Running title: *Access to soil moisture and seedling survival*

Rooting depth and soil moisture control woody seedling survival under Mediterranean drought

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Summary

1. Seedling survival is one of the most critical stages in a plant's life history often associated to a high mortality. In water-stressed habitats, drought and soil desiccation arise as the main causes. It has been hypothesized that root systems accessing moist soil layers are critical to become established; however, very little is known on seedling root growth in the field and root traits implications for survival, and such relationships have rarely been addressed directly.
2. We related seedling mortality and presence of deep roots in a field experiment in which we monitored soil moisture, root growth, and seedling survival in five Mediterranean woody species from the beginning of the growing season until the end of the drought season.
3. Species showed contrasting rooting patterns and survival rates. We found strong positive relationships between survival and rooting depth as well as between survival and soil moisture. Species with deep-rooted seedlings survived drought by growing roots into deep, moist soil layers, whereas species whose roots remained in shallow, drying soils died more frequently. However, biomass allocation to roots rather than to shoots was not related to establishment success. A minimum soil moisture value seems to control plant survival, so that plant establishment is prevented under such threshold.
4. Data showed that species able to keep roots in moist soil layers stand prolonged drought periods better. Access to moist soil horizons mechanistically explains species-specific survival rates and the existence of soil moisture thresholds that control establishment provides insights into plant population dynamics in dry environments.

Key-words: dry habitats, establishment, humidity threshold, Mediterranean species, root growth.

Introduction

Seedling recruitment is one of the most critical stages in a plant's life history since high mortality rates are often associated with the seedling phase (Fenner 1987). Seedlings of different species die from a wide variety of causes (Fenner & Kitajima 1999; Moles & Westoby 2004), including many biotic and abiotic factors such as pathogens, herbivory, high or low temperatures and radiation, allelopathy and competition. Drought and soil desiccation arise as the main causes preventing establishment in many environments (Moles & Westoby 2004). One such environment is Mediterranean-type ecosystems, where establishment after germination is greatly threatened by long, dry summer periods (Herrera 1992). In such areas seedlings are very drought-sensitive, and recruitment processes are often restricted to sporadic rainfall periods (Holmgren & Scheffer 2001; Pugnaire *et al.* 2006b) or privileged microsites (Padilla & Pugnaire 2006).

Deep-rooting is a trait long known as a means to withstand water stress, as large biomass allocation to roots may improve water uptake and increase probability of survival in Mediterranean communities (Lloret, Casanovas & Peñuelas 1999). Deep-rooted plants avoid dehydration by tapping stable water reserves, which allows for better water balance, higher photosynthetic rates and growth extended into the dry season (Nepstad *et al.* 1994). Deep roots might be also advantageous for seedling establishment, as species with deeper roots have a probability of surviving drought higher than shallow-rooted species (Donovan, Mausberg & Ehleringer 1993; Canadell & Zedler 1995). Contrasting seedling survival during drought is often due to varying tolerance to low soil humidity (Hasting, Oechel & Sionit 1989; Ackerly 2004). For instance, Davis (1989) found in the California chaparral that seedlings of drought-tolerant species, often shallow-rooted, survived water shortage better than seedlings of drought-sensitive species.

Although the role of deep root systems in plant establishment has long been acknowledged (Davis 1989; Enright & Lamont 1992; Canadell & Zedler 1995; Pugnaire, Chapin & Hardig 2006a), establishment failures due to lack of deep roots have rarely been specifically tested or quantified. Very little is known on root growth patterns in the field as related to soil water status, and many of the mechanisms controlling establishment success remain poorly understood (Hanley *et al.* 2004), particularly those related to root traits (but see Lloret, Casanovas & Peñuelas 1999). This question is particularly significant in dry environments and under global change scenarios with longer drought spells (IPCC 2001). Dryer conditions may alter the regeneration niche of many species (*sensu* Grubb 1977) and species abundance and occurrence may be restricted if seedlings are unable to deal with lower water availability (Brown, Valone & Curtin 1997, Schenk & Jackson 2002).

Here, we address how drought differently affected the establishment success of five perennial species and if such differences could be explained by rooting depth and access to soil moisture. We related seedling mortality and deep roots in a field experiment in which we monitored the soil moisture profile, root growth and survival of transplanted seedlings of woody Mediterranean species from the beginning of the growing season until the end of the drought period. We hypothesized that deep-rooted seedlings would attain higher survival after summer than shallow-rooted individuals by keeping roots in moister soil horizons.

