

# Counteractive biomass allocation responses to drought and damage in the perennial herb *Convolvulus demissus*

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**Abstract** Herbivory and water shortage are key ecological factors affecting plant performance. While plant compensatory responses to herbivory include reallocation of biomass from below-ground to above-ground structures, plant responses to reduced soil moisture involve increased biomass allocation to roots and a reduction in the number and size of leaves. In a greenhouse study we evaluated the effects of experimental drought and leaf damage on biomass allocation in *Convolvulus demissus* (Convolvulaceae), a perennial herb distributed in central Chile, where it experiences summer drought typical of Mediterranean ecosystems and defoliation by leaf beetles and livestock. The number of leaves and internode length were unaffected by the experimental treatments. The rest of plant traits showed interaction of effects. We detected that drought counteracted some plant responses to damage. Thus, only in the control watering environment was it observed that damaged plants produced more stems, even after correcting for main stem length (index of architecture). In the cases of shoot : root ratio, relative shoot biomass and relative root biomass we found that the damage treatment counteracted plant responses to drought. Thus, while undamaged plants under water shortage showed a significant increase in root relative biomass and a significant reduction in both shoot : root ratio and relative shoot biomass, none of these responses to drought was observed in damaged plants. Total plant biomass increased in response to simulated herbivory, apparently due to greater shoot size, and in response to drought, presumably due to greater root size. However, damaged plants under experimental drought had the same total biomass as control plants. Overall, our results showed counteractive biomass allocation responses to drought and damage in *C. demissus*. Further research must address the fitness consequences under field conditions of the patterns found. This would be of particular importance because both current and expected climatic trends for central Chile indicate increased aridity.

**Key words:** biomass allocation, compensation, drought, functional response, herbivory.

## INTRODUCTION

Herbivory and water shortage are key ecological factors affecting plant performance and survival in natural populations (Marquis 1992; Hawkes & Sullivan 2001; Holmgren *et al.* 2006). Plants have evolved mechanisms to tolerate damage by herbivores, that is, to reduce its negative effect on fitness, via compensatory responses (Rosenthal & Kotanen 1994; Strauss & Agrawal 1999; Stowe *et al.* 2000; Núñez-Farfán *et al.* 2007). Compensatory responses to damage include reallocation of biomass from below-ground to above-ground structures and activation of dormant meristems that increase branching, among other mechanisms (Strauss & Agrawal 1999; Hochwender *et al.* 2000; Tiffin 2000). Plant functional responses to decreased water availability, on the other hand, often

involve increased biomass allocation to roots and a reduction in the number and size of leaves (Schulze 1986; Sultan & Bazzaz 1993; Grace 1997).

Several studies show that tolerance of damage is limited by reduced water availability (Poiani & Pozo 1986; Fahnestock & Detling 1999; Hawkes & Sullivan 2001; Levine & Paige 2004; Acuña-Rodríguez *et al.* 2006; Wise & Abrahamson 2007; González *et al.* 2008; Atala & Gianoli 2009), but results are seldom discussed considering the interplay between plant functional responses to herbivory and to water stress (Valladares *et al.* 2007). On one hand, some compensatory responses to herbivory, such as increased resource allocation to shoots (Mabry & Wayne 1997; Huhta *et al.* 2000) to replace eaten biomass, counteract plant functional responses to drought, such as increased biomass allocation to roots (Blum 1996; Gianoli & González-Teuber 2005) to enhance water uptake. On the other hand, shoot damage may be beneficial to plant water economy during drought

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stress (Georgiadis *et al.* 1989) because the reduction of leaf area caused by herbivory coincides with a typical response of plants to diminish drought stress: the reduction of transpiring surface (Grace 1997).

In the present greenhouse study we evaluated the effects of experimental drought and leaf damage on biomass allocation in the perennial herb *Convolvulus demissus* (Convolvulaceae), which is endemic to the Andean slopes of central Chile and Argentina. This species is distributed in regions with temperate Mediterranean climate, with a distinctive drought during summer (Di Castri & Hajek 1976; Arroyo *et al.* 1981), which is the growth season for *C. demissus* in the high-mountain habitats where populations are found. In these populations, *C. demissus* individuals often suffer partial or total defoliation by leaf beetles and livestock (Gianoli *et al.*, pers. obs., 2004–2006). Earlier studies have reported that *C. demissus* shows phenotypic responses to drought (Quezada & Gianoli 2006) and shade (González & Gianoli 2004) via changes in physiological, morphological and allocation traits. Specifically, we investigated biomass allocation responses to drought and leaf damage considering the functional demands exerted by each factor. The three possible outcomes of the interplay between main effects – namely additivity, synergism or interference (Sokal & Rohlf 1995) – will be useful to estimate the relative importance of each of these ecological factors for the study species and to foresee how it would respond in a scenario of environmental changes.

