Original article

Leaf damage decreases fitness and constrains phenotypic plasticity to drought of a perennial herb

Ernesto Gianoli [a,b,c,*], Iván M. Quezada [a], Lorena H. Suárez [a,b]

[a] Departamento de Botánica, Universidad de Concepción, Casilla 160-C, Concepción, Chile
[b] Center for Advanced Studies in Ecology & Biodiversity (CASEB), Departamento de Ecología, P. Universidad Católica de Chile, Alameda 340, Santiago, Chile
[c] Departamento de Biología, Universidad de La Serena, Casilla 599, La Serena, Chile

ARTICLE INFO

ABSTRACT

Mediterranean-type ecosystems are increasingly prone to drought stress. Herbivory might limit plant functional responses to water shortage. This may occur as a result of plant resource depletion or due to the fact that leaf damage and drought may elicit opposite phenotypic responses. We evaluated the impact of herbivory on plant fitness in the field, and the effects of leaf damage on phenotypic plasticity to reduced soil moisture in a greenhouse. The study species was *Convolvulus demissus*, a perennial herb endemic to central Chile, which has a Mediterranean-type climate. Controlled herbivory by chrysomelid beetles (natural herbivores) in the field had a negative impact on plant fitness, estimated as number of fruits. Whereas reduced soil moisture alone did not affect seedling survival, damaged seedlings (simulated herbivory) had greater mortality when growing under water shortage. The hypothesis that herbivory would constrain phenotypic plasticity was supported by significant statistical interactions between leaf damage and soil moisture, followed by inspections of reaction norms. This was verified both overall (all phenotypic traits taken together, MANOVA) and in four of the six traits evaluated (ANOVAs). When plants were damaged, the reaction norms in response to low soil moisture of water use efficiency, root:shoot ratio and xylem water potential showed reduced slopes. While undamaged plants increased root biomass in response to low moisture, the opposite trend was found for damaged plants. The simultaneous occurrence of herbivory and drought events might curtail recruitment in plant populations of central Chile and other Mediterranean-type ecosystems due to the inability of damaged seedlings to show functional responses to low soil moisture. This finding is of ecological significance in view of current and projected trends of increased aridity in these ecosystems.

© 2009 Elsevier Masson SAS. All rights reserved.

1. Introduction

Environmental stress and herbivory may have additive and even synergistic detrimental effects on plant performance (Hawkes and Sullivan, 2001). Plants have evolved responses to these ecological factors and consequently show, in an ecological timescale, phenotypic adjustments that ameliorate their negative impact (Karban and Baldwin, 1997; Strauss and Agrawal, 1999; Schulze et al., 2005). Considerable research has addressed the interactive effects of herbivory and resource limitation on plant survival or reproductive output (reviewed in Gurevitch et al., 2000; Hawkes and Sullivan, 2001; Wise and Abrahamson, 2007). Less attention has been devoted to the mechanistic basis of such patterns. For instance,

comparatively little research has addressed whether herbivory may limit plant functional responses to the environment (but see Cipollini, 2004; Kurashige and Agrawal, 2005; Gianoli et al., 2007; Valladares et al., 2007).

Plants display phenotypic plasticity in response to environmental changes, and plasticity is often described using reaction norms (the plot of phenotypic trait expression against environmental variation) (Schlichting and Pigliucci, 1998). Adaptive phenotypic plasticity occurs when reaction norms are positively associated with plant fitness (Sultan, 1995; van Kleunen and Fischer, 2005). This may result from either correlated responses of plant phenotype and plant fitness, when there is enhanced exploitation of the environment, or phenotypic responses that allow fitness homeostasis (flat fitness reaction norms) when plants face challenging environments (Alpert and Simms, 2002). If damage by herbivores causes maladaptive modifications of plant reaction norms to the environment, then we will gain insights into proximate explanations for fitness losses due to herbivory. Plants exhibit phenotypic plasticity to drought that allows

* Corresponding author. Departamento de Botánica, Universidad de Concepción, Casilla 160-C, Concepción, Chile. Tel.: +56 41 2203418; fax: +56 41 2246005.
E-mail address: egianoli@udec.cl (E. Gianoli).

them to minimize water loss by transpiration and/or maximize water uptake. Typical phenotypic responses of plants to reduced soil moisture include an increase in the relative allocation of biomass to roots, a reduction in leaf area and number of leaves, enhanced water use efficiency (the ratio between carbon gain and water loss through stomata), and a decrease in the internal osmotic potential (Schulze, 1986; Sultan and Bazzaz, 1993; Grace, 1997; Chaves et al., 2002).

