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Allocation of biomass and photoassimilates in juvenile plants of six Patagonian species in response to five water supply regimes

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• *Background and Aims* The growth–differentiation balance hypothesis (GDBH) states that there is a physiological trade-off between growth and secondary metabolism and predicts a parabolic effect of resource availability (such as water or nutrients) on secondary metabolite production. To test this hypothesis, the response of six Patagonian Monte species (*Jarava speciosa*, *Grindelia chiloensis*, *Prosopis alpataco*, *Bougainvillea spinosa*, *Chuquiraga erinacea* and *Larrea divaricata*) were investigated in terms of total biomass and resource allocation patterns in response to a water gradient.
• *Methods* One-month-old seedlings were subjected to five water supply regimes (expressed as percentage dry soil weight: 13 %, 11 %, 9 %, 7 % or 5 % – field water capacity being 15 %). After 150 d, plants were harvested, oven-dried and partitioned into root, stem and leaf. Allometric analysis was used to correct for size differences in dry matter partitioning. Determinations of total phenolics (TP), condensed tannins (CT), nitrogen (N) and total non-structural carbohydrates (TNC) concentrations were done on each fraction. Based on concentrations and biomass data, contents of TP and CT were estimated for whole plants, and graphical vector analysis was applied to interpret drought effect.
• *Key Results* Four species (*J. speciosa*, *G. chiloensis*, *P. alpataco* and *B. spinosa*) showed a decrease in total biomass in the 5 % water supply regime. Differences in dry matter partitioning among treatments were mainly due to size variation. Concentrations of TP, CT, N and TNC varied little and the effect of drought on contents of TP and CT was not adequately predicted by the GDBH, except for *G. chiloensis*.
• *Conclusions* Water stress affected growth-related processes (i.e. reduced total biomass) rather than defence-related secondary metabolism or allocation to different organs in juvenile plants. Therefore, the results suggest that application of the GDBH to plants experiencing drought-stress should be done with caution, at least for Patagonian Monte species.

Key words: Drought, growth–defence trade-offs, *Larrea divaricata*, *Jarava speciosa*, *Chuquiraga erinacea*, *Prosopis alpataco*, *Bougainvillea spinosa*, *Grindelia chiloensis*, allocation, nitrogen, phenolics, total non-structural carbohydrates.

INTRODUCTION

Water deficit is often a key factor limiting plant growth, resource allocation patterns and survival, especially in semi-arid areas subject to episodes of prolonged drought (Boyer, 1982). Changes in the quality and quantity of plant biomass, particularly those linked to the accumulation of secondary metabolites, also affect herbivore feeding behaviour and thus trophic interactions in drought-affected ecosystems (Bardgett and Wardle, 2003; Huberty and Denno, 2004; Bezemer and van Dam, 2005). Under nutrient limitation or moderate drought, surplus carbon not used in plant growth can be allocated to carbon-based secondary compounds or to storage compounds in higher plants (Koricheva et al., 1998; Estiarte and Peñuelas, 1999) while under severe drought or very low resource levels, plants should be limited in both growth and photosynthetic capability (Bryant et al., 1983; Herms and Mattson, 1992; Stamp, 2003).

The growth–differentiation balance hypothesis (GDBH) predicts how plants allocate photoassimilates to growth or differentiation-related processes (i.e. defence) over a range of environmental conditions (Herms and Mattson, 1992; Stamp, 2003). The overall pattern of defence allocation predicted by the GDBH is curvilinear along a resource gradient (e.g. water availability), peaking at intermediate levels of resource supply. Experimental tests of this hypothesis in relation to water availability have generally been performed using two or three water regimes (Wilkens et al., 1996; Gindaba et al., 2005; Villagra and Cavagnaro, 2006), and only occasionally with four or more water regimes (see Li et al., 2009; Zavala and Ravetta, 2001). However, it has been demonstrated that two levels of resource availability are insufficient to properly test the GDBH's predictions (Wilkens et al., 1996; Wilkens, 1997) and Stamp (2004) notes that a minimum of five levels of a resource is required for a rigorous test of the GDBH.

Variation in allelochemical concentrations has often been interpreted as an indication of changes in the pattern of plant allocation to defence (Iason and Hester, 1993; Ward and Young, 2002; Gowda and Palo, 2003; Matsuki et al., 2004; Read et al., 2009). For example, higher concentrations of

total phenolics (TP) in young than in mature leaves (Gowda, 1997) could be interpreted as an allocation strategy to prioritize defence of the most valuable tissues. Similarly, ontogenetic changes in defensive allocation in seedling and juvenile plants may also be an evolutionary response to herbivory at this particularly vulnerable stage of a plant's life history (Boege and Marquis 2005; Elger *et al.*, 2009). Although frequently ignored, seedling and/or juvenile herbivory has important consequences for plant species composition in mature vegetation (Kelly and Hanley, 2005; Hanley and Sykes, 2009). Given that the effects of herbivory on plant survival and recruitment are more pronounced during the seedling/juvenile phase (Grubb, 1977; Hanley *et al.*, 2004), strong selection for chemical defences in the juvenile stages may be expected (Bryant *et al.*, 1983; Elger *et al.*, 2009; Barton and Koricheva, 2010).

It should be noted, however, that a decline in concentration of defences with leaf or plant age may well be the result of a dilution effect with growth instead of a defence allocation strategy. For this reason, absolute content could be a better measure of the total amount of resources allocated to defence than the use of concentrations. Koricheva (1999) suggested that even though concentrations are relevant estimates of plant quality for herbivores, plants produce molecules (quantities) rather than concentrations of allelochemicals and therefore content per plant should be the variable of primary interest in plant allocation studies. Vector diagrams are used to interpret ontogenetic variation in the biosynthesis and concentration of plant chemicals (Koricheva, 1999; Riipi *et al.*, 2002; Brenes-Arguedas *et al.*, 2006). The advantage of graphical vector analysis is that it enables one to distinguish between cases where shifts in concentrations are due to changes in compound uptake or synthesis and where shifts are simply the result of biomass accumulation.

The most recent climate change scenarios for South America (IPCC, 2007; Núñez *et al.*, 2009) suggest that northern Patagonia will experience a 10 % decrease in total precipitation by the end of the 21st century, mainly as a consequence of reduced summer precipitation, intensifying the trend already found in the last decades of the previous century (Labraga and Villalba, 2009). In addition, temperature will rise 2·5 °C with warming in simulations increasing linearly with time during this century over southern South America (IPCC, 2007). Although native plant species naturally exhibit drought tolerance, the ecophysiological response of these species to increased drought has not been previously studied.

The objective was to test GDBH by quantifying total biomass, its partitioning, and secondary metabolite production at the whole plant level across a gradient of five water supply regimes in juvenile plants of six Patagonian species. Specifically, the following questions are addressed. (*a*) Does water availability affect biomass production and partitioning in juvenile plants of six Patagonian species? (*b*) Are observed differences in patterns of biomass allocation due to water stress or to drought-induced size differences? (*c*) How well does the GDBH predict plant allocation to secondary metabolites and other plant nutritional qualities when the study species are subjected to drought?

