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Research paper

Local adaptation to aridity in a widely distributed angiosperm tree species is mediated by seasonal increase of sugars and reduced growth

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Trees in dry climates often have higher concentrations of total non-structural carbohydrates (NSC = starch + soluble sugars [SS]) and grow less than conspecifics in more humid climates. This pattern might result from the growth being more constrained by aridity than the carbon (C) gain, or reflect local adaptation to aridity, since NSC fuel metabolism and ensure adequate osmoregulation through the supply of SS, while low growth reduces water and C demands. It has been further proposed that C allocation to storage could come at the expense of growth (i.e., a growth–storage trade-off). We examined whether NSC and growth reflect the local adaptation to aridity in *Embothrium coccineum* J. R. Forst & G. Forst. (Proteaceae), a species with an exceptionally wide niche. To control for any influence of phenotypic plasticity on NSC and growth, we collected seeds from dry (46° 16′S, 71° 55′W, 500 mm year^{-1}) and moist (45° 24′S, 72° 40′W, >2500 mm year^{-1}) climates and grew seedlings in a common garden experiment for 3 years. We then compared the NSC and SS concentrations and pools (i.e., total contents) and the biomass of seedlings at spring, summer and fall. Seedlings from the dry climate had significantly lower biomass and similar NSC concentrations and pools as seedlings from moist climate, suggesting that reduced growth in arid environments does not result from a prioritization of C allocation to storage but that it confers advantages under aridity (e.g., lower transpiration area). Across organs, starch and NSC decreased similarly in seedlings from both climates from spring onward. However, root and stem SS concentrations increased during the growing season, and these increases were significantly higher in seedlings from the dry climate. The greater SS accumulation in seedlings from the dry climate compared with those from the moist climate demonstrates ecotypic differentiation in the seasonal dynamics of SS, suggesting that SS underlie local adaptation to aridity.

***Keywords*: common garden, drought resistance, intraspecific variation, niche breath, non-structural carbohydrates, Patagonia.**

Introduction

Trees in dry environments are often characterized by lower growth rates and higher concentrations of total non-structural carbohydrates (NSC) or of the soluble sugars (SS) fraction of NSC than do conspecifics in moister conditions (Sala and Hoch 2009, Piper et al. 2017, Cao et al. 2018, Liu et al. 2019, Fajardo and Piper 2021, Hao et al. 2021). Increased NSC concentrations and low growth rates in dry conditions could be due to the growth being more sensitive than photosynthesis to drought (Boyer 1970, Muller et al. 2011, Woodruff and Meinzer 2011). While this explanation has been supported by experimental and observational studies (e.g., Sala and Hoch 2009, Piper and Fajardo 2016, Piper et al. 2017), the high NSC and SS concentrations have been alternatively hypothesized as a survival adaptation to dry conditions, which would come at the cost of reduced growth, i.e., a growth–storage trade-off

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(e.g., Sala et al. 2012, Wiley and Helliker 2012, Dietze et al. 2014, Martínez-Vilalta et al. 2016, Fajardo and Piper 2021). High NSC concentrations could provide a survival advantage in dry conditions because NSC act as a buffer against the negative carbon (C) balance driven by prolonged water deficit (McDowell et al. 2008, Galiano et al. 2011). Additionally, SS—an important fraction of the NSC pool—confer dehydration tolerance in many species (Arndt et al. 2001, Zweifel et al. 2020, Aranda et al. 2021). On the other hand, a high biomass investment per unit of time (i.e., growth rate) would mean a high water expenditure and it is thus not favorable when water is limited. Although determining whether aridity promotes an increase in NSC at the expense of growth or as a result of growth limitations is especially important with regard to tree mortality and C sequestration, few studies have focused on this subject (Bachofen et al. 2018, Blumstein et al. 2022). In two studies using common garden experiments, NSC varied among populations across environmental gradients, but the NSC were not necessarily higher (nor was growth necessarily slower) in populations from harsher environments (Bachofen et al. 2018, Blumstein et al. 2022). Also, for both studies, the locations of the sampled populations differed in the precipitation, latitude, temperature and elevation, which could be selective drivers of changes in the NSC. Thus, the role of aridity as a determinant of high NSC and SS concentrations and low growth remains unclear.

The hypothesis that high NSC and SS concentrations may reflect a local adaptation to aridity is in line with theories about life history strategies (Grime 2006, Westoby et al. 2002). In this frame, stress-adapted plants are characterized by traits that favor resource conservation (e.g., NSC storage), while the traits of stress-sensitive plants favor the fast capture of resources (e.g., high growth rate). To determine the extent to which the storage of NSC in trees is part of a strategy to conserve resources, we need to consider the seasonality in the NSC dynamics concomitantly with changes in other drought-related traits. Species with a resource-conservative strategy have lower metabolic and growth demands than those with a resource-acquisitive strategy and also a lower seasonal remobilization of NSC (Signori-Müller et al. 2022). Drought-adapted plants are also expected to allocate more resources to roots, which would enable more soil to be explored for water (Grime 2006). Furthermore, the ratio of leaf area to sapwood area, defined as the Huber value, has been found to decrease with increasing aridity as the plant responds to avoid exceedingly low water potentials (Martínez-Vilalta et al. 2009, Zweifel et al. 2020). Plants with a low Huber value are considered to benefit from reduced transpiratory losses, thus favoring persistence in dry areas (Martínez-Vilalta et al. 2009). Leaf properties are determinants of the C gain under drought; small leaves reduce transpiratory losses, while a high leaf nitrogen (N) concentration has been linked to high water-use efficiency (Reich et al. 1989, Wright et al. 2001). Species with special root adaptations for nutrient uptake, such as cluster roots in some plant families like as Proteaceae, could be tolerant of dry environments since these structures increase the area of the root–soil interface. Although a theoretical framework of plant strategies has been proposed to account for the interspecific diversity (Reich 2014), local adaptation to dry conditions is well known to operate at the intraspecific level in plant species with broad climatic ranges (Volaire 1995, Giménez-Benavides et al. 2007, Carvajal et al. 2017, Barton et al. 2020).

