



Research paper

Divergent stem hydraulic strategies of *Caragana korshinskii* resprouts following a disturbance

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Received May 6, 2021; accepted August 5, 2021; handling Editor Maurizio Mencuccini

Resprouting plants are distributed in many vegetation communities worldwide. With increasing resprout age post-severe-disturbance, new stems grow rapidly at their early age, and decrease in their growth with gradually decreasing water status thereafter. However, there is little knowledge about how stem hydraulic strategies and anatomical traits vary post-disturbance. In this study, the stem water potential (ψ_{stem}), maximum stem hydraulic conductivity ($K_{\text{stem-max}}$), water potential at 50% loss of hydraulic conductivity ($K_{\text{stem}} P_{50}$) and anatomical traits of *Caragana korshinskii* resprouts were measured during a 1- to 13-year post-disturbance period. We found that the $K_{\text{stem-max}}$ decreased with resprout age from 1-year-old resprouts ($84.2 \text{ mol m}^{-1} \text{ s}^{-1} \text{ MPa}^{-1}$) to 13-year-old resprouts ($54.2 \text{ mol m}^{-1} \text{ s}^{-1} \text{ MPa}^{-1}$) as a result of decreases in the aperture fraction (F_{ap}) and the sum of aperture area on per unit intervessel wall area (A_{ap}). The $K_{\text{stem}} P_{50}$ of the resprouts decreased from 1-year-old resprouts (-1.8 MPa) to 13-year-old resprouts (-2.9 MPa) as a result of increases in vessel implosion resistance (t/b)², wood density (WD), vessel grouping index (GI) and decreases in F_{ap} and A_{ap} . These shifts in hydraulic structure and function resulted in an age-based divergence in hydraulic strategies i.e., a change from an acquisitive strategy to a conservative strategy, with increasing resprout age post-disturbance.

Keywords: drought, hydraulic safety, resprout age, stem hydraulic efficiency, wood anatomy.

Introduction

Drought is one of the most important factors limiting plant growth, development, survival and productivity (Baert et al. 2015, Mencuccini and Binks 2015, Epila et al. 2017, Semerci et al. 2017, Van Camp et al. 2018, Brodribb et al. 2020, Yang et al. 2021, Yao et al. 2021a), and the divergence in structures of stem xylem is tightly related to interspecific differences in drought resistance, species performance and distribution with drought (Brodribb et al. 2017, Venturas et al. 2017, Salomón et al. 2020). Stem structural traits, such as wood density (WD), vessel diameter and vessel wall and pit characteristics, facilitate a trade-off between stem hydraulic conductance (hereafter, 'hydraulic efficiency') and embolism resistance (hereafter, 'hydraulic safety') (Wheeler et al. 2005, Lens et al. 2011, Yin et al. 2018). Therefore, understanding

stem traits is an important way to enhance our knowledge of plant growth strategies and adaptation mechanisms (Brodribb 2009).

Resprouting is the initiation of new shoots from existing plant meristems in response to tissue loss following fire, clipping, herbivory or drought. It is common in many vegetation communities worldwide and enables the successful survival of plants after disturbance (Mostacedo et al. 2009, Pausas and Keeley 2014, Zeppel et al. 2015, Pausas et al. 2016, Yao et al. 2021c). In resprouting species, the water absorbed by roots is transported to less aboveground leaf area, which improves tissue water status and gas exchange at the early post-disturbance stage (Utsumi et al. 2010, Ramirez et al. 2012, Nolan et al. 2014, Schafer et al. 2014). The superior performance of younger resprouts relative to their more mature counterparts is usually

associated with their higher stem hydraulic conductivity (Genet et al. 2010, Fang et al. 2013, Schafer et al. 2014, Gričar et al. 2020). However, when resprouts recover, the increase in aboveground leaf area results in a decrease of water status with the increase in the resprout age (Mencuccini and Grace 1996, Fang et al. 2013, Bao et al. 2015, Schwikl et al. 2016, Yao et al. 2021c). To adapt to their declining water status, older resprouts exhibit stronger resistance to stem hydraulic losses through morphological and physiological adjustments, such as smaller vessel size (Mencuccini and Comstock 1997, Jacobsen et al. 2016, Gričar et al. 2020, Poulos et al. 2020). Moreover, it has shown that pit anatomical traits, such as smaller, thicker pit membrane and smaller pit aperture, favor resistance to drought-induced embolism across diverse species (Lens et al. 2011, Yin et al. 2018, Levionnois et al. 2021). How such vessel and pit characteristics are coupled to the hydraulic strategies of resprouts to adapt to declining water status is less studied in the field experiments.

The water potential at 50% loss of the maximum stem hydraulic conductivity ($K_{\text{stem}} P_{50}$) is widely used as an indicator of species resistance to losses of stem hydraulic efficiency (Choat et al. 2012, Salomón et al. 2017, Venturas et al. 2017, Gorel et al. 2019). There is a trade-off between xylem hydraulic efficiency and safety, i.e., an increase in one may decrease the other (Gleason et al. 2016, Pratt and Jacobsen 2017, Venturas et al. 2017). Thus, we propose that younger resprouts exhibit higher $K_{\text{stem-max}}$ and less negative $K_{\text{stem}} P_{50}$, while older resprouts exhibit lower $K_{\text{stem-max}}$ and more negative $K_{\text{stem}} P_{50}$. Furthermore, woody plants inhabiting water-limited environments usually exhibit a conservative strategy, with lower hydraulic efficiency and gas exchange rates and higher hydraulic safety; in contrast, woody plants from mesic environments often adopt an acquisitive strategy, with substantially higher hydraulic efficiency and gas exchange rates and lower hydraulic safety (Moreno-Gutiérrez et al. 2012, van der Sande et al. 2019, Gong et al. 2020, Guo et al. 2020). Therefore, we proposed that younger resprouts exhibit an acquisitive strategy, while older resprouts exhibit a conservative strategy.