The perennial herb *Convolvulus demissus* (Convolvulaceae) is endemic to Andean slopes in central Chile and Argentina. This is a region with temperate, Mediterranean-type climate, where plant populations often experience drought during the dry season (spring–summer) (Di Castri and Hajek, 1976; Arroyo et al., 1981). There is evidence of a trend of increased aridity in Central Chile (Pezoa, 2003). In general, likely scenarios of global climate change indicate that Mediterranean-type ecosystems would experience increased aridity (Valladares, 2004), and in the case of central Chile climate change projections consider a 20–30% decrease in precipitations during plant growth season (IPCC, 2007). Populations of *C. demissus* suffer herbivory mainly by leaf beetles (Chrysomelidae, Cassidinae) and occasionally by livestock, with common field levels of herbivory in the range of 20–60% defoliation (Gianoli et al., unpublished data). The occurrence of drought and leaf damage may be particularly critical for seedlings of *C. demissus*, which lack belowground reserves that might confer tolerance to herbivory and environmental stress. Seedlings of *C. demissus* have shown phenotypic plasticity to experimental variations in soil moisture (Quezada and Gianoli, 2006) and light intensity (González and Gianoli, 2004). In the present study with *C. demissus*, we first determined whether herbivory by chrysomelid beetles reduces plant fitness in the field. Secondly, we addressed the effect of leaf damage and soil moisture on seedling survival, and evaluated the effect of leaf damage on plant phenotypic plasticity to reduced soil moisture. We aimed at determining whether herbivory may constrain plant reaction norms of potential adaptive value in a stressful environment. This may be of increased importance given the expected accentuation of aridity in the study ecosystem.

2. Methods

C. demissus (Convolvulaceae) is a perennial prostrate herb distributed between 1200 m and 2700 m a.s.l. on the Andean slopes of Central Chile and Argentina (O'Donell, 1957; Herbarium Universidad de Concepción [CONC]). Stems are numerous and short (0.1–0.5 m long). Leaves are ovate to triangular and of small size (0.5–2.5 cm long). Flowers are hermaphroditic and solitary, with light pink funnel-like corollas (1–2 cm diameter). The fruit is a capsule with one to four seeds (4–7 mm long) (O'Donell, 1957). This species blooms throughout November, December and January. The plant dies back during February–March and remains dormant until the first rains of the next season (April–May). The dry season extends from October to March. In the study population (see below) we determined water availability in the soil at the start (October) and at the end (February) of the growing season. Soil matric water potential was measured with tensiometers (2725 Series Jet Fill, Soil Moisture, CO, USA) in bare ground adjacent to each of four plants of *C. demissus* randomly chosen. Measurements were made at a depth of 15 cm.

The field experiment was carried out in a population of *C. demissus* located at 2380 m a.s.l. in the Andes close to Santiago, Chile (33°21′S, 70°18′ W). The climate in this site is Mediterranean, with a cold and rainy winter and a dry and warm summer (Di Castri and Hajek, 1976; Arroyo et al., 1981). In October 2005 we located 80 plants of *C. demissus* at the vegetative stage. Selected plants had between one and three stems, and between six and ten leaves per stem. We randomly chose 40 plants to receive herbivory by larvae of *Chelymorpha varians* (Chrysomelidae, Cassidinae). In each of these plants two or three third-instar larvae were placed on leaves and the whole stem was immediately enclosed with a nylon mesh bag. This was done in such a way that approximately half of the leaves were exposed to beetles. A similar number of empty nylon mesh bags were put on each of the other 40 plants. We periodically checked for larval survival in order to replace them, and manually excluded other larvae naturally colonizing exposed stems of experimental plants. A wire cage covered each of the 80 plants in order to exclude herbivory by mammals. We withdrew beetles two weeks later and estimated the actual defoliation level of each plant, which ranged between 30% and 50%. Four months after the start of the experiment, we recorded the number of fruits and the number of seeds per fruit, and determined seed mass in the laboratory. Differences between damaged and undamaged plants were determined by a one-way ANOVA or by a Mann–Whitney *U* test, depending on whether data met parametric analysis assumptions or not.

Seedlings of *C. demissus* used in the greenhouse experiment were grown from seeds collected in the Andes close to Santiago at approximately 2400 m, in a population close to that described above. Seeds were collected from more than 30 widely spaced mother plants and pooled thereafter. In the lab, 300 seeds were subjected to acid scarification and then kept in the dark at 20 °C on wet papers to allow germination. On November 2005, we selected 120 seedlings of vigorous appearance. Seedlings were planted singly in 2 l plastic bags filled with potting soil and placed on benches of a greenhouse at Universidad de Concepción (central Chile). After four weeks, when seedlings had 10 leaves on average, the experimental treatments were applied. Experiments were performed during summer time, under approximately 1200 μmol m^{-2} s^{-1} PAR at noon, and maximum and minimum temperatures (daily ranges) of 22–29 °C and 10–14 °C, respectively.