Niche breadth is the range or variety of conditions that make up a species' realized niche (Sexton et al. 2017), and species with a wide niche can be key model systems to study regional responses of trees to climate. Wide-niche tree species are expected to have greater intraspecific variation in traits and therefore may better track environmental fluctuations (Fajardo and Siefert 2019). With these considerations in mind, to verify whether the potential differences in trait values (e.g., NSC and SS) are attributable to phenotypic plasticity or local adaptation, we chose an angiosperm tree species with an exceptionally wide niche, *Embothrium coccineum* J. R. Forst & G. Forst. (Proteaceae), which grows across a steep precipitation gradient in southern South America, from locations with >3000 mm of annual precipitation on the west side of the austral Andes to sites with <500 mm on the east side of the Andes. Its ample distribution and wide niche lend it to the study of attributes of populations of the species from contrasting climates when grown in a common environment.

In this study, we sought to determine whether a growth–storage trade-off for C allocation underlies local adaptation to contrasting levels of precipitation in *E. coccineum*. In a common garden experiment with seedlings grown from seeds collected from the populations in environments that differ more than threefold in mean annual precipitation (MAP) but with similar temperatures, latitudes and overall low elevation, we analyzed the role of NSC and growth in their local adaptation to aridity. Following the ecological definition of trade-off (Grubb 2016), if the local adaptation to climatic extremes involves a growth–storage trade-off, we expected that, when grown in the common garden, populations from the dry location should maintain higher seasonal NSC and SS concentrations and pools and have lower biomass compared with populations from the moist locations. Additionally, we aimed to explore whether the C storage and biomass vary concomitantly with other attributes of plant strategies in resource acquisition, as predicted for a survival–growth trade-off. Specifically, we explored the potential coordination of biomass and NSC—two iconic traits of resource allocation—with other drought-related traits that have been acknowledged to shape local adaptations to dry conditions, such as biomass distribution, specific leaf area, leaf size, leaf N concentration, root adaptations for nutrient uptake and the Huber value. We also analyzed the ratio of SS to NSC as a potential indicator of osmotic requirements.

Materials and methods

Study species

Embothrium coccineum J. R. Forst & G. Forst. (Proteaceae) is an evergreen, occasionally facultatively deciduous, short-statured tree (5–8 m mean height) endemic to southern South America. It extends over a wide latitudinal and altitudinal range throughout Chile and Argentina (Souto et al. 2009, Fajardo et al. 2019). In Chile, its distribution reaches from central Chile's Mediterranean climate (34°S) to Tierra del Fuego's sub-Antarctic climate (55°S); from very humid conditions (>3000 MAP) in the temperate rain forest to dry conditions (∼500 mm MAP) near the steppe-like environment along the border between Chile and Argentina (Souto et al. 2009, Piper et al. 2013, Fajardo and Piper 2021). With its showy bright red flowers (hence, its common name Chilean firebush), *E. coccineum* is bird- and insect-pollinated (Devoto et al. 2006, Chalcoff et al. 2012). Genetically distinct populations have been identified among climatically contrasting areas and are hypothesized to have resulted from past gene flow barriers (e.g., glaciations) reinforced by natural selection (Souto and Premoli 2007, Sepúlveda-Espinoza et al. 2022).

