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Re-introduction success of an autochthonous plant species, *Periploca angustifolia,* in the Natural Reserve of Oued Dekouk, Tunisia

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Abstract

The study evaluates the regeneration success of an autochthonous plant species, *Periploca angustifolia* Labill. (periploque), using spontaneous (pre-existing) and transplanted (re-introduced from a nursery) plants in the Natural Reserve of Oued Dekouk, southern Tunisia. We compared the growth status of both types of shrubs using morphological and ecophysiological parameters. The results show that spontaneous periploque was more vigorous and appears more adapted to aridity than transplanted periploque. Indeed, spontaneous shrubs have higher growth rates than transplanted ones. They also had higher leaf water potentials, indicating excellent plant water status recovery, and elevated photosynthetic assimilation rate. The better growth of spontaneous shrubs seems to be due to the efficacy of its aridity adaptive mechanisms. They limit water loss through a low leaf area and stomatal density and an efficient stomatal function. Furthermore, spontaneous plants have an efficient water-conducting system less vulnerable to embolism. Transplanted plants may have a less developed root system limiting their ability to absorb soil moisture. The present study indicates that the re-establishment of endangered plant species into areas with limited rainfall maybe more feasible by direct seeding or self regeneration, despite its slowness, than by the hardy and costly transplantation-technique.

Additional key words: aridity; endangered species; hydraulic conductance; periploque; transplantation.

Resumen

Éxito en la reintroducción en la Reserva Natural de Oued Dekouk, Túnez, de una planta autóctona, *Periploca angustifolia*

El estudio evaluó el éxito en la regeneración de una planta autóctona, *Periploca angustifolia* Labill. (cornical), introducida por la técnica de transplante en la Reserva Natural de Oued Dekouk (sur de Túnez), con un clima mediterráneo supra sahariano. Se llevó a cabo un estudio comparativo entre arbustos espontáneos (pre-existentes) y transplantados (a partir de un vivero) y se midieron varios parámetros morfológicos y ecofisiológicos. Los resultados muestran que los cornicales espontáneos fueron más vigorosos y están mejor adaptados a la aridez que los transplantados. De hecho, los arbustos espontáneos tienen mayores ratios de crecimiento que los transplantados. También mostraron mayor potencial hídrico en las hojas, lo que indica una excelente recuperación del estatus hídrico en la planta, y una elevada tasa de asimilación fotosintética. El mejor crecimiento de los cornicales espontáneos parece ser debido a la eficacia de sus mecanismos de adaptación a la aridez: limitan las pérdidas de agua mediante una menor área de la hoja y de densidad de estomas y una eficiente función de estos. Además, las plantas espontáneas tienen un sistema de conducción de agua eficiente menos vulnerable a la embolia. Las plantas transplantadas pueden tener un sistema de raíces menos desarrollado que limite su capacidad para absorber la humedad del suelo. El presente trabajo indica que la reintroducción de especies en peligro de extinción en áreas con precipitaciones limitadas puede ser más viable por siembra directa o por auto-regeneración, a pesar de su lentitud, que por la dura y costosa técnica del transplante.

Palabras clave adicionales: aridez; conductancia hidráulica; cornical; plantas en peligro de extinción; transplante.

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Abbreviations used: A (photosynthetic assimilation rate), E (leaf transpiration rate), g_s (stomatal conductance), K (hydraulic conductance), LA (leaf area), PLC (percentage loss of hydraulic conductivity), SD (stomatal density), VC (sulnerability curve), Ψ_{md} (mid-day leaf water potential), Ψ_{pd} (predawn leaf water potential), Ψ_{stem} (stem water potential).

Introduction

For several years, climate change had critical effects in several regions of the world. This can lead to catastrophic consequences on the sustainability of ecosystems, both natural and cultivated. In North Africa, the areas bordering the Sahara desert are frequently subjected to drought spells which destroy natural vegetation and lead to the advance of sand dunes. This precarious situation is aggravated by over-grazing by the herds of the nomadic population occupying the area (Zaafouri and Chaieb, 1999). Pre-saharian spontaneous vegetation, in southern Tunisia, is mainly limited to steppe vegetation (Floret *et al.*, 1983). This region is characterized by extreme climatic aridity, and is highly affected by various human activities (Le Floc'h, 1995). As a result, no virgin ecosystems exist in this region at the present time. Most ecosystems have reached a degradation threshold beyond repair and would require active human intervention to be restored to a more equilibrated state (Ferchichi and Neffati, 1992).