Four groups of 30 plants were arranged in a factorial design, crossing two levels of herbivory (undamaged vs. leaf damage) and two levels of soil moisture (regular watering vs. restricted watering). The resulting experimental groups were: control, drought, damage, and drought + damage. Plants from the control and damage groups were watered to field capacity every 3 days. Plants from the drought and the drought + damage treatments were watered to field capacity every 10 days. A simulated herbivory treatment was applied to plants of the damage and drought + damage treatments, which consisted in clipping with scissors half of the leaves along the mid vein (50% defoliation), simulating defoliation caused by herbivores. Simulated herbivory is a common practice in this kind of experiments (Tiffin and Inouye, 2000). We used it to standardize the defoliation level, avoiding deviations such as those observed in the field experiment. Plants of the four experimental groups were placed alternately on greenhouse benches in a systematic design in order to minimize micro-environmental biases, and were relocated over the benches every 10 d.

Two months after the onset of treatments, we recorded seedling mortality in each experimental group and the following traits were measured for each plant: main stem length, leaf area, instantaneous water use efficiency (WUE, the ratio of CO_2 assimilation and evapotranspiration), root biomass (dry weight), root:shoot biomass ratio, and xylem water potential. Leaf area was estimated from digital images of detached leaves using SigmaScan® (SPSS Inc., Illinois, USA). We sampled three leaves per plant to obtain the average leaf area of the individual. Xylem water potential was recorded using a Scholander pressure chamber (PMS 600, PMS Instruments, Corvallis, USA). In order to obtain the individual value of WUE, it was measured on three attached leaves per plant with a portable gas exchange system (LCi, ADC Bioscientific Ltd., Hertfordshire, UK) between 10:00 and 12:00. At the time of measurements PAR varied between 800 and 1200 μmol m^{-2} s^{-1}, which were assumed to be saturating light levels.

Seedling mortality was compared between groups using paired χ^2 tests. A two-way multivariate ANOVA was first applied to evaluate the effects on plant phenotype of leaf damage, soil moisture and their interaction. After this general analysis, we conducted univariate ANOVAs for each trait to evaluate particular responses to the main factors and their interaction. The significance of the interaction of main factors was used to test the hypothesis of differences in plasticity according to the damage treatment, in particular, whether leaf damage constrained plant phenotypic plasticity. All statistical analyses were done with Statistica (Statsoft Inc., Tulsa, USA).

3. Results

Plants suffering herbivory by leaf beetles in the field produced fewer fruits compared to undamaged plants ($Z = -1.999$, $p < 0.05$; Mann–Whitney U test) (Fig. 1). However, there were no differences
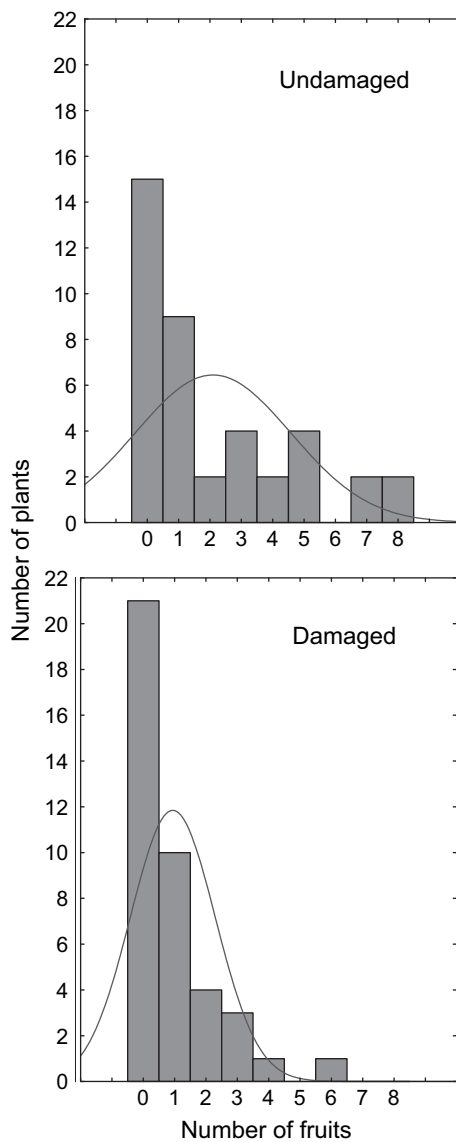
between groups in the number of seeds per fruit ($F_{1,42} = 0.024$, $p > 0.87$; one-way ANOVA) (undamaged plants $= 2.40 \pm 0.13$; damaged plants $= 2.43 \pm 0.11$; mean $\pm$ SE) and in seed mass ($F_{1,42} = 0.06$, $p > 0.80$; one-way ANOVA) (undamaged plants $= 29.40$ mg ± 1.08; damaged plants $= 28.99$ mg ± 0.51; mean $\pm$ SE). Both damaged and undamaged plants showed 100% survival. Soil matric water potential (mean $\pm$ SE) was almost the same at the start and at the end of the growing season: -28.5 ± 0.54 kPa in October and -28.7 ± 1.03 kPa in February. These values indicate that there was no drought stress during this season in the study population.