MATERIALS AND METHODS

Seedling establishment

Seeds of five species: *Jarava speciosa* (Trin. & Rupr.) Peñail. (Poaceae), *Prosopis alpataco* Phil. (Fabaceae), *Bougainvillea spinosa* (Cav.) Heimerl (Nyctaginaceae), *Chuquiraga erinacea* D. Don subsp. *hystrix* (Asteraceae) and *Larrea divaricata* Cav. (Zygophyllaceae) were collected from at least five plants from native populations in Chubut Province, north-eastern Patagonia, Argentina (42°39′S, 65°23′W) during the summer of 2005–2006 (November–February). These species are among the most abundant of the dominant plant growth forms of the Patagonian Monte phytogeographical district: *C. erinacea* and *L. divaricata* (evergreen shrubs), *B. spinosa* and *P. alpataco* (deciduous shrubs) and *J. speciosa* (perennial grass) (Campanella and Bertiller, 2008). Average precipitation is 235·9 mm with a high intra- and inter-annual variation and precipitation events are slightly concentrated in autumn–winter resulting in a soil profile which is wet down to 60 cm and dries slowly during spring and summer (Coronato and Bertiller, 1997). Seeds of the perennial shrub *Grindelia chiloensis* (Cornel.) Cabr. (Asteraceae) were collected from a population in Río Negro Province (39°38′S, 68°05′W). Although not present in the area where the seeds of all the other species were collected, *Grindelia chiloensis* is also native to the Monte regions of Argentina and was included for comparative purposes because it is among the most studied of Patagonian plants in terms of its responses to environmental factors (e.g. Zavala and Ravetta, 2001, 2002; Wassner and Ravetta, 2005).

Seeds were carefully sprinkled in 12-cm-diameter Petri dishes with 5 mL distilled water on top of two layers of filter papers (Whatman No.1) at room temperature. Immediately after germination seedlings were transplanted to 2·25-L pots (one seedling per pot) containing sieved soil (through a 2-mm mesh sieve) collected from the area where seeds of most species had been collected. Pots were placed randomly within a greenhouse under full sun conditions and, in order to facilitate the establishment of seedlings, they were maintained at field capacity for 1 month. Mean daily maximum/minimum temperatures during the whole experiment were 31·6 ± 0·4 °C/14·3 ± 0·3 °C.

Experimental design

When plants were one month old (November 2006) they were randomly assigned to one of five water supply regimes (13 %, 11 %, 9 %, 7 % or 5 %). To generate treatments, soil moisture content was checked daily: each pot was weighed and watered to achieve soil water content of 15 % (field capacity for this type of soil; Bisigato and Bertiller, 1999) whenever its soil moisture had dropped below the lowest level defined for the treatment (Pisani and Distel, 1998; Bisigato and Bertiller, 1999; Trillo and Fernández, 2005). Thus, treatments differed in the lowest soil water content reached by pots before re-irrigation took place (13 %, 11 %, 9 %, 7 % or 5 %; i.e. in the 5 % treatment pots were watered when soil moisture fell below 5 %). This experimental approach was considered an improvement over fixed-schedule irrigation regimes which do not take into account differences

in atmospheric demand as well as differential water-use by plants from different treatments and species (hereafter the term 'water stress' is used to refer to the experimental treatments).

Pot positions were re-randomized daily. At harvest, there were 4–15 plants per treatment depending on the species and survival (data not shown), each pot representing a single experimental unit.

Biomass assessment and chemical determinations

When plants were 150 d old (March–April 2007), all above- and below-ground biomass was harvested, obtaining the latter by carefully washing away the soil from the roots. Soon afterwards, they were oven dried at 45 °C to constant weight. Total biomass was partitioned into root, stem and leaf, and weighed. *Larrea divaricata* leaves and *G. chiloensis* leaves and stems were soaked in ethanol for 1 h to remove surface resins. Each fraction was milled prior to chemical analysis.

To test the predictions of the GDBH, TP and condensed tannins (CT) were quantified as indicators of the overall pattern of defence, since they have been widely used in herbivory studies (e.g. Häring *et al.*, 2007; Read *et al.*, 2009; Rafferty *et al.*, 2010). Also total non-structural carbohydrates (TNC) were measured because these storage compounds are an alternative to carbon-based secondary compounds for photoassimilates not used in growth. Finally, N-concentration was measured because it is a measure of the nutritional quality of plant tissues (Herms and Mattson, 1992). For TP and CT, dry powder was extracted with 50 % methanol. TPs were determined by the Folin–Ciocalteu method and CT assessed by the butanol–HCl assay (Waterman and Mole, 1994). Absorbance was measured with a Fisher spectrophotometer (S42669ND) at 740 nm and 550 nm, for TP and CT, respectively. In both cases, results are expressed in the form of milligrams of tannic acid equivalents. Nitrogen was determined by the micro-Kjedahl digestion procedure (Coombs *et al.*, 1985) and TNC evaluated by the anthrone method (Yemm and Willis, 1954); results for both were expressed as percentage of dry biomass. Where necessary, material of two or more individuals of the same species, fraction and treatment was pooled in order to obtain sufficient mass for chemical analysis.

It is important to note that terms such as allocation, partitioning and distribution are used differently in the literature; here 'dry matter partitioning' is used to indicate the absolute amount of biomass in each organ, whereas the term 'allocation' indicates the amount of biomass present in the various organs relative to total plant mass.

Statistical analysis

Where possible, data were analysed using one-way analysis of variance (ANOVA) with water supply regimes as a factor. Where significant treatment effects were apparent, *post-hoc* LSD tests were used to detect differences between treatment means. Data were first tested for homocedasticity and log-transformed when appropriate to meet this assumption. When ANOVA assumptions were not fulfilled, the nonparametric Kruskal–Wallis test was performed and multiple comparisons of mean ranks for all treatments applied.

Biomass allometric relationships between each biomass fraction and total plant weight were assessed through linear regression models, following Coleman *et al.* (1994) and Ploschuk *et al.* (2005). Partitioning coefficients were assessed as the slope of the linear regressions. Even though some models indicated some heterogeneity of the variance of the residuals, these deviations from the assumption of homogeneous variances seemed acceptable compared with the otherwise difficult interpretation of models involving variance-stabilizing transformations (Häring *et al.*, 2007).

Vector analysis

Because of the differences in total biomass of plants, a particular change in concentration or content may represent different things for each species or water supply regime. The changes in response to the different water supply regimes were plotted as a vector diagram (Haase and Rose, 1995; Koricheva, 1999). To build this diagram, mean whole-plant content of TP and CT were first estimated for each treatment by adding up the products of the concentrations and the biomass of individual fractions of a given species. Secondly, the mean concentration of TP and CT was estimated by dividing whole-plant contents by whole-plant biomass. Finally, the relative values of concentration and content were calculated using the 13 % water supply regime as the reference point. The employment of relative values made it possible to compare different photoassimilates, such as TP and CT, directly for each species. Detailed descriptions of vector analysis can be found in Haase and Rose (1995), Koricheva (1999) and Veteli *et al.* (2007).