The creation of protected territories such as biosphere reserves can enhance the sustainability of ecosystems and genetic heritage (*in-situ* conservation of species and varieties) (Ramade, 1997). Such reserves can be complementary to the efforts made for *ex-situ* conservation of threatened species (*e.g.* gene banks). To this end, Tunisia has established a number of national parks and natural reserves in different bioclimatic zones; among them the Natural Reserve of Oued Dekouk which is located in the southern part of the country and has an upper-saharian bioclimate.

In order to accelerate ecosystem recovery and prevent the extinction of several presently endangered autochthonous species, nurseries were set up and plantings of these species were established at several locations.

Periploque (*Periploca angustifolia* Labill.) is an autochthonous species of the Mediterranean region; it is well-adapted to arid climates and has a high pastoral and ecological value. It is a range shrub with continuous vegetative production when soil humidity allows it (Ferchichi, 1996). It grows on different types of soil but prefers calcareous substrates. In Tunisia, the species has a wide distribution extending from Jebel Ichkeul (sub-humid bioclimate) in the north to Dhiba (upper-saharian bioclimate) in the south. It commonly grows in areas where annual rainfall ranges from 100 to 400 mm (Chaieb and Boukhris, 1998).

The present work is a comparative study of spontaneous and transplanted (from a nursery) periploque shrubs using morphological and ecophysiological traits (shoot elongation, stomata density, plant water relations, gas exchange measurements and stem hydraulic conductivity). Its objective was to evaluate the growth status of transplanted shrubs compared to spontaneous ones.

Material and methods

Study area and plant material

The study was carried out in the Natural Reserve of Oued Dekouk (southern Tunisia, 10°32' E; 32°08' N), 37 km to the south of the town of Tataouine (Fig. 1). The area has an upper-Saharain Mediterranean climate. According to nearest weather stations, rainfall varied between 90 and 138 mm per year and January was the wettest month during the period 2000-2006. During the three months of 2007 spring, there was no rain except for 5 mm on 27 April. Average annual temperature and relative humidity were 20.4°C and 55%, respectively. The region is also subjected to at least 37 days of south-west dry and hot winds (called Sirocco). The predominant soil type is a raw mineral sandy.

Two types of periploque shrubs were used in this study: spontaneous (self-seeded) and transplanted (from a nursery). Seeds for the nursery were collected

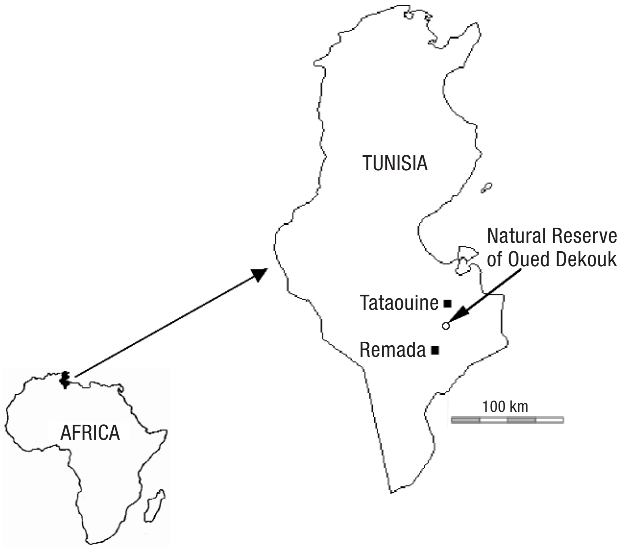

Figure 1. Location of the study area, the Natural Reserve of Oued Dekouk, between the towns of Tataouine and Remada, Tunisia.

from several locations in southern Tunisia. Therefore, the transplanted plants are assumed to be genetically identical to the spontaneous ones but they have started out their development, *i.e.,* their juvenile phase, in a nursery in 2002 before being transplanted to the park after one year. These seedlings were established near the spontaneous shrubs and were irrigated once a month during the first three months to help them endure transplantation stress. The young transplanted plants were partially sheltered with a couple of dry palms as a protection against south-west winds. These palms are generally over-grown or removed after a year or two. The spontaneous shrubs did not receive any care.

Six shrubs of each type (spontaneous and transplanted) comparable in age (about five years old) and size (about 80 cm high), growing in the same part of the park were used for the study. The shrubs' growth rate, plant water potentials, stomata density, gas exchange and stem hydraulic conductivity were measured on the 15th day of each month of spring 2007 (March, April, May).