Seed collection and common garden experiment

We collected seeds from adult trees in seven sites that were in two extremes along an abrupt west–east precipitation gradient in the Aysén Region, southern Chile (45–46°S). Here, due to the predominant western winds and the Andes Cordillera, precipitation decreases drastically from west to east at any given latitude, with little variation in temperature (Armesto et al. 1995). The four sites sampled to represent the moist climate were located ca 10 km from around the city of Puerto Aysén (45° 24′S, 72° 40′W, 15 m a.s.l.), where the mean annual and seasonal (September–April) precipitation is 2941 and 1421 mm, respectively (Luebert and Pliscoff 2017). The three sites that were sampled for the dry climate were located around the city of Puerto Ibáñez (46° 16′S, 71° 55′W, 210 m a.s.l.), where mean annual and seasonal precipitations are 508 and 261 mm, respectively (Dirección General de Aguas, Servicio Meteorológico de Chile) (Figure 1). Although precipitation has decreased in both the moist and the dry climate in the last 10 years (Boisier et al. 2018), the difference in precipitation between climates remains similar (Figure 1). Sites were separated from each other by at least 20 km. Sampling areas within each site were located at least 100 m from roads. At each site for seed collection, we selected four healthy-looking dominant individual trees (hereafter, the family) and avoided any that were close to obvious sources of water (e.g., creek and water-logged spots), particularly at the dry sites. *Embothrium coccineum* produces a large number of seeds. Seeds are contained in a follicle with ∼10–15 seeds inside. Seeds were collected in February 2017 and were cold-stratified at 4 °C for 3 months. The common garden experiment, seedling selection and transplanting were all done in an outdoor nursery at the Instituto Forestal of Chile (Infor) located in Coyhaique (45° 34′S, 72° 01′W, 345 m a.s.l.). Climatic conditions at the nursery location are shown in Figure S1 available as Supplementary data at *Tree Physiology* Online. Seeds were germinated in a germination bed in September 2017 and were transplanted to 15-cm^3 pots in speedling systems in November 2017. Seed germination was ca 80%. In November 2018, seedlings were transplanted into 500-cm^3 black plastic bags filled with a mixed substrate (Table S1 available as Supplementary data at *Tree Physiology* Online; chemical properties determined at the Laboratorio de Suelos, Universidad de Concepción, Chillán). Because *E. coccineum* grows slowly, each pot had enough volume for root exploration over the 3 years of the experiment. Seedling pots were randomly placed on nursery benches and were grown for 3 years with regular watering and full sun exposure. A subsample of seeds from each tree was counted and weighed, and the mean dry mass was calculated to account for potential maternal effects on the response variables (seedling biomass, NSC and companion traits).

Plant harvests and trait measurements

Seedlings were harvested for the determination of NSC and other traits in November 2020 (mid-spring), January 2021 (mid-summer) and May 2021 (late-autumn). As a part of a complementary study, we recorded the phenology for *E. coccineum* from the contrasting climates and we found that budbreak, leaf out and leaf shedding occur during the same period for the seedlings from the dry and the moist climates. A previous study on the seasonal dynamics of NSC concentrations in several species from the study area, including *E. coccineum*, found that NSC concentrations in evergreen species were highest in mid-spring and late-autumn and were lowest in mid-summer (Piper 2020). On each sampling date, 1 seedling per family was harvested (i.e., 8–10 seedlings were harvested per date for the dry climate and 16 seedlings were harvested for the moist). Because *E. coccineum* has cluster roots which exudate carboxylates and phosphatases that cause the substrate to be tightly adhered to the clusters (Avila et al. 2019), we gently washed the roots several times until all rhizospheric substrate was removed. Seedlings were then separated into roots, aboveground woody organs (stems and twigs), leaves from the previous season and new leaves. The different organs were placed in labeled paper bags, heated in a microwave for three 20-s cycles at 900 W to stop enzymatic activity of the living tissues (Popp et al. 1996) and were then dried in a forced-air oven at 70 °C for 72 h. Samples were then weighed on a precision balance (0.0001 g), and the biomass of each organ was recorded and summed to calculate the total seedling biomass. Biomass varied substantially among the harvest dates but was highest in the summer. Because most of the biomass

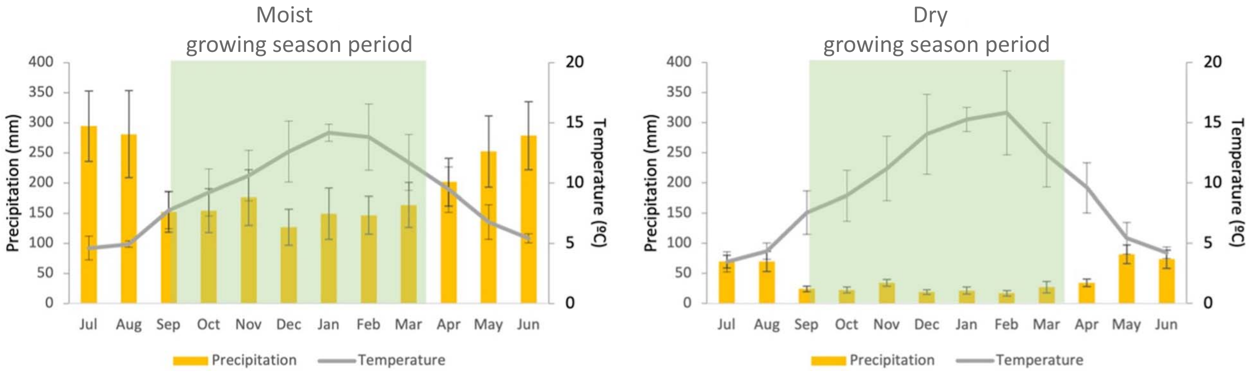

Figure 1. Monthly precipitation (mm) and mean air temperature (°C) for the period 2010–17 in two locations with historical contrasting precipitation in the Aysen Region, southern Chile. Data for the moist and dry climates are from the weather station at Puerto Chacabuco (45° 27′44″S, 72°48′57″W, 15 m a.s.l.) and at Puerto Ibanez (46°16′06″S, 71°59′19″W, 210 m a.s.l.), respectively. Error bars stand for standard errors.

increment in southern temperate evergreen species occurs from December onward (Fajardo and Siefert 2016, Löiez and Piper 2021), we harvested spring seedlings before the maximum seasonal biomass increase took place; hence, the seedlings were much smaller than in the summer.