Leaf damage caused plants to fail in maintaining fitness when subjected to water shortage in a greenhouse. Whereas reduced soil moisture alone did not have a significant effect on seedling survival (control vs. drought; $\chi^2 = 0.32$, $p > 0.57$), damaged seedlings had greater mortality when growing under reduced soil moisture (damage vs. drought + damage; $\chi^2 = 4.00$, $p < 0.05$) (Fig. 2).

Results of the MANOVA showed that, overall, seedling phenotype was affected by soil moisture, leaf damage, and their interaction (Table 1). Responses of C. demissus to water shortage such as the increase in root:shoot ratio, enhanced water use efficiency (WUE), and the decrease in xylem water potential (Fig. 3, Table 1), are considered functional phenotypic modifications that allow plants to improve water economy under conditions of reduced moisture availability. Leaf damage showed a significant effect in root biomass, root:shoot ratio and xylem water potential. However, this effect was only observed under low soil moisture (Fig. 3), which resulted in the significant interaction of main factors. The significance of the statistical interaction between leaf damage and soil moisture, together with the inspection of reaction norms, supported the hypothesis that simulated herbivory would constrain the expression of plant phenotypic plasticity. This was verified both at the general level (all phenotypic traits taken together) and, in particular, for four of the six traits considered (Table 1, Fig. 3). In the cases of WUE, root:shoot ratio and water potential, there was a clear reduction in the slope of the reaction norms, i.e., leaf damage caused seedlings to show a lesser increase in WUE and root:shoot ratio, and a lesser decrease in xylem water potential, in response to reduced soil moisture (Fig. 3). In the case of root biomass, there was a change in the direction of the reaction norm to soil moisture: while undamaged plants increased root biomass, the opposite trend was found for plants subjected to leaf damage (Fig. 3).
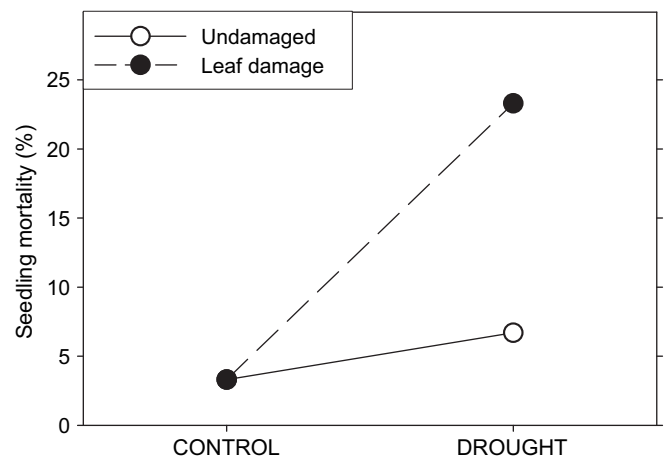

Fig. 1. Distribution of frequency of number of fruits in field plants of *Convolvulus demissus* (Andes of central Chile) at the end of the growing season. Damaged plants suffered 30–50% defoliation by chrysomelid beetles during early vegetative stages ($N = 40$ plants per group). Mean number of fruits: undamaged $= 2.08$; damaged $= 0.93$. Mean values and normal distribution curves are included only for reference because data were analyzed by a non-parametric test (see text).

Fig. 2. Mortality of damaged (50% defoliation) and undamaged seedlings of *Convolvulus demissus* subjected to regular watering (control) or restricted watering (drought) in a greenhouse. The initial number of seedlings was 30 per each of the four groups and mortality was evaluated two months after applying the treatments (leaf damage and contrasting watering regimes).

Table 1
Two-way multivariate and univariate ANOVAs for phenotypic traits of *C. demissus*. Main factors: soil moisture and leaf damage. *F*-values are shown along with statistical significance.

	Moisture (M)	Damage (D)	M × D
All traits	26.24*	10.87*	13.25*
Stem length	3.97*	0.01 ns	1.08 ns
Leaf area	1.82 ns	0.01 ns	0.28 ns
WUE	13.62**	0.52 ns	6.88*
Root biomass	0.01 ns	4.10*	7.91**
Root:Shoot	119.4***	130.5***	66.81***
Water potential	297.3***	64.72***	63.80***

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ns $p > 0.05$.

