



Epiphytic lichen growth in Mediterranean forests: Effects of proximity to the ground and reproductive stage

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Abstract

Reasonable lichen growth rates are required to maintain the important ecosystem functions provided by epiphytic lichen populations. Previously, lichen growth has mainly been quantified in boreal forests. Thus, there is a need for comparable studies from Mediterranean forests. We used reciprocal transplantation to compare relative biomass (RGR) and thallus area (RT_AGR) growth rates of the cephalolichen *Lobaria pulmonaria* and the cyanolichen *Lobarina scrobiculata* ($n = 720$ thalli) in two Mediterranean oak forests. Both juvenile and reproductive thalli were transplanted for 334 days at the base and at breast height of random trees in both forests. We measured functional traits, specific thallus mass (STM) and water holding capacity (WHC), to search for adaptation/acclimation patterns between regions and between local stands. Linear Mixed Models were used to assess the effects of (1) forest, (2) height on the trunk and (3) reproductive stage on RGR, RT_AGR , and functional traits. *Lobaria pulmonaria* grew faster than *L. scrobiculata*, consistent with its more flexible hydration traits. Growth rates in both species were fastest in juveniles at the trunk bases, consistent with trade-offs between (1) growth and reproduction and (2) humidity availability and distance from the ground. In *L. pulmonaria*, STM increased with increasing evaporative demands, consistent with acclimation mechanisms. Fundamental and realized niches differed in *L. scrobiculata*, indicating that high abundance does not necessarily coincide with optimal growth habitat. Both species grew as fast in drier and warmer Mediterranean forests as reported from boreal forests, presumably because local conditions during hydration periods differ less between macroclimate regions than average climatic parameters, and/or because dew formation is frequent, particularly near the ground. Furthermore, STM and WHC of lichens in Mediterranean forests were high, thus partly compensating for the drier conditions. This study improves our understanding of mechanisms underlying epiphytic lichen growth in Mediterranean climates.

Zusammenfassung

Um die wichtigen Ökosystemfunktionen, die von epiphytischen Flechten geleistet werden, zu erhalten, sind angemessene Wachstumsraten erforderlich. Bislang wurde das Flechtenwachstum hauptsächlich in borealen Wäldern untersucht. Es besteht somit ein Bedarf an entsprechenden Studien in mediterranen Wäldern. Wir nutzten reziproke Transplantation, um die relativen Wachstumsraten für Biomasse (RGR) und Thallusfläche ($RTAGR$) von *Lobaria pulmonaria* und *Lobarina scrobiculata* ($n = 720$ Thalli) in zwei mediterranen Eichenwäldern zu vergleichen. In beiden Wäldern wurden juvenile und reproduktive Thalli nahe der Stammbasis und in Brusthöhe auf zufällig ausgewählte Bäume für 334 Tage transplantiert. Wir maßen funktionale Merkmale, die spezifische Thallus-Masse (STM) und das Wasserspeichungsvermögen (WHC), um nach Mustern der Adaption bzw.

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Akklimatisierung zu suchen. Lineare gemischte Modelle wurden eingesetzt, um die Einflüsse von (1) Wald, (2) Höhe am Stamm und (3) reproduktivem Status auf RGR, RTAGR und die funktionalen Merkmale zu bestimmen. *L. pulmonaria* wuchs schneller als *L. scrobiculata*, was mit ihrem flexibleren Hydratationsverhalten übereinstimmt. Bei beiden Arten zeigten Juvenile an der Stammbasis die höchsten Wachstumsraten, was mit den Zielkonflikten zwischen Wachstum und Reproduktion und Wasserverfügbarkeit und Entfernung vom Boden übereinstimmt. Bei *L. pulmonaria* nahm die STM mit zunehmenden Evaporationsanforderungen zu, was mit Akklimatisierungsmechanismen erklärt werden kann. Die realisierte Nische von *L. scrobiculata* wich von der fundamentalen Nische ab, was anzeigt, dass hohe Abundanz nicht notwendigerweise mit dem Habitat optimalen Wachstums zusammenfällt. Beide Arten wuchsen in den trockeneren und wärmeren mediterranen Wäldern ebenso schnell wie das aus borealen Wäldern berichtet wird, vermutlich weil sich die lokalen Bedingungen während der Hydratationsperioden zwischen den makroklimatischen Regionen weniger voneinander unterscheiden als die durchschnittlichen Klimaparameter und/oder weil insbesondere in Bodennähe Taubildung häufig ist. Darüber hinaus waren STM und WHC der Flechten in den mediterranen Wäldern hoch, wodurch die trockeneren Bedingungen teilweise ausgeglichen werden konnten.

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Keywords: Functional traits; *Lobaria pulmonaria*; *Lobarina scrobiculata*; *Quercus* forest; Relative growth rate; Spain; Specific thallus mass; Vertical gradient; Water holding capacity

Introduction

Epiphytic lichens often represent a highly diverse biodiversity component in forests and play important functional roles in ecosystem functioning (reviewed by Ellis 2012). Previously, lichens were considered slow-growing (Grime 1979), but recent studies reported fast growth (e.g. Gaio-Oliveira, Dahlman, Máguas & Palmqvist 2004; Larsson, Solhaug & Gauslaa 2012, 2014; Bidussi, Gauslaa & Solhaug 2013). However, epiphytic lichen growth is mainly quantified in boreal and temperate climates (e.g. Hilmo 2002; Antoine & McCune 2004; Larsson et al. 2012; Shriver, Cutler & Doak 2012) but rarely in Mediterranean climates (see Gaio-Oliveira et al. 2004) with summer drought (Valladares, Camarero, Pulido & Gil-Pelgrín 2004). Interestingly, these dry and sunny areas share various epiphytic lichens with temperate and boreal regions. To improve our understanding of lichen functioning across contrasting ecosystems and environmental conditions, there is a need to quantify growth rates and functional traits also in Mediterranean forests.