The dried samples were then ground into a fine powder with a ball mill (Retsch® MM200; Haan, Germany) and were stored at 4 °C until NSC were determined. Additionally, we measured the leaf size and the leaf mass per area (LMA) of the new and previous season leaves of each seedling to estimate the total leaf area. Before placing leaves in the stove, new and previous season leaf cohorts were laid flat separately and were photographed with a reference square of known area; then, the total projected leaf area was calculated using SIGMAPROC image-processing software (Systat Software, Richmond, CA, USA). The LMA (g· m^{-2}) was computed for each leaf as its oven-dried mass divided by its total surface area.

For the spring and autumn harvests, the biomass of cluster roots was not quantified separately but was included in the root biomass. The cluster root biomass of this tree species is low (ca 5%, Piper et al. 2013), especially in the spring and fall due to either incipient formation (spring) or senescence (autumn). However, for the summer harvest, we quantified the number and biomass of cluster roots separately (relative to the whole-plant biomass). Additionally, we recorded the number of cluster roots in the autumn because even senescent cluster roots provide valuable information regarding the biomass investment in previous months. For the summer and autumn harvests, for each seedling, we also measured the basal diameter of the stem, bark thickness and pith diameter using a caliper and measured the seedling height using a measuring tape. We then estimated the sapwood area by subtracting the pith and bark areas from the total basal area. The bark area was calculated as the difference between the basal area with and without bark. The basal and pith areas were calculated using the formula of a circle. The Huber value was calculated for each seedling as the sapwood cross section divided by the total leaf area (Tyree and Ewers 1991). Previous season leaves were analyzed for their N concentrations using the micro-Kjeldahl method (Lang 1958). This analysis was performed for mature leaves collected in November.

NSC determination

Our assessment of C reserves was based on the determination of NSC concentrations, as the sum of the three most abundant low molecular-weight SS (glucose, fructose and sucrose) and starch. The NSC concentrations were analyzed following the procedure detailed by Hoch et al. (2002). Although this procedure has been modified to increase its precision (Landhäusser et al. 2018), it is still valid for comparing samples of a single species analyzed within the same laboratory (Quentin et al. 2015), as is the case in this study. Approximately 13 mg of dried powder from each sample were extracted with 1.6 ml of distilled water at 100 °C for 60 min using a food steamer. Part of the extract was used to determine SS after the enzymatic conversion of sucrose and fructose to glucose (respectively, invertase [I4504] and phosphoglucose isomerase [P5381] from *Saccharomyces cerevisiae*, Sigma Aldrich, St Louis, MO, USA). The concentration of free glucose was determined photometrically after the enzymatic conversion of glucose to gluconate-6-phosphate (Glucose Assay Reagent, G3293; Sigma Aldrich) using a 96-well multiplate reader. Following the degradation of starch to glucose using a purified fungal amylase (amiloglucosydase from *Aspergillus niger*; Sigma Aldrich 10115) at 45 °C overnight, NSC were determined in a separate analysis. The starch concentration was calculated as NSC minus SS. The NSC, SS and starch concentrations are presented on a percentage of dry matter basis. The ratio between SS and NSC concentrations was estimated for the stem and root of each seedling. Additionally, organ-specific NSC and SS pools (i.e., total content, or mass, in g) were calculated per seedling as the product of NSC or SS concentrations and the biomass of each organ. Organ-specific contents were then added to obtain whole-plant NSC and SS contents.

Table 1. Results of LMMs testing the effects of climate of seed origin (C: dry, moist), harvest date (D: spring, summer, autumn) and their interaction (C × D) on the total seedling biomass (g), root mass fraction, total NSCs, starch and SS concentrations (%) in leaf, stem and root, whole-seedling NSCs, starch and SS pools (g); and Huber value for 3-year-old seedlings of *E. coccineum* (Proteaceae). df stands for degree of freedom. In all cases, seed size was considered as a covariate.

Variable	Factors	df	*F*-ratio	*P*-value	Variable	df	*F*-ratio	*P*-value
Total biomass	C	1, 26	11.05	0.036	Root mass fraction	1, 23	6.34	0.019
	D	2, 71	39.75	<0.001		2, 70	35.3	<0.001
	C × D	2, 71	0.46	0.621		2, 70	0.56	0.600
Leaf NSC conc.	C	1, 14	0.01	0.904	Stem NSCs conc.	1, 29	0.02	0.888
	D	2, 68	35.37	<0.001		2, 71	191.9	<0.001
	C × D	2, 68	0.48	0.618		2, 71	0.68	0.510
Root NSC conc.	C	1, 15	4.32	0.055	Leaf SS conc.	1, 22	0.301	0.590
	D	2, 68	34.4	<0.001		2, 69	2.900	0.062
	C × D	2, 68	2.12	0.128		2, 69	0.675	0.512
Stem SS conc.	C	1, 14	1.776	0.203	Root SS conc.	1, 19	0.249	0.624
	D	2, 68	23.40	<0.001		2, 69	37.36	<0.001
	C × D	2, 68	3.164	0.048		2, 69	5.036	0.0091
Leaf starch conc.	C	1, 14	0.07	0.800	Stem starch conc.	1, 21	0.07	0.787
	D	2, 68	37.52	<0.001		1, 71	186.1	<0.001
	C × D	2, 68	0.51	0.604		1, 71	0.200	0.819
Root starch conc.	C	1, 14	4.57	0.051	Huber value	1, 21	0.696	0.413
	D	2, 68	46.82	<0.001		1, 47	3.322	0.075
	C × D	2, 68	1.04	0.360		1, 47	0.000	0.998
Whole-seedling NSC pool	C	1, 22	3.13	0.091	Whole-seedling starch pool	1, 19	2.84	0.108
	D	2, 69	16.24	<0.001		2, 69	23.82	<0.001
	C × D	2, 69	2.30	0.108		2, 69	2.47	0.092
Whole-seedling SS pool	C	1, 72	0.26	0.612				
	D	2, 72	10.43	<0.001				
	C × D	2, 72	0.202	0.818				