Methods

Species

We selected five native perennial woody species occurring in Mediterranean shrublands in semi-arid southeast Spain. Two of the species were nearly leafless shrubs with photosynthetic stems, including a gymnosperm, *Ephedra fragilis* Desf., and a legume, *Retama sphaerocarpa* (L.) Boiss. The other species included a succulent C_4, *Salsola oppositifolia* Desf., a large C_3 shrub, *Olea europaea* var. *sylvestris* Brot., and a tree species, *Pinus halepensis* Mill. Hereafter we refer to these species by their generic names only. *Ephedra, Olea* and *Pinus* are frequent in late-successional communities, whereas *Retama* and *Salsola* successfully colonize disturbed areas (Peinado, Alcaraz & MartínezParras 1992). Our selected species differ in drought tolerance strategy based on minimum pre-dawn water potential (Ψ_{pd}) reported. *Salsola* tolerates low water potentials, ($\Psi_{pd} \approx$ -5 MPa, Pugnaire, Armas & Valladares 2004). As for *Ephedra,* there are no data available, but preliminary measurements suggest high tolerance to low water availability ($\Psi_{pd} \approx$ -5.2 MPa, F.I. Pugnaire, unpublished data). Our other species could be considered as non-drought-tolerant based on less negative Ψ_{pd}; around -1.5 MPa for the deep-rooted *Retama* (Haase *et al.* 1999), -2.5 MPa for *Pinus* (Oliet *et al.* 2002), and -2.25 MPa for *Olea* (Faria *et al.* 1998). Germination of *Olea*, *Pinus* and *Retama* under Mediterranean conditions begins in winter (Rey & Alcántara 2000; Nathan & Ne'eman 2004; Pugnaire *et al.* 2006b). There are no accurate data for germination patterns of *Ephedra* and *Salsola*, but specific traits suggest that they germinate in winter too, because seeds disperse in late autumn or early winter (Rodríguez-Pérez, Riera & Traveset 2005) and seeds do not show dormancy (Navarro & Gálvez 2001).

Field site and experimental design

We tested our hypothesis by conducting a transplant experiment in a semi-natural field site rather than by monitoring seedling occurrence in nature. This minimized environmental heterogeneity and root losses at harvest, allowed roots to grow without soil impediments, and also allowed for comparisons of potential root growth under the same set of abiotic conditions. The experiment was set up in flat and homogeneous 15 x 15 m terrace for vegetable crop at the foothills of the Sierra Alhamilla range (Almería, Spain, 37º99'N, 02º99'W, 600 m elevation). The silt soil had been ploughed regularly for years, was free of rocks, and reached ca. 2 m in depth over a mica-schist bedrock. Fertility and water holding capacity were very low (Pérez-Pujalte 1989). Neither pesticides nor fertilizers were applied at the site for at least five years. The climate is typically Mediterranean semi-arid with a mean annual temperature of 17.3 ºC and mean annual precipitation of 282 mm and a marked drought period from May to September, though small rain events may occur. Temperatures are mild in winter and hot in late spring and summer.

In late winter 2004, eight 3 x 3 m plots spaced 1.5 m apart were laid out on the terrace in a 3 x 3 design. To homogenize soil and facilitate root growth, the soil in each plot was completely dug up to a depth of 0.5 m, using an auger (BT 120 C, Stihl AG & Co. KG, Germany) to drill 30 cm-wide holes adjoined to each other. One- to two-month-old seedlings were transplanted on early April, after heavy spring rainfalls. Seedlings were provided by local nurseries and seeds were collected in areas with similar ecological conditions. Care was taken to follow the natural recruitment dynamics of all species, and transplanting was done when seedlings of all species had already emerged in the field.

In each plot, ten bare-root seedlings of each species, similar in size and with intact root systems, were planted ca. 35 cm apart from each other and from the plot borders. The spatial arrangement of different species in each plot was fully random. The terrace was fenced to prevent herbivory, and each plot was watered once with 5 L (≈ 0.1 L of water/plant) immediately after transplanting. One plot was harvested (H, hereafter) every three weeks on average between April and September, encompassing the spring growing period and the summer drought. Initial data (referred to as H0) consisted of ten randomly harvested seedlings of every species before transplanting. The remaining harvests (H1 to

H8) were done 13, 28, 48, 66, 81, 97, 121, and 153 days after transplanting. On each harvest all living individuals in a randomly chosen plot were dug out carefully, and maximum rooting depth was accurately recorded. To take root systems out without serious damages and losses, a three-meter-long trench was dug around the periphery of the plot. Initially the trench was *ca.* 40 cm in width from the plot area and 30 cm deep, but the depth of the trench increased in successive harvests until reaching *ca.* 100 cm on the last sampling date. The front of the trench was gently crumbled from side to side with a hoe, which was replaced by a small punch when close to the base of the plant. Roots were carefully brushed and then manually extracted and stored in paper bags. Soil containing root that could not be separated in the field were carried to the laboratory. Similarly, broken roots and soil lumps suspected of having roots were also collected. Roots could be easily matched with individuals because all species had one or several major tap rots, grew vertically, and none spread horizontally. Fine roots attached to major tap roots were collected as well.

Trenches let us accurately register the maximum rooting depth reached by each individual. After collecting, samples were immediately carried to the laboratory for root extraction. Roots and soil were repeatedly submerged in water and finely sieved to retain fine roots. Soil particles attached to roots were manually separated with a fine brush. Shoot and root biomass were obtained after drying samples in a ventilated oven at 71 ºC for at least 48 hours. Root-to-shoot ratio (R:S) for each plant were obtained from these data.

