Research paper

Physiological differences between root suckers and saplings enlarge the regeneration niche in *Eucryphia cordifolia* Cav.

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Many clonal plants produce vegetative recruits that remain connected to the parent plant. Such connections permit resource sharing among ramets, explaining the high survival rates of vegetative recruits during establishment under suboptimal conditions for sexual regeneration. We propose that differences in the regeneration niches of sexual and vegetative recruits reflect different physiological adjustments caused by parental supply of resources to the ramets. We conducted ecophysiological measurements in saplings and root suckers of *Eucryphia cordifolia* Cav., a tree species of the temperate rainforest of southern South America. We compared the following traits of saplings and suckers: gas exchange at the leaf level, crown architecture, daily crown carbon balance, biomass allocation to above-ground tissues (leaf-to-stem mass ratio, leaf mass area and leaf area ratio), xylem anatomy traits (lumen vessel fraction, vessel density and size) and stem ring width. We also correlated the growth rates of saplings and suckers with relevant environmental data (light and climate). Saplings showed morphological, architectural and physiological traits that enhance daily crown carbon balance and increase water-use efficiency, in order to supply their growth demands while minimizing water loss per unit of carbon gained. The radial growth of saplings diminished under dry conditions, which suggests a strong stomatal sensitivity to water availability. Suckers have low stomatal conductance, likely because the carbon supplied by the parent plant diminishes the necessity of high rates of photosynthesis. The low responsiveness of sucker growth to temporal changes in water availability also supports the existence of parental supply. The physiological differences between sexual and vegetative recruits satisfactorily explain the ecological niche of *E. cordifolia*, with saplings restricted to more closed and humid sites.

Keywords: climate, daily crown carbon balance, parental supply, tree-ring growth, water-use efficiency.

Introduction

Many clonal plants produce vegetative recruits that remain connected, temporarily or permanently, to the parent plant. These connections permit the parent plant to share resources among ramets, in what is referred to as clonal integration (Alpert and Mooney 1986, de Kroon et al. 1996, Zhang et al. 2002, Saitoh et al. 2006, Pinno and Wilson 2014). The physiological integration among ramets explains the high competitiveness of some clonal plants with their neighbors (Otfinowski and Kenkel 2008, Roiloa et al. 2010, Liu et al. 2016). Clonal integration also permits ramets to colonize stressful microenvironments where sexual reproduction is unsuccessful (Pennings and Callaway 2000, Peltzer 2002, Escandón et al. 2013). Access to parental resources during establishment possibly increases the chances of survival of clonal recruits (Kirby 1980, Hartnett and Bazzaz 1983, Wiehle et al. 2009).

Parent subsidy likely modulates the physiological adjustments of the ramets, and thus they would differ physiologically from sexual recruits of a similar developmental stage. The supply from the parent plant might enhance the water and nutritional status of ramets, leading to higher gas exchange rates (Alpert 1990, Roiloa et al. 2014). Ramets that have access to parental photo-assimilates could hypothetically down-regulate photosynthesis due to the low requirements of their carbon sinks (Alpert and Mooney 1986, Zhang et al. 2002). Low carbon assimilation at high transpiration rates would lead to lower water-use efficiency in ramets compared with saplings of a similar size.

Sapling growth and survival relies entirely on the ability of the sapling to acquire carbon, water and mineral nutrients. It is to be expected that saplings will have high carbon assimilation rates in order to satisfy their high growth demands (Bond 2000, Thomas and Winner 2002). However, if high carbon gain is attained by increasing the stomatal conductance, saplings can reach low water potentials at dry microsites. Saplings are therefore at much greater risk of hydraulic failure than are ramets connected to larger parental root systems. Because of this, saplings are expected to have high stomatal responsiveness to water availability, with the known negative consequences in terms of carbon gain (McDowell et al. 2008). Consequently, the growth and survival of saplings will be more dependent upon environmental conditions.

Eucryphia cordifolia Cav. is a long-lived tree species of the temperate rainforest of southern South America that recruits both sexual and vegetatively (Escobar et al. 2006). Vegetative recruitment consists of the formation of root suckers that usually remain connected to the genet, and tend to occupy more open and drier microsites than the saplings (Escandón et al. 2013). Water and nutrient uptake by the root suckers is entirely dependent on the parent plant because the ramets lack an independent root system. The large root system of the parent plant protects root suckers of *E. cordifolia* from exposure to low water potentials, explaining their ability to colonize drier microhabitats (Escandón et al. 2013).

Sexual recruits of *E. cordifolia* lack prominent root systems during the sapling stage. Survival rates of saplings fall from 50% to 20% with a canopy openness factor of greater than 2%, with smaller plants suffering higher mortality. This result suggests that the higher evaporative demand of open microsites (vapor pressure deficit (VPD) $\geq$ 0.6 kPa) promotes drought-induced mortality (Escandón et al. 2013). Accordingly, significant mortality in *E. cordifolia* saplings has been reported after reduction of 37% of the soil water content in relation to the field capacity (Morales et al. 2014). These results suggest that saplings of *E. cordifolia* are highly sensitive to water stress, explaining why they occupy less open and more humid microsites than root suckers.