One reason to identify growth-regulating factors is that reproduction and fitness shaping population viability partly depend on growth (e.g. Martínez et al. 2012; Shriver et al. 2012). Although environmental factors determine lichen growth (e.g. Palmqvist 2000; Bidussi et al. 2013), internal factors such as reproductive effort also influence lichen growth by a trade-off between growth and reproduction (e.g. Gauslaa 2006). Lichens grow three-dimensionally: Area expansion enhances the interception surface for light, water, and nutrients; while dry mass gain per area unit e.g. improves water holding capacity (Larsson et al. 2012; Merinero, Hilmo & Gauslaa 2014). The specific thallus mass (STM = dry mass per area) is an important functional trait controlled by light exposure (Snelgar & Green 1981; Larsson et al. 2012) and/or nutrient availability (Asplund, Sandling & Wardle 2012). STM changes when area and dry mass do not increase concurrently, meaning that these processes are regulated differently.

Photosynthetic carbon gain shapes dry mass investments (Palmqvist 2000; Larsson et al. 2012), while fungal hyphae expansion drives area growth (Gaio-Oliveira et al. 2004; Larsson et al. 2012). STM is a driver of the water holding capacity (WHC); thick (Gauslaa & Coxson 2011) and large (Merinero, Hilmo et al. 2014) thalli retain more water. STM, and thus WHC, increase with increasing evaporative demands (e.g. Hilmo 2002; Gauslaa & Coxson 2011; Larsson et al. 2012; Merinero, Hilmo et al. 2014). Comparisons of WHC (and STM) in epiphytic lichens between open, dry Mediterranean sites (Valladares et al. 2004) and wet boreal sites including closed rain forests (e.g. Coxson & Stevenson 2007; Larsson et al. 2012) may thus give clues to understand links between site-specific hydration sources and lichen adaptation/acclimation traits.

The vertical distribution of lichens on tree trunks is influenced by e.g. solar radiation and humidity (reviewed by Ellis 2012). Air humidity increases with proximity to the ground, whereas light and wind exposure enhancing evaporation often increase with height (Geiger 1950). In Mediterranean sites, moisture availability is usually a limiting factor causing vertical gradients of lichens on tree trunks (Burgaz, Fuertes & Escudero 1994). The height on the trunk affects lichen performance, evidenced by height-dependent gradients in thallus size in lichen populations (e.g. Martínez et al. 2012).

We studied the cephalolichen *Lobaria pulmonaria* L. (Hoffm.) and the cyanolichen *Lobarina scrobiculata* (Scop.) Nyl. ex Cromb. They are widely distributed globally, but declining and red-listed in many European countries, including Spain (Martínez et al. 2003). In our study area, they inhabited two Mediterranean oak forests spaced by 7 km, although in contrasting proportions. We established reciprocal transplants to compare relative growth rates between species and between sites. In boreal canopies, both species acclimate to higher evaporative demand at greater heights by increasing their WHC (Merinero, Hilmo et al. 2014). To search for height-dependent growth and acclimation, we

transplanted lichens at trunk bases and breast height. By using juvenile and reproductive specimens, we tested whether the trade-off between growth and reproduction is consistent across regions and species. Furthermore, cyano- and cephalolichens have different hydration traits; the former require liquid water to activate photosynthesis, whereas cephalolichens additionally benefit from humid air (Lange, Kilian & Ziegler 1986). A comparison of such functional groups in Mediterranean and boreal climates (e.g. Hilmo 2002; Larsson et al. 2012, 2014) may improve our understanding of their functioning. We hypothesize that (1) relative growth rates in Mediterranean populations are lower than those reported in boreal ones due to drier climate; (2) *L. pulmonaria* with flexible hydration traits grows faster than *L. scrobiculata*; (3) both species grow faster near the trunk base than at the breast height; (4) juvenile specimens grow faster than reproductive ones; (5) both species maximize their growth in the forests with the highest respective abundance, consistent with similar fundamental and realized niches.

Materials and methods

Study area

The field experiment was done in two oak forests in central Spain with similar macroclimate, but yet different proportions of our target species (Table 1). The distance between the stands was 7 km. The Montejo stand (6.5 ha) is in Madrid (41°8'46"N, 3°29'33"W), and the Santuy stand (21 ha) in Guadalajara (41°5'44"N, 3°26'27"W). The oak forests are discontinuous, located in a matrix of pine plantations and grasslands. The climate (Table 1) is Mediterranean, characterized by 1.8 months of drought during summer, annual precipitation ≈800 mm, and mean annual temperature 9.5–9.7 °C (CLIMOEST; Sánchez-Palomares, Sánchez-Serrano & Carretero-Carrero 1999). Both forests were *Quercus pyrenaica*-dominated, accompanied by *Crataegus monogyna*, *Cytisus scoparius*, and *Rubus ulmifolius*. In Montejo, Atlantic species like *Ilex aquifolium* and *Erica arborea* also occurred. Montejo is a northern slope facing the Jarama river, while Santuy is on flatter land far from permanent water courses. Forest structure differed between the sites, mainly due to contrasting management in the past; the Montejo forest hosted larger but fewer trees than the Santuy forest (Table 1). Contrasts in orography and history may lead to the reported differences in microclimate and stand characteristics (Table 1). Many old-forest epiphytes occurred in Montejo (i.e. the *Lobarion*), whilst the pioneer epiphytic community *Parmelion* was common in Santuy.

Study species

We studied (1) the cephalolichen *Lobaria pulmonaria* with the green alga *Dictyochochloropsis reticulata* as its primary photobiont and the cyanobacterium *Nostoc* in scattered internal cephalodia and (2) the cyanolichen *Lobarina scrobiculata*

Table 1. Descriptive absolute or mean (± 1 SE) variables for each study site. *P* values from ANOVA are given. Summer = 21st June–20th September; Winter = 21st December–20th March.