Species survival was recorded at harvest by counting living individuals of each species in the harvested plot. Survival rate was obtained in relation to the number of plants that survived transplant after one week. Soil moisture and temperature at a depth of 5, 15, 30, 45, and 60 cm were continuously monitored during the experiment in the last plot harvested. One probe for measuring soil volumetric water content (ECH$_2$O, Decagon

Devices, Inc., Pullman, WA, USA) and temperature (Onset Computers, Pocasset, MA, USA) was inserted at each depth. Readings were taken every ten minutes and averaged daily. Soil water content at any given depth within an interval was determined through interpolation between neighboring readings, assuming that water content in the interval changed linearly. To draw the evolution of a particular soil moisture value with time and depth we inferred soil depth corresponding to such particular moisture value. To achieve this, soil depth having such moisture value was back-interpolated from readings of neighboring probes that comprised moisture both above and below the target value.

Growth analysis and statistics

Mean relative growth rate (RGR) for each species during the monitoring period was calculated from observed values between H8 and H0, using the Hunt *et al.* (2002) spreadsheet tool. Growth curves were analyzed using ANCOVA on log-transformed observed values with number of days after transplanting as a covariate. Differences among species were considered significant when the species x time interaction was significant. Relative root extension rates (RER) between two consecutive harvests were obtained for each species from fitted polynomial curves using the HPcurves v.3.0 software (Pooley *et al.* 2003). Differences in biomass and maximum rooting depth among species at H8 were tested using one-way ANOVA followed by Scheffé post-hoc comparison tests. Heteroscedastic variables were log-transformed to meet ANOVA assumptions. Differences in seedling survival among species in September were compared through simple binary logistic regression where survival was the dependent variable and species the predictor factor (Agresti 2002). Regression analyses were performed to test correlation strength between variables, using adjusted R^2 to correct for the degrees of freedom. All analyses were conducted with SPSS v13.0 (SPSS Inc., Chicago, IL, USA) and differences were significant at $P< 0.05$. Sample size in all analyses was 4-10 for each species, with the exception of *Pinus* at H8, when only two plants remained alive. Data are presented as means ± one standard error.

Results

In our field site, a rainy spring (205 mm *vs.* 101 mm average in the 1967-1997 period, Confederación Hidrográfica del Sur) was followed by a summer with no rainfall (Fig. 1S). Survival in spring –April to June– was 100% in all species except *Ephedra*, whose seedlings reached a mortality rate of 12.5% by mid-June, and increased to 40% in September. By contrast *Retama* and *Salsola* maintained maximum survival rates (100%) throughout the season, whilst *Olea* reached 80% and *Pinus* 20% (Fig. 1). Moisture decreased quickly in top soil layers as the drought period progressed, reaching values in September of 1.5 % and 11.5 % at 5 and 15 cm respectively; soil humidity remained at ~21 % from mid-June onwards at 45 and 60 cm. Thus soil moisture in September showed a strong gradient, increasing with depth (Fig. 1).

Species differed significantly in plant size (Table 1). Significant differences were found in the maximum depth reached by roots in September (one-way ANOVA $F_{4,23} = 11.7$, $P<0.001$). The two early colonizers, *Salsola* and *Retama*, rooted deepest and also had the highest mean root extension rates (RER_{mean}, Table 1). By contrast, the shallowest roots were found in *Ephedra* and *Pinus*, which also had the lowest mean root extension rates. In September, at least one tap root of *Olea*, *Retama* and *Salsola* reached well below 35 cm, whereas roots of *Ephedra* and *Pinus* did not surpass 25 cm (Fig. 1). While the root-to-shoot ratio in all species was below 0.5, significant differences were detected regarding allocation patterns (one-way ANOVA $F_{4,23} = 29.3$, $P<0.001$). *Pinus* allocated the most to roots, followed by *Olea*, *Retama* and *Ephedra*. By contrast, allocation to roots relative to shoots was rather small in *Salsola* (R:S < 0.1). Root-to-shoot ratios did not increase in response to increasing drought but on the contrary decreased over the course of the season in *Olea, Pinus* and *Salsola,* and remained relatively constant in *Ephedra* and *Salsola* (Fig. 2S).

At the end of the drought season there were significant differences in seedling establishment among species (logistic regression $\chi^2 = 24.2$, df = 4, P<0.001). There were strong positive relationships between seedling survival and maximum rooting depth (logistic function, R_{adj}^2=0.99, P<0.01, Fig. 2a), and between survival and the soil moisture estimated at the maximal rooting depth of the species at final harvest (logistic function, R_{adj}^2=0.97, P<0.02, Fig. 2b), showing that the probability of establishment success strongly related to increasing rooting depth and therefore soil moisture. Species whose roots accessed soil layers below 45 cm had 100% survival rates (*Salsola* and *Retama*), whereas much lower rates (20-40%) were found in species that rooted in shallower, dryer soil layers (*ca.* 22.5 cm, *Ephedra* and *Pinus*). Species rooting in layers with < 12% soil moisture established poorly (*Pinus*, *Ephedra*). On the contrary, full establishment was exhibited by species whose roots reached soil layers above ~18 % moist (*Retama* and *Salsola*). There was, however, no relationship between survival at final harvest and R:S ratio (linear regression, R_{adj}^2=0.12, P>0.38, Fig. 2c). Initial plant size did not correlate with maximum rooting depth at H8 nor RGR, (linear regression, *P*>0.57 and *P*>0.3, respectively) or survival at H8 (*P*>0.3 for all fitted functions).