Data analyses

To test how the climate of origin and the season affected the concentrations of NSC, SS and starch in the two seedling groups (dry and moist climates), we used linear mixed-effects models (LMMs). We ran separated models for each organ. We considered climate, harvest date and their interaction as fixed factors and the site of seed origin as the random factor. Similar models were used to test the effect of the climate of the seedlings on total plant biomass, organ-specific biomass and the whole-seedling and organ-specific pools of NSC, starch and SS. To test for the effects of climate origin and of season on the ratio between the concentrations of NSC and SS, we used generalized mixed-effects models, considering climate, harvest date and their interaction as fixed factors and the site of seed origin as the random factor. To test for an effect of climate of origin on the Huber value, bark thickness, cluster root biomass, leaf N concentration, LMA and root mass fraction, we used LMMs by considering climate as the fixed factor and site of origin as the random factor. In this analysis, we pooled the data from each sampling date to obtain the Huber value and cluster root number. In all these analyses, seed mass was used as a covariable to account for any maternal effects. JMPpro version 16.0 (SAS Institute, Cary, NC, USA) was used for all analyses.

Results

Biomass

Total biomass of seedlings from the seeds from the moist climate (hereafter, moist-climate seedlings) was higher than for those from the dry climate (hereafter, dry-climate seedlings) (Table 1 and Figure 2). Although a similar difference in biomass was found for each organ, it was only significant for new leaves (Table S2 and Figure S2 available as Supplementary data at *Tree Physiology* Online). Both seedling biomass and the biomass of each organ were highest in the summer and lowest in the spring, except for old leaves which had minimal values in the autumn (Figure 2 and Figure S2 available as Supplementary data at *Tree Physiology* Online).

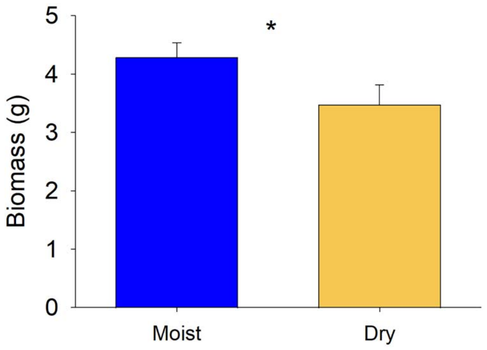

Figure 2. Whole-seedling biomass (in g) in 3-year-old seedlings of *E. coccineum* (Proteaceae) from dry and moist climates grown under common garden conditions in Coyhaique, Chile. Bars are the mean values from harvest of spring (November 2020), summer (January 2021) and autumn (May 2021). Asterisk indicates statistically significant differences among seasons at $P < 0.05$ for both pooled origins.

NSC concentrations and pools

The NSC, starch and SS concentrations in the different organs were, in general, similar between dry- and moist-climate seedlings, except for marginally significant trends for higher root NSC and starch concentrations in moist-climate seedlings (climate effect: $P = 0.055$ [NSC], $P = 0.051$ [starch], respectively) (Table 1 and Figure 3). The NSC, starch and SS concentrations were much more affected by seasonality (i.e., date) than by the climate of seed origin. Regardless of the climate of seed origin, the NSC and starch concentrations were highest in the spring and decreased afterward (Figure 3), although the NSC concentrations in the stems and roots did not significantly differ in summer and autumn. By contrast, variations in stem and root SS concentrations were significantly dependent on the climate of seed origin (i.e., significant climate × date interaction, Table 1). Thus, stem SS concentrations increased from summer to autumn in both moist- and dry-climate seedlings, but the increase was only significant in dry-climate seedlings (Figure 3). Likewise, the root SS concentrations increased significantly from spring to summer and from summer to autumn in both moist- and dry-climate seedlings ($P < 0.001$). However, this trend was largely driven by the dry-climate seedlings, for which the increases from spring to summer and from summer to autumn were consistently significant. By contrast, in moist-climate seedlings, the SS concentrations of the roots were significantly different only between autumn and spring (Figure 3). Root SS/NSC ratio also varied during the season, and this variation was marginally dependent on the climate of seed origin (Table S2 available as Supplementary data at *Tree Physiology* Online). Specifically, both moist- and dry-climate seedlings increased their root SS/NSC ratio from spring to summer and autumn when the ratio for dry-climate seedlings was highest (Figure 4). After repeated analyses, we found that leaf SS concentrations were zero for spring and autumn in seedlings of both climates, and starch concentrations were also zero in autumn for moist-climate seedlings (Figure 3).