Variable	Montejo	Santuy	<i>P</i>
<i>Climate and orography</i>			
Annual precipitation (mm)	818	801	
Mean annual temperature (°C)	9.5	9.7	
Altitude (m a.s.l.)	1263	1235	
<i>Microclimate</i>			
Mean annual temperature (<i>T</i> , °C)	9.2 \pm 0.1	10.2 \pm 0.1	0.000
Mean <i>T</i> in summer (°C)	17.4 \pm 0.1	19.5 \pm 0.1	0.000
Mean <i>T</i> in winter (°C)	2.3 \pm 0.1	2.7 \pm 0.1	0.001
Mean relative humidity (RH, %)	74.1 \pm 0.2	69.6 \pm 0.3	0.000
<i>n</i> hours (days)	1026 (100)	759 (88)	
RH \geq 96.5% in total			
<i>n</i> hours (days)	14 (2)	35 (5)	
RH \geq 96.5% in summer			
<i>n</i> hours (days)	425 (45)	214 (29)	
RH \geq 96.5% in winter			
<i>Forest structure</i>			
Tree density (trees in 1/4 ha)	158	720	
Tree DBH (cm)	22.9 \pm 1.1	12.3 \pm 0.1	0.000
Tree base diameter (cm)	66 \pm 1.7	18.1 \pm 0.2	0.000
Trunk slope (°)	81.5 \pm 0.6	82 \pm 0.2	0.487
Bark roughness (mm)	8.6 \pm 0.4	8.4 \pm 0.1	0.555
Bark pH	6.2 \pm 0.1	5.8 \pm 0.1	0.002
Bryophyte cover (%) <1 m	20.5 \pm 1.3	21.1 \pm 0.5	0.587
Bryophyte cover (%) 1–2 m	11.9 \pm 1.0	6.8 \pm 0.4	0.000
Canopy openness (%)	29.1 \pm 0.1	30.6 \pm 0.1	0.150
Total light (%)	27.1 \pm 0.1	29.7 \pm 0.1	0.046
<i>Study species</i>			
<i>n</i> individuals of <i>Lobaria pulmonaria</i>	2385	15	
<i>n</i> individuals of <i>Lobarina scrobiculata</i>	8	10,546	

Bold values indicate significant effects at $P < 0.05$.

with *Nostoc* as its only photobiont. The abundance of both species (Table 1) had been recorded in two 50 m \times 50 m plots within both sites (unpublished data). Three-hundred-sixty *L. pulmonaria* lobes were randomly collected in Montejo, and the same number of *L. scrobiculata* was taken in Santuy on 4 May 2012. One-hundred-and-eighty lobes were juvenile and another 180 reproductive with 10–30% cover of soredia and/or isidia. Thalli were air-dried in the laboratory on the collection day and kept at room temperature protected from high light. They were cleaned from debris and randomly labelled. Mean (\pm SD) lobe area for *L. pulmonaria* and *L. scrobiculata* were 13.0 \pm 4.1 and 8.6 \pm 4.0 cm², respectively.

Experimental design

The transplantation unit, a 20 cm × 20 cm nylon net, had four lobes, one juvenile and one reproductive of each species (see Appendix A: Fig. 1). These lobes were randomly assigned to each net, fastened with polyester thread in a way allowing the lobes to curl (Barták, Solhaug, Vráblíková & Gauslaa 2006). Each net was randomly assigned to forest and tree trunk; 45 trees in each forest were randomly chosen. We stapled two nets per tree, one at the base of the trunk (25 cm height), the other at breast height (150 cm). We placed all nets on the north side of the trunks. A total of 90 nets (360 lobes) were placed in each forest. In Santuy five nets were lost, so we collected 85 nets in each forest at the end of the experiment. The transplants stayed in the field from 24 May 2012 to 22 April 2013.

Lichen area, mass, growth, and fluorescence measurements

Before and after transplantation, we fully hydrated each lobe by deionised water, and flattened them under a piece of glass before taking photographs (Canon 550D equipped with a Tanrom AF-18-200 mm lens). Lobe area (A) was assessed in Photoshop 6.0 (Adobe Systems, San Jose, CA). Immediately thereafter, thalli were gently blotted with filter paper before measuring wet mass (WM) to the nearest mg. The maximum photochemical efficiency of photosystem II (F_v/F_m) was measured with a fluorometer (Plant Efficiency Analyser; Handsatech, King's Lynn, Norfolk, UK) after 15 min dark adaptation and after 1 h hydration. Then, lichens were air-dried 48 h before recording air-dry mass (DM). Ten control thalli similarly treated, were repeatedly weighed together with the transplants and no changes in mass were detected. These controls were oven-dried (60 °C for 72 h) before their DM was determined, and oven-dry DM of transplants was computed by using the weight reduction factor of these sacrificed thalli. Specific thallus mass was calculated as STM (mg DM cm⁻²) = DM/ A ; the water holding capacity (WHC in mg H₂O cm⁻²) = (WM – DM)/ A . Growth during the transplant period ($\Delta t = 334$ days) was quantified as follows (cf. Evans 1972):

Relative growth rate (RGR, mg g⁻¹ day⁻¹)

$$= \frac{\ln(DM_{\text{end}}/DM_{\text{start}})}{\Delta t} \times 1000$$

Relative thallus area growth rate

$$(RT_{\text{A}}GR, \text{ mm}^2 \text{ cm}^{-2} \text{ day}^{-1}) = \frac{\ln(A_{\text{end}}/A_{\text{start}})}{\Delta t} \times 100$$

Percent changes in STM and WHC were quantified as:

$$\Delta STM (\%) = \frac{STM_{\text{end}} - STM_{\text{start}}}{STM_{\text{start}}} \times 100$$

$$\Delta WHC (\%) = \frac{WHC_{\text{end}} - WHC_{\text{start}}}{WHC_{\text{start}}} \times 100$$

Besides the lost nets, 49 transplants were lost (22 *L. pulmonaria* and 27 *L. scrobiculata*) from mainly the base of trunks, likely due to detachment by cattle or wildlife. The loss was similar in the two sites. Furthermore, lobes with area and/or dry mass reductions >10% were excluded (*L. pulmonaria*: 3.9%; *L. scrobiculata*: 10.3% lost or fragmented). Thus, the final sample size for statistical analyses comprised 326 *L. pulmonaria* and 303 *L. scrobiculata*.