4. Discussion

Herbivory by leaf beetles in the field had a moderate negative impact on plant fitness. Whereas damaged plants of *C. demissus* showed a reduction in fruit production, the number of seeds per fruit and seed mass were not affected by beetle herbivory. This indicates that the main effect of early leaf damage was a reduction in the number of reproductive structures, a pattern reported for several other plant–herbivore interactions (Marquis, 1992). The null mortality of damaged field plants might be a consequence of tolerance mechanisms associated with reallocation of resources from belowground tissue (Stowe et al., 2000). Similar tolerance levels were observed in damaged seedlings under regular watering in the greenhouse. In contrast, seedlings subjected to leaf damage and water shortage did not show such a tolerance. Interestingly, 2005 – the year in which experiments were carried out – was a wet year for Santiago city (39% excess of rainfall compared to an average year). This might also partly explain the null mortality of damaged field plants. In fact, we recorded during the dry season soil water potentials (ca. −29 kPa) that are not associated with drought stress. It might be conceived that in dry years populations of *C. demissus* would experience drought stress, as has been shown for other species at similar altitudes in the Andes of central Chile (Cavieres et al., 2006), and consequently the effects of leaf damage on plant fitness could be of greater magnitude. In years 2003 and 2007 there was a 31% and 46% deficit of rainfall in Santiago, respectively. Foreseen climatic scenarios indicate increased aridity for this region (IPCC, 2007).

Simulated herbivory constrained the capacity of seedlings of *C. demissus* to show phenotypic plasticity to reduced soil moisture. Undamaged plants were able to modify their morphology, physiology and biomass allocation in response to water shortage, as has been shown before in *C. demissus* and congeneric species (Gianoli, 2004; Gianoli and González-Teuber, 2005; Quezada and Gianoli, 2006). In contrast, damaged plants tended to show less steep (or even flat) reaction norms of phenotypic traits to reduced soil moisture, and hence were less able to maximize water capture and reduce water loss by transpiration. This was reflected in the lower survival of damaged plants in low soil moisture. Seedling mortality in this group was ≈25% after only two months of drought treatment. Although this figure is not particularly high, it is likely that greater mortality would be observed after a more prolonged stress in natural conditions.

In our analysis, herbivory-mediated constraints on plasticity are inferred when there are major departures from the reaction norm of undamaged plants, which are thus assumed to be the "appropriate" responses of the plant to the environment. In some cases these assumptions might be questioned. For instance, the divergence of reaction norms of root biomass in undamaged and damaged seedlings could be interpreted as a functional response of damaged seedlings to the need of increased allocation of resources to the shoot, as has been reported in other systems (compensatory responses; Mabry and Wayne, 1997; Stowe et al., 2000). Likewise, the less steep
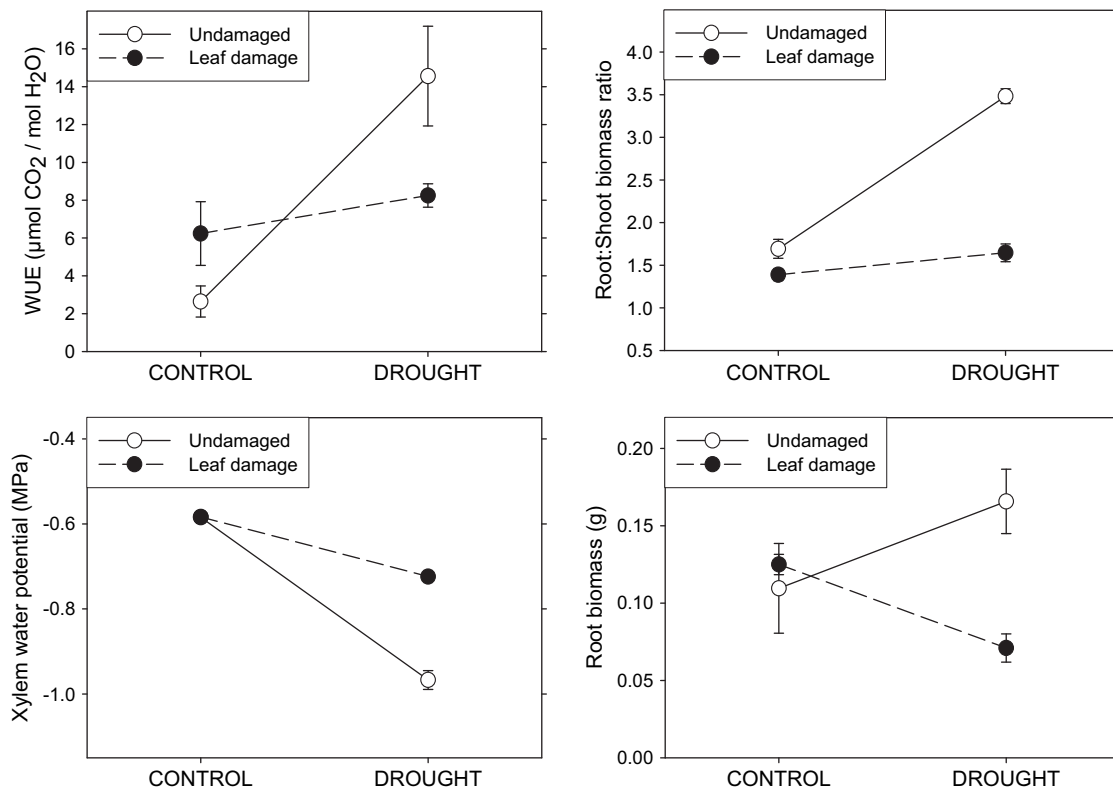