Measurements

Shoot extension was measured monthly on six plants of each type; on each plant, six stems were measured. Water status was evaluated in mid-May by measuring predawn (Ψ_{pd}) and mid-day (Ψ_{md}) leaf water potentials and stem water potential (Ψ_{stem}) with a Scholander pressure chamber (The PMS instrument Company, Albany, Oregon, USA). The Ψ_{stem} was measured using the covered leaf technique. The terminal part of the shoot was enclosed in an aluminium bag for at least 2 h before the measurement to allow leaf and xylem water potentials to equilibrate. Ψ_{stem} was measured on 1-year-old leafy twigs because the petioles were too short to allow the leaves to be put individually in the Scholander pressure chamber.

Gas exchange measurements were carried out under saturating light conditions between 09:00-10:00 h with an LCpro+ portable photosynthesis system (ADC, BioScientific Ltd., Hoddensdon, UK). Air temperature ranged between 20°C and 30°C. The measurements were conducted in replicates of four readings on each of three leaves per plant. Six plants per shrub type were used each time.

To determine the stomata density (SD), a thin layer of nail polish was applied to the upper and lower sides of the leaf after its trichomes were first removed with adhesive tape. Once dry, the polish layers were carefully peeled-off with adhesive tape, then fixed on a microscope slide and examined under a light microscope (Micros Austria, Vienna, Austria) equipped by camera (CAM2800-XP 3.0, Micros Austria, Vienna, Austria) interfaced to a computer. The number of stomata per leaf area unit was determined. Five leaves from each shrub were used, and three random counts were carried out on each side of a leaf.

Xylem embolism was quantified by measuring the percentage loss of hydraulic conductivity (PLC) at several levels of plant water potential (Ψ_{stem}). Vulnerability curves (VCs) were constructed by plotting PLC values against Ψ_{stem} according to the bench drying technique. PLC, due to an air blockage, is an indirect estimate of the percentage of cavitated vessels (Cochard *et al.*, 2000). For hydraulic conductivity measurements, branches were excised under water to prevent air embolism caused by sample collection. The samples were immediately placed in black plastic bags to prevent excessive transpirational water loss during transport to the laboratory. The hydraulic conductivity was then measured by the technique described by Sperry *et al.* (1988). The technique involves measuring the hydraulic conductance (K) of 2-cm-long branch segments before (K initial, K_i) and after (K maximum, K_m) water refilling. The branch segments were excised under water and their cut ends were polished with a sharp razor blade. One of the cut ends was then hermitically attached to the hydraulic apparatus. The flow rates were measured using an analytical balance (±0.1 mg, Mettler Toledo AB204-S) interfaced to a computer. The measurement solution was a 10 mM KCl, and the delivery pressure was approximately 5 KPa. The solution flowed from a beaker sitting on the balance and through the branch segments, which was kept under water during the measurement. K_i was measured at low pressure (5 KPa). To measure K_m, air obstructing stem xylem vessels was removed by applying a series of 10-s hydraulic pressure flushes (0.2 MPa) until measured values of K_m remained constant between flushes. PLC was calculated as:

$$\mathrm{PLC} = (1 - K_i/K_m) \times 100$$

Statistical analysis

Data variance was analysed using ANOVA procedure in the SAS statistical software version 6.12 (SAS Institute, Cary, NC, U.S.A) for a completely randomi-

Table 1. Predawn and mid-day leaf water potentials (Ψ_{pd}, Ψ_{md}), internode length, leaf area (LA) and leaf stomata density (SD) of transplanted and spontaneous shrubs of *Periploca angustifolia.* Each value represents the mean (± SE) of at least three replicates

Type of plants	Ψ_{pd} (MPa)	Ψ_{md} (MPa)	Internode length (mm)	LA (mm^2)	SD (No. mm^{-2})	
					Lower side	Upper side
Transplanted	–1.57 ± 0.02	–4.13 ± 0.03	28 ± 2	99 ± 3	82 ± 1	81 ± 4
Spontaneous	–1.45 ± 0.04	–3.33 ± 0.07	16 ± 4	91 ± 2	47 ± 5	56 ± 5

zed design. All measurements were replicated at least three times.