RESULTS

Total biomass and dry matter partitioning

Water stress reduced total biomass of four species (Fig. 1). There were significant differences between total biomass produced in the slight water stress of 13 % and the most severe water stress treatment of 5 % in plants of *J. speciosa* [$H_{(4)} = 39.39$, $P < 0.0001$; non-parametric Kruskall–Wallis test], *G. chiloensis* [$F_{(4,33)} = 18.79$, $P < 0.0001$; ANOVA], *P. alpataco* [$F_{(4,46)} = 3.97$, $P = 0.0075$; ANOVA] and *B. spinosa* [$F_{(4,33)} = 8.062$, $P = 0.0001$; ANOVA]. Of these four species, only *G. chiloensis* displayed a decline in total biomass production following water stress over the intermediate (7–11 %) treatments. Total biomass of *C. erinacea* and *L. divaricata* was unaffected by water supply regime [$F_{(3,23)} = 0.83$, n.s.; $F_{(4,25)} = 1.18$, n.s.; ANOVA].

The effects of water stress on patterns of dry matter partitioning between plant structures was most pronounced between the 13 % and 5 % water treatments (Table 1). Significant differences among water treatments were found in: root and leaf of *J. speciosa* [$H_{(4)} = 35.59$, $P < 0.0001$; $H_{(4)} = 46.07$, $P < 0.0001$; non-parametric Kruskall–Wallis test]; root, leaf and stem of *G. chiloensis* [$F_{(4,33)} = 12.26$, $P < 0.0001$; $F_{(4,33)} = 17.99$, $P < 0.0001$; $F_{(4,33)} = 14.32$, $P < 0.0001$; ANOVA]; root, leaf and stem of *P. alpataco* [$F_{(4,46)} = 4.036$, $P = 0.0068$; $F_{(4,46)} = 9.03$, $P < 0.0001$;
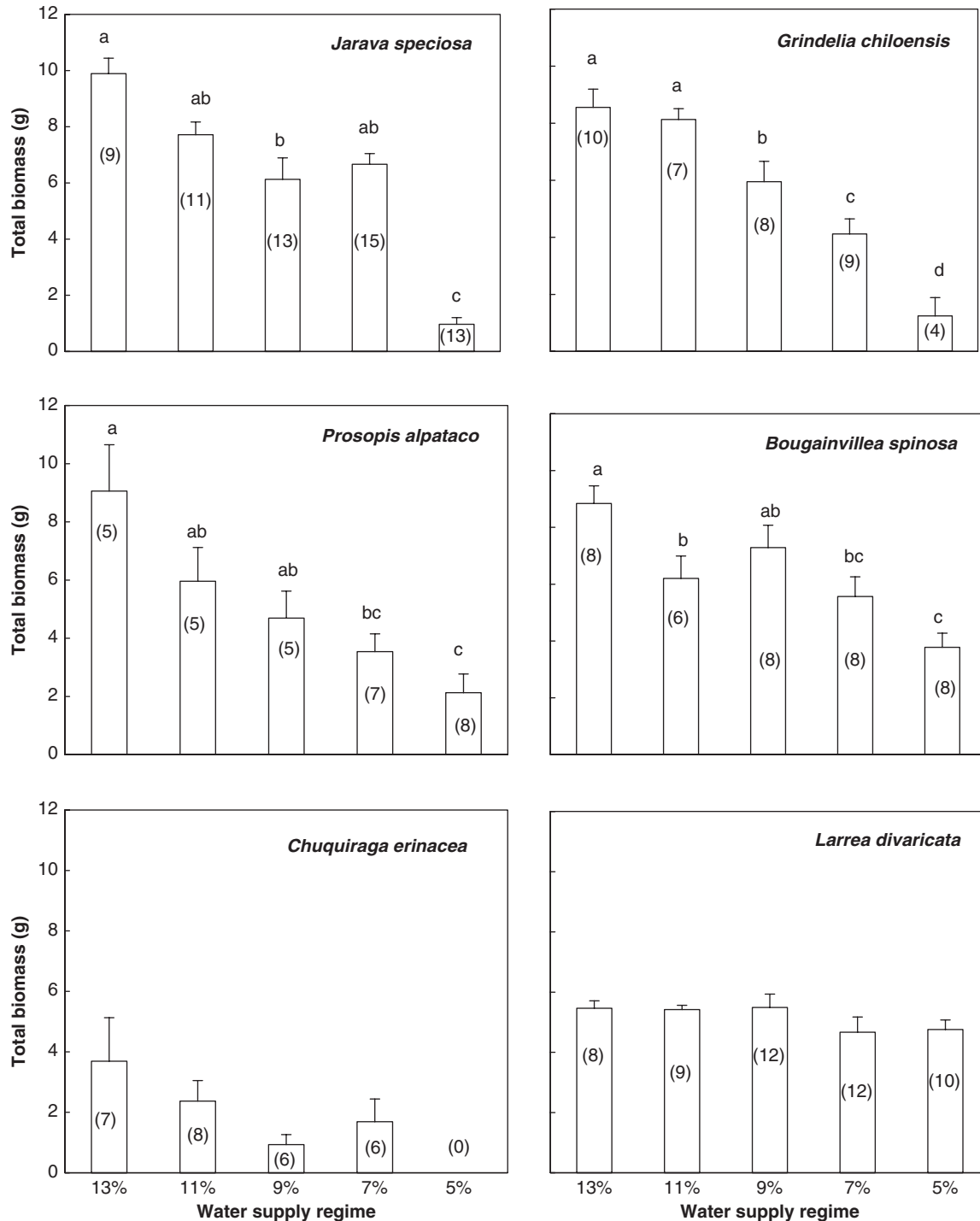
FIG. 1. Total biomass of 150-d-old *J. speciosa*, *G. chiloensis*, *P. alpataco*, *B. spinosa*, *C. erinacea* and *L. divaricata* plants in response to five water stress regimes (15 % = field capacity). Standard errors are indicated, and *n* is given in parenthesis. Different letters indicate significant ($P < 0.05$) differences across the five water supply regimes according to LSD test or multiple comparisons of mean ranks.

$F_{(4,46)} = 3.737$, $P = 0.0102$; ANOVA] and root, leaf and stem of *B. spinosa* [$F_{(4,33)} = 5.034$, $P = 0.0027$; $F_{(4,33)} = 3.274$, $P = 0.0228$; $F_{(4,33)} = 7.207$, $P = 0.0002$; ANOVA].

In contrast, *L. divaricata* plants only changed dry matter partitioning to stem in response to water availability, decreasing stem biomass when water stressed with significant differences among the 13 % water supply regime and the 7 % and 5 % treatments [$F_{(4,25)} = 3.924$, $P = 0.0131$; ANOVA; Table 1].

Thus, it is concluded that water stress exerts a significant effect on biomass accumulation and dry matter partitioning in the majority of the study species.