Species also differed in root growth patterns over spring and summer (ANCOVA species x time $F_{4,333}$ = 18.4, P<0.001, Fig. 3S). Roots of *Salsola* displayed a parabolic growth curve characterized by rather low RER values early in the season, followed by a period of increasing growth rate until the onset of the drought season in mid-June, at which point RER started to decrease. In contrast, *Ephedra*, *Olea* and *Retama* grew at a constant rate from April to September. *Ephedra* and *Olea* shared nearly identical RER, whereas *Retama* exhibited larger values. Growth rate of *Pinus* decreased from the beginning, showing the highest value in the first harvest and the lowest in the last one.

Discussion

The effect of summer drought on seedling establishment has long been acknowledged in Mediterranean environments (Herrera 1992); however, to our knowledge, the direct links between rooting depth, soil moisture, and establishment were never quantified. We found that survival was related to keeping roots within a minimum soil humidity value that control plant establishment. Our data show that the ability to develop deep roots was decisive for seedlings to survive summer drought, regardless of species-specific drought tolerance. Deep-rooted seedlings either from a drought-tolerant species (based on minimum Ψ_{pd} reported) such as *Salsola* or a drought-sensitive species such as *Retama* had consistent access to moist soil layers and showed the greatest survival rates. By contrast, shallow-rooted seedlings of *Ephedra* (a drought-tolerant species) and *Olea* and *Pinus* (more drought-sensitive species) relied on water from shallower soil layers and died as summer drought progressed.

Climate change scenarios predict for western Mediterranean basin reduced annual precipitation, shifts in seasonal rainfall patterns (decreasing in spring, summer and autumn) and extended drought periods (IPCC 2001). Here, we looked at species ability to extend their roots fast enough to keep pace with retreating soil humidity and showed that deep-rooted seedlings were best fitted for establishing under a very dry growing season due to such capacity, suggesting that these species may be favored over species of shallow-rooted seedling under extended drought periods. Whether shallow-rooted species would decrease in abundance or be confined to more humid patches or microsites still remains unknown

(Schenk & Jackson 2002); however, it is worth noticing that shifts in regional climates are currently leading to changes in vegetation type dominance, e.g., the encroachment of shrubs into grasslands (Brown, Valone & Curtin 1997) most likely because new conditions favor establishment of deep-rooted species (Schenk & Jackson 2002).

The relationship reported between soil moisture and survival suggests the existence of a threshold in soil humidity that control plant establishment (Figure 3). In our system, very low establishment rates were achieved by species that kept roots in shallow soil layers with moisture around 12 % (e.g., *Ephedra* and *Pinus*), and according to our data, no establishment would occur for plants rooting in layers drier than ~8%. On the other hand, higher establishment rates were found for deep-rooted species reaching soil layers wetter than 15 % (*Retama* and *Salsola*), and full establishment would be related to rooting in soil layer moister than ~20 %. It is likely that something similar occurs in natural systems, where obviously the threshold will vary depending on soil properties and the species involved. Rainfall-dependent recruitment dynamics reported in dry environments can be interpreted under such operating thresholds. Kitzberger, Steinaker & Veblen (2000) and Holmgren *et al.* (2006) showed that recruitment in dry years is almost lacking, whereas rainy years constitute a window of opportunity for establishment. We suggest that plants in dry habitats may establish easily in wet years without deep roots because soil moisture is kept above the critical threshold along the soil profile (Fig. 3). Conversely, when moisture in the soil profile is below the threshold (m_0), it is not enough to maintain seedlings alive. Soil moisture between both extremes would pivot around the critical threshold, and rooting ability and drought tolerance of the different species would explain variation in establishment patterns.

The lack of rain during our experiment produced a vertical soil moisture gradient, but summer rains could have replenished soils and altered the gradient, most likely changing the final outcome. This is supported by research evidencing that alleviation of summer drought by watering boots establishment success in Mediterranean environments (Castro *et al*. 2005), and work conducted in a nearby area with the species we used here showed that irrigation during summer did considerably raise survival in all species (Sánchez *et al.* 2004). Our interpretation is that small rainfalls (like watering) keep soil moisture above certain thresholds and that rain events in summer may determine seedlings' fate.