In this study, we evaluate ecophysiological differences between suckers and saplings of *E. cordifolia*, considering the reported differences in the niche regeneration between both recruit types,

and the possible parental supply to the suckers. We predict that saplings have morphological, architectural and physiological traits that enhance the daily crown carbon balance, whereas root suckers assimilate comparatively lower amounts of carbon and have lower water-use efficiency. We also expect that the growth responses to environmental variability depend on the recruit type, with the above-ground growth of saplings being more limited by light and water than that of root suckers.

Materials and methods

Study site

This study was conducted in a 30 ha temperate rainforest located in south-central Chile (Katalapi Park: 41°31′8″S, 72°45′2″W, elevation ~90 m above sea level). Katalapi Park hosts young regenerating forests with remnants of old-growth forest, and has been protected during the last 27 years from anthropogenic alterations (logging and cattle) to promote its natural regeneration. The most frequent tree species are *Nothofagus nitida* (Phill.) Krasser, *Laureliopsis philippiana* (Looser) Schodde, *Caldcluvia paniculata* Cav., *E. cordifolia* Cav., *Drimys wintery* J.R. et G. Forster, and several Myrtaceae and Proteaceae (Lusk and Corcuera 2011).

This area has a temperate maritime climate. Annual rainfall of ~1900 mm is concentrated from April to November, with a mild dry season from December to March (see Figure S1 available as Supplementary Data at *Tree Physiology* Online). During the dry season, the mean air temperature reaches 15 °C, the minimum relative humidity of the air ranges between 45% and 55% and a 15-day-long dry period frequently occurs every summer. The maximum photosynthetic photon flux density (PPFD) ranges between 1500 µmol of photons m^{-2} s^{-1} and 7 µmol of photons m^{-2} s^{-1} at (respectively) 35% and 5% of canopy openness (i.e., the percentage of unobscured sky over a given point). For more details of the study site see Escandón et al. (2013).

Plant material and field measurements

In November 2012, we established a 50 × 50 m^2 plot in the study site where *E. cordifolia* recruits both sexual and vegetatively. The canopy openness of the plot ranged between 0.7% and 13.4% averaging 5.3 ± 3.3% (mean ± SD), which is within the regeneration niche breadth of this species (Escandón et al. 2013). All *E. cordifolia* recruits under 1.5 m in height within the plot were identified as either root suckers or saplings. In order to classify the origin of the recruits, the root collar was carefully dug, and the root systems were observed. Specimens were identified as suckers when they were connected to a large woody lateral root, and as saplings when no subsidiary root connection or root scar indicating past connection was observed (Escandón et al. 2013). We identified 26 suckers and 29 saplings in the plot. Although it was not possible to identify the parental tree of each of the suckers (as a

deep excavation could damage the recruits), we verified that all adult trees within the plot produced root suckers. This was done by digging one lateral root from each root collar until vegetative recruits were encountered.

The light environment of each recruit was estimated by means of canopy openness. A hemispherical photograph was taken over each plant apex during homogeneous, overcast conditions (Chazdon and Field 1987). The photographs were taken using a Coolpix 4500 digital camera equipped with a FC-E8 fisheye lens (Nikon, Tokyo, Japan). The camera was hand leveled and oriented so that the top of the image faced north. The photographs were analyzed for the percentage of canopy openness with the Gap Light Analyzer 2.0 software (Frazer et al. 1999). The canopy openness was slightly higher for saplings (4.6 ± 2.7 and 6.4 ± 3.3% for suckers and saplings, respectively), but it did not significantly differ among recruit types ($F_{1, 53}$ = 4.10, $P = 0.067$; response variable log-transformed). The homogeneity in the light environment allow us to compare ecophysiological measurements between the saplings and the root suckers growing within the plot.

The measurements described in the next sections were conducted in May 2013, at the end of the growing season for the study area.

Leaf gas exchange, chlorophyll content and optical properties measurements

Leaf gas exchange was measured with an IRGA Li-6400 (Li-Cor, Inc., Lincoln, NE, USA) during the morning (9:30–14:30 h), within the period when the maximum photosynthetic rate occurs for this species (Morales et al. 2014). We performed photosynthetic light response curves (A–Q) on fully expanded (1-year-old) leaves of both suckers and saplings growing within the study plot (three individuals per recruit type, one leaf per individual). Ten different light intensities between 1000 and 0 μmol photons $m^{-2} s^{-1}$ were used at 400 μmol CO_2 mol^{-1} air, 17 ± 0.7 °C (leaf temperature), and 65 ± 5% relative humidity within the leaf cuvette. Leaf photosynthesis was previously stimulated with ~200 μmol photons $m^{-2} s^{-1}$ (light quality proportion of 85%, 10%, 2% and 3% red, blue, orange and yellow, respectively) using an LED lamp. Due to the low photosynthetic rates of this species and the small area (2 cm^2) of the cuvette, the flow rate was adjusted from 100 to 200 ml min^{-1} to ensure that CO_2 differentials between the reference and the sample IRGA were >4 μmol mol^{-1} air. Photosynthesis software (Li-Cor Inc.) was used to determine the following A–Q curve parameters for each recruit: maximum net assimilation rate based on area (A_{max}), maximum quantum yield (AQE), light compensation and saturation points (LCP and LSP), and curvature factor (θ). These parameters were averaged for each recruit type and used to model the daily crown assimilation (see below).