TABLE 1. *Dry matter partitioning, total phenolics (TP), condensed tannins (CT), total non-structural carbohydrates (TNC) and nitrogen (N) for the six species subjected to the five water supply regimes*

Water supply	Dry biomass (g)			TP (mg g^{-1} d. wt)			CT (mg g^{-1} d. wt)			TNC (%)			N (%)		
	R	S	L	R	S	L	R	S	L	R	S	L	R	S	L
J. speciosa															
13 %	5.76[a] (0.44)	–	4.14[a] (0.18)	5.01[a] (0.75)	–	21.78[n.s.] (1.56)	0.05[n.s.] (0.04)	–	0.87[n.s.] (0.13)	19.57[n.s.] (3.44)	–	16.04[n.s.] (0.59)	0.43[n.s.] (0.09)	–	0.87[a] (0.04)
11 %	4.29[b] (0.31)	–	3.43[b] (0.18)	5.90[a] (0.28)	–	21.40[n.s.] (1.93)	0.05[n.s.] (0.02)	–	0.76[b] (0.05)	17.39[n.s.] (3.18)	–	16.88[n.s.] (0.84)	0.61[n.s.] (0.05)	–	0.88[a] (0.06)
9 %	3.56[b] (0.54)	–	2.57[b] (0.25)	4.75[ab] (0.41)	–	27.54[n.s.] (3.99)	0.07[n.s.] (0.05)	–	0.86[n.s.] (0.14)	19.18[n.s.] (3.53)	–	16.35[n.s.] (1.52)	0.63[n.s.] (0.04)	–	1.24[b] (0.10)
7 %	4.01[b] (0.31)	–	2.66[bc] (0.10)	5.39[a] (0.37)	–	24.10[n.s.] (2.59)	0.07[n.s.] (0.04)	–	1.22[n.s.] (0.09)	19.19[n.s.] (3.07)	–	18.01[n.s.] (0.60)	0.62[n.s.] (0.05)	–	1.17[n.s.] (0.13)
5 %	0.57[b] (0.15)	–	0.40[c] (0.09)	3.42[b] (0.33)	–	16.58[n.s.] (1.95)	0.06[n.s.] (0.04)	–	0.92[n.s.] (0.26)	11.62[n.s.] (1.43)	–	15.09[n.s.] (0.72)	0.85[n.s.] (0.12)	–	2.29[c] (0.21)
G. chiloensis															
13 %	4.72[a] (0.49)	1.07[a] (0.09)	2.77[a] (0.17)	46.20[a] (3.65)	16.04[n.s.] (1.70)	34.32[ac] (3.01)	0.94[n.s.] (0.17)	0.24[n.s.] (0.03)	0.81[n.s.] (0.18)	13.17[n.s.] (0.45)	14.74[n.s.] (1.24)	14.37[n.s.] (0.55)	0.53[ab] (0.07)	0.49[n.s.] (0.05)	1.24[b] (0.08)
11 %	4.74[ab] (0.33)	0.92[a] (0.14)	2.47[a] (0.21)	47.36[a] (4.68)	19.62[n.s.] (1.27)	42.94[a] (4.83)	1.14[n.s.] (0.25)	0.15[n.s.] (0.03)	1.04[n.s.] (0.11)	12.97[n.s.] (0.42)	14.28[n.s.] (0.72)	15.96[n.s.] (1.11)	0.47[n.s.] (0.04)	0.52[n.s.] (0.04)	1.13[b] (0.05)
9 %	3.53[b] (0.47)	0.55[b] (0.10)	1.88[b] (0.20)	44.08[a] (1.71)	19.66[n.s.] (1.43)	40.18[a] (4.22)	1.06[n.s.] (0.07)	0.23[n.s.] (0.04)	0.79[n.s.] (0.12)	11.23[n.s.] (0.26)	13.74[n.s.] (1.10)	15.39[n.s.] (1.17)	0.56[ab] (0.05)	0.72[ab] (0.12)	1.31[a] (0.15)
7 %	2.22[c] (0.32)	0.37[b] (0.07)	1.54[b] (0.16)	24.76[b] (4.43)	14.86[n.s.] (1.17)	20.80[b] (2.19)	0.55[n.s.] (0.12)	0.16[n.s.] (0.13)	0.81[n.s.] (0.05)	19.78[n.s.] (3.64)	13.03[n.s.] (0.41)	13.09[n.s.] (1.00)	0.81[n.s.] (0.04)	1.04[b] (0.20)	2.00[b] (0.20)
5 %	0.73[d] (0.40)	0.08[c] (0.05)	0.43[c] (0.20)	21.15[a] (5.75)	*14.73[n.s.]*	*18.30[bc]*	*0.73[n.s.]* (0.12)	–	0.55[n.s.] (0.05)	23.99[n.s.] (1.52)	*12.19[n.s.]*	*12.97[n.s.]*	1.20[ab] (0.59)	*2.22[c]*	*2.03[b]*
P. alpataco															
13 %	4.59[a] (0.87)	2.99[a] (0.60)	0.73[a] (0.11)	21.40[n.s.] (2.02)	11.22[n.s.] (0.93)	25.04[a] (0.80)	18.20[n.s.] (1.23)	0.61[n.s.] (0.06)	3.22[n.s.] (1.05)	37.43[n.s.] (4.86)	26.96[n.s.] (1.50)	18.24[n.s.] (1.92)	1.36[n.s.] (0.12)	1.19[n.s.] (0.10)	2.43[n.s.] (0.18)
11 %	2.87[ab] (0.56)	2.00[ab] (0.43)	0.54[ab] (0.10)	22.44[n.s.] (4.09)	11.44[n.s.] (1.03)	25.00[a] (1.59)	17.36[n.s.] (2.80)	0.68[n.s.] (0.12)	2.34[ab] (0.53)	44.69[n.s.] (4.37)	28.74[n.s.] (2.14)	20.77[n.s.] (3.95)	1.19[n.s.] (0.08)	1.52[n.s.] (0.16)	2.73[n.s.] (0.24)
9 %	2.47[ab] (0.54)	1.55[bc] (0.31)	0.33[bc] (0.07)	17.94[n.s.] (1.80)	11.27[n.s.] (0.31)	30.37[bc] (1.62)	12.93[n.s.] (1.46)	1.00[n.s.] (0.20)	1.11[n.s.] (0.38)	46.82[n.s.] (3.85)	29.78[n.s.] (2.03)	21.49[n.s.] (2.05)	1.09[n.s.] (0.12)	1.38[n.s.] (0.18)	2.27[n.s.] (0.27)
7 %	1.74[bc] (0.29)	1.36[bc] (0.27)	0.21[cd] (0.04)	16.78[n.s.] (2.19)	11.78[n.s.] (0.93)	36.05[b] (1.35)	14.61[n.s.] (1.54)	0.97[n.s.] (0.19)	2.25[c] (0.02)	39.31[n.s.] (7.06)	28.29[n.s.] (1.48)	15.24[n.s.] (1.60)	1.30[n.s.] (0.12)	0.98[n.s.] (0.06)	3.00[n.s.] (0.35)
5 %	1.08[c] (0.32)	0.77[c] (0.24)	0.13[d] (0.05)	16.10[n.s.] (1.71)	11.04[n.s.] (0.83)	*27.90[ac]*	13.27[n.s.] (0.99)	0.82[n.s.] (0.13)	*2.64[n.s.]*	38.85[n.s.] (2.95)	33.23[n.s.] (3.50)	*20.93[n.s.]*	1.22[n.s.] (0.11)	1.41[n.s.] (0.31)	*2.46[n.s.]*