Canopy and microclimate measurements

Canopy cover and light exposure at each net were estimated from hemispherical photographs (Englund, O'Brien & Clark 2000). Digital photographs were taken from a horizontal position close to each transplantation net, at breast height and at base, using a horizontally levelled digital camera (Nikon Coolpix 4500, Nikon, Japan) aimed at the zenith, using the fish-eye lens FCE8, Nikon. Photographs were analyzed using Gap Light Analyser (GLA) software ver. 2.0 (Frazer, Canham & Lertzman 2000).

Air temperature and relative humidity 1.60 m above the ground were recorded every hour in both forests with four HOBO® H8 Pro Series data loggers (Onset, USA) along one transect within each forest and during the transplant period (Table 1). Liquid water likely occurred at relative air humidity >96.5%, because humidity sensors had an accuracy of <±3.5% (Onset Corporation 2009). Because climatic data were not replicated at the tree scale, they were not used in statistical models.

Bark pH measurement

Bark from 14 trees in each forest was taken. Three to four bark pieces from each tree constituted one sample. Bark samples were ground in a coffee mill; pH measurements (CRISON GLP21 pH meter; Alella, Barcelona, Spain) followed Kricke (2002).

Statistical analyses

Linear Mixed Models (LMMs) with a Laplace approximation (Bolker et al. 2009) and Gaussian error were fitted for each species to test the effect of site (Montejo-Santuy), height on the trunk (base-breast), the interaction site × height, reproductive stage (juvenile-reproductive), and the total light (%) on RGR, RT_AGR, and changes in STM and WHC. We used total light (%) because it highly correlated with canopy openness (Fig. 1, Table 1). Thallus area at start (A_{start}) was included as a covariate because growth is size-dependent (e.g. Larsson & Gauslaa 2011). To disentangle the correlation between A_{start} and reproductive stage, we run a Generalized Linear Model (GLM) with reproductive stage as the response

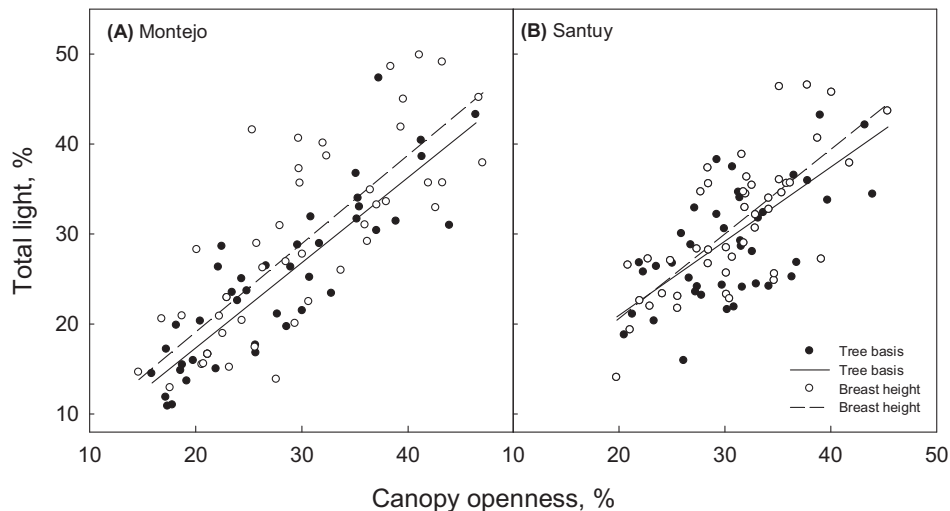


Fig. 1. Correlation between canopy openness and total light in (A) Montejo and (B) Santuy forests for each transplanted net. Overall Pearson correlation coefficient $r=0.82$, $P<0.001$ in Montejo and $r=0.69$, $P<0.001$ in Santuy.

variable and A_{start} as a predictor. Residuals of these models were included in the LMMs as the reproductive stage variable (Legendre & Legendre 1988, Chapter 20). We used mixed effects analyses because our data had an unbalanced and hierarchical structure with “lobes” nested within “tree” as nets on the same tree might be spatially dependent. Thus, data were analyzed from a multi-level approach, considering “tree” as random factor and introducing the explanatory variables as fixed factors (Bolker et al. 2009). Fixed factors were examined by Wald F -statistic tests. Models were finally checked for residuals normality and homoscedasticity. Statistical analyses were done in R version 2.15.2 (R Foundation for Statistical Computing, Vienna, Austria). We used the package nlme (Pinheiro, Bates, DebRoy & Sarkar 2008) for mixed effect models. Mean values \pm SD are reported in the text.

Results

Forest characteristics: microclimate, canopy openness, bark pH

Macroclimate was similar in both sites (Table 1). However, microclimate differed significantly because Montejo had higher relative humidity and lower temperature than Santuy (Table 1). In total, Montejo had more hours with liquid water than Santuy, except in summer, although humid days were few in both sites (Table 1). Santuy had 4.5 times more trees per area than Montejo that had more heterogeneous mean canopy openness (15–48%; Fig. 1). Nevertheless, the canopy openness did not differ significantly between sites. Neither did canopy openness differ between heights on the trunk (data not shown). Total light was slightly, but significantly higher in Santuy (Table 1; Fig. 1), and slightly higher at breast height ($29.2 \pm 1.6\%$ of above canopy light) than

at the tree base ($25 \pm 1.3\%$; t -test; $P=0.045$) in Montejo (Fig. 1). Besides, mean bark pH was higher in Montejo than in Santuy (Table 1).