Instantaneous light-saturated assimilation rate (A_{SAT}) and stomatal conductance (g_s) were measured at 500 μmol photons $m^{-2} s^{-1}$ and 400 μmol CO_2 mol^{-1} air; such PPFD is above the LSP and is not photoinhibitory (see A–Q curves, Figure 1A). The leaves used to measure A_{SAT} were then kept in the dark for 60 min and dark respiration (R_d) was measured at 0 μmol photons $m^{-2} s^{-1}$. These measurements were conducted on 11 suckers and nine saplings over three fully expanded 1-year-old leaves, using the same leaf temperature and relative humidity within the cuvette as used to construct the A–Q curves. A_{SAT}, g_s and R_d were standardized by the IRGA leaf cuvette area (i.e., expressed on a leaf area basis). The intrinsic water-use efficiency ($_iWUE$) was calculated as the ratio between A_{SAT} and g_s.
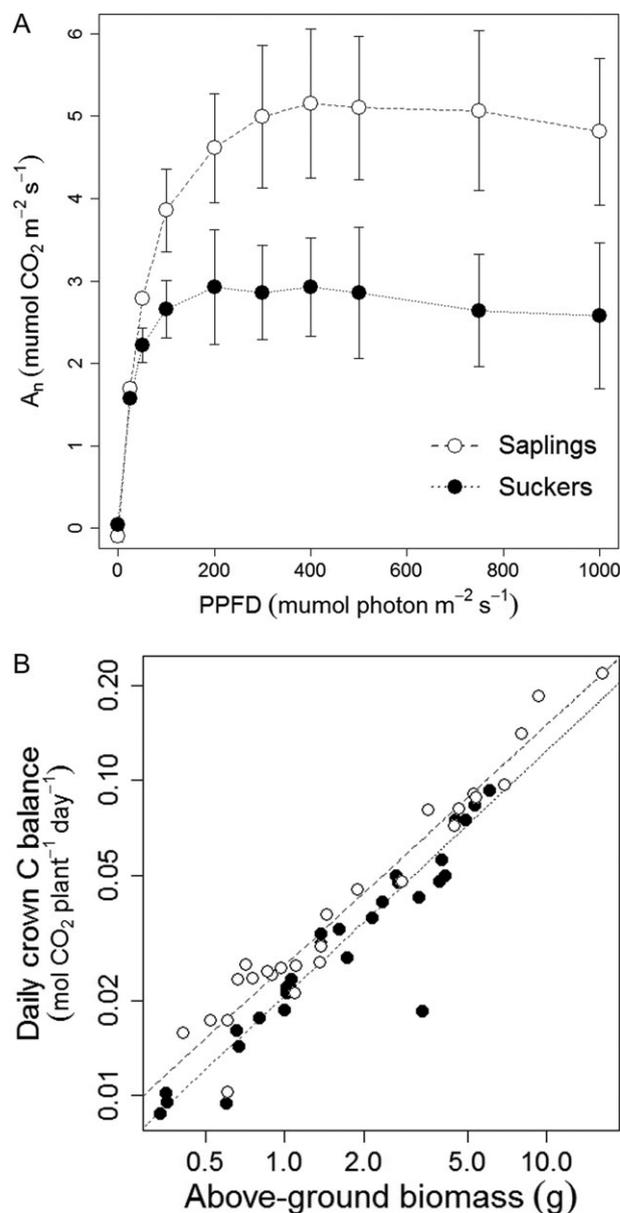

Figure 1. Gas exchange at leaf and crown level of root suckers and saplings of *E. cordifolia*. (A) Light response curve (mean ± SD). (B) Relationship between the daily crown carbon balance and the above-ground biomass.

The relative chlorophyll content of three fully expanded leaves uniformly distributed along the stem was measured using an SPAD-502Plus (Konica Minolta Optics, Inc., Osaka, Japan). Leaf optical properties (transmittance, reflectance and absorbance) were measured with the light source of the IRGA cuvette using a spectroradiometer (HR2000CG-UV-NIR; Ocean Optics Inc., Dunedin, FL, USA) following Gago et al. (2013).

Plant crown architecture

The northern side of each recruit within the study plot was marked in order to later simulate the light environment. The recruits were carefully excavated with ~$30 \times 30 \times 25$ cm^3 of soil in order to extract a substantial part of the root system. Plants were then immediately put into containers and watered to field capacity in order to maintain their architectural traits. The plants were then carried to the field station (located 500 m from the plot) to be digitized. Measurements were taken of the diameter stem ends, both basal and apical. Petiole diameter was measured of leaves at low, middle and high positions in the crown. All measurements were taken with a digital caliper. We then created an average leaf shape on a flat surface over cartesian coordinates centered in the leaf blade base, considering at least 25 points of the leaf border. This leaf model was used to populate the nodes of each virtual plant (see below). Finally, the position (3D coordinates) of each branch and leaf node in the crown was recorded using the 3D FASTRACK®-digitizer (Polhemus, Colchester, VT, USA), with the FLORADIG software (CSIRO Entomology, Brisbane, Australia). Virtual plants were constructed with the YplantQMC package of the R software (Duursma and Cieslak 2012), which uses the same 'plant' and 'leaf' files as the 3D plant model YPLANT (Pearcy and Yang 1996).