B. spinosa															
13 %	3.49[a] (0.31)	2.89[a] (0.48)	1.23[a] (0.06)	6.60[n.s.] (0.74)	25.74[n.s.] (5.24)	22.94[n.s.] (2.66)	0.28[n.s.] (0.11)	8.49[ac] (0.93)	1.19[n.s.] (0.15)	10.72[ac] (0.84)	11.32[n.s.] (0.83)	8.27[n.s.] (1.84)	0.67[n.s.] (0.02)	0.65[n.s.] (0.03)	1.13[ab] (0.06)
11 %	2.41[bc] (0.31)	2.00[ab] (0.54)	0.90[abc] (0.08)	6.78[n.s.] (1.09)	23.55[n.s.] (4.82)	22.20[n.s.] (4.24)	0.40[n.s.] (0.12)	5.04[b] (0.29)	1.62[n.s.] (0.44)	13.09[abc] (1.10)	12.95[n.s.] (1.25)	6.37[n.s.] (0.43)	0.74[n.s.] (0.10)	0.70[ab] (0.12)	1.46[bc] (0.15)
9 %	2.61[b] (0.35)	2.60[a] (0.27)	1.04[ab] (0.16)	8.58[n.s.] (0.63)	28.72[n.s.] (1.92)	27.74[n.s.] (5.14)	0.53[n.s.] (0.08)	7.14[ab] (0.66)	1.20[n.s.] (0.26)	13.71[a] (0.97)	11.66[n.s.] (0.76)	5.75[n.s.] (0.37)	0.68[n.s.] (0.05)	0.54[n.s.] (0.03)	0.80[a] (0.13)
7 %	2.33[bc] (0.35)	1.53[b] (0.22)	0.85[bc] (0.11)	7.08[n.s.] (0.87)	29.36[n.s.] (3.41)	22.72[n.s.] (1.44)	0.77[n.s.] (0.22)	10.24[c] (1.05)	1.56[b] (0.18)	15.84[b] (1.53)	13.65[n.s.] (0.50)	6.47[n.s.] (0.26)	0.64[n.s.] (0.03)	0.64[b] (0.01)	1.22[b] (0.12)
5 %	1.59[a] (0.21)	0.77[c] (0.12)	0.71[c] (0.10)	7.84[n.s.] (0.49)	29.16[n.s.] (1.26)	18.32[n.s.] (3.68)	0.52[n.s.] (0.15)	8.12[ac] (0.69)	1.35[n.s.] (0.11)	9.73[c] (1.32)	11.28[n.s.] (0.93)	6.62[n.s.] (0.63)	0.81[n.s.] (0.03)	0.87[b] (0.11)	1.76[n.s.] (0.10)
C. erinacea															
13 %	0.85[n.s.] (0.32)	0.64[n.s.] (0.28)	1.10[n.s.] (0.42)	24.85[n.s.] (2.73)	12.23[n.s.] (2.56)	17.38[n.s.] (1.95)	0.19[n.s.] (0.14)	0.01[n.s.] (0.01)	0.78[n.s.] (0.10)	11.15[n.s.] (2.11)	9.78[n.s.] (0.16)	1.28[n.s.] (0.21)	0.19[n.s.] (0.14)	0.72[n.s.] (0.12)	0.78[n.s.] (0.10)
11 %	0.51[n.s.] (0.11)	0.30[n.s.] (0.12)	0.78[n.s.] (0.23)	18.25[n.s.] (3.87)	9.15[n.s.] (0.45)	16.90[n.s.] (2.77)	0.07[n.s.] (0.04)	0.03[n.s.] (0.02)	0.91[n.s.] (0.17)	15.39[n.s.] (3.80)	10.50[n.s.] (0.78)	1.38[n.s.] (0.14)	0.07[n.s.] (0.04)	0.65[n.s.] (0.06)	0.91[n.s.] (0.17)
9 %	0.31[n.s.] (0.11)	0.08[n.s.] (0.04)	0.27[n.s.] (0.09)	12.75[n.s.] (1.15)	*9.70[n.s.]*	20.20[n.s.]	0.09[n.s.] (0.06)	*0.00[n.s.]*	*0.75[n.s.]*	22.00[n.s.] (3.59)	–	1.35[n.s.] (0.09)	0.09[n.s.] (0.06)	*0.65[n.s.]*	*0.75[n.s.]*
7 %	0.54[n.s.] (0.22)	0.20[n.s.] (0.13)	0.48[n.s.] (0.21)	14.90[n.s.] (1.50)	9.65[n.s.] (0.95)	18.30[n.s.] (4.80)	0.05[n.s.] (0.05)	0.00[n.s.] (0.00)	1.10[n.s.] (0.39)	24.61[n.s.] (0.99)	12.68[n.s.] (0.80)	1.22[n.s.] (0.26)	0.05[n.s.] (0.05)	0.83[n.s.] (0.29)	1.10[n.s.] (0.39)
5 %	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
L. divaricata															
13 %	3.19[n.s.] (0.22)	1.35[a] (0.03)	0.93[n.s.] (0.06)	12.30[a] (0.85)	68.38[n.s.] (13.22)	72.08[n.s.] (7.33)	5.04[a] (1.64)	23.38[n.s.] (4.79)	17.95[n.s.] (3.04)	9.25[n.s.] (1.03)	13.35[n.s.] (1.24)	14.86[n.s.] (0.88)	0.73[n.s.] (0.04)	23.38[n.s.] (4.79)	17.95[n.s.] (3.04)
11 %	3.51[n.s.] (0.09)	1.04[ab] (0.08)	0.87[n.s.] (0.06)	9.25[b] (0.99)	51.05[n.s.] (3.19)	47.88[n.s.] (6.89)	3.87[ab] (1.15)	22.67[n.s.] (3.21)	32.63[n.s.] (10.07)	7.80[n.s.] (1.01)	13.79[n.s.] (1.37)	16.19[ab] (1.63)	0.68[n.s.] (0.01)	22.67[n.s.] (3.21)	32.63[n.s.] (10.07)
9 %	3.44[n.s.] (0.37)	1.11[ab] (0.13)	0.94[n.s.] (0.03)	8.28[bc] (0.67)	48.88[n.s.] (12.84)	52.34[n.s.] (12.59)	1.50[bc] (0.29)	23.97[n.s.] (6.93)	19.57[n.s.] (1.18)	7.71[n.s.] (0.89)	14.36[n.s.] (0.88)	15.66[n.s.] (1.11)	0.62[n.s.] (0.05)	23.97[n.s.] (6.93)	19.57[n.s.] (1.18)
7 %	2.88[n.s.] (0.35)	0.91[b] (0.13)	0.87[n.s.] (0.07)	6.18[c] (0.52)	62.88[n.s.] (17.19)	53.16[n.s.] (9.19)	0.60[c] (0.15)	12.99[n.s.] (2.46)	12.25[n.s.] (2.72)	8.37[n.s.] (2.18)	15.59[n.s.] (0.63)	14.99[n.s.] (1.42)	0.64[n.s.] (0.06)	12.99[ab] (2.46)	12.25[n.s.] (2.72)
5 %	2.99[n.s.] (0.29)	0.85[b] (0.07)	0.92[n.s.] (0.05)	7.15[bc] (1.20)	77.92[n.s.] (11.28)	43.33[n.s.] (7.76)	0.76[c] (0.46)	13.22[n.s.] (3.52)	13.05[n.s.] (2.27)	6.15[n.s.] (0.46)	15.40[n.s.] (0.30)	17.67[n.s.] (1.43)	0.68[n.s.] (0.04)	13.22[b] (3.52)	13.05[n.s.] (2.27)

