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Early root growth plasticity in seedlings of three Mediterranean woody

species

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Francisco M. Padilla[*], Juan de Dios Miranda and Francisco I. Pugnaire

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Estación Experimental de Zonas Áridas, Consejo Superior de Investigaciones

Científicas, C/ General Segura 1, E-04001 Almería, Spain, Phone +34 950281045, Fax

+34 950277100

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[*] fpadilla@eeza.csic.es

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3

4 **Abstract**

5 Since very young seedlings are sensitive to dehydration, soil desiccation is often

6 responsible for seedling death in water-stressed environments. Roots play a major role

7 in overcoming water stress and plant establishment, thus early root development in

8 response to limited water availability becomes a strategy that may ensure seedling

9 recruitment. We explored whether different water availabilities altered growth patterns

10 of very young seedlings, focussing on root elongation, and hypothesized that seedling

11 responses would depend on species-specific drought tolerance and seed size. We carried

12 out a greenhouse experiment exposing two-week-old seedlings of three Mediterranean

13 shrubland species, the drought-tolerant and small-seeded *Genista umbellata* (L'Hér.)

14 Dum. Cours. and *Lycium intricatum* Boiss., and the drought-sensitive, large-seeded

15 *Retama sphaerocarpa* (L.) Boiss., to two watering quantities and monitored plant and

16 root growth weekly in glass cases for five weeks. We found that at such early stages,

17 reduced water quantity enhanced root growth in all three species, regardless of drought

18 tolerance and seed size, although root plasticity was the highest in the small-seeded and

19 drought-tolerant *Genista*. In contrast, shoot elongation and mass allocation, root-to-

20 shoot mass (R:S) ratio, was unaffected by watering. Seedlings responded to lower water

21 availability with faster root elongation rate and greater absorptive root surface, which

22 can account for the enhanced relative growth rate (RGR) of the small-seeded *Genista*

23 and *Lycium* under reduced watering. By contrast, a larger root absorptive surface did not

24 lead to higher RGR in the large-seeded *Retama* probably because of its greater

25 independence from external mineral resources. Our data evidence the importance of

1   water availability on the initial developmental stages of these three species regardless of

2   seed size and drought tolerance. Root growth can be interpreted as an adaptive strategy

3   to deal with drying soils and decreasing soil moisture since larger roots enable to exploit

4   unexplored soil areas of soil, which may ensure recruitment success.

5

1 **Introduction**

2   Plant communities are shaped by germination and recruitment processes

3 (Donovan et al. 1993), which ultimately affect community composition and structure

4 (Grubb 1977; Harper 1977). Plants do not actively choose the habitat they grow in

5 (Bazzaz 1991); rather, habitat choice is first imposed on plants by seed dispersal, and

6 then by environmental factors which constrain seed survival, germination, seedling

7 establishment and growth (Schupp 1995). After seed dispersal, germination does give

8 way to the most critical phase in the regeneration process, seedling establishment

9 (Fenner and Kitajima 1999). Very young seedlings are susceptible to many hazards,

10 such as extreme temperatures and radiation, competition, pathogens, herbivory or

11 drought (Moles and Westoby 2004a), and as a result high mortality rates are often

12 associated to this stage (Fenner 1987). An important determinant of successful seedling

13 recruitment is the microsite where the seed is placed, often a safe site providing

14 conditions and resources required for germination and establishment (i.e., the

15 regeneration niche *sensu* Grubb 1977; Fenner 1987). However, seed-seedling conflicts

16 may arise when environmental conditions promoting seed germination are not

17 favourable for seedling survival and growth (Schupp 1995), e.g., conditions good

18 enough for triggering germination may not be as good for seedling growth. Eventually,

19 seedling's fate and recruitment success will depend on the seedling's ability to cope

20 with limiting environmental conditions.

