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Short research contribution

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SIMULATED HERBIVORY LIMITS PHENOTYPIC RESPONSES TO DROUGHT IN *CONVOLVULUS DEMISSUS* CHOISY (CONVOLVULACEAE)

ABSTRACT: Drought and herbivory are important stress factors for plants. When plants are subjected to any form of stress, phenotypic responses are elicited to reduce damage. Responses to drought include a decrease in leaf area and aerial biomass, and an increase in root/shoot ratio of biomass allocation. If plants are subjected to drought and herbivory at the same time, constrained responses are expected due to resource limitation. In a greenhouse experiment we analyzed the effect of simulated herbivory on the ability to respond to drought stress in seedlings of the Chilean perennial herb *Convolvulus demissus* (Convolvulaceae), which in natural populations may experience water deficit during dry summers as well as grazing by mammals. Plants subjected to drought showed the phenotypic responses theoretically expected. In contrast, plants subjected to a combined drought + herbivory treatment did not show those responses, being phenotypically similar to control plants. It is suggested that herbivory may limit responses to drought in *C. demissus*, hence magnifying the negative consequences on plant fitness of such abiotic factor.

KEY WORDS: perennials, phenotypic plasticity, resource availability, water deficit

Phenotypic plasticity is a mean by which plants may respond to environmental challenges. Low water availability may be a stress factor for plants. At low soil moisture, a reduction of leaf area allows plants to minimize water loss by transpiration and to allocate more biomass to roots, thus maximizing water uptake (Grace 1997, Pedrol *et al.* 2000). For instance, Maldonado *et al.* (2003) detected plastic responses to water deficit in *Lycopersicon chilense* Dunal (Solanaceae), which included a decrease in leaf biomass and leaf area. Another important stress factor for plants is herbivory. Removal of plant biomass by herbivores may affect fundamental functions such as water uptake and photosynthesis rate, hence reducing plant fitness (Karban and Strauss 1993, Gurevitch *et al.* 2002, Ehrlén 2003).

Drought may make plants more suitable for insect growth (Mattson and Haack 1987, Larsson 1989). There is some evidence that when water availability is low plants are less able to show compensatory responses to herbivory (Coughenour 1985, Levine and Paige 2004), which reduce the impact of damage on plant fitness (Stowe *et al.* 2000). In Chile, Poiani and Del Pozo (1986) showed that plants of *Colliguaja odorifera* Mol. (Euphorbiaceae) are highly influenced by water availability in their capacity to tolerate herbivory. On the other hand, there is little information on the effects of herbivory on the ability of plants to show functional responses to abiotic stress. Using *Chenopodium album* L. (Chenopodiaceae) as a model species, Kurashige and Agrawal (2005) found no effect of herbivory on plant phenotypic responses to light competition.

The goal of this study was to evaluate in a greenhouse the effect of simulated herbivory on the responses to drought of *Convolvulus demissus* Choisy (Convolvulaceae), a perennial herb that grows in the Andean slopes of Central Chile. In this region, with predominance of Mediterranean-type climate, plant populations may experience drought during dry summers (Arroyo *et al.* 1981). *C. demissus* may suffer aboveground herbivory by mammals and chrysomelid beetles (Gianoli *et al.* – unpublished). We hypothesized that plant functional responses to drought would be limited by herbivory.

Convolvulus demissus is a perennial herb, which grows prostrate and is only dispersed by seed. Stems are numerous and short (between 0.1–0.5 m long). Leaves are ovate to triangular and small (0.5–3 cm long). This species is native from Chile and Argentina and grows in the Andean slopes between 29–35°S, generally between 1200 and 2700 m (O'Donell 1957, Herbarium Universidad de Concepción [CONC]). Seeds of *C. demissus* were collected from a population (~20 individuals) in the Andean slopes of Santiago, Chile ("Road to Farellones", 1900 m). Seeds were scarified by immersion in concentrated H_2SO_4 for 30 minutes and then washed in running tap water for 5 minutes. After this procedure, seeds where kept in the dark inside boxes with wet filter paper to allow germination, in a room at 19°C. After germination, seedlings were raised in 500 ml plastic bags filled with potting soil. After 4 weeks seedlings were transplanted to 2 l plastic bags and the experimental treatments were applied. The experiment was carried out in a greenhouse located at an outdoor experimental plot at Universidad de Concepción, Concepción, Chile.