Growth rates

Lobaria pulmonaria grew significantly faster than *L. scrobiculata* across sites, heights and lichen life stages (Fig. 2A–D; ANOVAs, $P<0.001$). Both species grew significantly faster in Montejo, and on tree bases in both forests (Fig. 2A–D, Table 2). In Montejo, the RGR of *L. pulmonaria* was 0.99 ± 0.37 vs 0.79 ± 0.33 $\text{mg g}^{-1} \text{day}^{-1}$ in Santuy. $\text{RT}_{\text{A}}\text{GR}$ was also significantly higher in Montejo than in Santuy (0.07 ± 0.03 and 0.06 ± 0.03 $\text{mm}^2 \text{cm}^{-2} \text{day}^{-1}$, respectively). *Lobarina scrobiculata* grew significantly faster (RGR = 0.50 ± 0.37 $\text{mg g}^{-1} \text{day}^{-1}$; $\text{RT}_{\text{A}}\text{GR} = 0.05 \pm 0.03$) in Montejo where it was rare, than in Santuy where it was abundant (RGR = 0.32 ± 0.31 $\text{mg g}^{-1} \text{day}^{-1}$; $\text{RT}_{\text{A}}\text{GR} = 0.04 \pm 0.03$ $\text{mm}^2 \text{cm}^{-2} \text{day}^{-1}$; Table 2, Fig. 2B and D). Height on the trunk and reproductive stage strongly influenced growth rates (Table 2, Fig. 2A–D). In both species and sites, the highest growth rates occurred in juveniles at the trunk bases (Table 2, Fig. 2A–D). Reproductive *L. pulmonaria* thalli grew significantly slower than juvenile ones (Table 2, Fig. 2A and C). By contrast, the reproductive structures on *L. scrobiculata* significantly reduced the area expansion, but not the dry mass gain (Table 2, Fig. 2B and D).

$\text{STM}_{\text{start}}$ was similar for *L. pulmonaria* (12.9 ± 1.6 mg cm^{-2}) and *L. scrobiculata* (12.9 ± 1.9 mg cm^{-2}), but reproductive lobes had significantly higher $\text{STM}_{\text{start}}$ than juvenile ones in both species (Fig. 2K and L; ANOVAs, $P<0.001$). Nevertheless, area and dry mass investments significantly differed between species (Fig. 3A and B). In *L. pulmonaria*, the dry mass gain increased more than the area expansion, resulting in a mean STM increase of $\sim 10\%$.

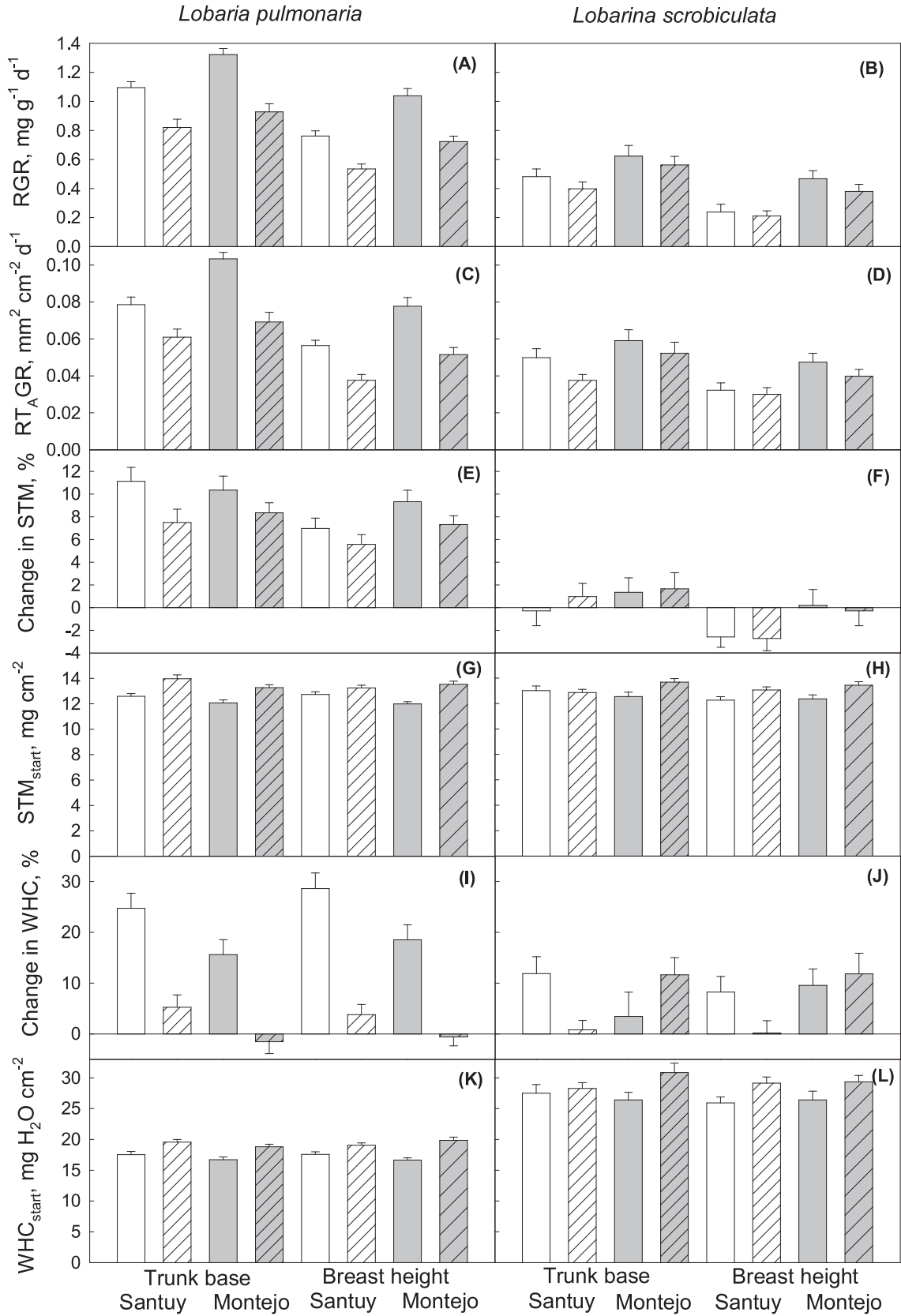


Fig. 2. Relative growth rates (RGR), relative thallus area growth rates (RT_AGR), changes in specific thallus mass (STM) and water holding capacity (WHC), and start values of STM and WHC (mean ± SE) in *Lobaria pulmonaria* (n = 326) and *Lobarina scrobiculata* (n = 303) across two forest stands (white columns: Santuy; grey columns: Montejo), and two heights on the trunk (base and breast height). Hatched columns: reproductive lobes; open columns: juveniles.