21   Because emerged seedlings are much more sensitive to dehydration than seeds

22 or juvenile individuals (Evans and Etherington 1991), drought is often the main cause of

23 seedling death in many environments (Moles and Westoby 2004a). This is particularly

24 true in water-stressed Mediterranean ecosystems, where a dry, long summer season

25 jeopardizes recruitment of seedlings emerged in winter and spring (Herrera 1992). In

1   addition, seedlings in arid environments are exposed to highly variable rainfall, both in

2   duration and amount, being characteristic the presence of dry periods interspersed

3   between rain events (Lázaro et al. 2001). Establishment success in such areas greatly

4   depends on seedling ability to overcome water shortage (Davis 1989), and root systems

5   play a major role. Large biomass allocation to roots is often related to higher survival

6   rates through improved water and nutrient uptake (Lloret et al. 1999, Pugnaire et al.

7   2006) linked to reaching moister soil layers and exploring larger soil volumes (Davis

8   1989; Donovan et al. 1993; Leishman and Westoby 1994a). Consequently, deep-rooted

9   seedlings have a probability of surviving summer drought higher than shallow-rooted

10   seedlings (Padilla and Pugnaire 2007). Species-specific drought tolerance, however, is a

11   main factor for seedling survival in drying soils (Ackerly 2004), and Davis (1989) and

12   Hasting *et al.* (1989) found in the California chaparral that seedlings of drought-tolerant

13   species, usually shallow-rooted, survived water shortage better than seedlings of

14   drought-avoider species, often deep-rooted, because of the greater tolerance to low soil

15   water potentials of tolerant species. Seed size has also been related to successful

16   recruitment in dry habitats (Leishman and Westoby 1994a; Moles and Westoby 2004b).

17   Large-seeded species have storage reserves in cotyledons that sustain growth during

18   unfavorable periods, and are more likely to have large seedlings and longer roots than

19   small-seeded species (Buckley 1982; Jurado and Westoby 1992; Fenner and Kitajima

20   1999), traits shown to be related to a higher probability of survival by allowing access

21   to soil moisture at deeper levels (Donovan et al. 1993).

22   Given the typically unpredictable and variable rainfall in arid environments and

23   Mediterranean ecosystems, and the fact that climate change scenarios forecast for the

24   western Mediterranean Basin a mean annual precipitation reduced by ~30% and shifts

25   in the frequency of rain events, i.e., greater, less frequent events followed by longer

1   drought periods (IPCC 2001), understanding seedling responses to changes in water

2   availability is important. Here, we explored whether differences in watering altered

3   growth patterns of seedlings at the very early stages of development, with cotyledons

4   still attached. We carried out an experiment in mini-rhizotrons, subjecting very young

5   seedlings of three perennial woody species of Mediterranean shrubs to reduced

6   watering, monitoring plant and root growth. We reduced the amount of water supplied

7   and its frequency expecting that pulses of water of different magnitude have different

8   effects on plants, even if the amount of water provided is kept constant. Research has

9   shown that roots grow towards resource patches (Reader et al. 1993; Cahill and Casper

10   1999; Rajaniemi and Reynolds 2004; Eapen et al. 2005), showing an elongation

11   response in low moisture (Evans and Etherington 1991). Furthermore, it is widely

12   accepted that plants adjust to resource imbalance by allocating biomass to organs that

13   acquire the limiting resource (Chapin et al. 1987). Therefore, we expected larger

14   biomass allocation to roots relative to shoots and larger root elongation rates in response

15   to drought as a means to overcome water shortage. We hypothesized that 1) seedling

16   responses would depend on species' water stress tolerance, so that drought-sensitive

17   species would show stronger responses to drought than drought-tolerant species as a

18   means to overcome their lower capacity of dealing with low water availability and,

19   following Leishman and Westoby (1994a) 2) root growth would be positively

20   associated to seed size, so that large-seeded species would show stronger responses to

21   drought than small-seeded species because cotyledons allow plant to growth under

22   unfavorable conditions.