Plants may adjust to resource imbalance by allocating biomass to organs that acquire the limiting resource (Chapin *et al.* 1987), so that a higher allocation to roots (i.e., higher root-to-shoot ratios) could be expected under water stress. We found, however, no relationship between survival and R:S ratio, and paradoxically the most successful survivor, *Salsola*, allocated relatively the least to roots, and the species with most failures, *Pinus*, had the highest R:S ratio. Overall we did not find substantial changes in R:S during the growth period, in contrast to reports that found shifts in dry mass partitioning between shoots and roots during plant growth (Klepper 1991). Biomass allocation to roots did not increase in any species in response to drought, suggesting that large R:S ratios may not be enough to compensate for the deepening of moisture along the soil profile in summer. Rather, the ability to alter rates, timing and placement of root proliferation may have greater importance for plant success than changes in biomass allocation between roots and shoots (Reynolds & D'Antonio 1996). Lloret, Casanovas & Peñuelas (1999), however, reported a large positive correlation between R:S and seedling survival in a Mediterranean shrubland, but in their field site roots rarely reached below 10 cm and the high summer rainfall in the reported years kept shallow soil horizons moist. Under such circumstances greater biomass allocation to roots may allow for more efficient water uptake.

Some species from dry environments have dual root systems with shallow lateral roots that exploit small rainfall events which hardly penetrate into the soil, and deep roots that tap deep water sources (Canadell & Zedler 1995). However, dual systems often develop as the plant matures, and the absence of lateral branches is frequent in seedlings from xeric habitats (Canadell *et al.* 1999; Nicotra, Babicka & Westoby 2002). Our observations agree with these patterns, since roots of the five species grew vertically and none spread horizontally. This suggests a primary investment to develop root systems that penetrate into deeper, more reliable water sources rather than allocating biomass to develop both surface and deep roots, because moisture in the soil surface is unreliable (Ehleringer & Dawson 1992).

In conclusion, our work contributes to understand plant strategies to deal with seasonal drought at the seedling stage and underlines the importance of rooting depth in plant survival. In the absence of other constraints on establishment like dispersal, seed germination triggers, or herbivory, the ability to reach deeper, moister soil horizons is critical to cope with water stress at such early stage and become established. Our data suggests that species able to keep roots in moist soil layers are better prepared to withstand drought. Also, soil moisture thresholds seem to control plant survival, so that plant establishment may be restricted if soil moisture is below certain levels, which has direct implications for population and community dynamics.

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Tables

Table 1. Final plant size, mean relative growth rate (RGR_{mean}) and mean root extension rate (RER_{mean}) between initial and final plant sizes; maximum rooting depth and root-to-shoot ratio (R:S) of each species. Significant differences among species are indicated by F values, *** $P<0.001$. Different letters in a row show significant differences among species at $P<0.05$ (one-way ANOVA, Scheffé's test). Values are means ± 1SE.

Species	*Ephedra*	*Olea*	*Pinus*	*Retama*	*Salsola*	$F_{4,23}$
Total mass (g)	0.267 ± 0.06^{ac}	1.572 ± 0.326^{b}	0.152 ± 0.024^{c}	1.221 ± 0.285^{ab}	75.012 ± 17.011^{d}	67.963^{***}
RGR_{mean} (mg g^{-1} day^{-1})	17.1±3.4	13.7±0.5	4.4±1.9	25.5±7.8	48.0±5.1	-
RER_{mean} (mm cm^{-1} day^{-1})	5.3±3.3	5±1.3	1.9±1.2	9.3±3.8	10.8±2.5	-
Rooting depth (cm)	20.6 ± 3.1^{a}	35.2 ± 2.3^{ab}	15.9 ± 2.7^{a}	47.3 ± 6.9^{b}	59.5 ± 6.7^{b}	11.651^{***}
R:S ratio	0.22 ± 0.03^{a}	0.43 ± 0.02^{a}	0.49 ± 0.07^{a}	0.31 ± 0.04^{a}	0.08 ± 0.01^{b}	29.319^{***}