Table 2. Summary of the linear mixed models fit by a Laplace approximation used to examine the effects of environmental and intrinsic variables on the relative growth rates of *Lobaria pulmonaria* and *Lobarina scrobiculata* transplanted in two Mediterranean sites (Data shown in Fig. 2). The data comprise the Wald-type *F*-statistic value (*F*), degrees of freedom (df), coefficient of the variable in the model (Estimate), standard error of the estimator (SE), significance value (*P*), and random effects standard deviation (SD).

	RGR ^a				RT _A GR ^b		
	df	Estimate (SE)	<i>F</i>	<i>P</i>	Estimate (SE)	<i>F</i>	<i>P</i>
<i>Lobaria pulmonaria</i>							
<i>Fixed effects</i>							
Site (S)							
Santuy	88	−0.16 (0.05)	29.9	0.000	−0.02 (0.004)	29.6	0.000
Height (H)							
Breast	231	−0.25 (0.04)	89.4	0.000	−0.02 (0.004)	63.2	0.000
Life stage							
Reproductive	231	−0.07 (0.007)	98.1	0.000	−0.006 (6×10^{-4})	69.6	0.000
<i>A</i> _{start} ^c	231	−0.01 (0.004)	9.04	0.003	− 7×10^{-4} (3×10^{-4})	4.7	0.031
Total light	231	− 3×10^{-4} (0.002)	0.0	0.950	− 9×10^{-5} (2×10^{-4})	0.3	0.598
S × H	231	−0.06 (0.06)	0.9	0.340	−0.001 (0.006)	0.0	0.849
<i>Random effects</i>							
Tree				SD			SD
Residuals				0.11			0.01
				0.26			0.02
<i>Lobarina scrobiculata</i>							
<i>Fixed effects</i>							
Site (S)							
Santuy	88	−0.167 (0.062)	15.2	0.000	−0.013 (0.005)	10.5	0.002
Height (H)							
Breast	208	−0.178 (0.051)	34.9	0.000	−0.012 (0.004)	18.1	0.000
Life stage							
Reproductive	208	−0.013 (0.007)	3.2	0.070	− 1.6×10^{-3} (6×10^{-4})	6.4	0.012
<i>A</i> _{start} ^c	208	−0.001 (0.005)	0.1	0.740	− 4×10^{-5} (4×10^{-4})	0.0	0.922
Total light	208	−0.001 (0.002)	0.2	0.630	2×10^{-5} (2×10^{-4})	0.0	0.840
S × H	208	−0.032 (0.068)	0.2	0.640	2×10^{-5} (0.006)	0.0	0.905
<i>Random effects</i>							
Tree				SD			SD
Residuals				0.16			0.01
				0.29			0.02

Bold values indicate significant effects at $P < 0.05$.

^aRelative growth rate ($\text{mg g}^{-1} \text{day}^{-1}$).

^bRelative thallus area growth rate ($\text{mm}^2 \text{cm}^{-2} \text{day}^{-1}$).

^cThallus initial size (mm^2).

The increased STM was mainly attributable to the effect of height on the trunk and reproduction (Figs. 2E and 3C, Table 3); STM became significantly lower in the reproductive lobes at breast height due to slow growth (Fig. 2E and F, Tables 2 and 3). In *L. scrobiculata* there were no changes in STM due to balanced area and dry mass gains (Figs. 2F and 3D), and no explanatory variables influenced STM change (data not shown).

The $\text{WHC}_{\text{start}}$ was significantly higher in *L. scrobiculata* than in *L. pulmonaria* (27.9 ± 7.5 vs 18.2 ± 3.0 $\text{mg H}_2\text{O cm}^{-2}$; ANOVA, $P < 0.001$). However, the WHC of *L. pulmonaria* significantly increased in Santuy, particularly in juvenile lobes (Fig. 2I; Table 3). By contrast, the WHC of *L. scrobiculata* responded less (Fig. 2J) without consistent patterns and/or significant effects of tested variables (data not shown). Change in STM was significantly coupled to change

in WHC in both species, particularly in *L. scrobiculata* (see Appendix A: Fig. 2).

The mean $F_V/F_{M \text{ start}}$ was 0.71 ± 0.03 for *L. pulmonaria* and 0.48 ± 0.08 for *L. scrobiculata*. Both species had slightly higher F_V/F_M after the transplant period in both sites; F_V/F_M of *L. pulmonaria* did not differ between forests (0.72 ± 0.05), but *L. scrobiculata* showed slightly higher means in Santuy (0.57 ± 0.04) than in Montejo (0.55 ± 0.05) (ANOVA; $P = 0.003$).

Discussion

This is the first experimental study that compares annual growth rates of the threatened epiphytic lichens *L. pulmonaria* and *L. scrobiculata* in Mediterranean forests and