## METHODS

*Convolvulus demissus* Choisy (Convolvulaceae) is a perennial herb native to Chile and Argentina. In Chile it grows in the Andean slopes between 29°S and 35°S, at altitudes between 1200 and 2700 m (O'Donnell 1957; Herbarium Universidad de Concepción (CONC)). This species grows prostrate, stems are numerous and short (between 0.1 and 0.5 m long), and it is only dispersed by seeds. *Convolvulus demissus* does not produce flowers in the earlier stages of development, which are very susceptible to drought (Gianoli *et al.* 2009). Shoot biomass could be a good estimator of reproductive output in *C. demissus* because it displays small shoots together with a great load of reproductive buds per unit of shoot mass; the ratio of reproductive/vegetative biomass in mature plants may be as high as 0.8 (Gianoli, pers. obs., 2005). Plants used in this experiment were grown from seeds collected in the Andean slopes of Santiago, Chile (33°S) at altitudes between 1900 and 2400 m. The climate in the site of origin is Mediterranean, with a cold winter season with rain and snowfall, and a dry and warm summer season (Di Castri & Hajek 1976; Arroyo *et al.* 1981).

Collected seeds ( $n = 300$ ) were scarified by immersion in concentrated  $H_2SO_4$  for 30 min and then washed in running tap water for 5 min. After this procedure seeds were placed in boxes with moistened filter paper to induce germination and kept 7 days in a dark room at 19°C. When cotyledons were

fully evident, 120 seedlings were transplanted to plastic bags (2 L) filled with commercial potting soil, and grown in a greenhouse located in the Universidad de Concepción campus (Concepción, central Chile) at natural environmental conditions (Photosynthetically Active Radiation at noon about  $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ , 23°C average temperature). After 4 weeks, experimental procedures were applied.

Plants were grouped into two watering treatments: Control and Drought. Control and Drought plants were watered to field capacity every 3 and 10 days, respectively. Half of the plants from each of the watering treatments were subjected to simulated herbivory (Damage group, 50% defoliation) just after the beginning of the experiment. We used scissors to remove 50% of leaf area of all leaves, which were cut along the mid-vein. This level of damage corresponds roughly to the upper levels of defoliation found in the field (Suárez & Gianoli, pers. obs., 2004–2006). Undamaged plants were left intact and had no sign of herbivory at the end of the experiment. There were four experimental groups ( $n = 30$  seedlings per group) after the combination of control/drought conditions and damaged/undamaged treatments. Plants from the different treatments had similar initial size ( $P > 0.63$ , one-way ANOVA, data not shown).

Two months after treatments were initiated, the following traits were determined for each plant: number of stems, number of leaves, internode length, index of architecture (number of stems/main stem length), shoot : root biomass ratio, the relative shoot and root biomass (shoot/total biomass and root/total biomass) and total plant biomass. Measured variables were analysed by a two-way ANOVA (fixed factors: Damage and Water). To evaluate plant responses to drought and damage both separately and jointly, attention was focused on comparisons of means among groups using a Tukey test. These *a posteriori* analyses were only conducted when both Damage and Water effects were statistically significant or when the Damage–Water interaction was significant in the two-way ANOVA. Analyses were performed using STATISTICA.

## RESULTS

Several biomass allocation traits showed Damage and Water significant effects or significant Damage–Water interaction in the ANOVA, thus being suitable to evaluate the interplay between plant responses to drought and herbivory; only the number of leaves and internode length were unaffected by the experimental treatments (Table 1). None of those plant traits showed additivity of effects as indicated by the significant interaction term in the ANOVA (Table 1).