Fig. 3. Contrasting reaction norms to reduced soil moisture in undamaged (control) and damaged (50% defoliation) seedlings of *Convolvulus demissus*. Traits shown (mean ± SE) are those that had a significant moisture × damage interaction in Table 1: instantaneous water use efficiency (WUE, the ratio of CO_2 assimilation and evapotranspiration), root biomass (dry weight), root:shoot biomass ratio, and xylem water potential.

reaction norms found for WUE and water potential in damaged seedlings might be interpreted as a consequence of a lower demand of water (fewer sinks for water loss), compared to undamaged seedlings, due to the inevitable reduction of leaf area caused by the damage treatment. In particular, water potential is often taken as an inverse indicator of plant stress and hence our interpretation could be misleading. However, in an ecological scenario of water-limiting conditions, the lower the water potential achieved by a plant genotype, variety, or species, the greater the power to extract water from the soil. This active response to water shortage is accomplished by changes in osmotic potential via solute concentration (osmotic adjustment, Hsiao et al., 1976). Summarizing, the fact that the combined effect of herbivory and water shortage reduced survival suggests that the reaction norms of damaged plants reflect constrained phenotypic expression rather than functional responses to two concurrent challenging factors. In other words, the observed lack of tolerance of damage in plants under reduced soil moisture compared to those grown with regular watering (vertical analysis of Fig. 2) makes unlikely the adaptive interpretation of departures from the reaction norm of undamaged plants.

There are several reports on the interactive effects of herbivory and water shortage on plant fitness (Hawkes and Sullivan, 2001; Wise and Abrahamson, 2007; Gonzáles et al., 2008). To our knowledge, however, very few studies have explicitly addressed the effect of herbivory on the reaction norm of plants to reduced soil moisture (see Valladares et al., 2007). Using a non-factorial design, we recently showed in *C. demissus* that, compared to the phenotypic expression of plants growing in experimental drought, plants subjected to both drought and defoliation failed to attain the target phenotype (Quezada and Gianoli, 2006). Although a possible interpretation of these results is that leaf damage limited the expression of plasticity to drought, the experimental design utilized does not allow drawing a clear-cut conclusion. Such non-factorial designs, in which the performance or phenotype of plants grown in a control environment is contrasted with that of plants subjected to a single stress factor and to a combination of two stress factors, are suitable for testing whether plants perform worse when they face stress factors simultaneously. However, these designs are not useful to detect phenotypic trends and lack information on the relative contribution of the two concurrent factors to the patterns observed (Gurevitch et al., 2000; Hawkes and Sullivan, 2001). In the present approach, we were able to identify how reaction norms to reduced soil moisture were affected by simulated herbivory, and hence we might anticipate the behaviour of plant functional responses in foreseen environmental scenarios.

We have shown that simulated herbivory limits the expression of putatively adaptive phenotypic plasticity in *C. demissus*. Such phenotypic plasticity is termed adaptive because it allowed fitness homeostasis under a restrictive environment: low water availability (see Alpert and Simms, 2002). Patterns observed are hardly explained by direct consequences of early leaf biomass removal in the herbivory treatments. For instance, the initial 50% defoliation applied would tend to cause an increase of the root:shoot ratio of biomass allocation. However, damaged seedlings showed a decrease in root:shoot ratio. Specific mechanistic explanations for the detected patterns of constrained plasticity are out of the scope of the present study. Nonetheless, we could consider two probable, non-exclusive causes. On one hand, leaf clipping may trigger the onset of compensatory responses in growth and/or differentiation (Strauss and Agrawal, 1999; Stowe et al., 2000; González-Teuber and Gianoli, 2007; Atala and Gianoli, 2009) that may be opposite to those adequate for a low soil moisture environment, such as the differential aboveground vs. belowground biomass allocation. On the other hand, herbivory might induce defensive traits whose synthesis may be either costly (Gianoli et al., 1996; Collantes et al., 1997), and

thus indirectly limit resources available for plastic responses, or may directly compete with precursors of hormones or stress metabolites involved in plant responses to drought, as has been shown for chemical defences and hormones associated with plant responses to shading (Cipollini, 2004).