23   **Materials and Methods**

24   *Species*

1   Three perennial woody species co-occurring in open Mediterranean semiarid

2   shrublands of southeast Spain were selected; *Genista umbellata* (L'Hér.) Dum. Cours.,

3   *Lycium intricatum* Boiss., and *Retama sphaerocarpa* (L.) Boiss. Hereafter we refer to

4   these species by their generic names only. Two of the species were nearly leafless

5   legumes with photosynthetic stems, the small shrub *Genista* and the large shrub

6   *Retama*, whereas *Lycium* was a thorny shrub with drought-deciduous succulent leaves.

7   Our species differed in drought-tolerance strategy based on rooting depth and minimum

8   pre-dawn water potential (Ψ_{pd}) measured in the field during the water shortage. *Retama*,

9   a very deep-rooted species accessing stable water sources through the year (Haase et al.

10   1996), may be considered as drought–avoider given the usually high Ψ_{pd} reported ($\approx$ -

11   1.5 MPa, Haase et al. 1999). The other two species can be classified more properly as

12   drought-tolerant. *Lycium* stands very low water potentials ($\approx$ -5 MPa, Tirado 2003) and

13   its drought-deciduous habit evidences shallow rooting depth. There are no data available

14   for *Genista umbellata*, a shallow-rooted species (< 0.75 m, pers. obs.), but a closely

15   related species, *G. hirsuta*, showed high tolerance to Mediterranean stress, reaching Ψ_{pd}

16   under -6 MPa (Lansac et al. 1994). Species also differed in seed mass. *Genista* and

17   *Lycium* are relatively small-seeded species, whereas *Retama* is a larger-seeded species

18   with very heavy seed coat (up to 35 mg, Table 1).

19

20   *Experimental design*

21   Freshly collected seeds of the three species were sown separately in germination

22   trays containing type III vermiculite (Verlite®, Vermiculita y Derivados SL, Gijón,

23   Spain) in laboratory at room temperature and light on 22 Mar. 2005. Seeds were

24   collected in the field or provided by local nurseries. All seeds germinated within two

25   weeks, and very young seedlings were transferred to glass cases on 13 Apr. 2005, once

1   that cotyledons had fully emerged from seed coats. Six randomly selected seedlings of

2   every species were harvested before transplanting (Table 1). Four transparent glass

3   cases, 129 cm length, 43 cm depth, 3 cm width set at a 30º angle from the vertical, were

4   filled with vermiculite and placed in the greenhouse (Fig. 1). Because of the narrow

5   design of the cases, we selected vermiculite because its lower compaction and greater

6   oxygenation than other growing media. The case bottom was perforated to allow for

7   water drainage. At transplant, individuals of each species were placed completely at

8   random 8 cm from each other and near the lower side of each case. Given the small

9   seedling size, the lack of lateral roots and the short monitoring period, this distance

10   seemed enough to prevent competition. The lower side of the glass case was covered by

11   a black canvas so that roots grew in darkness on this side and root growth could be

12   monitored through the glass. The other side was left uncovered. Each individual was

13   watered with 40 ml every three days during the first week following transplant. After

14   acclimation, on 19 Apr. 2005, seedlings were allocated to treatments following a

15   factorial design with two factors and two levels each. Watering quantity included a

16   control (20 ml every time) and a watering of 30% less than the control (14 ml). A

17   second factor included frequency of watering, and comprised a 'normal' level (two

18   waterings per week) and half the number of events (one per week). Each of the four

19   combinations comprised five replicates per species. All waterings were done with a

20   syringe to prevent flooding. Seedlings grew in a greenhouse sheltered from direct

21   radiation for five weeks without fertilization and the cases position was rearranged

22   weekly. The mean daily temperature in the sheltered area was 18.9 ± 0.3 ºC, and the

23   mean maximum and minimum were 23.9 ± 0.4 ºC and 13.7 ± 0.3, respectively.