The climate of seed origin had no significant effect on the whole-seedling or organ-specific NSC, starch or SS pools, which nonetheless differed significantly between dates (Table 1, and Table S2 and Figure S3 available as Supplementary data at *Tree Physiology* Online). The NSC and starch pools decreased progressively from spring to autumn, while the SS pool peaked in summer, was lowest in spring and intermediate in autumn (Figure S3 available as Supplementary data at *Tree Physiology* Online). The variation among dates was similar between moist- and dry-climate seedlings (i.e., no significant climate × date interaction; Table 1).

Companion traits

Huber values were similar between the climates of seed origin and across dates (Table 1). The root mass fraction was significantly higher in dry-climate seedlings (0.524 ± 0.022) than in the moist climate (0.520 ± 0.01), and it increased similarly from spring to autumn regardless of origin (climate: $F_{1,23} = 6.34$, $P = 0.019$; date: $F_{2,70} = 35.31$, $P < 0.0001$). The cluster root number was similar for moist- and dry-climate seedlings ($F_{1,13} = 2.401$, $P = 0.144$) and was higher in the summer (6.42 ± 0.83) than in the autumn (1.033 ± 0.21) (date: $F_{1,46} = 48.45$, $P < 0.0001$; climate × date interaction: $F_{1,46} = 1.86$, $P = 0.180$). The biomass of cluster roots was very low and was similar between moist- and dry-climate seedlings (0.005 ± 0.002 and 0.006 ± 0.003 g, for the moist and dry provenances, respectively: $F_{1,16} = 0.0004$, $P = 0.983$). The LMA was similar between moist- and dry-climate seedlings (131.2 ± 8.2 and 130.4 ± 6.5 g m^{-2} for moist and dry climates, respectively: $F_{1,75} = 0.34$, $P = 0.564$). Likewise, leaf N concentrations were similar between moist- and dry-climate seedlings ($F_{1,15} = 0.18$, $P = 0.675$), reaching 0.55 ± 0.03 for the moist-climate seedlings and 0.54 ± 0.02% for the dry.

Discussion

No evidence for NSC mediating local adaptation to dry climate

In our common garden experiment, 3-year-old seedlings of the widely distributed species *E. coccineum*, that were grown from seeds harvested in the dry climate, had significantly lower biomass but similar NSC concentrations and pools compared with moist-provenance seedlings (Figures 2 and 3, and Figures S2 and S3 available as Supplementary data at *Tree Physiology* Online). Seedlings from both climates had also similar leaf N concentrations, which is a proxy for photosynthetic capacity (Evans 1989). Because the two provenances were compared in the common garden conditions, the pattern of C reserves found here does not support the hypothesis that aridity selects for higher NSC concentrations at the expense of

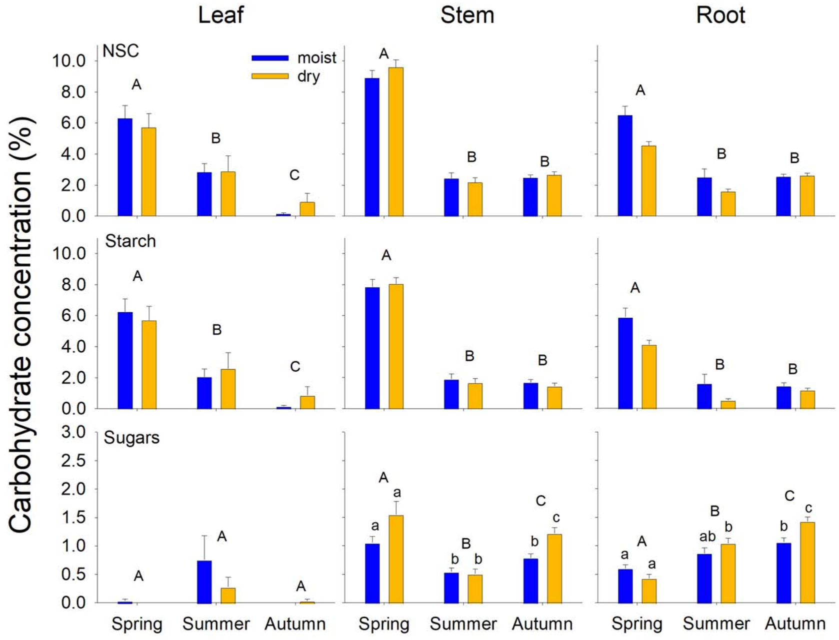

Figure 3. Organ-specific concentrations of total NSC, starch and low molecular weight SS (as percentage of dry mass) in 3-year-old seedlings of *E. coccineum* grown from seed from either a dry or moist climate in a common garden in Coyhaique, Chile. Data are shown for spring (November 2020), summer (January 2021) and autumn (May 2021). Different uppercase letters above means indicate significant differences among seasons at $P < 0.05$ for both ecotypes combined. Different lowercase letters above means indicate significant differences among seasons for each ecotype separately.