From a general perspective, our results suggest that the detrimental effects of herbivory on plant fitness may be of greater magnitude in changing environments. There is evidence of an increase of the stressfulness of Mediterranean-type ecosystems associated with global climate change, mainly due to more prolonged events of drought and greater unpredictability of precipitations (Valladares, 2004). In the case of central Chile, both current (Pezoa, 2003) and expected (IPCC, 2007) climatic trends indicate increased aridity. Earlier work has described the synergistic negative effects of herbivory and water shortage on both plant performance and population parameters in several species native to these ecosystems (Torres et al., 1980; Poiani and Del Pozo, 1986; Gutiérrez and Meserve, 2000; Holmgren et al., 2006). In the case of the study species, which is a perennial herb endemic to Mediterranean-type ecosystems, the simultaneous occurrence of herbivory and drought events might significantly curtail recruitment in natural populations due to the inability of damaged seedlings to show functional responses to low soil moisture.

Acknowledgments

References

Alpert, P., Simms, E.L., 2002. Relative advantages of plasticity and fixity in different environments: when is it good for a plant to adjust? Evol. Ecol. 16, 285–297.
Arroyo, M.T., Armesto, J.J., Villagrán, C., 1981. Plant phenological patterns in the high Andean cordillera of central Chile. J. Ecol. 69, 205–223.
Atala, C., Gianoli, E., 2009. Effect of water availability on tolerance of leaf damage in tall morning glory, *Ipomoea purpurea*. Acta Oecol. 35, 236–242.
Cavieres, L.A., Badano, E.I., Sierra-Almeida, A., Gómez-González, S., Molina-Montenegro, M.A., 2006. Positive interactions between alpine plant species and the nurse cushion plant *Laretia acaulis* do not increase with elevation in the Andes of central Chile. New Phytol. 159, 59–69.
Chaves, M.M., Pereira, J.S., Maroco, J., Rodrigues, M.L., Ricardo, C.P.P., Osorio, M.L., Carvalho, I., Faria, T., Pinheiro, C., 2002. How plants cope with water stress in the field. Photosynthesis and growth. Ann. Bot. 89, 907–916.
Cipollini, D., 2004. Stretching the limits of plasticity: can a plant defend against both competitors and herbivores? Ecology 85, 28–37.
Collantes, H.G., Gianoli, E., Niemeyer, H.M., 1997. Effect of defoliation on the patterns of allocation of a hydroxamic acid in rye (*Secale cereale*). Environ. Exp. Bot. 38, 231–235.
Di Castri, F., Hajek, E., 1976. Bioclimatología de Chile. Ediciones Pontificia Universidad Católica, Santiago.
Gianoli, E., 2004. Plasticity of traits and correlations in two populations of *Convolvulus arvensis* (Convolvulaceae) differing in environmental heterogeneity. Int. J. Plant Sci. 165, 825–832.
Gianoli, E., Papp, M., Niemeyer, H.M., 1996. Costs and benefits of hydroxamic acids-related resistance in winter wheat against the bird cherry-oat aphid, *Rhopalosiphum padi* (L.). Ann. Appl. Biol. 129, 83–90.
Gianoli, E., Molina-Montenegro, M.A., Becerra, J., 2007. Interactive effects of leaf damage, light intensity and support availability on chemical defences and morphology of a twining vine. J. Chem. Ecol. 33, 95–103.
Gianoli, E., González-Teuber, M., 2005. Environmental heterogeneity and population differentiation in plasticity to drought in *Convolvulus chilensis* (Convolvulaceae). Evol. Ecol. 19, 603–613.
Gonzáles, W.L., Suárez, L.H., Molina-Montenegro, M.A., Gianoli, E., 2008. Water availability limits tolerance of apical damage in the Chilean tarweed *Madia sativa*. Acta Oecol. 34, 104–110.
González, A.V., Gianoli, E., 2004. Morphological plasticity in response to shading in three *Convolvulus* species of different ecological breadth. Acta Oecol. 26, 185–190.
González-Teuber, M., Gianoli, E., 2007. Tolerance to simulated herbivory in two populations of *Convolvulus chilensis* (Convolvulaceae). Acta Oecol. 32, 119–123.
Grace, J., 1997. Plant water relations. In: Crawley, M.J. (Ed.), Plant Ecology. Blackwell, London, pp. 28–50.
Gurevitch, J., Morrison, J.A., Hedges, L.V., 2000. The interaction between competition and predation: a meta-analysis of field experiments. Am. Nat. 155, 435–453.

Gutiérrez, J.R., Meserve, P.L., 2000. Density and biomass responses of ephemeral plants to experimental exclusions of small mammals and their vertebrate predators in the Chilean semiarid zone. J. Arid Environ. 45, 173–181.