Three groups of 24 plants each were arranged. Each plant was set on a separate plastic bag and all of them were randomly interspersed. The first group was the control treatment (C), the second group was assigned to drought treatment (D) and the third group was assigned to the combined drought and herbivory treatment (D+H). Control plants were watered at field capacity every 3 days. Plants from groups D and D+H were watered at field capacity every 10 days. A simulated herbivory treatment was applied to plants of the D+H group: *ca.* 50% of the aerial biomass from each plant was removed just before starting the drought treatment. Biomass was removed cutting half of the main stem, simulating browsing by large herbivores. Simulated herbivory is a common practice in this kind of experiments, and allows standardizing damage to the plant avoiding deviations due to herbivore behavior (Tiffin and Inouye 2000).

After four months (early December 2004–late March 2005), treatments ended and the following traits were measured for each plant: survival, root/shoot biomass ratio, basal root diameter (mm), average leaf area (cm^2), number of branches and leaves, and plant water potential (bar). Root diameter was measured using a digital caliper. Water potential was recorded using a Scholander pressure bomb. Leaf area was estimated from

Table 1. Comparison of phenotypic responses to drought of control and damaged seedlings of *C. demissus*. Planned contrasts: C *vs.* D (control *vs.* drought) and C *vs.* D+H (control *vs.* drought + herbivory). *P* values after LSD tests are shown.

Trait	C *vs.* D	C *vs.* D+H
Water potential	0.006	0.03
Basal root diameter	ns	ns
Branch number	0.069	ns
Leaf number	ns	ns
Root/shoot ratio	0.027	ns
Leaf area	0.002	ns

digital images using SigmaScan® software. A one-way ANOVA was applied to evaluate the significance of the treatments. Afterwards, two planned contrasts (LSD test) were performed in order to test the hypothesis, comparing groups C *vs.* D (response to drought of undamaged plants) and C *vs.* D+H (response to drought of damaged plants). We only report statistical results of the planned contrasts because only these are relevant for the hypothesis.

Undamaged plants subjected to drought showed an increase in root/shoot ratio and water potential, and a decrease in basal root diameter, leaf area, number of branches and leaves (Fig. 1). However, not all of