Results

Morphological traits and growth rate

Spontaneous periploque plants were more vigorous than transplanted ones. They had denser canopies and greener leaves. Most of the leaves of transplanted shrubs turned yellow at the end of the observation period and started to fall indicating a severe water stress. Spontaneous plants had shorter internodes and smaller leaves with lower stomata densities (Table 1). The two sides of a leaf had similar stomata densities regardless of shrub type.

Shoot extension of spontaneous shrubs started earlier and was fastest in March (Fig. 2). The shoot reached 50% of their final length by mid-March. Shoot extension of transplanted plants was only one fourth of that of spontaneous ones.

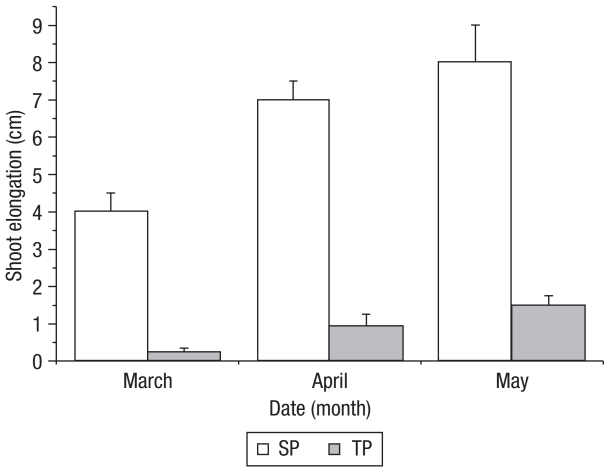

Figure 2. Cumulative shoot elongation of spontaneous (SP) and transplanted (TP) shrubs of *Periploca angustifolia.* Bars represent standard errors (n = 6).

Plant water relations

The transplanted shrubs had lower Ψ_{pd} and Ψ_{md} than spontaneous ones (Table 1) possibly suggesting that the former plants were water stressed.

Gas exchange

The evolution of stomatal conductance (g_s) shows that the types of shrubs behave differently (Fig. 3a). In March and May, spontaneous shrubs had higher g_s than transplanted ones. This difference was no longer significant in April.

The photosynthetic assimilation rate (A) showed a trend similar to that of g_s for types of shrubs (Fig. 3b). The A of transplanted plants was highest in March, and then decreased gradually during the two following months. Spontaneous shrubs had higher A than transplanted ones in March. However, in April, A of the former plants decreased sharply and reached values slightly lower than those of transplanted periploque. In May, A of spontaneous shrubs increased to values equal to those recorded in March and higher than those of transplanted ones.

Leaf transpiration rate (E) evolved differently in the two types of shrubs (Fig. 3c). The transplanted plants maintained a stable E during the period of study with only a slight decrease in May. However, in spontaneous shrubs, E is high in March and May and low in April. In April, spontaneous shrubs had lower E than transplanted shrubs.

Embolism vulnerability curves

The embolism vulnerability curves (VCs) obtained by plotting the percentage loss of hydraulic conductivity (PLC) in the shoot xylem against xylem water potential (Ψ_{stem}) show the same trend in both type