If varying hydraulic strategies exist in the same resprouting species during regrowth, it is useful to obtain an understanding of the physiological basis for age-related changes in hydraulics (Chave et al. 2009, Reich 2014, Yin et al. 2018). Species increase their hydraulic safety by reducing the risk of embolism by developing small-diameter vessels; however this, approach comes at a cost of lower hydraulic efficiency (Christman et al. 2012, Lens et al. 2013, Kitin and Funada 2016, Gorel et al. 2019). The higher wood density (WD) due to the smaller-diameter vessels and thicker conduit walls enhances xylem cavitation resistance (Jacobsen et al. 2007, Onoda et al. 2010). Additionally, pit characteristics, such as the small pit membrane area and small pit aperture, may favor a lower risk of embolism but result in lower water transport efficiency between two

adjacent vessels (Lens et al. 2011, Venturas et al. 2017, Yin et al. 2018). Finally, water has to be transported horizontally among stem xylem vessels when moving from plant roots to leaves (Steppe 2018). The network of vessel elements, i.e., vessel grouping index (GI), would have significantly effect on xylem hydraulics (Lens et al. 2011, Scholz et al. 2013b, Levionnois et al. 2021, Wason et al. 2021). Some studies have shown that a higher degree of vessel grouping facilitates bypassing of embolisms in the xylem by water, resulting in greater xylem resistance to embolism (Carlquist 1984, 2009, Lens et al. 2011, Levionnois et al. 2021). However, other studies have shown that vessel connectivity increases hydraulic conductivity by providing alternative pathways for water movement and therefore increases vulnerability to embolism by facilitating the spread of embolisms (Loepfe et al. 2007, Martínez-Vilalta et al. 2012, Yin et al. 2018). Overall, these stem xylem structures had been reported previously to have determined hydraulic efficiency and safety in the woody plants (Hacke et al. 2001, Lens et al. 2011, Yin et al. 2018, Wason et al. 2021) that are correlated with woody plant hydraulic strategies (van der Sande et al. 2019, Gong et al. 2020, Guo et al. 2020). However, the xylem-structure basis of hydraulic strategies remains under debate, and the significance of xylem structure to hydraulic strategies is not well understood.

C. korshinskii is a long-lived shrub (>50 years) native of arid environments in Northwest China that possess a high economic and ecological value (Fang et al. 2017, Waseem et al. 2021, Yao et al. 2021c). Its shoots are often subjected to branch removal and herbivory, and rapidly recover by resprouting during the initial regrowth stage, but regrowth becomes slow with increasing age post-disturbance (Fang et al. 2008, 2013). Previous research has shown that intraspecific gradual decrease in sapwood area-specific hydraulic conductivity occurred in *C. korshinskii* resprouts with increasing age (Fang et al. 2013). The progressive loss of stem hydraulic conductivity with height growth decreased the leaf water potential in *C. korshinskii* resprouts, and induced a decrease in leaf photosynthesis, which may have contributed to the cessation of height growth (Fang et al. 2013). However, there is little knowledge about how stem hydraulic strategies and anatomical traits vary post-disturbance. In this study, we tested whether younger (1- to 3-year-old) resprouts exhibited an acquisitive strategy, with higher xylem hydraulic conductivity and less xylem resistance to embolism, and whether older (11- to 13-year-old) resprouts displayed a conservative strategy, with lower hydraulic conductivity and greater resistance to embolism. Then we ascertained the structural basis underlying the age-based difference in resprout hydraulic strategies by assessing the aperture fraction, the sum of aperture area on per unit intervessel wall area, vessel implosion resistance, WD, and vessel grouping index. To achieve these objectives, the stem water potential, maximum stem hydraulic conductivity, and vulnerability curves as well

as anatomical features were measured in 1- to 13-year-old *C. korshinskii* resprouts.

Materials and methods

Study site and species

C. korshinskii is a dominant xerophytic shrub that has been widely applied in the revegetation and reconstruction of fragile ecosystems in arid and semiarid regions on the Loess Plateau, NW China. The study was carried out at the Semiarid Ecosystem Research Station (104°25'E, 36°20'N; 2,400 m altitude) located in Zhonglianchuan village in the northern mountainous region of the Loess Plateau, northern Yuzhong County, Gansu Province, NW China. In this area, *C. korshinskii* shoots are harvested by local farmers to use as fuel in winter or to feed sheep in late winter before new grass grows. At this site, the mean annual precipitation is 320 mm (70% occurs from May to October), but the annual evaporation is 1326 mm (Fang et al. 2008, 2013).

The experiment was designed as described in Fang et al. (2013). Ten plants were randomly selected for sampling, and the aboveground biomass was clipped to 10 mm above the soil surface at the end of winter (15–28 March) in 2007, 2009, 2011, 2013, 2015, 2016, 2017, 2018 and 2019. The area was then fenced to avoid trampling or mammalian herbivory. In August 2019, 1-year-old (2019) to 13-year-old resprouts (2007) were sampled for hydraulic and anatomical measurements.

Plant water status

The leaf water potential (Ψ_{leaf} , MPa) was measured with a pressure chamber (PMS Instrument Company, Albany, OR, USA) on continuously sunny and windless days. Two healthy leaves were excised from each of five individuals in each age group before dawn (4:00–6:00) to measure the predawn Ψ_{leaf} . To estimate the midday stem water potential (midday Ψ_{stem}), terminal branches from five individuals per age group were wrapped in plastic bags and aluminum foil in the evening before the measurement days. Then, leaves from the bagged stems were collected at midday (12:00–14:00), and Ψ_{leaf} was measured to estimate the midday Ψ_{stem} , as the leaves were assumed to be in equilibrium with the Ψ_{stem} (Zhang et al. 2017).

Hydraulic conductivity and vulnerability curves

Five branches per age group were collected from different individuals near where the shoots emerged from the basal buds before dawn. The stem segments were immediately recut under water, transported to a nearby laboratory, kept in water, and rehydrated for 8 h. The maximum vessel length (MVL) was determined by the air injection method (Cohen et al. 2003). Stem segments (0.3–1.0 m) longer than the MVL

were used to measure the stem hydraulic conductivity following the method described by Sperry et al. (1988). In brief, the stem segments were flushed at a pressure of 0.1 MPa with 10 mM potassium chloride (KCl) solution for 30 min in the laboratory (20–25 °C) to remove native embolisms. An elevated water reservoir with a KCl solution generated gravity-induced hydrostatic pressure (5.0 kPa) and was connected to one end of the stem segment. The water flow rate (mmol/s) through the stem segment was measured by a pipette (0.1 mL, BOMEX, Beijing, China) connected to the opposite end of the segment. The hydraulic conductivity per unit pressure gradient (K_h , mol m s⁻¹ MPa⁻¹) is equal to the water flux rate through the stem segment divided by the pressure gradient causing the flow. Thereafter, the sapwood of the stem was flushed with a methyl blue solution, and the cross-sapwood area was calculated as the mean of the cross-section of the two ends of the stem. The maximum stem hydraulic conductivity (K_s , mol m⁻¹ s⁻¹ MPa⁻¹) is equal to the ratio between K_h and the cross-sapwood area.