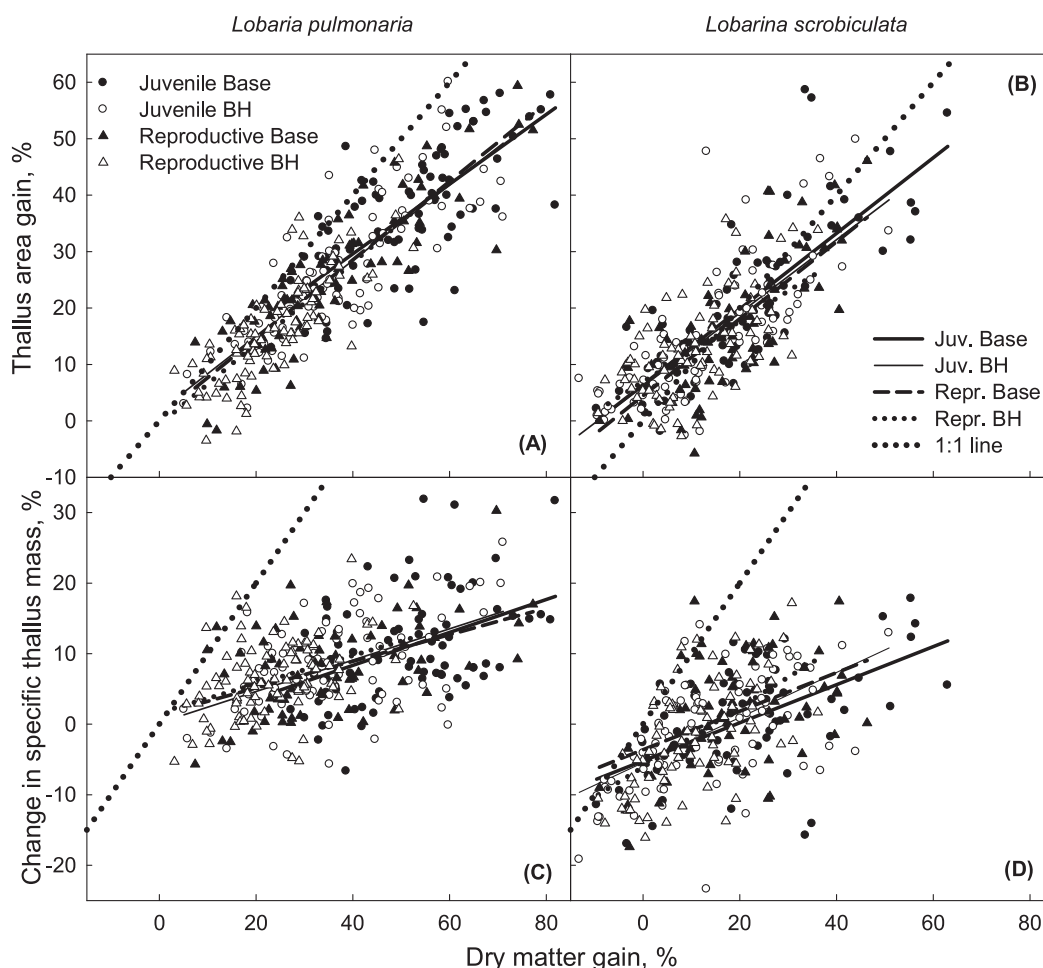


Fig. 3. Relationships between dry matter gain and (A and B) area gain, and (C and D) change in specific thallus mass (STM) for *Lobaria pulmonaria* and *Lobarina scrobiculata*. Filled symbols: thalli at trunk base; open symbols: thalli at breast height (BH). Circles refer to juvenile thalli, triangles to reproductive thalli.

quantifies height-on-trunk-effects on growth and performance of juvenile and reproductive stages. Contrary to our hypothesis, both species can grow as fast in dry Mediterranean climates as in moist boreal sites (e.g. Hilmo 2002; Coxson & Stevenson 2007; Larsson et al. 2012, 2014). However, *L. pulmonaria* grew slower than in inland British Columbia (Gauslaa & Goward 2012) and in Portugal (Gaio-Oliveira et al. 2004), and much slower than juveniles in boreal rain forests (Larsson & Gauslaa 2011). There are fewer studies on *L. scrobiculata*, but our specimens gained area three times faster than in boreal clearcuts (Larsson et al. 2014), and at similar rates as in suboceanic Norwegian spruce forests (Hilmo 2002), whereas juveniles in boreal rain forests grew three times faster than our juveniles (Larsson & Gauslaa 2011). Moreover, our lichens showed adaptation/acclimation mechanisms with respect to STM and WHC. STM is higher in *L. pulmonaria* from Spain than from Norway and British Columbia, whereas *L. scrobiculata* varies little between dry and wet forests (Table 4). Both species have higher WHC in Mediterranean forests (Table 4).

Light availability recorded at a lichen site level did not influence lichen growth. Thus, water availability is likely a more important limiting factor than light for lichens in Mediterranean forests (Burgaz et al. 1994). By contrast, light scarcity strongly limits lichen growth in moist boreal forests (e.g. Gauslaa, Lie, Solhaug & Ohlson 2006). Mediterranean deciduous forests are open (e.g. Gaio-Oliveira et al. 2004), providing sufficient light for lichen growth in all seasons, especially in winter when trees are leafless. Recent studies showed that lichen growth may be limited by nutrient availability (reviewed by Palmqvist 2000). However, such mechanism might be less important for the growth rates of our lichens in our forests with relatively high bark-pH.

Our results highlight the flexibility of *L. pulmonaria* with faster growth than *L. scrobiculata* in Mediterranean (Fig. 2A–D) as in boreal forests (Larsson & Gauslaa 2011; Larsson et al. 2012). This higher flexibility is probably facilitated by its green algal photobiont that can utilize also humid air to activate photosynthesis (Lange et al. 1986). Máguas, Griffiths and Broadmeadow (1995) showed that at 90%

Table 3. Results of the linear mixed models used to examine the effects of environmental and intrinsic variables on the changes in specific thallus mass (STM) and water holding capacity (WHC) (%) of *Lobaria pulmonaria* transplanted in two Mediterranean forests (Data shown in Fig. 2). The data comprise the Wald-type *F*-statistic value (*F*), degrees of freedom (df), coefficient of the variable in the model (Estimate), standard error of the estimator (SE), significance value (*P*), and random effects standard deviation (SD).

	<i>Lobaria pulmonaria</i>						
	Change in STM (%)				Change in WHC (%)		
	df	Estimate (SE)	<i>F</i>	<i>P</i>	Estimate (SE)	<i>F</i>	<i>P</i>
<i>Fixed effects</i>							
Site (S)							
Santuy	88	−0.08 (1.07)	2.2	0.141	7.75 (2.75)	18.6	0.000
Height (H)							
Breast	231	−1.43 (1.01)	8.8	0.003	1.08 (2.68)	0.72	0.398
Life stage							
Reproductive	231	−0.52 (0.17)	9.3	0.003	−4.8 (0.45)	112.2	0.000
^a A _{start}	231	−0.16 (0.09)	3.6	0.058	−0.67 (0.23)	8.61	0.004
Total light	231	0.03 (0.04)	0.8	0.371	0.08 (0.11)	0.59	0.444
S × H	231	−1.78 (1.41)	1.6	0.206	−0.12 (3.72)	0.001	0.975
<i>Random effects</i>							
				SD			SD
Tree				1.24			0.002
Residuals				6.27			16.65

Bold values indicate significant effects at $P < 0.05$.