The crown architecture of each virtual plant (see examples in Figure 2) was described by means of the following variables: total plant leaf area (A_L), total surface area of the 3D convex hull wrapped around the leaf cloud (A_C), and projected (A_P) and displayed (i.e., exposed) leaf area (A_D) averaged over the entire hemisphere. We calculated the self-shading (SS) as $(A_P - A_D)/A_P$ (Lusk et al. 2011) and the crown density (CD) as the ratio of
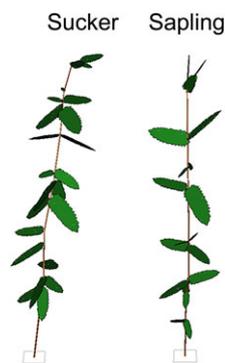

Figure 2. Examples of three-dimensional reconstruction of root sucker and sapling crowns of *E. cordifolia*. Scale bar = 10 cm.

A_L to A_C (Duursma and Cieslak 2012). The light interception efficiency was estimated as the 'silhouette to total area ratio' ($\overline{STAR}$) that is the ratio of A_D to A_L averaged over the entire sky hemisphere. Finally, we calculated the leaf dispersion (LD) as the average ratio of the observed mean distance from each leaf to the five nearest leaves, divided by the expected value if the leaves were randomly located (see Duursma et al. 2012 for more details).

Daily crown assimilation modeling

The daily crown carbon assimilation was estimated for each recruit by means of the package YplantQMC run using the R software (Duursma and Cieslak 2012). We used the crown architecture, the hemispherical photography data, and the leaf optical properties of each recruit as inputs. As light response parameters we used the mean values of A_{max}, AQE and θ for each recruit type, (i.e., suckers and saplings) obtained from their respective A–Q curves. For the environmental conditions, minimum and maximum temperature and daily photosynthetically active radiation (PAR) (i.e., integrated quantum flux) above the canopy were set according to data registered at 4–6% of canopy openness during an average clear day (i.e., PAR >1500 μmol photons m^{-2} s^{-1}). This canopy openness represents the average value for all of the studied plants. These data were obtained from a H21-002 HOBO meteorological station connected to S-LIA-M003 and S-THA-M0xx sensors (Onset, Bourne MA, USA). Clear days were identified by the PAR recorded with a Li-1400 data logger connected to Li 250, Li 1400-104 and Li 1400-106 sensors (Li-Cor Inc.) at 100% canopy openness. The daily crown carbon balance of each recruit was determined as the difference between the daily crown carbon assimilation and the daily R_d values (the latter values were retrieved from the A–Q curves; see above).

Xylem anatomy

After digitization, plants were harvested, and the basal portion of the stem (~2 cm length) was collected in 15 suckers and 15 saplings. The samples were soaked in distilled water for 24 h. After soaking, a Sakura Accu-Cut SRM™ 200 rotary microtome (Zoeterwoude, The Netherlands) was used to obtain thin (40 μm) fresh transverse sections of the basal stems. Sections were stained with a safranin solution (0.05%) for 3 min, washed with distilled water, dehydrated in increasing concentrations of ethanol (50%, 70%, 80%, 90%, 96%, and 100%; 5 min each), and permanently mounted on glass slides with Neo-mount (Merck, Darmstadt, Germany) after removal of the ethanol with Neo Clear Clarification™ (EMD Chemicals Inc., Gibbstown, NJ, USA). The cross sections were photographed at 40× of magnification with a digital camera (Moticam 2500 5.0 MP; MOTIC™) attached to a binocular microscope (Olympus CX21-FS1; Olympus Corporation, Tokyo, Japan). Recruit age was estimated from the number of xylem rings. The annual ring width was

measured with the MoticImage Plus 2.0 software (Motic China Group Co., Ltd, Xiamen, China). The cross section photographs were converted to binary (black and white) images, and the xylem vessels of one-fourth of each cross section were counted. Individual vessel lumen diameter was then determined using ImageJ 1.47q (Wayne Rasband/NIH, Bethesda, MD, USA) software. The percentage of the xylem area occupied by total vessel lumen area, the number of vessels per unit of xylem area and the average vessel diameter (hereafter vessel lumen fraction, vessel density and vessel size, respectively) were calculated. Huber values were calculated as the vessel lumen fraction divided by the total leaf area.

Biomass allocation

After crown digitization, leaves were removed and scanned. Leaf area was determined from each scanned image using ImageJ 1.47q software. Leaves and stems (including the portion of the stem remaining after the anatomical analysis) were dried in a forced air oven for 72 h at 60 °C. Leaf mass per area (LMA), leaf area ratio (LAR) and leaf to stem mass (LSR) were determined. The LMA was calculated by dividing leaf dry mass by leaf area; LAR is the relationship between leaf area and total above-ground plant dry weight; LSR is the ratio between leaf and stem dry mass.

Climatic data

To evaluate the effect of climate on the radial growth of both suckers and saplings, we compiled a meteorological time series from the nearest weather station with the most complete climate record for the 2004–2013 period (Tepual Airport, 41°25′S, 73°05′85′W; Meteorological Office of Chile, http://www. meteochile.gob.cl/). This period includes the 3 years preceding the year of birth of the youngest sampled plant, until the year in which the sampling took place. Therefore, this climate time series permits us to evaluate the reported delay of the effect of climate on radial tree growth (Fritts 1976). We compiled monthly rainfall, air temperature and relative humidity data for this period. Using this data we estimated the monthly VPD according to Murray (1967). The annual values (sum of rainfall, mean temperature and mean VPD) were calculated using the monthly records of April of a given year through March of the following one, coinciding with the hydrological year in the region (Lara et al. 2008). We also calculated the spring (September to November), summer (December to March) and growing season (September to March) climatic values.