R, root; S, stem; L, leaf.

Values given are mean ± s.e. (in parenthesis). –, No data. Note that *J. speciosa* is a grass and consequently there are no data for the stem, and all individuals of *C. erinacea* in the 5 % water supply regime died. Pooled samples are shown in italics (see Materials and methods).

Different letters indicate significant differences among treatments (ANOVA $P < 0.05$; Kruskall–Wallis $P < 0.05$) for each organ and species according to LSD test ($P < 0.05$); n.s. = not significant (ANOVA $P > 0.05$; Kruskall–Wallis $P > 0.05$).

Allometric comparisons

No departures from linearity were found for any allometric relationship between organ biomass fractions and total plant biomass across the five water stress regimes (Fig. 2; Supplementary data, available online); i.e. dry matter partitioning increased linearly with plant size rather than in relation to ontogenetic shifts in plant defence requirements. The allometric relationship between root, leaf or stem and total
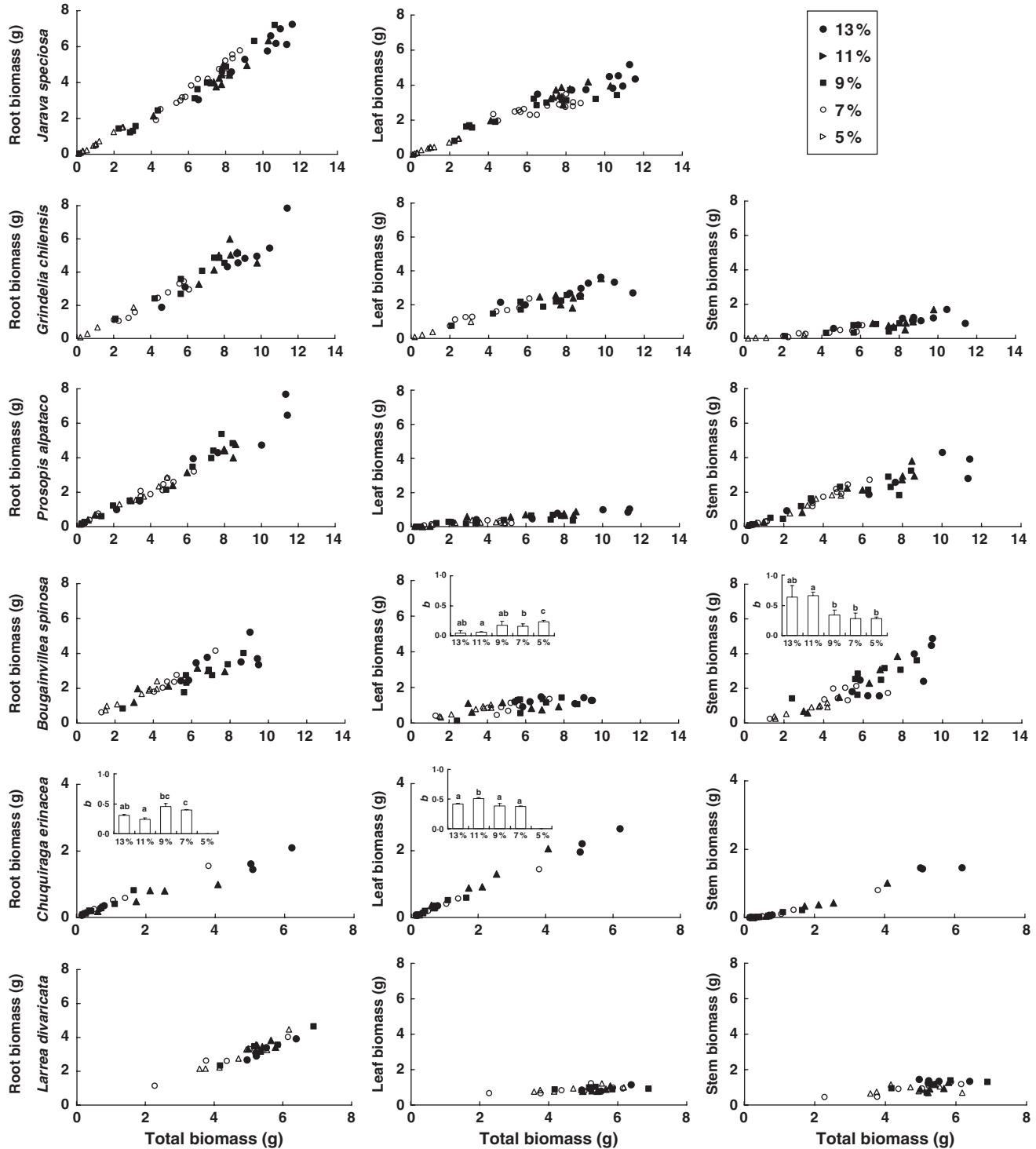
FIG. 2. Allometric relationships between organ biomass fractions and total plant biomass for *J. speciosa*, *G. chiloensis*, *P. alpataco*, *B. spinosa*, *C. erinacea* and *L. divaricata* plants grown in five water stress regimes (15 % = field capacity) until 150 d old. Different symbols indicate the five water supply regimes. One point denotes one plant. When regression coefficients from linear-fitting slopes (*b*) differed among treatments, they are shown in the top left corner of the corresponding panel. Different letters indicate significant (*P* < 0·05) differences between slopes in distinct treatments for each biomass fraction.

biomass showed a similar allocation irrespective of treatments in plants of *J. speciosa* [root: $F_{(4,51)} = 1·414$; n.s.; leaf: $F_{(4,51)} = 1·414$, n.s.; ANCOVA], *G. chiloensis* [root: $F_{(4,28)} = 0·511$, n.s.; leaf: $F_{(4,28)} = 0·558$, n.s.; stem: $F_{(4,28)} = 0·721$, n.s.; ANCOVA], *P. alpataco* [root: $F_{(4,41)} = 0·897$, n.s.; leaf: $F_{(4,41)} = 0·933$, n.s.; stem: $F_{(4,41)} = 0·455$, n.s.; ANCOVA] and *L. divaricata* [root: $F_{(4,20)} = 1·263$, n.s.; leaf: $F_{(4,20)} = 1·378$, n.s.; stem: $F_{(4,20)} = 2·054$, n.s.; ANCOVA] (Fig. 2). Thus it is concluded that the partitioning coefficients (assessed as the slope of those relationships; data not shown) were unrelated to different water supply regimes.

Biomass allocation between leaf and stem in *B. spinosa*, however, was affected by water stress [leaf: $F_{(4,28)} = 3·392$, $P = 0·0220$; stem: $F_{(4,28)} = 2·799$, $P = 0·0450$; ANCOVA]. Plants in the low water supply regime (5 %) allocated more biomass towards leaf than those growing in all the other treatments (Fig. 2; partitioning coefficients in the top left corner of *B. spinosa* leaf). However, the higher partitioning coefficients in the 5 % water supply regime were produced at the expense of a lower allocation to stem (Fig. 2; partitioning coefficients in the top left corner of *B. spinosa* stem). No significant differences were apparent for biomass partitioning coefficients to root [$F_{(4,28)} = 1·192$, n.s.; ANCOVA].