^aThallus initial size (mm²).

of relative humidity, *L. pulmonaria* efficiently assimilated carbon, whereas *L. scrobiculata* released carbon, indicating inactive photosynthesis and active respiration. On average, *L. pulmonaria* gained dry mass faster than area, whereas in *L. scrobiculata* these processes were similar (Fig. 3A and B), consistent with the view that area and dry mass gains are regulated differently and in species-specific ways (Larsson et al. 2012).

An important finding is the faster growth rates at trunk bases than at 1.5 m, consistent with the much higher abundance of these lichens at stem bases in Spain (Martínez et al. 2012; Merinero, Rubio-Salcedo, Aragón & Martínez 2014). Studies in North America either did not find significant effects of height on lichen growth rates along a vertical gradient of 3 m (Bidussi & Gauslaa 2015), or did so, but along a larger

gradient (3–43 m; Antoine & McCune 2004). Thus, the effect of height on lichen growth rates depends on climate and forest types. In open Mediterranean forests, the fast growth near the ground is likely boosted by nocturnal cooling forming strong temperature profiles and, thus, humidity gradients (Geiger 1950). Moreover, the base is less vertical than the stem itself, and likely catches more rain/dew. Despite lacking height-dependent differences in canopy openness, light was slightly higher at breast height than at the base in Montejo, probably increasing desiccation rates and reducing growth. Excess light during defoliated months (≈October–March) and low humidity in summer presumably exclude our study species from the upper canopy (Merinero, Rubio-Salcedo et al. 2014; M. Rubio-Salcedo, S. Merinero & I. Martínez, unpublished data). To compensate for scarcity of water, we expected both

Table 4. A comparison of available data (means ± SE) for specific thallus mass (STM) and water holding capacity (WHC) in *Lobaria pulmonaria* and *Lobarina scrobiculata* taken from: ¹Bidussi et al. (2013), ²Larsson et al. (2012), and ³Gauslaa and Coxson (2011).

	<i>L. pulmonaria</i>	<i>L. scrobiculata</i>
STM (mg cm^{−2})		
Mediterranean oak forest, Spain, at start ($n = 303$ – 326)	12.90 ± 0.09	12.88 ± 0.11
Mediterranean oak forest, Spain, at end ($n = 303$ – 326)	13.94 ± 0.09	12.82 ± 0.11
Boreal rain forest, Norway ($n = 194$ – 197) ¹	9.10 ± 0.10	11.67 ± 0.10
Boreal rain forest, Norway ($n = 480$) ²	9.75 ± 0.06	13.22 ± 0.08
Inland rain forest, British Columbia ($n = 120$ – 122) ³	8.50 ± 0.13	10.73 ± 0.22
WHC (mg H₂O cm^{−2})		
Mediterranean oak forest Spain, at start ($n = 303$ – 326)	18.20 ± 0.17	27.92 ± 0.43
Mediterranean oak forest Spain, at end ($n = 303$ – 326)	20.06 ± 0.19	29.13 ± 0.39
Inland rain forest, British Columbia ($n = 120$ – 122) ³	13.12 ± 0.18	20.90 ± 0.36

species to increase their STM and WHC at breast height and in Santuy. However, only *L. pulmonaria* significantly increased its WHC in Santuy, despite higher STM gain at the breast height in both forests. Acclimation was strongest in juvenile *L. pulmonaria*, because reproductive specimens already had high WHC. Neither forest site nor height on the trunk influenced STM or WHC in *L. scrobiculata*, consistent with low cyanolichen flexibility.

The slower area growth in reproductive lobes than in juvenile ones is consistent with a trade-off between growth and reproduction in both species. Indeed, STM_{start} was significantly higher for the reproductive lobes than for the juvenile ones, suggesting that reproduction may increase with increasing thallus thickness and presumably with ageing, or that reproduction competes with growth for carbon (see Gauslaa 2006).

Contrary to our hypothesis, *L. scrobiculata* grew faster in Montejo where it was rare. The high cyanolichen growth is likely explained by the slightly higher liquid water availability in Montejo (Table 1), located in a northern slope near a river. A valley position, such as in Montejo, likely causes cold air-ponding from elevated position and favours dew formation during nocturnal cooling (Oke 1987). Discrepancy between abundance and growth rates of *L. scrobiculata*, suggests that realized and fundamental niches do not coincide. The paradox with highest cyanolichen growth rates in Montejo where it rarely occurs naturally is intriguing and calls for explanations. Mixed effects of dispersal limitation (low propagule pressure) and environmental filtering in the establishment phase can be underlying mechanisms. By contrast, realized and fundamental niches of *L. pulmonaria* coincided. Small *L. scrobiculata* thalli have significantly higher WHC than those of *L. pulmonaria* (Merinero, Hilmo et al. 2014), a trait that probably allows *L. scrobiculata* to cope with drier conditions and compensate its inability to be activated by humid air.

Conclusions

Overall growth rates of *L. pulmonaria* and *L. scrobiculata* were fairly similar in Mediterranean and boreal climates, showing that dry macroclimate does not necessarily reduce lichen growth rates of established thalli. High STM and WHC of Spanish lichens, consistent with adaptation/acclimation mechanisms to drier conditions, may have contributed to the similar growth rates across climatic regions. However, growth-stimulating effects of increasing proximity to the ground highlight the importance of microhabitat differentiation. Forestry practices should be addressed to maintain the forest structure that allows the required microhabitat and microclimate differentiation for our lichen species. Finally, our results emphasize the higher flexibility of *L. pulmonaria* than of *L. scrobiculata* due to species-specific hydration traits, and the strong trade-off between reproductive effort and growth.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.baae.2015.01.007>.

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