Hawkes, C.V., Sullivan, J.J., 2001. The impact of herbivory on plants in different resource conditions: a meta-analysis. Ecology 82, 2045–2058.

Holmgren, M., López, B.C., Gutiérrez, J.R., Squeo, F.A., 2006. Herbivory and plant growth rate determine the success of El Niño Southern Oscillation-driven tree establishment in semiarid South America. Glob. Change Biol. 12, 2263–2271.

Hsiao, T.C., Acevedo, E., Fereres, E., Henderson, D.W., 1976. Water stress, growth, and osmotic adjustment. Phil. Trans. R. Soc. Lond. B 273, 479–500.

IPCC, 2007. Synthesis Report of Key Findings and Uncertainties Contained in the Working Group Contributions to the Fourth Assessment Report. http://www.ipcc.ch/pdf/assessment-report/ar4/syr/ar4_syr.pdf (Valencia, Spain).

Karban, R., Baldwin, I.T., 1997. Induced Responses to Herbivory. University of Chicago Press, Chicago.

van Kleunen, M., Fischer, M., 2005. Constraints on the evolution of adaptive phenotypic plasticity in plants. New Phytol. 166, 49–60.

Kurashige, N.S., Agrawal, A.A., 2005. Phenotypic plasticity to light competition and herbivory in Chenopodium album (Chenopodiaceae). Am. J. Bot. 92, 21–26.

Mabry, C.M., Wayne, P.W., 1997. Defoliation of the annual herb Abutilon theophrasti: mechanisms underlying reproductive compensation. Oecologia 111, 225–232.

Marquis, R.J., 1992. Selective impact of herbivores. In: Fritz, R.S., Simms, E.L. (Eds.), Plant Resistance to Herbivores and Pathogens. University of Chicago Press, Chicago, pp. 301–325.

O'Donell, C.A., 1957. Convolvuloideas chilenas. Bol. Soc. Arg. Bot. 6, 143–184.

Pezoa, L., 2003. Recopilación y análisis de la variación de las temperaturas (período 1965-2001) y las precipitaciones (período 1931–2001) a partir de la información de estaciones meteorológicas de Chile entre los 33° y 53° de latitud Sur. Licentiate Thesis, Universidad Austral de Chile, 122 pp.

Poiani, A., Del Pozo, A., 1986. Efectos de la defoliación experimental sobre la producción de semillas y el crecimiento de Colliguaya odorifera Mol., Euphorbiaceae. Rev. Chil. Hist. Nat. 59, 39–45.

Quezada, I.M., Gianoli, E., 2006. Simulated herbivory limits phenotypic responses to drought in Convolvulus demissus (Convolvulaceae). Pol. J. Ecol. 54, 499–503.

Schlichting, C.D., Pigliucci, M., 1998. Phenotypic Evolution: A Reaction Norm Perspective. Sinauer, Sunderland.

Schulze, E.D., 1986. Whole-plant responses to drought. Aust. J. Plant Physiol. 13, 127–141.

Schulze, E.D., Beck, E., Müller-Hohenstein, K., 2005. Plant Ecology. Springer, Berlin.

Stowe, K.A., Marquis, R.J., Hochwender, C.G., Simms, E.L., 2000. The evolutionary ecology of tolerance to consumer damage. Annu. Rev. Ecol. Syst. 31, 565–595.

Strauss, S.Y., Agrawal, A.A., 1999. The ecology and evolution of plant tolerance to herbivory. Trends Ecol. Evol. 14, 179–185.

Sultan, S.E., 1995. Phenotypic plasticity and plant adaptation. Acta Bot. Neerl. 44, 363–383.

Sultan, S.E., Bazzaz, F.A., 1993. Phenotypic plasticity in Polygonium persicaria. II. Norms of reaction to soil moisture and the maintenance of genetic diversity. Evolution 47, 1032–1049.

Tiffin, P., Inouye, B.D., 2000. Measuring tolerance to herbivory: accuracy and precision of estimates made using natural versus imposed damage. Evolution 54, 1024–1029.

Torres, J.C., Gutiérrez, J.R., Fuentes, E.R., 1980. Vegetative responses to defoliation of two Chilean Matorral shrubs. Oecologia 46, 161–163.

Valladares, F., 2004. Global change and radiation in Mediterranean forest ecosystems: a meeting point for ecology and management. In: Arianoutsou, M., Papanastasis, V. (Eds.), Ecology, Conservation and Sustainable Management of Mediterranean Type Ecosystems of the World. Millpress, Rotterdam, pp. 1–4.

Valladares, F., Gianoli, E., Gómez, J.M., 2007. Ecological limits to plant phenotypic plasticity. New Phytol. 176, 749–763.

Wise, M.J., Abrahamson, W.G., 2007. Effects of resource availability on tolerance to herbivory: a review and assessment of three opposing models. Am. Nat. 169, 443–454.