24

25   *Measurements*

1   Shoot height and root length of each plant were measured weekly during the

2   manipulation period. Shoot height was measured with a calliper and new root segments

3   and trajectories were drawn on the glass surface using different colour markers. At the

4   end of the experiment, root length marks on the glass were traced to acetate sheets and

5   digitalized with a portable scanner (Epson GT7000, Seiko Epson Corp., Nagano, Japan)

6   at 300 dpi. Root length was measured from digitalized traces using the macro

7   RootMeasure v.1.80 (Kimura and Yamasaki, 2003) implemented on the software Scion

8   Image Beta v. 4.02 (Scion Corp., Maryland, USA). We calculated mean root and shoot

9   elongation rates for each plant between the initial and final lengths. Growth curves were

10   obtained by plotting cumulative root length data against time. Maximum rooting depth

11   was recorded before harvesting. At harvest, on 24 May 2005, shoots of each species

12   were clipped at surface level, stored in paper bags, dried at 71ºC for at least 48 hours in

13   a ventilated oven and weighed. Glass cases were then emptied out gently so as not to

14   break root systems and vermiculite particles attached to root hairs were removed by

15   gently washing and brushing them out. Roots were labelled, placed into wet paper

16   towels and kept cool in zip bags in a refrigerator until they were scanned. Root length

17   and root area of each plant were digitalized and measured following the procedure

18   described above for traced roots. Root biomass was obtained after drying samples as

19   with shoots, and root-to-shoot mass ratio (R:S ratio) for each plant was calculated from

20   these data. Specific root length (SRL, cm g^{-1}) on the entire root system was computed

21   from total root length and mass.

22

23   *Growth analysis*

24   Relative growth rate (RGR, mg g^{-1} day^{-1}) during the monitoring period was

25   calculated from data at harvest (W_2) and transplant (W_1) following:

1
$$RGR = \frac{(\log W_2 - \log W_1)}{(t_2 - t_1)} \qquad (1)$$

2 where $t_2 - t_1$ was 41 days, using the Hunt et al. (2002) spreadsheet tool. We calculated

3 water-use efficiency (WUE, mg L^{-1}) as the ratio between biomass gained and water

4 received during the experiment, taking into account averaged initial biomass at

5 transplant (Kikvidze et al. 2006). From seedling root length in reduced and control

6 water levels at harvest, we calculated for each species the relative interaction index (RII,

7 Armas et al. 2004) as an index of root plasticity to reduced watering, expressed as:

8
$$RII = \frac{(R_r - R_c)}{(R_r + R_c)} \qquad (2)$$

9 where R_r and R_c were root length and shoot mass in reduced and control plants,

10 respectively. Although this is not a specific plasticity index, its strong mathematical and

11 statistical properties make it appropriate for comparisons between plants growing in two

12 treatment groups, in this case control and reduced.

13

14 *Statistics*

15   Data were exploratory analyzed as a two-factor design (watering quantity and

16 frequency), however, analyses showed no differences in any variable between normal

17 watering and half the number of events in the frequency factor. Likely, pulses of water

18 of different magnitude while keeping constant the amount of water provided did not

19 affect soil moisture in our conditions. For this reasons we excluded the frequency factor

20 from analyses to gain statistical power since some plants died after transplant, and those

21 data were pooled either into corresponding control or reduced quantity level since the

22 amount of water provided was kept constant within the frequency factor (i.e. plants in

23 the control water quantity received 40 ml per week in one (half events) or two events

1 (normal frequency), and similarly in the reduced water quantity (28 ml distributed in a

2 single or two 14 ml events per week).

3   Data were then analyzed as a factorial design with two factors, species and water

4 quantity. Differences in mean growth rate, total root length, root area, maximum rooting

5 depth, biomass, SRL, R:S ratio and WUE were tested using two-way analysis of

6 variance (ANOVA) for each variable followed by Tukey HSD post-hoc comparison

7 tests. For total root length analysis we used length of traced roots instead of length of

8 scanned roots since the former data were more homoscedastic. Differences in root

9 length measurements between the two procedures were not significant (paired t-test,

10 P=0.47). Because of the unequal sample size, we used type III sum of squares.

11 Heteroscedastic variables were transformed to meet ANOVA assumptions. When

12 variables were still heteroscedastic (as in WUE), we ran for each species separately the

13 non-parametric Mann-Whitney U test (M-W U). Comparisons in plasticity index (RII)

14 among species were conducted from standard errors since all replicates belonging to a

15 treatment were integrated in computation.