We detected interference of effects in the case of the number of stems and index of architecture, because drought counteracted plant responses to damage. Thus, only in the control watering environment was it observed that plants subjected to simulated herbivory produced more stems, even after correcting for main stem length (index of architecture) (Fig. 1). In the case of shoot : root ratio, relative shoot biomass and relative root biomass we found that the damage treatment

**Table 1.** Two-way ANOVA for biomass allocation traits of *Convolvulus demissus* seedlings

	Damage (D)	Water (W)	D × W
Internode length	0.540 ns	2.304 ns	0.235 ns
Number of leaves	0.021 ns	2.583 ns	0.000 ns
Number of stems	31.71***	5.288*	13.38***
Index of architecture	23.21***	0.015 ns	14.64***
Shoot : root ratio	42.19***	48.51***	15.76***
Relative shoot biomass	72.37***	71.54***	28.30***
Relative root biomass	70.35***	69.11***	27.29***
Total plant biomass	0.000 ns	0.008 ns	34.93***

\* $P < 0.05$ ; \*\*\* $P < 0.001$ ; ns  $P > 0.05$ . Main factors: Damage, Water.  $F$ -values are shown along with statistical significance.

counteracted plant responses to drought. Thus, while undamaged plants under water shortage showed a significant increase in root relative biomass and a significant reduction in both shoot : root ratio and relative shoot biomass, none of these responses to drought was observed in plants subjected to simulated herbivory (Fig. 1). Total plant biomass increased in response to damage under regular watering, apparently due to greater shoot size, and also increased in response to drought in undamaged plants, presumably due to greater root size. However, damaged plants under experimental drought had the same total biomass than undamaged, well-watered plants (Fig. 1).

## DISCUSSION

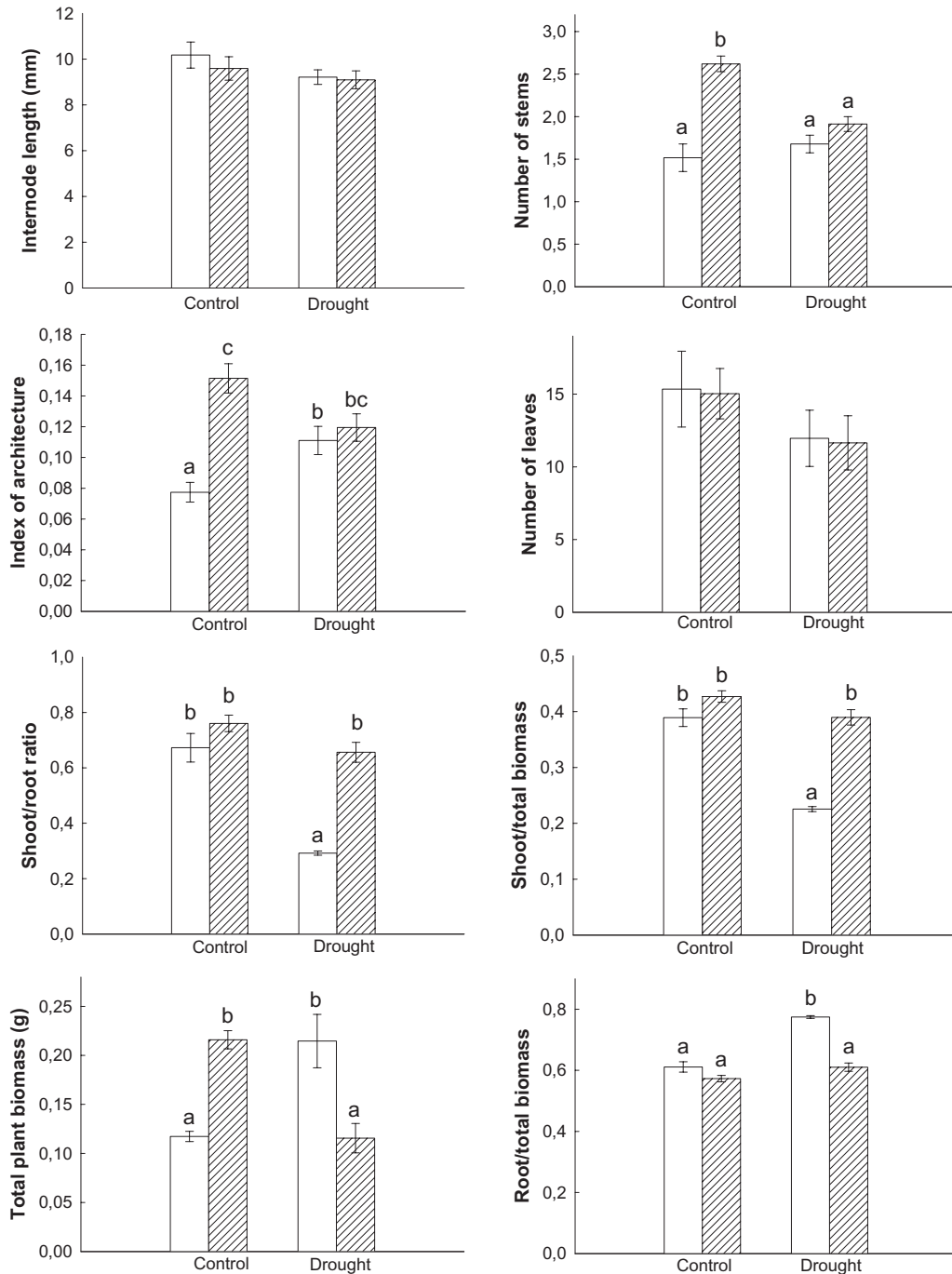
Plants of *C. demissus* in natural populations often face reduced soil moisture and herbivore damage simultaneously during the growth season. We found that plants subjected to simulated herbivory did not show increased branching, a common compensatory response to shoot damage (Strauss & Agrawal 1999; Stowe *et al.* 2000), when exposed to water shortage. We also found that the typical relative increase in root biomass allocation in response to reduced water availability (Hsiao *et al.* 1976; Schulze 1986; Grace 1997) did not occur in damaged plants. These counteractive effects of drought and damage on biomass allocation might explain why the co-occurrence of these ecological factors often results in decreased plant fitness, as has been reported for herbs (Gutiérrez & Meserve 2000), forbs (González *et al.* 2008) and shrubs (Torres *et al.* 1980; Poiani & Pozo 1986) distributed in semi-arid habitats of central Chile.