Biomass allocation between root and leaf in *C. erinacea* was also affected by water stress treatment [root: $F_{(3,19)} = 8·031$, $P = 0·0011$; leaf: $F_{(3,19)} = 1·441$, $P = 0·0007$; ANCOVA]. The allometric relationship indicated higher biomass allocation to root (i.e. higher partitioning coefficients) at 9 % and 7 % water supply regimes than at 11 % (Fig. 2; partitioning coefficients in the top left corner of *C. erinacea* root). The higher partitioning coefficients for root in those treatments were also produced at the expense of reduced allocation to leaf biomass (Fig. 2; partitioning coefficients in the right corner of *C. erinacea* leaf); i.e. partitioning coefficients for leaf were lower in the 13 %, 9 % and 7 % water supply regime in comparison with the 11 % water regime. Biomass allocation to stem was unaffected by water stress [$F_{(3,19)} = 2·374$, n.s.; ANCOVA].

Therefore, allometric analyses revealed that four of the species did not alter allocation patterns in response to water availability; i.e. when compared at the same total biomass, *J. speciosa*, *G. chiloensis*, *P. alpataco* and *L. divaricata* in different water supply regimes had comparable root, leaf and stem biomass.

Water stress and secondary metabolite accumulation

Aside from the significantly lower TP concentration in the 5 % compared with 13 % water treatments in roots of *J. speciosa*, *G. chiloensis* and *L. divaricata* [$F_{(4,20)} = 3·539$, $P = 0·0243$; $F_{(4,17)} = 8·774$, $P = 0·0004$ and $F_{(4,20)} = 6·719$, $P = 0·0013$; ANOVA], TP and CT concentrations varied little according to plant fraction or water regime (Table 1). Differences were apparent in TP concentration in *G. chiloensis* leaves, where the 11 % and 9 % treatments exhibited higher concentrations than 7 % and 5 % treatments [$F_{(4,17)} = 4·975$, $P = 0·0076$; ANOVA]. Similarly, for *P. alpataco* significant differences in TP concentration in leaves were found among the 13 % and 7 % treatments, but with the highest concentration in leaves of plants grown at

7 % water supply [$F_{(4,11)} = 7·728$, $P = 0·0032$; ANOVA]. Stem TP concentration was unaffected by water stress for any species (Table 1). Variation in CT concentrations was low; root CT concentration in *L. divaricata* decreased steadily with water stress [$F_{(4,20)} = 6·736$, $P = 0·0013$; ANOVA], while for *B. spinosa* stem CT concentration was higher at 7 % than at 11 % water supply regime [$F_{(4,19)} = 5·273$, $P = 0·0049$; ANOVA; Table 1].

N and TNC showed little variation in relation to water supply regime (Table 1). Where significant differences in N content were apparent, these were explained by an increase in the more stressing water treatments [e.g. *J. speciosa* leaf: $F_{(4,20)} = 21·445$, $P < 0·0001$; ANOVA; *G. chiloensis* root, leaf and stem: $H_{(4)} = 12·535$, $P = 0·0138$; non-parametric Kruskall–Wallis test; $F_{(4,17)} = 6·428$, $P = 0·0024$; $F_{(4,17)} = 7·55$, $P = 0·0010$; ANOVA; *B. spinosa* leaf and stem: $F_{(4,19)} = 10·351$, $P = 0·0001$; $F_{(4,19)} = 2·908$, $P = 0·0492$; ANOVA; and *L. divaricata* stem: $F_{(4,20)} = 4·252$, $P = 0·0118$; ANOVA]. For all these species and fractions, significant differences were found between the 13 % and 5 % water treatments. TNC did not vary in response to water regimes with the sole exception of *B. spinosa* root [$F_{(4,19)} = 4·345$, $P = 0·0115$; ANOVA]; concentration increasing steadily from the 13 % to 7 % water treatment, and then decreasing at 5 % (Table 1).

At the whole plant level, graphical vector analysis was used to visualize the allocation patterns in total phenolics and condensed tannins (Fig. 3). Vertical changes represent passive dilution (decrease) or concentration (increase) due to changes in total biomass alone. Horizontal changes represent biosynthesis and accumulation (increase) or transformation or translocation (decrease).

According to the GDBH, an enhanced allocation to TP or CT at the expense of a lower biomass production with water stress would be expected, since water deficit would limit growth more than photosynthesis. In contrast, severely water-stressed plants in this study reduced allocation to TP and CT with respect to the 13 % water supply regime. Only *G. chiloensis* increased both TP and CT contents in the 11 % water supply regime and decreased them in all the other treatments, as predicted by the GDBH. This increase in contents of TP and CT in *G. chiloensis* was accompanied by an increase in concentrations and a reduction in biomass (note that concentrations increased more than contents) (Fig. 3).

DISCUSSION

Artificially imposed drought reduced total plant biomass in four of the six species studied, a result supporting previous work showing a detrimental effect of water stress on Patagonian plant species (Zavala and Ravetta, 2001; Villagra and Cavagnaro, 2006; Yahdjian and Sala, 2006). These observations strongly suggest that Patagonian Monte vegetation will be severely impacted by predicted reductions in annual precipitation in the region as a consequence of anthropogenic climate change (IPCC, 2007). However, it is also noted that field studies are required to assess more directly how a combination of increased temperature, elevated CO_2 and drought would simultaneously influence plant growth in the region.
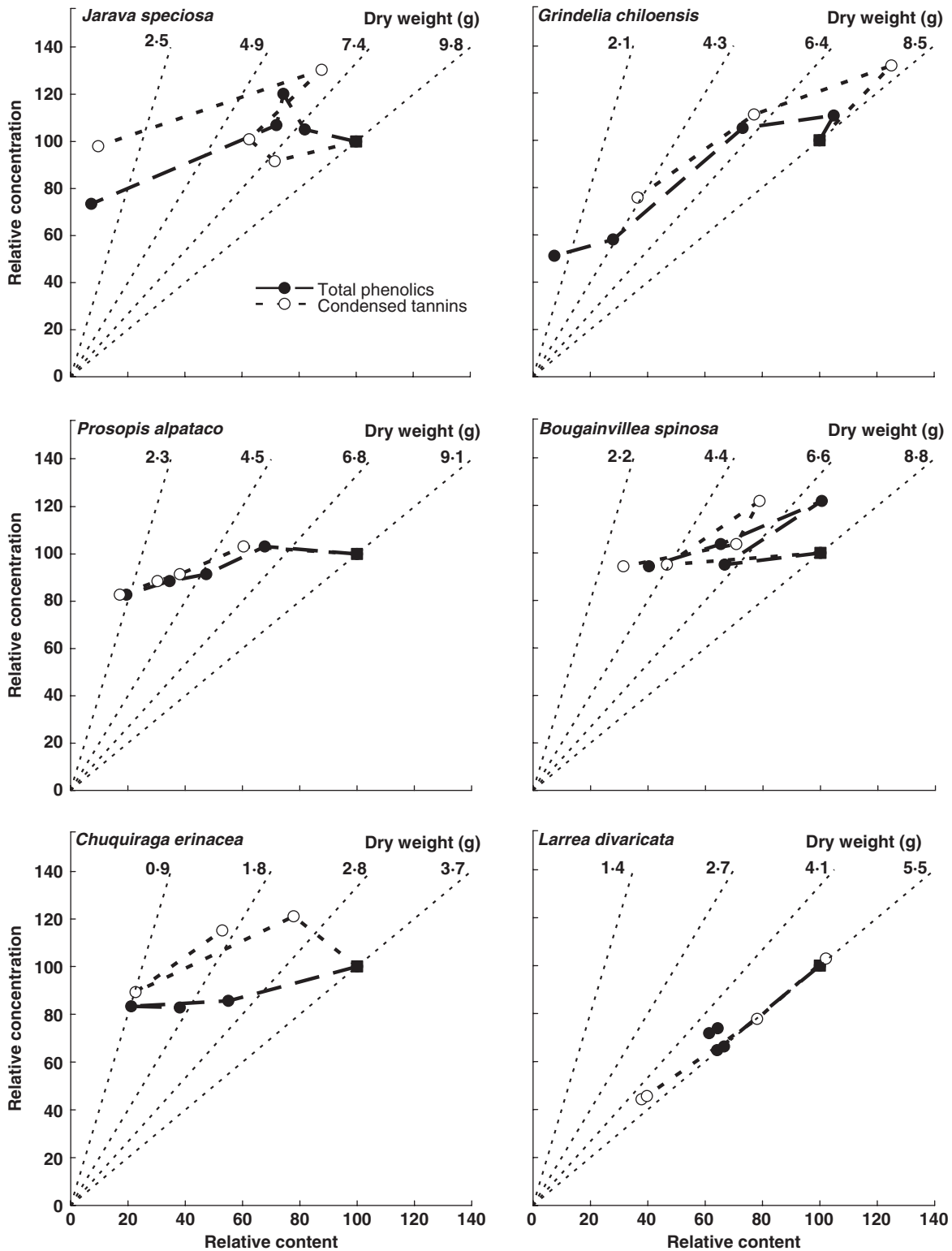
FIG. 3. Graphical vector analysis showing the different accumulation of total phenolics and condensed tannins, as indicated, for *J. speciosa*, *G. chiloensis*, *P. alpataco*, *B. spinosa*, *C. erinacea* and *L. divaricata* plants grown in five water stress regimes (15 % =field capacity) until 150 d old. For each compound, relative concentration (*y*-axis) is plotted against corresponding relative content (*x*-axis) of whole plants. Diagonal dotted lines correspond to the mean total biomass (dry weight) of whole plants. Values are changes relative to the 13 % water supply regime (black square). Dotted lines connecting circles indicate the changes from the 13 % water supply regime to the following treatment (11 % water supply regime) and so on.