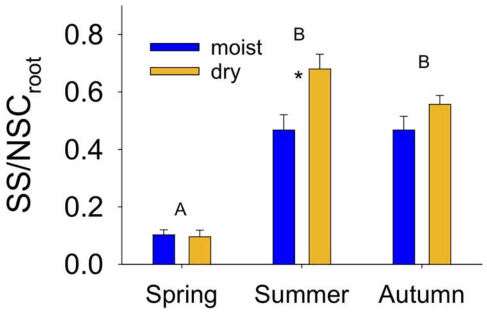

Figure 4. Ratio between root concentrations of SS and total NSC in 3-year-old seedlings of *E. coccineum* (Proteaceae) from dry and moist climates grown under common garden conditions in Coyhaique, Chile. Data are shown for spring (November 2020), summer (January 2021) and autumn (May 2021). Different upper case letters stand for statistically significant differences among seasons at $P < 0.05$ for both ecotypes combined.

reduced growth (Sala et al. 2012, Wiley and Helliker 2012, Dietze et al. 2014). According to the principle of resource allocation (Grubb 2016), if C is resource-limiting for growth and storage, the less the C is invested in biomass, the more the C is invested in storage. However, we found that lower biomass was not translated into greater NSC stores. Assuming that the C gain was similar between dry- and moist-origin seedlings (similar leaf N concentrations and LMA between climates), our results do not support a growth–storage trade-off as a feasible explanation for the intra-specific previously reported pattern of reduced growth rates and higher NSC concentrations under natural conditions of increasing aridity. It is likely that in environmental conditions like those in our study, C is not a limiting resource and the allocations to growth and storage do not compete for C availability in seedlings of *E. coccineum*. Empirical evidence supporting the growth–storage trade-off was found when studying a fast-growing species (*Populus trichocarpa*) (Blumstein et al. 2022). *Embothrium coccineum* is a short, rather slow-growing species. Thus, even in the moist climate, C demands for growth in *E. coccineum* may be low and seedlings may not need to channel C away from storage. Consequently, our results using a wide-niche tree species lead us to suggest that the reduced growth of trees inhabiting arid environments is not likely due to prioritized allocation of C to storage at the expense of growth.

A widely reported increase in the NSC and SS concentrations along with reduced growth rates under dry conditions have led to a long debate regarding whether C allocation to storage competes with growth or rather C storage starts once growth demands have been met (e.g., Dietze et al. 2014, Palacio et al. 2018, Huang et al. 2019). Some studies have suggested that high NSC concentrations and reduced growth could be an adaptation to thrive in dry environments (Sala et al. 2012, Wiley and Helliker 2012). Based on our results, reduced growth of trees in arid environments is likely adaptative. Small plant size can be advantageous under arid conditions because it implies a lower demand of C and water as well as a lower transpiratory area (Westoby et al. 2002, Grime 2006). However, in our study, reduced growth was unlikely due to prioritized allocation of C to storage at the expense of growth. In that case, the previously reported patterns of higher NSC and less growth in dry environments than in more humid ones are likely the result of the higher sensitivity of growth than photosynthesis to drought (Körner 2003, Muller et al. 2011), a more plastic response. In the same line, a recent study found that concentrations of NSC and SS, and the fraction of NSC represented by SS, significantly increased with increasingly dry conditions at the time of sampling (Blumstein et al. 2023). However, the same study found no relationship between the site aridity and the seasonal maximum NSC or SS concentrations, suggesting no adaptative role of NSC to such environments.

The seasonal dynamics of NSC and starch levels were similar between moist- and dry-climate seedlings (Figure 3 and Table 1). Thus, for both moist- and dry-climate seedlings, concentrations of NSC and starch were the highest in spring, which is consistent with photosynthesis from older leaves benefiting from sunny, warm days during late winter and early spring onward (Hoch 2015). The NSC concentrations in leaves of *E. coccineum* in the spring was indeed entirely represented by starch, supporting the role of leaves as the main site of storage in evergreen species (Piper and Fajardo 2014, Palacio et al. 2018). Thus, leaf SS concentrations were zero in spring, which is an infrequent value. Other studies, however, have also reported zero values for SS in other species (Dünisch and Puls 2003, Wong et al. 2003, Würth et al. 2005). Such values might relate to the facultative deciduous character of *E. coccineum*, or to the use of SS as metabolic source to support the growth of new leaves (Landhausser and Lieffers 2012). The NSC and starch concentrations and pools decreased in summer when the seedling biomass increased, thus reflecting increased C demands associated with growth and metabolism (Hoch et al. 2003, Furze et al. 2019, Piper 2020). Given that leaf lifespan in *E. coccineum* is c. 13 months (Lusk and Contreras 1999), and that leaves are shed in late summer and autumn (Fajardo and Piper 2015), by the end of the growing season, seedling biomass likely decreased from tissue turnover. We also found a significant root mass reduction through the growing season, which is indicative of root turnover (Berntson and Bazzaz 1996). Stem biomass of seedlings was also reduced, which might reflect a smaller initial seedling size when we randomly assigned seedlings for each harvest date.