Statistical analyses

Variables related to biomass allocation (LSR, LMA and LAR), xylem anatomy (vessel lumen fraction, vessel density, vessel size and Huber value) and crown architecture (LD, A_L/A_C and SS) were compared between the two recruit types by means of one-way ANOVAs. Relative leaf chlorophyll content and variables related to gas exchange at the leaf level (A_{SAT}, g_s and $_iWUE$) were compared between suckers and saplings by means of

linear mixed models, where the individual was included as a random factor nested within the recruit type and the three measured leaves considered as pseudoreplicates. Model fit and estimation of dispersion were conducted using an analysis of variance. Parameter estimation was obtained by means of maximum likelihood. The significance of the contribution of the recruit type on the variability of the mixed model was calculated by comparing (by means of a likelihood ratio test) the null model (including random factor only) with the alternative one that incorporated the recruit type as an explanatory variable. These analyses were conducted with the lm4 library of the R package (Bates et al. 2014). The daily crown carbon balance was compared between recruit types by means of a one-way ANCOVA, including above-ground biomass as a covariate.

We evaluated changes in growth rates between saplings and suckers by comparing the above-ground biomass between recruit types by means of a one-way ANCOVA, where age was considered as a covariate. We also compared changes in the cumulative ring width with age between the two recruit types by means of a linear mixed model, where the individual was included as a random factor nested within the recruit type, and the annual rings measured were considered as pseudoreplicates. We used the same type of analysis to assess gas exchange at the leaf level. We then evaluated the effect of environmental variables on growth rate. For this purpose, we first compared the effect of canopy openness on the changes of above-ground biomass with age; this analysis was conducted separately for suckers and saplings by means of two independent two-way ANOVAs. For the case of suckers, we also evaluated the effect of the parent root diameter (as a rough proxy of parental supply) on changes of the above-ground biomass with age by means of a two-way ANOVA. Finally, to evaluate the effect of the regional climate on radial growth rate, we first calculated the median annual ring width by year and recruit type. Then, for each recruit type, we cross-correlated the median of the annual ring width with the climate time series (rainfall, temperature and VPD) of the corresponding and the three preceding years (i.e., time lag effect tested from 0 to 3 years). We conducted these time lag analyses because (at least in adult trees) carbon storage might demonstrate a delay in the relationship between climate and tree growth (Fritts 1976).

Results

Biomass allocation and xylem anatomy

Whereas suckers allocated more biomass to leaves than to stems, saplings allocated nearly equal amounts of biomass to both leaves and stems (i.e., the confidence interval of LSR included one; Table 1). However, because of the higher LMA in suckers (Table 1), the leaf area to shoot biomass ratio (LAR) was not different between the two recruit types (Table 1).

We also found differences in the xylem anatomy. The vessel lumen fraction was higher for suckers than it was for saplings (Table 1). This pattern was mostly the result of the slightly higher vessel density in suckers, rather than being due to differences in the mean vessel diameter between the two recruit types (see details on vessel size distribution in Figure S2 available as Supplementary Data at *Tree Physiology* Online). The Huber value was higher in suckers than it was in saplings (Table 1), due to the higher vessel lumen fraction.

Gas exchange and crown architecture

Net photosynthetic rate measured at the leaf level (A_{SAT}) was higher in saplings (Table 2), despite their lower LMA (Table 1). The higher A_{SAT} in saplings could be explained by the higher g_s

and relative chlorophyll content of saplings as compared with suckers (Table 2). For the same g_s, saplings had higher A_{SAT} and consequently showed higher $_iWUE$ than suckers (Table 2).

Leaf dispersion (LD) did not significantly differ among recruit types (Table 1). The lower quartile of LD was higher than one for both suckers and saplings [interquartile ranges: (1.06, 1.15) and (1.02, 1.19) respectively], indicating a leaf distribution more regular than random (Duursma et al. 2012). Saplings showed lower crown density (A_L/A_C) and self-shading (SS) than suckers, and consequently a higher light interception efficiency ($\overline{STAR}$; Table 1).

Saplings showed higher daily crown carbon balance when compared with suckers of the same total above-ground biomass ($F_{1, 50} = 23.62$, $P < 0.001$; Figure 1B; Table S2 available as

Table 1. Summary of the results of the one-way ANOVAs comparing variables related to the biomass allocation, xylem anatomy and crown architecture between saplings and root suckers of *E. cordifolia*. Mean values (±SD) for each variable and recruit type are also shown. The full ANOVA results are shown in Table S1 available as Supplementary Data at *Tree Physiology* Online.