Stem vulnerability curves were derived from the pneumatic method (Pereira et al. 2016, Bittencourt et al. 2018). The 0.5 m- to 1.0 m-long basal branches collected from different individuals were rehydrated for 8 h in black plastic bags, and the basal end of each branch was connected to a pneumatic apparatus through a three-way stopcock including a syringe as a vacuum source and a pressure sensor (142PC05D, Honeywell International Inc., Charlotte, NC, USA). Before measurement, a leak test was performed by running the system without connecting it to the branch. Once the branch end was connected to the pneumatic tubing system, the tubing was not replaced, and the branch ends were not shaved before the measurements were taken. The rigid plastic tube between the branch end and the pressure sensor functioned as a vacuum reservoir. First, the branch-vacuum reservoir pathway was closed, i.e., the branch end was left open to the atmosphere. A pressure of ~40 kPa was obtained in the vacuum reservoir by pulling the syringe plunger. Then, the vacuum reservoir-syringe pathway was closed, and the branch-vacuum reservoir pathway was opened. The initial pressure was recorded immediately after connecting the end of the branch to the vacuum reservoir. After extracting air from the branch to the vacuum reservoir for 2.5 min, the final pressure was recorded. Finally, the branches were enclosed in a black plastic bag for 20 min to equilibrate the xylem pressure. The water potential of two cut leaves was measured with a pressure chamber (PMS Instrument Company, Albany, OR, USA) as was the stem xylem water potential. Glue (Loctite 431, Henkel Technology Center-Asia Pacific, Yantai, China) was placed on the branch where the leaf was cut to prevent air from entering the plant tissues. The branch was then detached from the apparatus and dried on the bench. The drying time was between 0.5 and 2 h for the two measurement intervals. The volume of the reservoir was adjusted by changing the reservoir tube length according to the branch size.

Measurements were concluded when the stem water potential was approximately -6 MPa. The percentage of air discharged (PAD, %), which is analogous to the percentage loss of conductivity (PLC), and the water potential at 50% loss of conductivity ($K_{\text{stem}} P_{50}$) were calculated as proposed by Pereira et al. (2016).

Xylem anatomical traits

The basal portion of each stem used for the hydraulic measurements was also used to determine WD and xylem anatomical traits (Scholz et al. 2013a, Yin et al. 2018). For WD, the bark was removed from 5-cm-long stems, and the stems were soaked in water for 24 h to remove gases from the stems. The saturated volume of the stems was measured using the water displacement method (Hacke et al. 2000), i.e., by determining the mass displaced by samples submerged in water. The stems were dried at 80°C for 48 h, and their dry mass was determined. The WD was calculated as the ratio of the dry mass to the saturated volume.

For the anatomical measurements at the vessel level, 5-cm-long segments were soaked in 1:1 (v/v) ethanol and glycerol solutions for 3 months after the removal of the bark. Transverse sections of $30\text{ }\mu\text{m}$ thickness from five segments per age group were made using a sliding microtome (Leica RM2245, Heidelberg, Germany). All sections were stained with 1% safranin ethanol solution for 1 h, washed with 85% ethanol for 5 min, and stained with 0.5% fast green ethanol solution for 30 s again to enhance the visual contrast. Afterward, the sections were dehydrated in an ethanol series (95 and 100%) for 5 min, treated with xylene to increase transparency, and mounted in Canada balsam. The images were taken under a light microscope (Axio Scope Al: HBO100, Carl Zeiss MicroImaging GmbH, Gottingen, Germany) with an inbuilt digital camera connected to a computer. Three digital images per sample were taken from the inside to the outside of the transverse xylem sections. The values of the anatomical characteristics were calculated with ImageJ software (National Institutes of Health, Bethesda, MD, USA). The vessel density (VD, no. mm^{-2}) was determined as the number of vessels per xylem area. The vessel grouping index (GI) was equal to the ratio of the total number of vessels to the total number of vessel groupings (incl. solitary and grouped vessels); higher values indicate greater degrees of vessel grouping (Carlquist 2001, Scholz et al. 2013a). The vessel diameter (D) was measured as the diameter of a circle with the same area as the vessel lumen. The vessel implosion resistance ($(t/b)^2$) was the square of the ratio of the vessel wall thickness to the vessel lumen diameter (Hacke et al. 2001).

To measure the anatomical traits of the xylem at the pit level, tangential sections of $50\text{ }\mu\text{m}$ thickness were cut using a Leica RM2245 sliding microtome after the bark was removed from 1.5-cm-long stems. The sections were dehydrated in an ethanol

series (30-50-70-90-100%) for 20 min at each step and dried for 8 h in a freeze-dryer (Hitachi, ES-2030, Ibaraki, Japan). The dried, untreated sections were fixed on aluminum stubs with an electron-conductive carbon sticker and coated with 12.5–15 nm gold in an ion sputtering system (Hitachi, E-1010, Ibaraki, Japan) for 2 min. The sections were observed with a scanning electron microscope (Hitachi, S-3400 N, Ibaraki, Japan) at an accelerating voltage of 15 kV. Images were taken at $\times 3000$ magnification with ImageJ software to analyze the pit-level structures. The aperture fraction (F_{ap}) and the sum of aperture area on per unit intervessel wall area (A_{ap}) were measured on at least 100 pits from several vessels per resprout age group.

Statistical analysis

Before the statistical analyses, all of the data were tested for normality and equality of variance. One-way ANOVA and t -tests were used to test for trait differences across resprout age groups. Because we were specifically interested in investigating, which structural traits were predictive of hydraulic function, principal component analysis (PCA) was performed in CANOCO 5.0 to examine the multivariate associations among the measured functional traits (Table 1), and the samples were arranged by their scores on the first two principal components. Statistical analyses were performed with SPSS 22 (IBM, Armonk, New York, NY, USA), and figures were generated in SigmaPlot 14 (Systat Software Inc., Erkrath, Germany).

Results

Age-related changes in hydraulic traits

The predawn Ψ_{stem} was similar, about -0.5 MPa, across resprout ages, and the midday Ψ_{stem} decreased gradually, from -1.5 MPa in the 1-year-old resprouts to -2.2 MPa in the 13-year-old resprouts (Figure 1, Table S1 available as Supplementary data at *Tree Physiology Online*). $K_{\text{stem-max}}$ decreased gradually with increasing resprout age, from $84.2\text{ mol m}^{-1}\text{ s}^{-1}\text{ MPa}^{-1}$ in the 1-year-old resprouts to $54.2\text{ mol m}^{-1}\text{ s}^{-1}\text{ MPa}^{-1}$ in the 13-year-old resprouts (Figure 2a, Table S1 available as Supplementary data at *Tree Physiology Online*). $K_{\text{stem}} P_{50}$ showed a similar change pattern, decreasing from -1.8 MPa in the 1-year-old resprouts to -2.9 MPa in the 13-year-old resprouts (Figure 2b, Figure S1 available as Supplementary data at *Tree Physiology Online*; Table S1 available as Supplementary data at *Tree Physiology Online*). We found a significant trade-off relationship between hydraulic safety (i.e., $K_{\text{stem}} P_{50}$) and hydraulic efficiency (i.e., $K_{\text{stem-max}}$) across resprout ages (Figure 2c).

Age-dependent modifications of xylem anatomical traits

The anatomical traits of resprout xylem varied dramatically across resprout ages at the tissue, pit and vessel network levels

Table 1. The list of major functional variables with definitions and units.