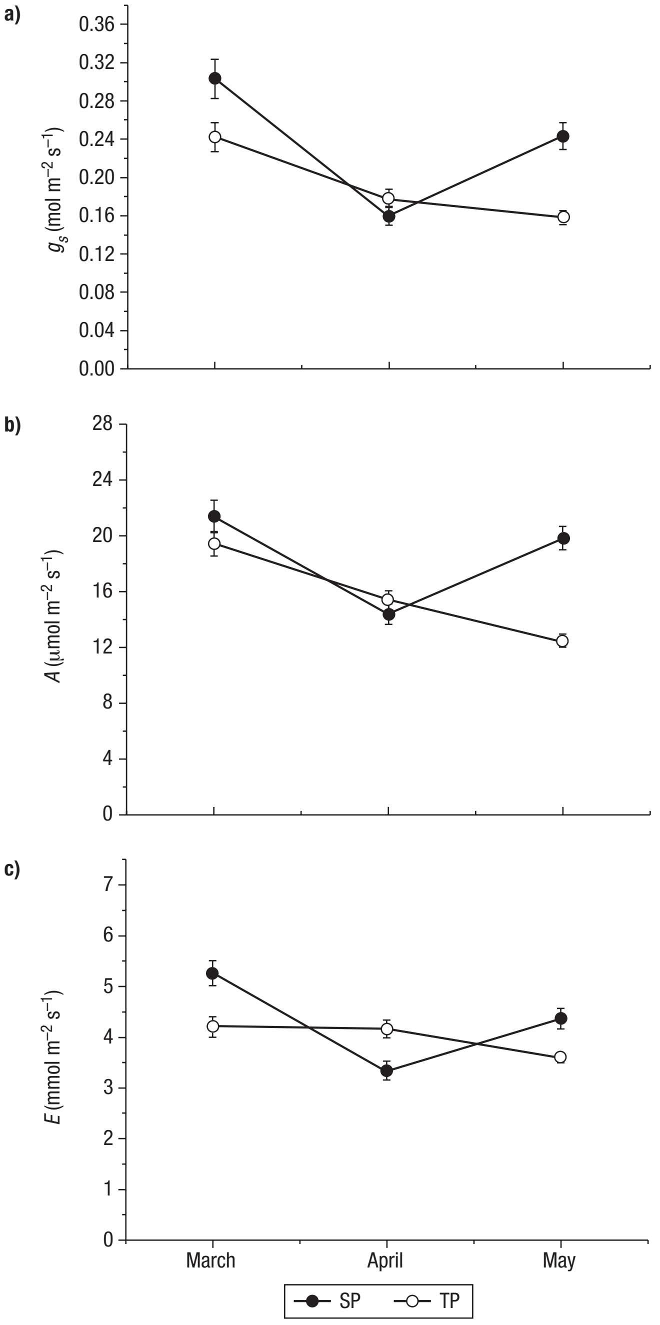

Figure 3. Gas exchanges parameters in spontaneous (SP) and transplanted (TP) shrubs of *Periploca angustifolia.* (a) stomatal conductance, g_s; (b) net photosynthetic assimilation rate, A; and (c) transpiration rate, E. Each point represents the mean (± SE) of six replicates.

shrubs (Fig. 4). The PLC started relatively low and increased as Ψ_{stem} decreased.

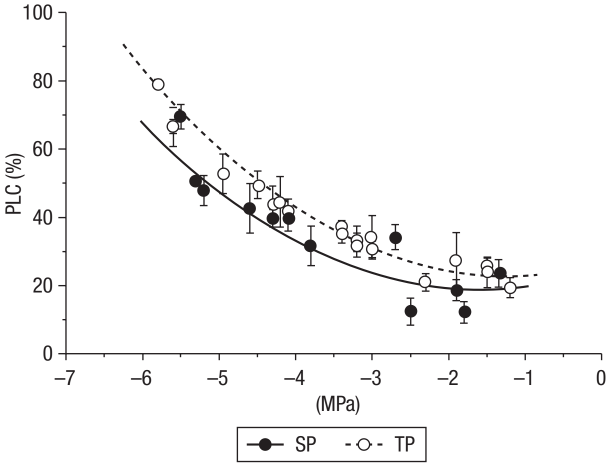

Figure 4. Xylem cavitation vulnerability curves for spontaneous (SP) and transplanted (TP) shrubs of *Periploca angustifolia* represented as the percentage loss of hydraulic conductivity (PLC) as a function of stem water potential (Ψ_{stem}). Bars represent standard errors (n = 5).

Both types of shrubs show relatively high native PLCs (20%). This may reflect drought-induced cavitation suffered by the shrubs during their development in this arid environment. The xylem water potential which induced a PLC of 50% ($\Psi_{50\%}$) was –4.5 MPa for transplanted plants and –5.3 MPa for spontaneous shrubs. Therefore, transplanted shrubs were more vulnerable to embolism than spontaneous ones.

Discussion

The biological cycle of *Periploca angustifolia*, a species well adapted to arid environments, is tightly linked to precipitation events. Water availability appears to be the most determining factor controlling the species development. The spontaneous shrubs were more vigorous with well hydrated green leaves. Whereas, transplanted shrub were showing signs of water stress (yellowish and rolled leaves). Shoot extension was also more important on spontaneous plants; final cumulative shoot growth of transplanted periploque plants was only about 25% that of spontaneous ones. Plant growth and development reflect the species compatibility with its environment through the integration of various morphological and physiological adaptations (Chaves *et al.*, 2003).