The fact that internode length and the number of leaves and stems of undamaged plants were not affected by water availability indicates that, in contrast to what has been observed in other *Convolvulus* species (Gianoli 2004, Gianoli & González-Teuber 2005), shoot traits are not responsive to water shortage in *C. demissus*. In view of this, and considering that shoot : root ratio decreased dramatically under

drought conditions, it is suggested that functional responses to reduced soil moisture in *C. demissus* mainly occur via increased growth of roots, as has been shown for other species (Malik *et al.* 1979; Sharp & Davies 1979; Blum 1996). There is also evidence that the study species is not consistently responsive to drought in terms of reducing leaf area (Quezada & Gianoli 2006; Gianoli *et al.* 2009). Therefore, the hypothetical beneficial effect of herbivory on plants under drought conditions via the reduction of transpiring leaf surface (see Introduction) is unlikely for this species. Thus, in this case herbivory would remove leaf tissue that could be important for whole-plant photosynthesis instead of being disposable in the context of adjustments in leaf area for water economy.

In general, we detected interference of effects between water shortage and leaf damage. In no case did we find a significant main effect of one of the factors and no effects of the other factor and of the interaction of factors. Consequently, we did not detect in any plant trait that responses to drought were identical in undamaged or damaged plants, or that responses to damage were the same regardless of water availability. This would suggest that, at least for biomass allocation, both ecological factors have similar importance for *C. demissus*. Interestingly, in terms of total plant biomass the effects of water shortage and leaf damage apparently cancelled each other because damaged plants under experimental drought had the same total biomass as undamaged and well-watered plants. Fitness measures after field experiments including more levels of each factor are needed to test the relative importance of water availability and herbivory for this species.

Overall, our results show counteractive biomass allocation responses to drought and damage in *C. demissus*, a perennial herb that often deals simultaneously with herbivory and reduced soil moisture in natural populations in central Chile. Further research must address the fitness consequences under field conditions of the patterns found. This would estimate whether this species has evolved the capacity to show functional integrative responses to such a combination of biotic and abiotic factors, as has been reported for a related



**Fig. 1.** Effect of experimental drought and leaf damage on biomass allocation traits of *Convolvulus demissus* seedlings. Hatched bars: damaged plants; open bars: undamaged plants. Means  $\pm$  SE are shown. Bars sharing a letter are not significantly different ( $P > 0.05$ ; Tukey test).

*Convolvulus* species that is also endemic to Mediterranean-type ecosystems in Chile (González-Teuber & Gianoli 2007, 2008). Results of such investigations would be of particular importance because both current and expected climatic trends for central Chile indicate increased aridity (Fuenzalida *et al.* 2007).

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