Variable	Acronym	Definition	Units
Hydraulic efficiency	$K_{\text{stem-max}}$	Maximum stem hydraulic conductivity	$\text{mol m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$
Hydraulic safety	$K_{\text{stem}} P_{50}$	Stem xylem water potential at 50% loss of conductivity	MPa
Huber value	Hv	The ratio of the sapwood area to leaf area	$\text{cm}^2 \text{m}^{-2}$
Wood density	WD	The ratio of the dry mass to the saturated volume	g cm^{-3}
Vessel implosion resistance	$(t/b)^2$	Square of intervessel wall thickness to vessel lumen diameter ratio	No units
Vessel density	VD	The number of vessels per wood area	no. mm^{-2}
Arithmetic vessel diameter	D	The simple average of the equivalent circle diameters	μm
Aperture fraction	F_{ap}	The pit aperture area divided by pit membrane area	No units
The sum of aperture area on per unit intervessel wall area	A_{ap}	Percentage of total aperture area in the unit intervessel wall area	No units
Vessel grouping index	GI	The ratio of the total number of vessels to the total number of vessel groupings (incl. solitary and grouped vessels)	No units

LM, light microscopy; SEM, scanning electron microscopy

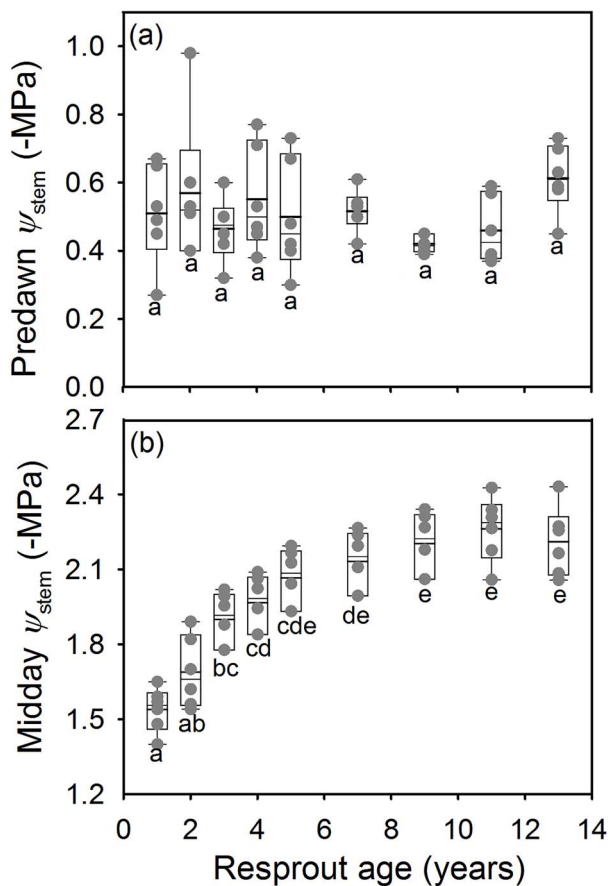


Figure 1. Stem water potential in resprouts with age. (a) Predawn stem water potential (Ψ_{stem}) were similar, but (b) midday stem water potential (Ψ_{stem}) were more negative across resprouts with the increase in the resprout age. Different letters indicate significant differences between species (one-way ANOVA; $P < 0.05$, $n = 5$).

(Figure 3; Figure S2 available as Supplementary data at *Tree Physiology Online*; Table S1 available as Supplementary data at *Tree Physiology Online*). The WD, $(t/b)^2$ and VD in the younger resprouts were lower, and they exhibited a gradually increasing

trend with the increase in the resprout age (Figure 3; Figure S2 available as Supplementary data at *Tree Physiology Online*; Table S1 available as Supplementary data at *Tree Physiology Online*). However, D did not change significantly across resprout ages (Table S1 available as Supplementary data at *Tree Physiology Online*). At the pit level, younger resprouts had significantly higher F_{ap} and A_{ap} (0.12, 0.064) than older resprouts (0.06, 0.031), which meant that the pit aperture area decreased from 1- to 13-year-old resprouts (Figure 3; Figure S2 available as Supplementary data at *Tree Physiology Online*; Table S1 available as Supplementary data at *Tree Physiology Online*). For the vessel networks, GI showed a gradually increasing trend with the increase in the resprout age; the vessels tended to be less grouped (GI, 1.65) in the younger resprouts and more grouped (GI, 3.13) in the older resprouts (Figure 3; Figure S2 available as Supplementary data at *Tree Physiology Online*; Table S1 available as Supplementary data at *Tree Physiology Online*).

Coordination of xylem hydraulics and anatomical traits

We found that $K_{\text{stem-max}}$ and $K_{\text{stem}} P_{50}$ were tightly associated with some structural characteristics (Figures 4 and 5). Across resprout ages, $K_{\text{stem-max}}$ was positively correlated with F_{ap} ($r^2 = 0.80$, $P < 0.01$; Figure 4d) and A_{ap} ($r^2 = 0.49$, $P < 0.05$; Figure 4e), negatively correlated with $(t/b)^2$ ($r^2 = 0.46$, $P < 0.05$; Figure 4b) and VD ($r^2 = 0.49$, $P < 0.05$; Figure 4c), and not correlated with WD or GI (Figure 4a and f). $K_{\text{stem}} P_{50}$ was positively correlated with WD ($r^2 = 0.46$, $P < 0.05$; Figure 5a), $(t/b)^2$ ($r^2 = 0.57$, $P < 0.05$; Figure 5b), VD ($r^2 = 0.82$, $P < 0.01$; Figure 5c), and GI ($r^2 = 0.65$, $P < 0.01$; Figure 5f) but negatively correlated with F_{ap} ($r^2 = 0.68$, $P < 0.01$; Figure 5d) and A_{ap} ($r^2 = 0.76$, $P < 0.01$; Figure 5e).