The spontaneous shrubs had smaller leaves (microphylla), shorter internodes and denser clumps; these are features of sclerophyllous species well adapted to arid environments (Lo Gullo and Salleo, 1988; Gratani and Varone, 2004). The stomata distribution in both leaf sides is another criterion of adaptation in several species (Nevo *et al.*, 2000; Bacelar *et al.*, 2004). The spontaneous shrubs had lower SD than transplanted ones; this should minimize water loss by stomatal transpiration. These morphological traits should improve water use efficiency especially in spontaneous plants (Bacelar *et al.*, 2007).

The leaf water potentials (Ψ_{pd} and Ψ_{md}) of spontaneous shrubs were higher than those of transplanted ones. This suggests that spontaneous shrubs have a higher capacity to recover their water potentials because it is generally admitted that a wider Ψ_{pd} variation means a lower capacity to recover after a transient lowering of the plant's water potential (Angelopoulos *et al.*, 1996; Gratani and Varone, 2004). This behaviour indicates that transplanted plants are more affected by drought than spontaneous ones.

The gas exchange measurements are useful tools to evaluate the plant's ability to adapt to its environment (Chaves *et al.*, 2003; Marchi *et al.*, 2008). Gas exchange parameters are well correlated with the plant's water status (Ennajeh *et al.*, 2008). In the current study, in early spring (March), when environmental conditions were less stressful, spontaneous plants had higher g_s and A than transplanted plants (Fig. 3). In April, when soil water deficit increased, g_s and A decreased in spontaneous shrubs. These rates recovered to near their March level after it rained at the end of April. This confirms what was reported about *Periploca angustifolia*'s ability to quickly resume vegetative growth after periods of rainfall regardless of the season or the phenological stage (Ferchichi, 1996). However, in the case of transplanted plants, A and g_s decreased in April and May and did not recover despite the rain of the end of April. This suggests that transplanted plants had less developed root systems, therefore less capable of taking advantage of occasional light rains.

Plants respond to a water deficit by first closing their stomata thus decreasing their gas exchange rates (Chaves, 1991; Fernández *et al.*, 1997). In periploque, A was highly correlated with g_s ($r = 0.94$, $p < 0.01$). When soil moisture decreased in April, A, gs as well as E were affected. Stomatal transpiration is the most important phenomenon of water loss in a plant. It is influenced by various internal and external factors such as soil moisture and stomata density and distribution (Chaves, 1991). Spontaneous plants had higher E values than transplanted plants in March and May, when there was some soil moisture. However, in mid-April under more severe water deficit conditions, E in spontaneous shrubs was lower than in transplanted ones. This suggests that the former type has a better stomatal function in addition to its lower leaf SD.

Previous reports indicate that periploque is less vulnerable to embolism compared to several other Mediterranean species (Hacke *et al.*, 2000; Brodribb *et al.*, 2003; Cochard *et al.*, 2004). We report here that spontaneous shrubs are more resistant to embolism than transplanted ones as indicated by the lower $\Psi_{50\%}$ of the former type (–5.3 MPa) compared to the latter type (–4.5 MPa) (Fig. 4). Therefore, spontaneous shrubs have more efficient anatomical and physiological adaptive mechanisms to withstand embolism than transplanted plants.

Spontaneous shrubs were also more vigorous and appear more adapted to aridity. This advantage could be explained by more efficient defence mechanisms against environmental constraints. These defences may include small leaf area, shorter internodes, low leaf SD, good stomatal function and efficient water conducting-system. Furthermore, transplantation may have disrupted the plants' root systems limiting their ability to absorb soil moisture. Self-seeded plants have generally deeper root systems than transplanted ones. Deeper rooted plants can better survive occasional drought spells (Sayar *et al.,* 2007).

Several factors can influence regeneration success of plant species. The environmental conditions under which the zygote develops into a sporophyte can influence the phenotype of the offspring following germination (Schmid and Dolt, 1994).

Previous studies demonstrated that environmental maternal effects may exert a greater influence on offspring than genetic maternal effects (Schmid and Dolt, 1994; Byers *et al.,* 1997). Our results suggest a similar influence of environmental conditions during the juvenile phase on the capacity of a plant to adapt to its environment after transplantation.

In conclusion, the present study suggests that environmental effects during the juvenile phase can influence the ability of *Periploca angustifolia* plants to adapt to the post-transplantation environment. Furthermore, transplanted plants may have a less developed root system limiting their ability to absorb soil moisture and take advantage of occasional light rains. In such ecosystem rehabilitation programs, the success of the plant's transplantation and establishment depends largely on cultural practices at the nursery.

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