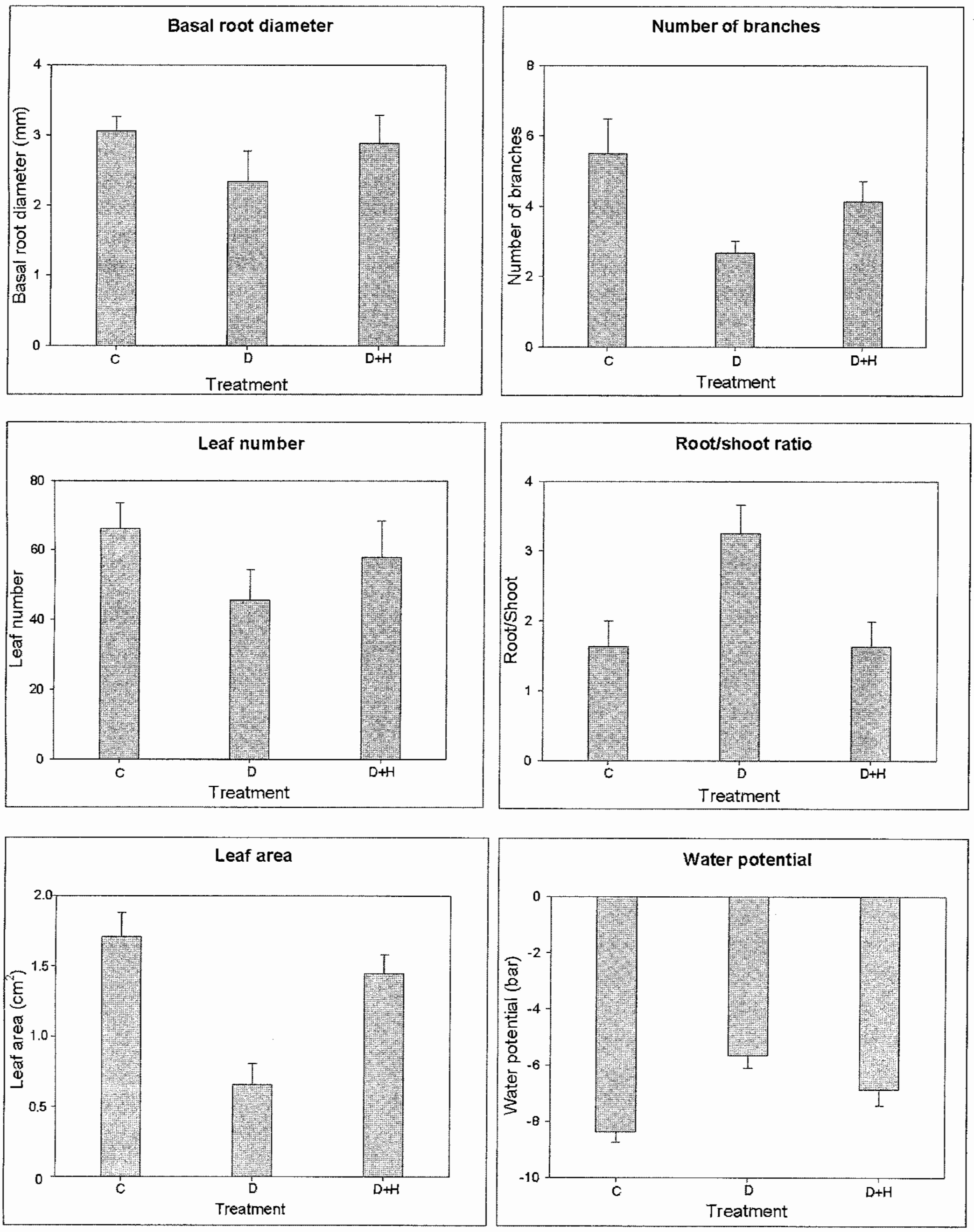

Fig. 1. Variation in morphological and physiological traits of seedlings of *C. demissus* in three experimental treatments: control (C), drought (D) and drought + herbivory (D+H). Means and S.E. are shown.

these variations were significant (Table 1). On the other hand, damaged plants exposed to drought showed no variation compared to control plants in almost all the measured traits, with the exception of water potential, which showed a significant decrease (Table 1, Fig. 1).

Plants of the genus *Convolvulus* have shown significant variations in morphological traits under different environmental regimes. These plant species exhibit plasticity to drought: a decrease in basal root diameter, leaf area and leaf number, and an increase in root/shoot biomass ratio (Gianoli 2004, Gianoli and González-Teuber 2005). Likewise, plasticity to shading in *C. demissus* and other two congeneric species includes an increase in leaf area and stem length, and a decrease in the number of branches (González and Gianoli 2004). General trends observed in this study support the hypothesis that responses of plants to abiotic stress may be limited by herbivory. In the drought treatment, individuals of *C. demissus* showed significant variation in some morphological traits (higher root/shoot ratio, lower branch number and lower leaf area), all of them expected responses to water deficit (Grace 1997). In contrast, in the combined treatment (drought plus herbivory), plants did not show the responses or trends observed in the drought treatment, compared to the control group. It seems that herbivory would impair the capacity of plants to show plastic responses because of a shortage in resource acquisition (reduction of photosynthetic area). Alternatively, it might be speculated that plant compensatory responses to damage involve trait expression that is opposite to that observed after reduced water availability. In other systems it has been shown that branch and leaf number, root/shoot ratio and leaf area increase after herbivory (Houle and Simard 1996, Tiffin 2000).

The results of the present study are preliminary due to the short term of the experiments and the phenological stage of the evaluated plants (seedlings of a perennial plant species). Nevertheless, they strongly suggest that a biotic stress such as herbivory may significantly affect the expression of phenotypic plasticity in natural plant populations.

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