The results of the PCA of the hydraulic and anatomical traits showed that PC1 and PC2 explained 67.2 and 18.4% of the total variation, respectively (Figure S3 available as Supplementary data at *Tree Physiology Online*). The structural traits associated with hydraulic efficiency (i.e., $K_{\text{stem-max}}$, A_{ap} , F_{ap}) clustered

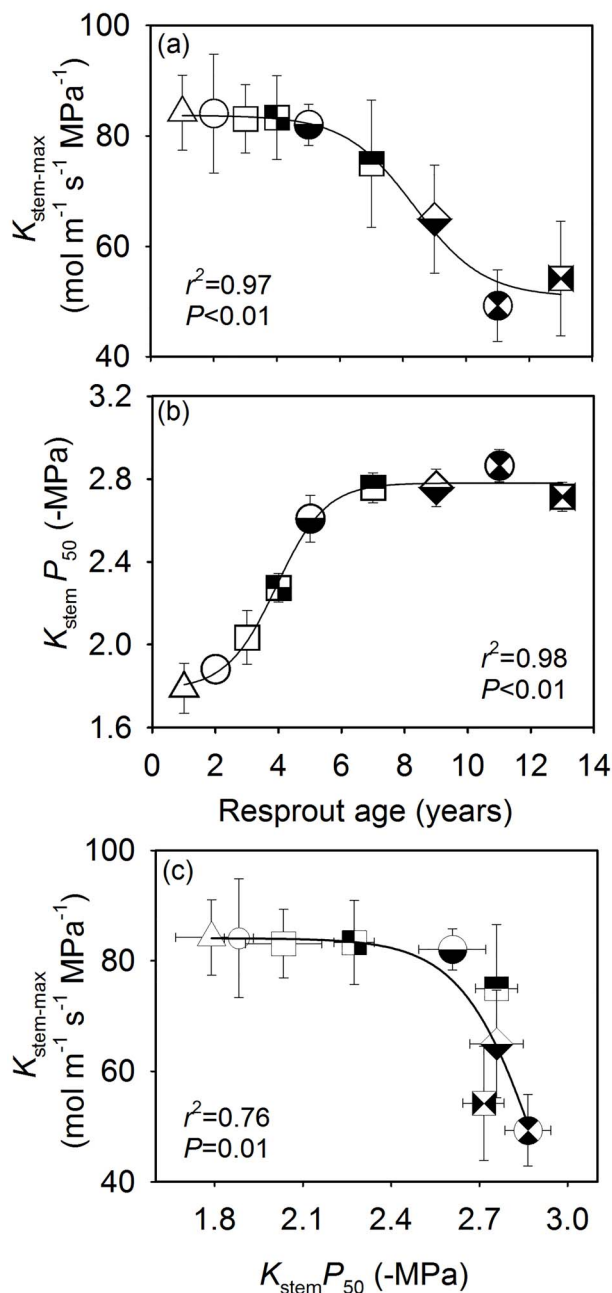


Figure 2. Mean values of $K_{\text{stem-max}}$ and $K_{\text{stem}} P_{50}$ and their correlations in resprouts with age. (a) Maximum stem hydraulic conductivity ($K_{\text{stem-max}}$), (b) stem xylem water potential corresponding to a 50% loss of hydraulic conductivity ($K_{\text{stem}} P_{50}$) (c) correlations among $K_{\text{stem-max}}$ and $K_{\text{stem}} P_{50}$ in resprouts with age. Symbols: Triangle, one-year-old; circle, 2-year-old; square, 3-year-old; checker square, 4-year-old; semi-filled circle, 5-year-old; semi-filled square, 7-year-old; semi-filled diamond, 9-year-old; hourglass circle, 11-year-old; and hourglass square, 13-year-old resprouts. Values are means \pm SEs ($n = 5$). The correlation coefficients (r^2) and probability (P) of the fitted sigmoidal regressions are provided. Significant correlations are indicated with solid lines. Error bars represent standard errors.

on the positive side of PC 1, whereas the traits contributing to stronger resistance to cavitation (i.e., $K_{\text{stem}} P_{50}$, WD, VD, $(t/b)^2$, GI) were clustered on the negative side of PC 1 (Figure S3

available as Supplementary data at *Tree Physiology* Online). The resprouts were clearly separated by age in the space defined by the two PC axes, with the 1- to 3-year-old resprouts showing greater hydraulic efficiency but lower resistance to cavitation and the 11- to 13-year-old resprouts showing greater resistance to cavitation but lower hydraulic efficiency. The locations of the resprouts of other ages on the PC plot indicate a transition between those two extreme hydraulic trait groups (Figure S3 available as Supplementary data at *Tree Physiology* Online).

Discussion

The importance of hydraulic strategies to the survival of plants has been well documented across ontogenetic growth (Ishida et al. 2005, Schafer et al. 2014, Zeppel et al. 2015, Resco de Dios et al. 2020) and along a water availability gradient (Schenk et al. 2008, Choat et al. 2012, Rosas et al. 2019, Yao et al. 2021b). In this study, we observed significant age-related trends in stem xylem anatomical and hydraulic traits in resprouts of *C. korshinskii* (Figure 2 and Figure S2 available as Supplementary data at *Tree Physiology* Online; Table S1 available as Supplementary data at *Tree Physiology* Online). The younger resprouts had higher F_{ap} and A_{ap} and consequently achieved higher stem hydraulic conductivity ($K_{\text{stem-max}}$) than the older resprouts (Figures 2 and 4). In contrast, the stem resistance to embolism ($K_{\text{stem}} P_{50}$) decreased from 1-year-old resprouts to 13-year-old resprouts, which was associated with higher $(t/b)^2$, WD, GI and lower F_{ap} , A_{ap} (Figures 2 and 5). The results indicate that *C. korshinskii* resprouts display a strategy spectrum, from an acquisitive strategy to a conservative strategy, with increasing age post-disturbance to adapt to its decreasing water status.

Divergence in hydraulic strategies post-disturbance

In meta-analyses of 757 angiosperm and 16 gymnosperm species, a significant stem hydraulic safety–efficiency trade-off was observed worldwide (Gleason et al. 2016, Liu et al. 2021). This relationship was also supported by a study across seven *Acer* taxa (Lens et al. 2011). During ontogenetic growth of *P. abies*, it has been shown that both hydraulic conductivity and safety increased (become less negative), and a safety–efficiency trade-off was observed (Couvreur et al. 2018, Prendin et al. 2018). In resprouting species, it has shown that resprout were less resistant to embolism than adults, but hydraulic efficiency presented low variability between resprouts and adults (Jacobsen et al. 2016, Schwilk et al. 2016). Here, a significant trade-off between hydraulic safety and efficiency was observed in *C. korshinskii* resprouts with increasing age, and this correlation clearly resulted in divergent hydraulic strategies post-disturbance.