Variables	ANOVA results		Saplings	Suckers
Biomass allocation[1]				
Leaf stem ratio (LSR; g g^{-1})	$F_{1,52} = 10.16$	$P = 0.002$	1.19 ± 0.54	1.77 ± 0.76
Leaf mass area (LMA; g m^{-2})	$F_{1,52} = 5.97$	$P = 0.018$	54.41 ± 10.8	62.42 ± 13.08
Leaf area ratio[2] (LAR; cm^2 g^{-1})	$F_{1,52} = 0.007$	$P = 0.933$	93.66 ± 29.13	94.24 ± 21.79
Xylem traits				
Vessel lumen fraction (%)	$F_{1,28} = 24.32$	$P < 0.001$	5.5 ± 1.9	9.0 ± 2.0
Vessel density[3] (μm^{-2})	$F_{1,28} = 3.03$	$P = 0.092$	2.7 ± 1.4	3.7 ± 2.3
Vessel size[3] (μm^2)	$F_{1,28} = 1.43$	$P = 0.242$	242 ± 108	288 ± 120
Huber value (mm^2 m^{-2})	$F_{1,28} = 5.02$	$P = 0.033$	2.4 ± 1.3	3.6 ± 1.7
Crown architecture				
Leaf dispersion[4] (LD)	$F_{1,53} = 1.36$	$P = 0.249$	1.11 ± 0.13	1.15 ± 0.14
Crown density (A_L/A_C; cm^2 cm^{-2})	$F_{1,53} = 16.47$	$P < 0.001$	0.14 ± 0.04	0.17 ± 0.06
Self-shading (SS; cm^2 cm^{-2})	$F_{1,53} = 9.27$	$P = 0.004$	0.12 ± 0.04	0.15 ± 0.04
Silhouette to total area ratio ($\overline{STAR}$; cm^2 cm^{-2})	$F_{1,53} = 8.44$	$P = 0.005$	0.44 ± 0.02	0.43 ± 0.02

[1]One outlier was discarded from the analyses.
[2]LAR was calculated considering only the above-ground biomass.
[3]Log-transformation prior to the analyses.
[4]Reciprocal transformation prior to the analysis.

Table 2. Results of the linear mixed models comparing relative chlorophyll content and instantaneous gas exchange variables at leaf level between saplings and root suckers. Mean values (±SD) for each variable and recruit type are also shown. AIC, Akaike information criterion; BIC, Bayesian information criterion; LogLik, Log-likelihood.

	AIC	BIC	logLik	Likelihood ratio test			Saplings	Suckers
				χ^2	df	P		
Relative chlorophyll content (SPAD units)								
Null	1364.6	1374.8	−679.31					
+ Recruit type	1327.6	1341.2	−659.8	39.02	1	<0.001	45.6 ± 4.5	35.5 ± 6.1
Carbon assimilation rate at light saturation (A_{SAT}; μmol CO$_2$ m^{-2} s^{-1})								
Null	−15.041	−8.758	10.521					
+ Recruit type	−41.025	−32.648	24.512	27.98	1	<0.001	2.7 ± 0.6	0.8 ± 0.5
Stomatal conductance (g_s; mmol H$_2$O m^{-2} s^{-1})								
Null	−465.25	−458.97	235.63					
+ Recruit type	−474.89	−466.51	241.44	11.64	1	<0.001	53.3 ± 0.6	26.6 ± 0.5
Intrinsic water-use efficiency ($_iWUE$; μmol CO$_2$ mmol H$_2$O^{-1})								
Null	401.68	407.96	−197.84					
+ Recruit type	392.64	401.02	−192.32	11.04	1	<0.001	0.054 ± 0.016	0.028 ± 0.015

Supplementary Data at *Tree Physiology* Online), which is consistent with the higher A_{SAT} and the higher $\overline{STAR}$ of saplings compared with suckers.

Growth rate

There were no statistical differences in above-ground biomass between recruit types of the same age ($P = 0.132$; Figure 3A; Table S4 available as Supplementary Data at *Tree Physiology* Online). Canopy openness explained a significant portion of the variability in the above-ground biomass for saplings, but not for suckers (Table 3). However, above-ground biomass was positively affected by average root diameter of the parent roots

feeding the suckers. The explained variance by parent root diameter was even higher than that by sucker age (Table 3).

The cumulative ring width for a given plant age was higher in saplings than in suckers ($P = 0.049$; Figure 3B; Table S5 available as Supplementary Data at *Tree Physiology* Online). On the other hand, the annual ring width of saplings was positively correlated with the spring rainfall of the corresponding year (Table 4). No significant correlation was found between temperature or VPD and annual ring width of saplings. In the case of suckers, the annual ring width was positively correlated with the mean temperature of the growing season, the spring and the summer of the corresponding year, as well as with the annual VPD of the previous year (Table 4).

Discussion

As predicted, *E. cordifolia* saplings showed morphological, architectural and physiological traits that enhance the daily crown carbon balance when compared with root suckers. Even with the same total leaf area per above-ground biomass, the crown arrangements of saplings permit a higher light interception efficiency, and the incident light is rapidly captured due to their high chlorophyll content. In addition, saplings have a higher g_s, which enhances CO_2 supply at the carboxylation site, thus increasing carbon assimilation at the leaf level (Lambers et al. 2008). Suckers showed higher LMA than saplings, which was unexpected considering their lower carbon assimilation rate (on area basis) and chlorophyll content (Wright et al. 2005, Lambers et al. 2008). Higher LMA in root suckers compared with neighboring saplings was also reported in *Fagus grandifolia*, but contrary to our results, no differences were found in gas exchange parameters at the leaf level in that study (Farahat and Lechowicz 2013). Leaves with higher LMA and thicker palisade parenchyma were reported in *E. cordifolia* saplings exposed to high

Table 3. Summary of the results of the two-way ANOVAs comparing changes in the above-ground biomass (log-transformed) with age, canopy openness and the parent root diameter (the latter for root suckers only). The full ANOVA results are shown in Table S3 available as Supplementary Data at *Tree Physiology* Online.