FIGURES

Figure 1

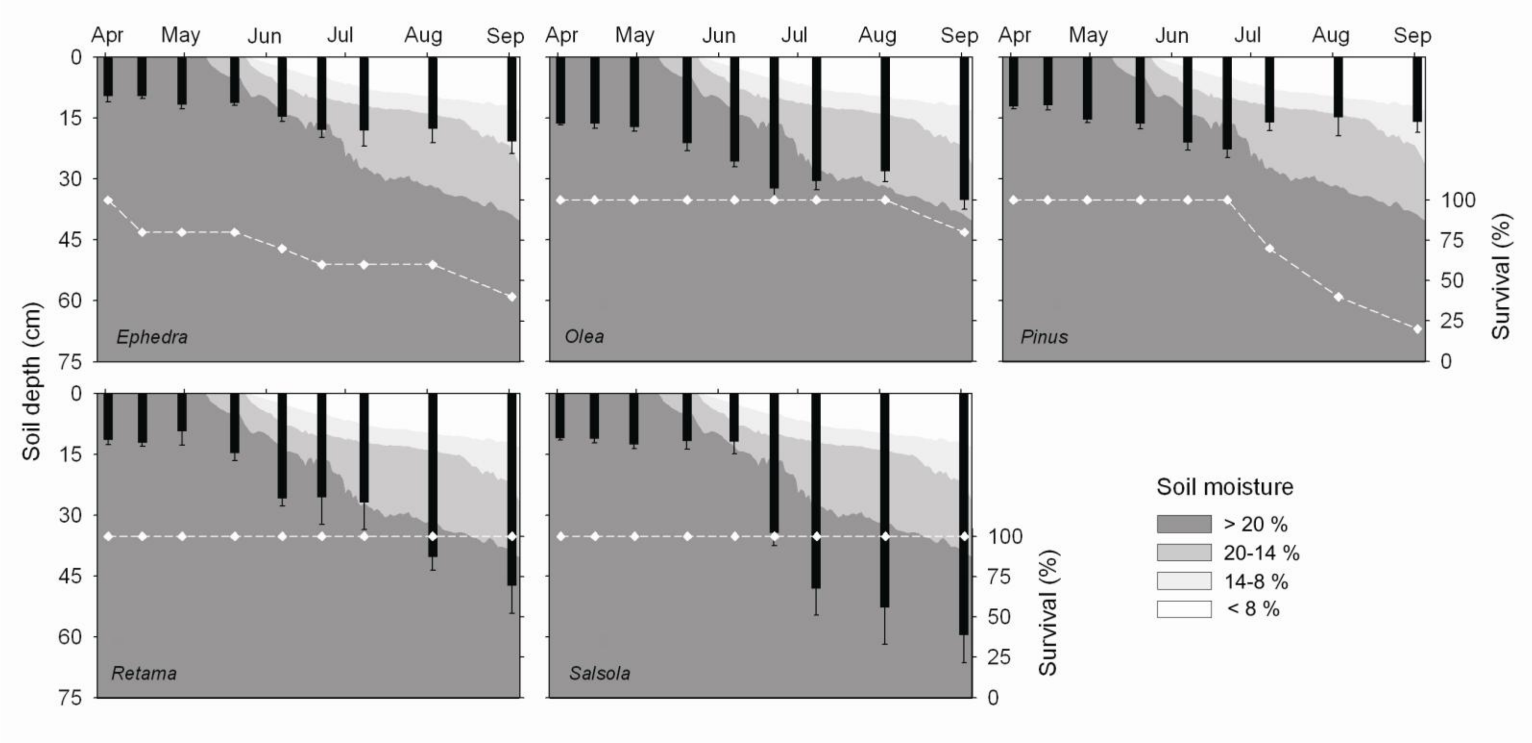

Figure 1. Maximum rooting depth (solid bars) and isoclines of soil moisture (shaded areas) on left Y-axis and seedling survival (white dots) on secondary right Y-axis. Soil layers with moisture above 20% are represented by dark grey; in grey area moisture ranged 14-20%; pale grey area indicate 8-14%, and white area below 8%. Values of soil moisture in the interval 0-5 cm depth were extrapolated due to the lack of humidity probes within this range. Values of rooting depth are means ± 1SE.

Figure 2

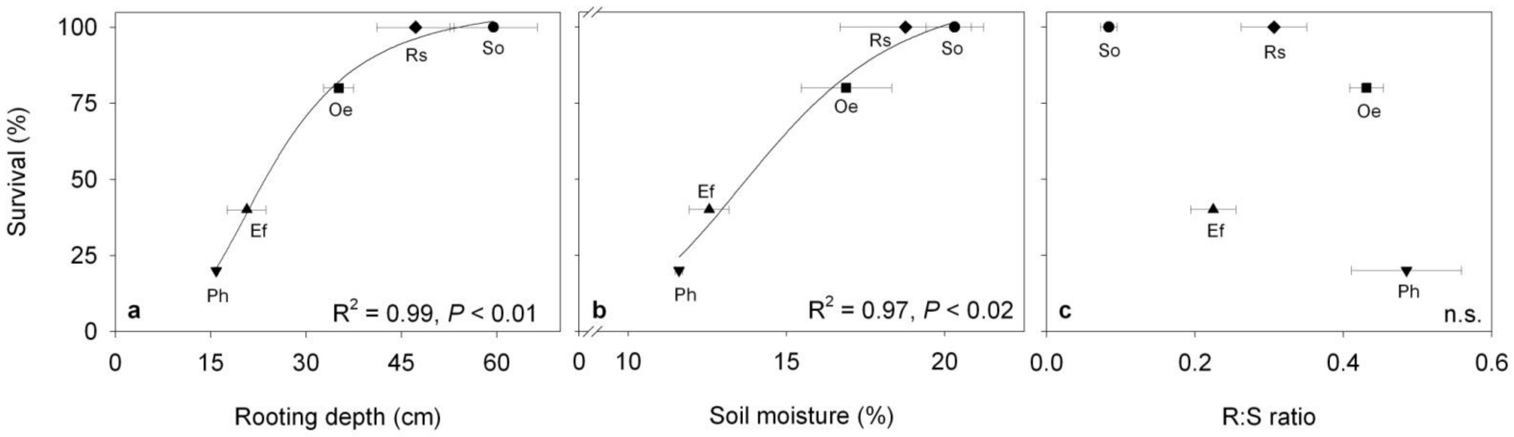

Figure 2. Relationships between survival and, maximum rooting depth (a), moisture at the deepest soil layer reached by roots (b), and R:S ratios in September (c), after the summer drought. Values are means ± 1SE, with the exception of survival. Ef, *Ephedra*; Oe, *Olea*; Ph, *Pinus*; Rs, *Retama*; So, *Salsola*. n.s.= not significant correlation at P<0.05.