Younger resprouts (1- to 3-year-old) usually exhibit the typical traits of an acquisitive strategy, i.e., higher hydraulic

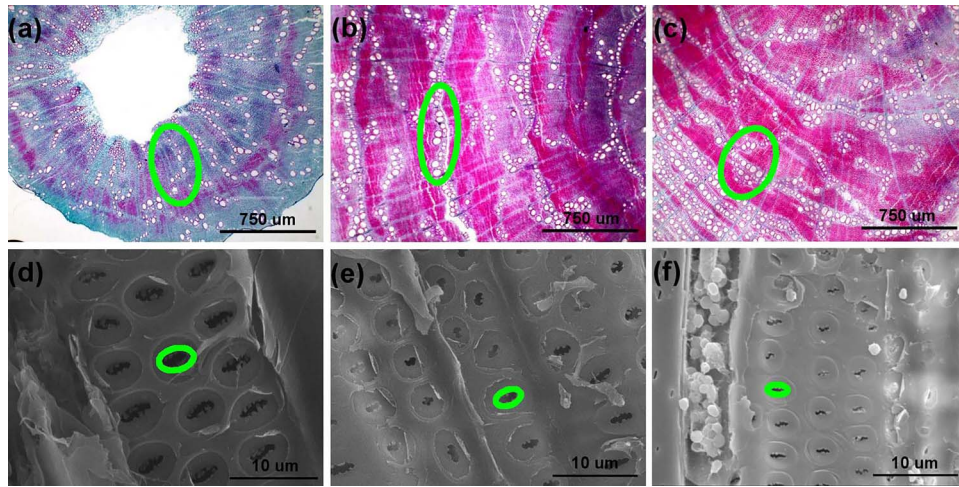


Figure 3. Images taken with (a–c) light microscopy and (d–f) scanning electron microscopy of the stem xylem structure of the representative resprouts by age. The vessel grouping patterns and pit aperture are indicated by green ellipse. (a, d) one-year-old resprouts were characterized by a lower vessel density and GI and a larger pit aperture. (b, e) five-year-old resprouts were characterized by an intermediate vessel density, GI, and pit aperture. (c, f) thirteen-year-old resprouts were characterized by a higher vessel density and GI and a smaller pit aperture.

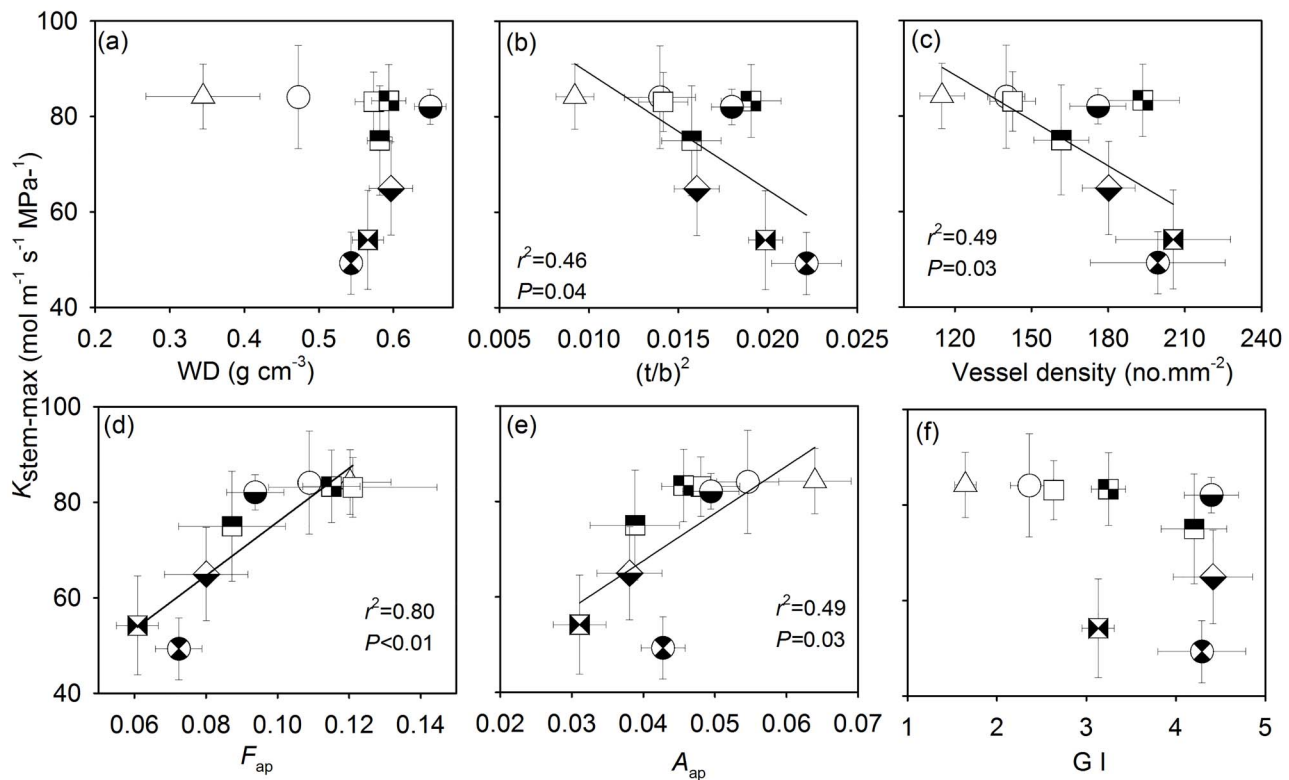


Figure 4. Correlations between $K_{\text{stem-max}}$ with WD, $(t/b)^2$, VD, F_{ap} , A_{ap} , and GI across the resprouts with age. The relationships between maximum stem hydraulic conductivity ($K_{\text{stem-max}}$) and (a) wood density (WD), (b) vessel implosion resistance ($(t/b)^2$), (c) vessel density (VD), (d) aperture fraction (F_{ap}), (e) the sum of aperture area on per unit intervessel wall area (A_{ap}), (f) vessel grouping index (GI). Values are means \pm SE ($n = 5$). Symbols: Triangle, one-year-old; circle, 2-year-old; square, 3-year-old; checker square, 4-year-old; semi-filled circle, 5-year-old; semi-filled square, 7-year-old; semi-filled diamond, 9-year-old; hourglass circle, 11-year-old; and hourglass square, 13-year-old resprouts. Values are means \pm SE ($n = 5$). The correlation coefficient (r^2) and probability (P) of the fitted linear regressions are listed. Significant linear correlations are represented with solid lines. Error bars represent standard errors.