	ANOVA results		Explained variance (%)
Saplings			
Age (A)	$F_{1,21} = 18.65$	$P = 0.000$	39.2
Canopy openness (CO)	$F_{1,21} = 5.29$	$P = 0.032$	11.1
A × CO	$F_{1,21} = 2.62$	$P = 0.120$	5.5
Suckers			
Age (A)	$F_{1,21} = 5.52$	$P = 0.027$	16.7
Canopy openness (CO)	$F_{1,21} = 0.08$	$P = 0.781$	0.2
A × CO	$F_{1,21} = 2.40$	$P = 0.134$	7.3
Suckers			
Age (A)	$F_{1,21} = 6.89$	$P = 0.015$	16.7
Parent root diameter (PRD)	$F_{1,21} = 9.20$	$P = 0.006$	22.3
A × PRD	$F_{1,21} = 0.09$	$P = 0.765$	0.2

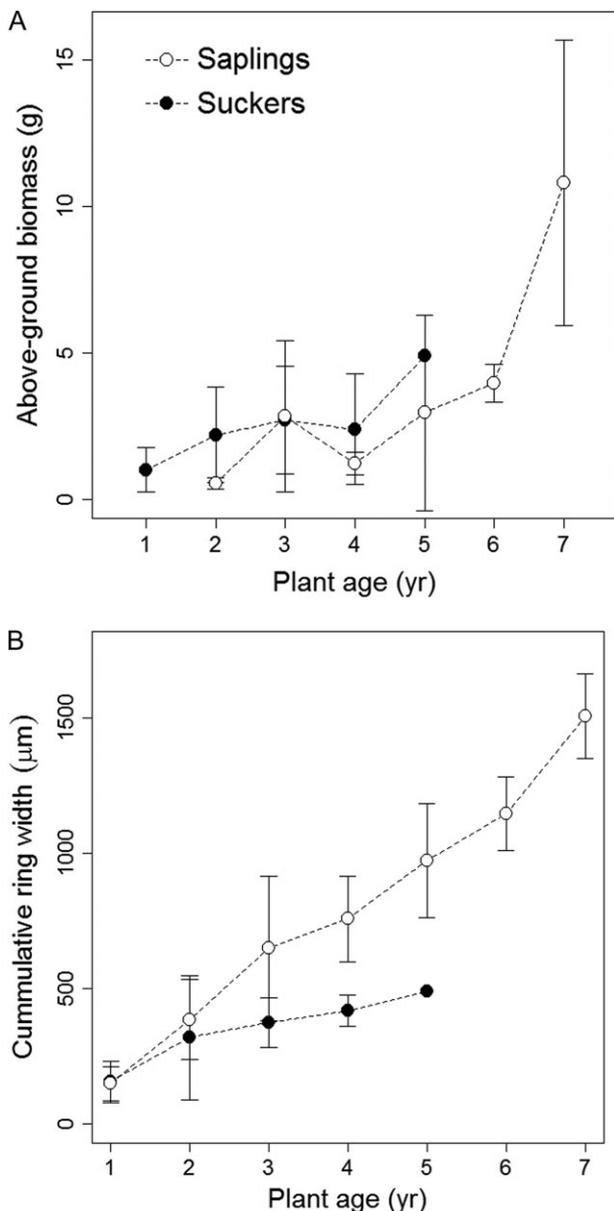Figure 3. Growth rate of root suckers and saplings of *E. cordifolia*. (A) Changes in above-ground biomass (mean ± SD) with age. (B) Changes in cumulative ring width (mean ± SD) with age.

Table 4. Cross-correlation coefficients for the median ring width and several climatic variables for the complete year (April to March), the growing season (September to March), spring (September to November) and summer (December to February). Significant correlations at 95% confidence are indicated with an asterisk.

Lag	Saplings				Suckers			
	Annual	Growing season	Spring	Summer	Annual	Growing season	Spring	Summer
Rainfall								
−3	−0.34	−0.34	−0.12	−0.39	0.19	−0.03	−0.12	0.05
−2	0.00	−0.08	−0.31	0.04	0.67	0.38	0.12	0.63
−1	0.45	0.47	0.24	0.55	−0.75	0.34	0.71	−0.04
0	−0.64	0.30	0.78(*)	0.07	−0.34	−0.72	−0.57	−0.76
Mean temperature								
−3	0.25	0.29	0.15	0.27	0.17	0.03	0.07	−0.01
−2	−0.09	−0.10	−0.15	−0.04	0.12	−0.39	−0.22	−0.49
−1	0.06	−0.40	−0.17	−0.50	−0.83	−0.20	−0.28	−0.05
0	−0.43	−0.15	−0.29	−0.11	0.60	0.99(*)	0.94(*)	0.97(*)
Vapor pressure deficit (VPD)								
−3	0.38	0.37	0.28	0.39	0.01	−0.02	0.06	−0.03
−2	−0.18	−0.09	−0.15	−0.06	−0.53	−0.60	−0.39	−0.61
−1	−0.34	−0.44	−0.19	−0.46	−0.17	−0.06	−0.41	−0.02
0	−0.25	−0.23	−0.51	−0.19	0.89(*)	0.78	0.84	0.77