Figure 3

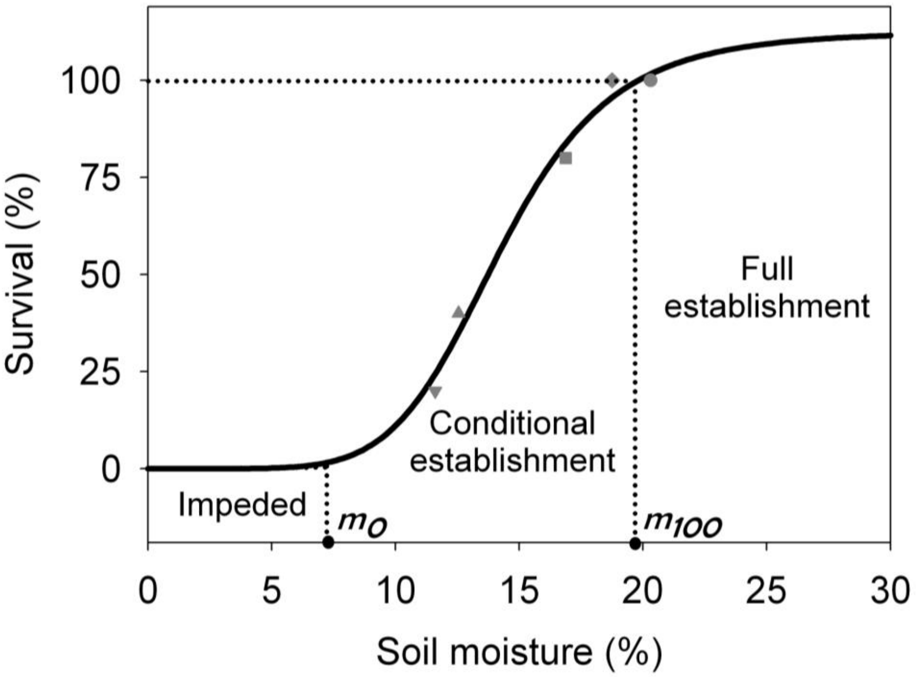

Figure 3. Proposed control of soil moisture on seedling establishment. There is a point below which seedling establishment is impeded due to insufficient moisture (m_0) and another point above which full establishment is reached (m_{100}). Conditional establishment occurs between m_0-m_{100} depending on rooting depth and species-specific drought tolerance. Grey dots show survival of our species.

Supplementary material

Figure 1S

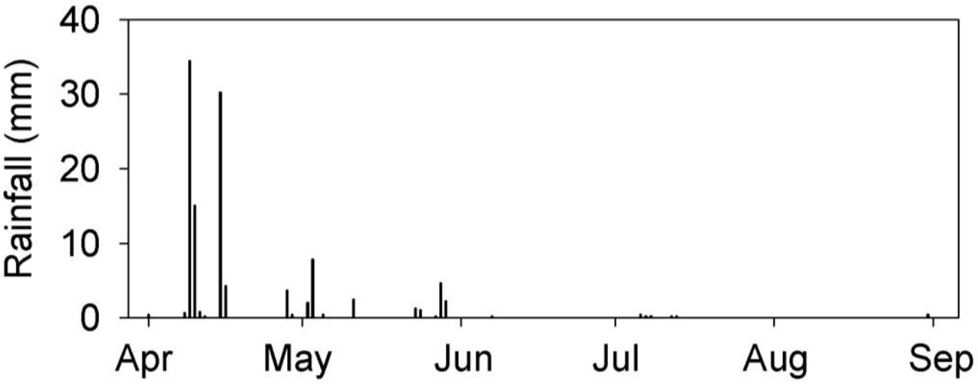

Figure 1S. Daily rainfall between April and September 2004.