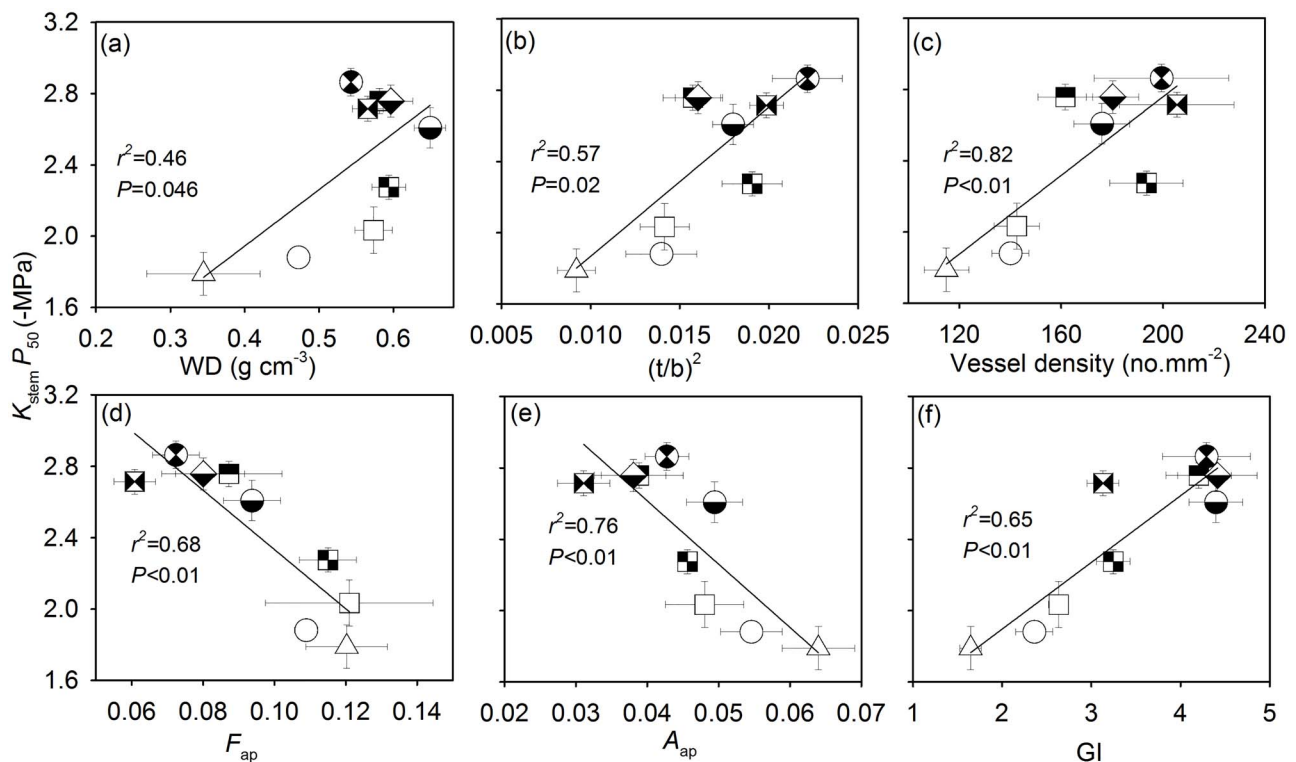


Figure 5. Correlations between $K_{\text{stem}} P_{50}$ with WD, $(t/b)^2$, VD, F_{ap} , A_{ap} , and GI across the resprouts with age. The relationships between stem xylem water potential corresponding to 50% loss of hydraulic conductivity ($K_{\text{stem}} P_{50}$) and (a) wood density (WD), (b) vessel implosion resistance $((t/b)^2)$, (c) vessel density (VD), (d) aperture fraction (F_{ap}), (e) the sum of aperture area on per unit intervessel wall area (A_{ap}), (f) vessel grouping index (GI). Symbols: Triangle, one-year-old; circle, 2-year-old; square, 3-year-old; checker square, 4-year-old; semi-filled circle, 5-year-old; semi-filled square, 7-year-old; semi-filled diamond, 9-year-old; hourglass circle, 11-year-old; and hourglass square, 13-year-old resprouts. Values are means \pm SE ($n = 5$). The correlation coefficient (r^2) and probability (P) of the fitted linear regressions are listed. Significant linear correlations are represented with solid lines. Error bars represent standard errors.

efficiency, stomatal conductance and photosynthetic capacity (Fang et al. 2013, van der Sande et al. 2019, Gong et al. 2020). It is an advantage for younger resprouts to possess an acquisitive strategy in terms of water transport and use that contribute to its rapid regrowth (Santiago et al. 2004). Our findings are consistent with previous studies stating that younger resprouts must grow rapidly, with high stomatal conductance and hydraulic conductance, to ensure the rapid re-establishment of the population and to compete for nutrients, light, and space post-disturbance (Clemente et al. 2005, Utsumi et al. 2010, Fang et al. 2013, Schafer et al. 2014, Jacobsen et al. 2016). However, younger resprouts are more vulnerable to dehydration post-disturbance, as indicated by their less negative $K_{\text{stem}} P_{50}$ in this study. Indeed, younger resprouts are less resistant to embolism and may suffer easily from presumable hydraulic failure. For example, it has reported that significantly high resprout mortality occurred when intense drought occurred in the year following a fire during shrub recovery; similar mortality was not observed in the same species near mature stands in California chaparral shrubs (Ramirez et al. 2012, Pratt et al. 2014, Jacobsen et al. 2016, Pausas et al. 2016); moreover, post-fire

resprouts are particularly susceptible to drought compared with mature plants in upland trees in western Texas (Schwilk et al. 2016). Indeed, xylem that is more hydraulically vulnerable to cavitation and mechanically and structurally less robust would benefit for producing more tissues and facilitating rapid growth (Chave et al. 2009, Poorter et al. 2010), and the acquisitive strategy creates the priority of hydraulic efficiency over safety in the younger resprouts.

On the other hand, owing to their lower root-to-shoot ratio, older resprouts experience a declining water status compared with younger resprouts. Thus, older resprouts (11- to 13-year-old) have low, stable hydraulic conductivity and relatively high hydraulic safety (Figure 2; Table S1 available as Supplementary data at *Tree Physiology* Online). The typical traits of a conservative strategy (Markesteijn et al. 2011, van der Sande et al. 2019), high WD, huber values and low gas exchange rates, were also observed in older *C. korshinskii* resprouts (Fang et al. 2013, Guo et al. 2020). It is an advantage for older resprouts to possess a conservative strategy as following two reasons: (i) drought imposes strong stresses on the hydraulic function integrity of plants, and

conservative strategy maintain hydraulic integrity by avoiding catastrophic hydraulic failure (Engelbrecht et al. 2007, Fang et al. 2014, Zhang et al. 2017, Guo et al. 2020); and (ii) the conservative strategy, although it comes at the cost of reduced carbon assimilation and growth, can contribute to decreasing water consumption in arid environments due to its lower stem hydraulic conductivity (Fang et al. 2013, Gong et al. 2020, Guo et al. 2020). The results suggest that the maintenance of hydraulic integrity, rather than growth, becomes more important in older resprouts. The 4- to 10-year-old resprouts exhibited a transitional strategy between those two extreme strategies, with moderate $K_{\text{stem-max}}$ and $K_{\text{stem}} P_{50}$ values. This obvious divergent adaptation strategy in resprouts is also supported by the results of the PCA (Figure S3 available as Supplementary data at *Tree Physiology* Online). Overall, the 1- to 13-year-old resprouts show a comprehensive spectrum, from an acquisitive strategy to a conservative strategy, in this post-disturbance arid environment.