light, compared with those growing in shade (Morales et al. 2014). However, there were no significant differences in canopy openness among the studied suckers and saplings (see Materials and methods section), discarding dissimilarities in the light environment as a cause of the differences in LMA among recruit types. One explanation of the high LMA in suckers might be related to the reported ontogenetic changes that increase LMA in trees. These changes likely have a genetic basis that allows the leaves to cope with harsh canopy conditions like high radiation, water deficit and wind (Thomas and Winner 2002). In fact, in the tropical tree *Macaranga gigantean*, the morpho-anatomical characteristics of the leaves of stump suckers are intermediate between those of saplings and adult trees; the high LMA values of stump suckers and adults in this species are related to leaf traits providing mechanical resistance to damage, rather than enhancing carbon gain (Ishida et al. 2005). Accordingly, the higher LMA of *E. cordifolia* suckers could be due to anatomical changes that increase leaf toughness (thicker vascular and sclerenchymatic tissues), but do not enhance the rate of photosynthesis based on area (de la Riva et al. 2016).

Despite the fact that we did not directly measure carbon translocation among *E. cordifolia* ramets, our results suggest that the maintenance and construction costs of root suckers were subsidized by parent photoassimilates, similar to other studies of clonal species (e.g., Alpert and Mooney 1986, Zhang et al. 2002). Firstly, the low carbon gain (at both leaf and crown level) in the root suckers can be explained by a possible down-regulation of photosynthesis induced by the low sucker requirements if their carbon necessities were supplied by the parent tree (for down-regulation of photosynthesis by sinks see Herold 1980, Watson and Casper 1984, Paul and Foyer 2001). Secondly, whereas above-ground biomass for a given age in saplings depends on

light availability, it was strongly related to parent root diameter in suckers. This suggests that the higher the parental supply, the higher the growth rate of the root suckers. Finally, despite the greater carbon balance of saplings as compared with suckers, the two recruit types did not differ in terms of biomass. The similar growth rates were achieved by different biomass allocation strategies: saplings allocated more carbon to stem radial growth and suckers to producing denser and/or thicker leaves (high LMA).

In spite of their higher g_s, saplings have a higher $_iWUE$, indicating that they adjust gas exchange in order to maximize the rate of carbon assimilation (to supply their growth demands), minimizing water loss per unit of carbon gained. Despite the fact that A_{SAT} was three times greater in saplings than in suckers, g_s was only twice as great. These results suggest a higher level of stomatal control in saplings than in root suckers, which is to be expected considering the high risk of hydraulic collapse due to both the small root systems of saplings and the high water potential at the turgor loss point for this species (Jiménez-Castillo et al. 2011). The high stomatal control in response to water availability can also be inferred from the climate–growth relationship. The annual ring width in saplings was significantly affected by the water availability in the spring (i.e., the lower the rainfall, the smaller the ring width; Table 4). Lower rainfall during the spring could induce stomatal closure in saplings (in order to decrease water loss by transpiration), and thus diminish carbon availability to be allocated to growth (Fritts 1976).

Root suckers are potentially able to conduct the high amounts of water supplied by the parent root system, due to their high vessel lumen fraction in relation to the transpiration surface (i.e., high Huber values). The enhanced water status of suckers due to the parental supply would explain their less efficient carbon assimilation rate in terms of water loss. Both the lower $_iWUE$ and

the weak relationship between rainfall and ring width support the hypothesis of poor stomatal control of suckers in response to environmental water availability. On the other hand, the radial growth of root suckers was positively correlated with the temperature of the corresponding year of growth. Mean temperatures were within the range of values for which increasing temperatures stimulate carbon gain, as indicated by studies conducted in a congeneric species inhabiting the Australian temperate rainforest (Hill et al. 1988). If this is the case for *E. cordifolia*, the question that arises is why changes in temperature did not affect saplings in a similar way. An increase in temperature also enhances the evaporative demand (Murray 1967), and thus warmer conditions would depress the carbon gain in saplings by stimulating stomatal closure in order to save water. This gas exchange regulation in response to warmer and drier conditions probably does not occur in root suckers, which are less susceptible to water limitation due to their connection to the large parent root system. In fact, radial growth in suckers was also positively related to VPD, reflecting the positive relationship between this climate variable and air temperature (Murray 1967).

Conclusions

In the present study, we showed that root suckers and saplings of *E. cordifolia* differ in functional traits related to carbon gain and hydraulic architecture. Specifically, saplings are able to assimilate more carbon with higher water-use efficiency, allowing them to supply their growth demands while minimizing water loss per unit of carbon gained. However, the stomatal response to water availability likely diminishes growth rates under dry conditions and ultimately might compromise survival, explaining why saplings are restricted to more closed and humid microsites (Escandón et al. 2013). On the other hand, suckers can maintain a low stomatal conductance, likely because carbon supply by the parent plant reduces the necessity of high rates of photosynthesis. Despite their low transpiration rates, root sucker leaves have access (through a large vessel lumen fraction) to high amounts of water, provided by the parent root system. Parental supply satisfactorily explains the low responsiveness of sucker growth to temporal changes in water availability, and their capacity to occupy more open and drier microsites (Escandón et al. 2013).

Supplementary Data

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

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