Figure 2S

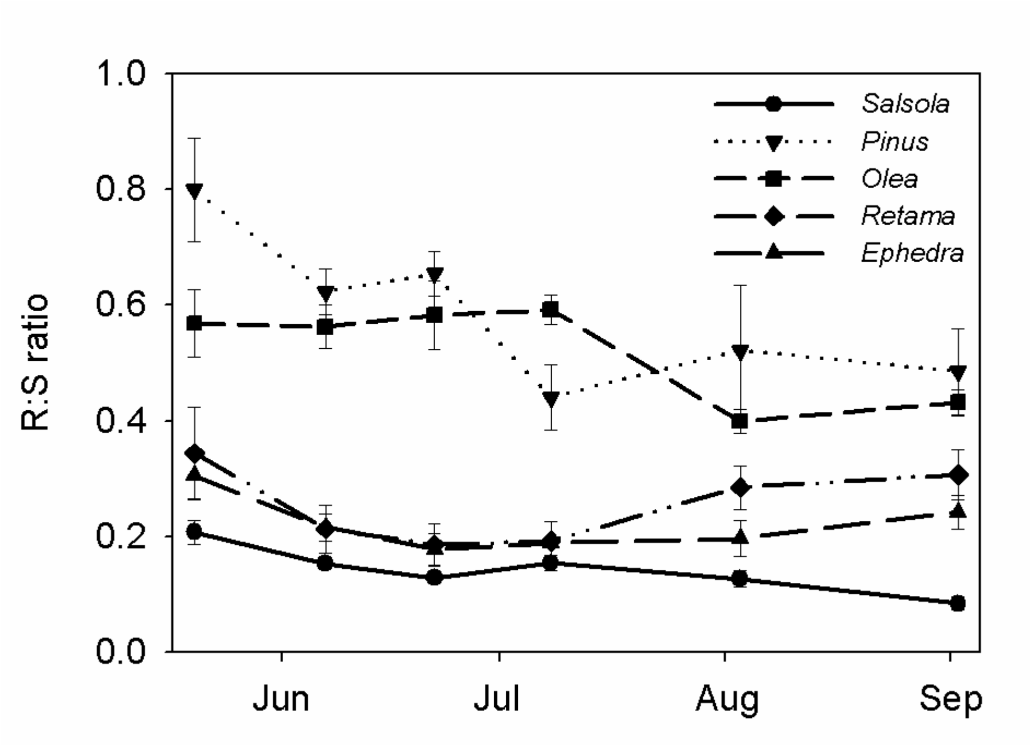

Figure 2S. Root-to-shoot ratio (R:S) for each species. Values are means ± 1SE.

Figure 3S

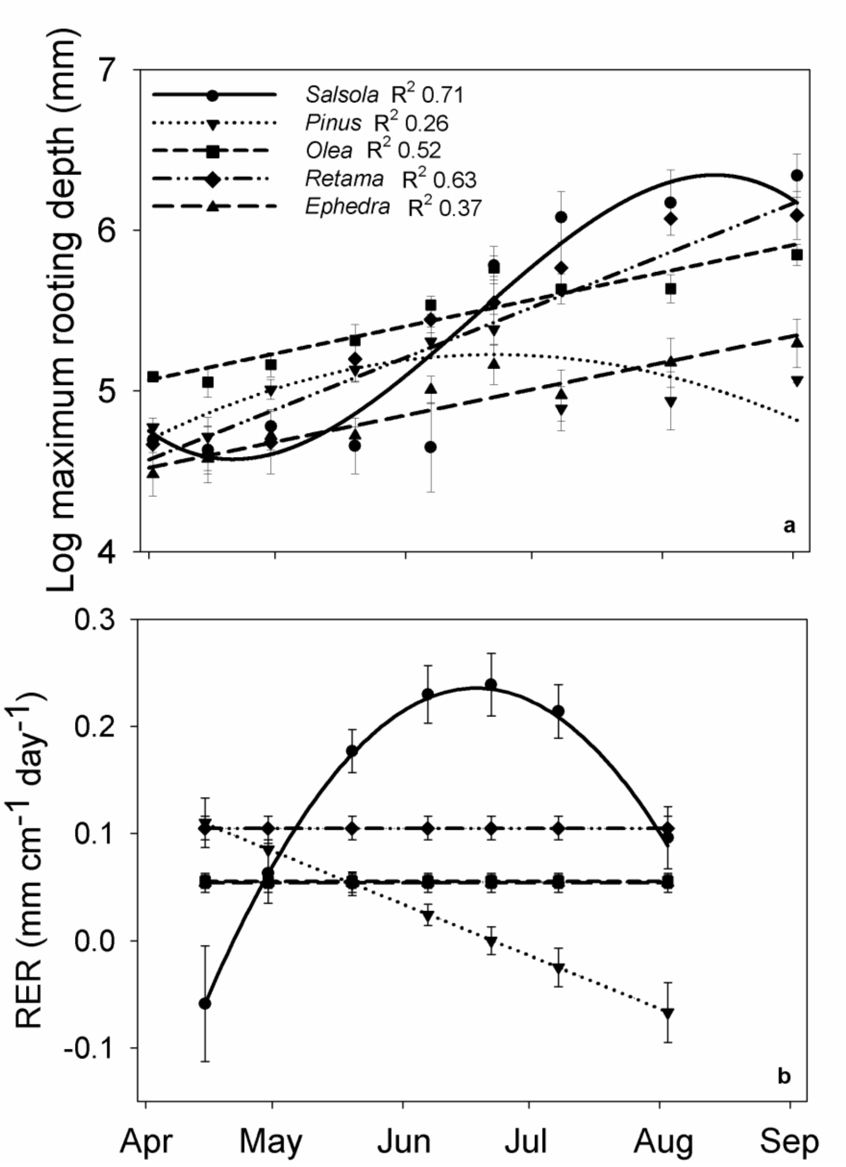

Figure 3S. (a) Log-transformed maximum rooting depth (mm) at each harvest (symbols) and fitted functions (lines). R^2 shows regression coefficient at $P < 0.05$. (b) Root extension rate (RER, mm cm^{-1} day^{-1}) for each harvest and species. Symbols and lines of *Olea* and *Ephedra* overlap. Symbols are means ± 1 SE. Initial and final RER values not shown for clarity due to widening of confidence limits.