C.C.H., H.S.T., E.D.M., M.I.P., M.E.C., and P.T.S. collected data, managed data, developed methods, and edited the manuscript.

DATA AVAILABILITY

Data are available in Appendix S8.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

APPENDIX S1. Temperature and precipitation for field sites.

APPENDIX S2. Phylogeny and authority for sampled taxa.

APPENDIX S3. Mean and spread of traits pooled by sites.

APPENDIX S4. Scatterplot between parenchyma area and fiber area.

APPENDIX S5. Scatterplots analyzing starch per unit volume of sapwood.

APPENDIX S6. Scatterplot of starch expressed on a per dry mass vs. as per volume basis.

APPENDIX S7. Scatterplots analyzing starch per unit volume of sapwood with cell types.

APPENDIX S8. Data used for analyses in manuscript.

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