Shifts in anatomical traits that account for hydraulic strategies

The differences in xylem hydraulic strategies across resprouts can be attributed to the biophysical constraints of xylem 'design' (Figure 3). Our results point to pit structure playing a role in driving lower $K_{\text{stem-max}}$ in the older resprouts, as the decreasing F_{ap} and A_{ap} contribute to reducing water supply capacity ($K_{\text{stem-max}}$) (Jacobsen et al. 2016, Yin et al. 2018), and decreasing F_{ap} and A_{ap} were tightly associated with lower $K_{\text{stem-max}}$ across the resprouts (Figures 4d and e). Similar results have also been reported in seven *Acer* taxa (Lens et al. 2011). However, WD, $(t/b)^2$ or VD were not related to the decrease in $K_{\text{stem-max}}$ across resprouts. The reasons are that (i) the decreasing $K_{\text{stem-max}}$ was not associated with WD in *C. korshinskii* resprouts with the increase in the resprout age (Figure 4a), and WD maybe, is influenced more by fibers rather than by vessels (Jacobsen et al. 2005); (ii) although decreasing $K_{\text{stem-max}}$ was tightly associated with increase in $(t/b)^2$ in older *C. korshinskii* resprouts, but vessel diameter was similar across resprouts, and the increase in $(t/b)^2$ in older *C. korshinskii* resprouts was due largely to increases in vessel wall thickness (Table S1 available as Supplementary data at *Tree Physiology* Online); (iii) it has reported that increasing VD could contribute to the increase in the $K_{\text{stem-max}}$ (Yin et al. 2018), but in older *C. korshinskii* resprouts, they had low $K_{\text{stem-max}}$ accompanied by increasing VD (Figure 4c). It has been shown that higher GI increases the maximum hydraulic conductivity by providing alternative pathways for water movement (Loepfe et al. 2007, Martínez-Vilalta et al. 2012). However, our data showed that decreasing $K_{\text{stem-max}}$ had no relation with GI with the increase in the resprout age, as older resprouts had low $K_{\text{stem-max}}$, but had higher GI (Figure 4f; Figure S2f available as Supplementary data at *Tree Physiology* Online).

Contrary to the idea that low $K_{\text{stem-max}}$ was associated with the shifts of pit traits, the more negative $K_{\text{stem}} P_{50}$ in older *C. korshinskii* resprouts appears to be driven by changes at the tissue level (WD, $(t/b)^2$, VD), pit level (F_{ap} , A_{ap}) and network traits (GI) (Figure 5). Many studies have shown that high WD contributed to enhanced hydraulic safety in diverse species (Hacke and Sperry 2001, Jacobsen et al. 2007, Janssen et al. 2020), and the same results was also observed here, as older resprouts exhibiting higher WD and increasing hydraulic safety-more negative $K_{\text{stem}} P_{50}$ (Figure 5a). Indeed, higher WD usually arise from higher fraction fibers in angiosperms (Jacobsen et al. 2005), which can assist adjacent vessels in resisting partial implosion, thus preventing the stretching or rupture of the pit membranes and lowering the risk of embolism (Pratt and Jacobsen 2017, Hillbrand et al. 2019). Furthermore, our results showed that the more negative $K_{\text{stem}} P_{50}$ could be achieved through high $(t/b)^2$ across resprouts (Figure 5b). Indeed, studies have shown that $K_{\text{stem}} P_{50}$ is related to the ability of the xylem vessels to resist bending stresses during water transport, and $(t/b)^2$ was a much better structural correlate with $K_{\text{stem}} P_{50}$ (Hacke et al. 2001, Pittermann et al. 2006). In addition, our data suggest that VD played a role in the increase hydraulic safety (Figure 5c), as the high VD reduces the overall impact of cavitation in a single vessel (Ewers et al. 2007, Pratt and Jacobsen 2017). For the pit-level traits, our results point to lower F_{ap} and A_{ap} playing a role in increasing hydraulic safety across resprouts (Figure 5d and e). Indeed, studies have shown that the pit resistance is estimated to represent ca. 50% of the total plant hydraulic resistance (Wheeler et al. 2005, Choat et al. 2008, Kaack et al. 2019), and the smaller pit aperture (lower F_{ap} , A_{ap}) can prevent air in already damaged conduits from entering functional conduits and 'air-seeding' cavitation (Zimmermann 1983, Lens et al. 2011, Yin et al. 2018). For the vessel network, the relationship between $K_{\text{stem}} P_{50}$ and GI remains controversial. Some researchers have shown that with a high GI, water can be carried through the same pathways by alternate vessels if one or several vessels in a group are incapacitated by embolism; therefore, the plants have greater hydraulic safety (Carlquist, 1984, Carlquist, 2009, Lens et al. 2011, Arx et al. 2013). But others have shown that GI increases vulnerability to embolism by facilitating the spread of embolism (Loepfe et al. 2007, Martínez-Vilalta et al. 2012); thus, species with higher GI have lower hydraulic safety. In this study, a tight relationship between GI and $K_{\text{stem}} P_{50}$ suggest that higher GI could have helped increase hydraulic safety in the older *C. korshinskii* resprouts (Figure 5f). The higher GI maybe result from the increasing vessel density and longer vessel length with the increase in the resprout age, as in denser and longer vessels, connections with other vessels can be made over shorter axial distances, resulting in a higher GI (Lens et al. 2011, Levionnois et al. 2021).

Conclusion

Our results demonstrate that the stems of *C. korshinskii* resprouts have a universal hydraulic strategy spectrum, from the acquisitive strategy in younger resprouts to the conservative strategy in older resprouts, to adapt to their declining water status during regrowth. This shift in strategy is considered an efficient way for the resprouts to contribute to the recovery of aboveground biomass during their initial regrowth stage and improve resistance to drought as they approach their final size and experience a decrease in water status, and a shift in stem xylem architecture account for the specific hydraulic strategy. Given that resprouts with increasing age exhibit decreasing photosynthesis, which is constrained by leaf specific anatomical traits and mesophyll conductance (Carriqui et al. 2015, 2019, 2020), future studies should test whether leaf anatomical features and mesophyll conductance are linked to the shift in hydraulic strategy during the resprout regrowth.

Supplementary data

Supplementary data for this article are available at *Tree Physiol* Online.

Acknowledgments

The authors thank Jing-Zhong Zhang and Feng-Ping Li for their assistance with measurements in the field. We also thank the Experimental Center for Life Science Research, School of Life Sciences, Lanzhou University.

Conflict of interest

None declared.

Funding

The research was partially supported by Second Tibetan Plateau Scientific Expedition and Research Program, Grant/Award Number: (STEP)(2019QZKK0301); the National Natural Science Foundation of China (nos 31971406, 31670404, 31860176); Key Research & Development Program of Shaanxi (2020ZDLSF06-06); Key Research & Development Program of Gansu (20YF3FA037).

Authors' Contributions

XWF and ZFN designed the experiments; ZFN and ZQL conducted most of the experiments; GQY and MHB performed data and statistical analyses; JATdS, and T-PG provided study guidance and interpretation of data and experimental results; XWF and XQT prepared the figures; XWF, ZFN, and JATdS co-wrote and edited the manuscript.

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