Seasonal dynamic of SS and local adaptation to aridity

Seedlings from contrasting climates differed in the seasonal dynamics of their SS concentrations. These differences were consistent with local adaptation to aridity; dry-origin seedlings showed a steeper increase in root SS concentrations from spring to autumn, and in stem SS concentrations from summer to autumn, than in moist-origin seedlings (Figure 3). Dry-origin seedlings also stored a greater proportion of total root NSC as SS compared moist-origin seedlings (Figure 4). A high concentration and a high fraction of SS during the growing season could be locally advantageous for *E. coccineum* to thrive in the dry climate, particularly during the driest period of the season (late summer) (Fajardo and Piper 2021). The SS help to increase cell turgor by reducing the osmotic potential (Morgan 1984, Sapes et al. 2021), which in turn promotes dehydration tolerance (Carpenter et al. 2008, Turner 2018). Accordingly, drought-adapted plants have a lower constitutive osmotic potential than their more drought-sensitive counterparts (Bartlett et al. 2012). It is, nonetheless, intriguing that SS levels in dry-climate seedlings were not always higher than those from the moist climate. A similar SS dynamic has been found in observational studies in other ecosystems. In a study across contrasting precipitation regimes in Amazonia, the fractions of leaf NSC represented by SS were remarkably similar across communities with contrasting precipitation regimes during the wet season, but these increased greatly in the driest sites during the dry season for almost all species (Signori-Müller et al. 2021). Similarly, in a global-scale study of the seasonal NSC dynamics of plants from different biomes, Mediterranean woody species had a peak in SS concentrations in the summer unlike plants from other biomes (Martínez-Vilalta et al. 2016). Our results show that increases in SS concentrations at the driest period of the year by seedlings adapted to aridity take place even when they are watered well, suggesting that the SS dynamics underlie local adaptation to aridity by promoting high tissue turgor under conditions that otherwise could cause dehydration.

The differences in the seasonal variation of SS concentrations found between moist- and dry-climate seedlings were most likely explained by differential allocations to starch rather than differences in C gain. Seedlings from the dry climate had significantly lower starch concentrations in their roots (Figure 3), suggesting that the higher stem and root SS exhibited by the dry-climate seedlings in the summer and autumn came at the cost of some starch synthesis or enhanced starch degradation. Considering that starch is one of the main energetic reservoirs in angiosperm (Rosell et al. 2021, Signori-Müller et al. 2021, 2022, Blumstein et al. 2022), and that starch concentrations may be predictive of survival under C stress (Barker Plotkin et al. 2021, Piper et al. 2022), our results suggest that the higher SS requirements in seedlings from the dry climate may limit their capacity to respond to natural disturbance events that cause C stress. Starch reserves fuel tree regrowth after defoliation, fire, frost damage and other disturbances (Piper and Fajardo 2014, Piper and Paula 2020); thus, the recovery capacity of trees to such disturbances might be more limited in ecotypes from dry than moist climates. On the other hand, significantly higher root starch levels in the moist-origin ecotypes could reflect an adaptation to tolerate shade and herbivory (Myers and Kitajima 2007), two factors that constrain C assimilation in plant communities of moist ecosystems, such as rainforests.

Weak coordination between NSC and other drought-related traits

For most of the companion traits measured in this study, seedlings from the moist and dry climates did not differ. An exception is root mass fraction, for which dry-origin seedlings had a higher value than their moist-origin counterparts (Table 1). This result is consistent with the advantage that more extensive roots provide for accessing deeper water. The lack of difference between seedlings from both climates found for the other traits can be explained by two factors. First, the traits measured could be more important at more advanced ontogenetic stages. For example, the Huber value, which is a measure of the investment of vascular tissue per unit of leaf area fed, could be limited in seedlings that are likely still developing sapwood area. Second, while the measured companion traits are related to strategies of drought avoidance, plants may resist dehydration through non-exclusive mechanisms of avoidance or tolerance (Morgan 1984, Pausas et al. 2016). For plants facing recurrent and severe water deficit, dehydration tolerance has been proposed to be more common than dehydration avoidance (Volaire 2018). If the main strategy against drought in dry-origin populations of *E. coccineum* is dehydration tolerance, ecotypic differences in traits related to drought avoidance would not be expected.

Limitations of the study

Our results must be interpreted under the assumption that the NSC in *E. coccineum* are mostly the ones that our method can assess, i.e., the sum of starch, glucose, fructose and sucrose. More complex sugars, lipids and other non-structural carbon compounds have been identified as C stores susceptible to seasonal remobilization in other tree species (Schoonmaker et al. 2021), and thus any of them could potentially play some role in the C reserve dynamics of *E. coccineum*. However, in the few studies that examined a wide range of nonstructural carbon compounds in angiosperm trees, the NSC considered by our protocol were also found to be quantitively and functionally the most significant (Hoch et al. 2003). Along the same line, sucrose, glucose and fructose have also been reported as the most abundant sugars in angiosperm trees (Hoch et al. 2003, Wong et al. 2003).

Conclusions

Considering the continuing increases of atmospheric CO_2 concentrations and more severe and frequent drought events, understanding how trees will respond to these forcing drivers is even more important. In our common garden experiment to compare ecotypes from contrasting precipitation regimes, reduced growth was indeed constitutive of dry-origin plants, though it was not necessarily accompanied by correspondingly high NSC concentrations. This result suggests a lack of competition between the growth and storage for carbohydrates. Therefore, the previously reported patterns of higher NSC concentrations in trees from drier locations compared with more mesic ones is apparently driven by a drought-induced C surplus (Sala and Hoch 2009, Piper et al. 2017, Fajardo and Piper 2021), a mere phenotypic plasticity response. By contrast, our common garden experiment revealed that increasing SS concentrations in response to drought, as well as slow growth, can be adaptative responses of plants to a dry environment.

Supplementary data

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

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Conflict of interest

None declared.

Data availability statement

Data will be available from the Dryad Digital Repository upon acceptance of the manuscript.

Authors' contributions

A.F. and F.I.P. conceived the research idea; A.F. designed methodology and executed the sampling and experiment; F.I.P. and A.F. collected and analyzed the data; F.I.P. led the writing of the manuscript.

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