In addition to total biomass, dry matter partitioning to root, leaf and stem also changed as a response to drought stress in four of the study species, although allometric relationships between root, leaf or stem and total biomass showed no differences in allocation for these species (i.e. *J. speciosa*, *G. chiloensis*, *P. alpataco* and *L. divaricata*). However, for *B. spinosa* (deciduous shrub), higher allocation to leaves was apparent at the expense of reduced allocation to stems; while in *C. erinacea* (evergreen shrub), higher allocation to roots was produced at the expense of reduced allocation to leaves. Consequently, allocation patterns did not differ between different plant life forms, since neither *P. alpataco* (deciduous shrub) nor *L. divaricata* (evergreen shrub) showed changes in biomass allocation. These results support a previous study evaluating phenology, leaf traits and leaf litterfall in Patagonian Monte species, which reported inter-specific differences but high overlap among life forms (Campanella and Bertiller, 2008).

According to the GDBH, growth reduction by means of water stress can be expected to increase the content of secondary compounds such as total phenolics or condensed tannins. However, the GDBH does not take into account alternative competing sinks for carbon such as storage (Estiarte and Peñuelas, 1999; Stamp, 2003). This fact justifies their evaluation in this study, in the form of TNC. Contrary to expectations, no clear pattern in the production of total phenolics or condensed tannins in root, leaf and stem emerged in response to the water supply regimes, supporting the meta-analysis of Koricheva *et al.* (1998) which reported that drought stress had no significant effect on carbon-based secondary compounds. Furthermore, the relative proportion of TNC did not vary in a consistent manner; only in the case of N was there a slight tendency in some of the species (*J. speciosa*, *G. chiloensis*, *B. spinosa* and *L. divaricata*) towards an increase in N with water stress.

It has been proposed that tests of the predictions derived from the GDBH should focus on whole plants instead of particular fractions (Stamp, 2004), in particular because whole plant analysis facilitates a clear emphasis on resource trade-offs between defence and growth (Häring *et al.*, 2007). In such cases, vector analysis can be used to interpret environmental changes in concentrations of secondary defence metabolites and it is particularly valuable for comparing the effects of different resource stress treatments as is the case here (Haase and Rose, 1995). Juvenile *Grindelia chiloensis* plants showed increased TP and CT content in the 11 % water supply regime in comparison with the 13 % water supply regime, a result in agreement with predictions derived from the GDBH. For all the other species studied, TP and CT content and concentrations diminished with water stress. Therefore, in general, the present findings do not support the GDBH prediction that defence allocation is curvilinear, peaking at intermediate levels along the water supply gradient.

The theory of a functional equilibrium states that plants shift allocation towards roots at a low level of below-ground resources, such as nutrients and water (Poorter and Nagel, 2000). In the present experiment, *J. speciosa*, *G. chiloensis*, *P. alpataco* and *B. spinosa* plants decreased dry matter partitioning to roots in response to the water supply regime of 5 % soil moisture content. However, following allometric analysis differences in dry matter partitioning to roots for those species disappeared because these were due to inherent size differences and not to water treatment *per se*. Water stress only increased biomass allocation to roots in *C. erinacea* but at the expense of reduced allocation to leaves. A reason for this general lack of response could be that species adapted to low-nutrient habitats have a lower phenotypic plasticity in the root : shoot ratio than rapidly growing species from high-nutrient habitats (Chapin, 1980). Moreover, plant responses to water scarcity are complex, involving adaptive changes and/or deleterious effects and, under field conditions, are synergistically or antagonistically modified by the superimposition of other stresses such as high temperature or light, extreme climatic events and/or herbivory (Chaves *et al.*, 2002).

This study focused on juvenile plants because the regeneration phase is pivotal particularly to our understanding of plant recruitment (Hanley *et al.*, 2004). The results are consistent with a previous study employing a dynamic modelling approach on apple, beech and spruce saplings (Gayler *et al.*, 2008) where growth processes had priority over allocation towards secondary compounds and where N deficiency had stronger consequences for growth-related rather than defence-related secondary metabolism. Nonetheless, in order to understand the ontogeny of plant defence, further research is needed on allocation patterns at different developmental stages such as seedling, juvenile and mature individuals (Boege and Marquis, 2005; Hanley *et al.*, 2007; Barton and Koricheva, 2010).

In summary, this study shows that water stress significantly limits growth-related processes resulting in reduced total biomass for some species. However, and contrary to the predictions of the GDBH, defence-related secondary metabolism was little affected by drought. While observed plasticity in root/shoot partitioning with plant age (Gedroc *et al.*, 1996) limits extrapolation of the results to con-specific adults, nonetheless this study shows that reductions in juvenile growth could be expected for some species in the warmer and drier conditions projected for Patagonia in the coming decades (IPCC, 2007), with potentially important consequences for the regeneration of important plant species in this region.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjouanls.org and provide the linear regression coefficients associated with the regression lines in Fig. 2 for each plant fraction and species.

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