16   Since plotted data of cumulative root length against time showed a linear trend,

17 growth curve analyses were conducted by fitting individual data to a linear function $Y =$

18 $mX + b$, where Y was length (cm), X was time (days), m was the slope and b the y-

19 intercept. Differences in growth curves between species and water treatment were tested

20 by comparing regression slopes of each plant (m) through ANOVA. We could not

21 perform repeated-measures and multivariate ANOVA to test growth responses because

22 our data violated statistical assumptions (Von Ende 2001). Only those individuals

23 whose roots could be seen through the glass case from the beginning of the experiment

24 were included into root growth analysis.

1   All tests were conducted with Statistica v. 6.0 (Statsoft Inc, Tulsa, OK, USA)

2   and differences were considered significant at $P<0.05$. Data are presented as means $\pm$

3   one standard error.

4

5   **Results**

6   Cumulative root length over time was best adjusted to a linear function. Growth

7   curves were statistically different between control and reduced water quantity in all

8   species (P=0.014), with roots under drought growing faster (Fig. 2). This was reflected

9   in root elongation rate ($ANOVA_{water}$ P=0.013); plants subjected to lower watering

10   elongated more than control plants (8.58±0.74 *vs.* 6.74±0.65), regardless of species

11   identity ($ANOVA_{speciesxwater}$ P=0.99, Fig. 3a). We found significant differences in mean

12   root elongation rate among species, with *Lycium* having the highest rate (10.57±0.58

13   mm day^{-1}), followed by *Retama* (6.66±0.66) and *Genista* (4.67±0.49, Table 2). As for

14   root plasticity, all species responded to reduced watering by developing longer roots (as

15   reflected by positive values of RII), though *Genista* showed the strongest response

16   (0.142±0.024), whereas in *Lycium* and *Retama* it was lower (0.078±0.011 and

17   0.083±0.017, respectively).

18   Total root length and root area at harvest differed among species, decreasing

19   *Lyicum > Retama > Genista* (Table 2, Fig. 3b). There were also significant differences

20   in root length and root area between water treatments ($ANOVA_{water}$ P<0.01), regardless

21   of species ($ANOVA_{speciesxwater}$ P>0.6, Fig. 2 and 3b). When compared to control, plants

22   supplied with reduced water quantity showed longer roots (28.65±2.26 *vs.* 35.75±2.57

23   cm) and greater root area (2.69±0.26 *vs.* 4.02±0.47 cm^2). On the contrary, we only

24   detected a tendency to root deeper in response to lower water availability (25.55±2.03

25   for reduced *vs.* 22.45±1.71 cm for control plants, $ANOVA_{water}$ P=0.13, Tables 2 and 3).

1   Roots of *Lycium* and *Genista* had higher SRL than *Retama*, although no significant

2   adjustment in response to altered watering quantity was detected in any species

3   (P_{water}=0.86, $P_{speciesxwater}$=0.31). Root-to-shoot mass ratio was below 0.6 in all species

4   (Table 3), ranging from 0.47±0.05 in *Genista* and 0.41±0.03 in *Lycium* to 0.28±0.02 in

5   *Retama*. We did not detected significant effects of water quantity on R:S ratio in any

6   species (ANOVA$_{water}$ P=0.42).

7   We found differences among species in plant, shoot and root mass at harvest

8   (ANOVA$_{species}$ P<0.001, Table 2), in contrast, no differences were observed in mean

9   shoot elongation in any species in response to drought (M-W U$_{water}$, P>0.25). The

10   effects of watering quantity on biomass depended on species, as revealed by the species

11   x water interaction (ANOVA P<0.03); plants supplied with lower water quantity tended

12   to exhibit larger mass than those in control in *Lycium* and *Genista*, whereas *Retama*

13   performed nearly the same both in control and reduced levels. The same pattern was

14   observed if plant growth was considered with respect to initial plant size (i.e., RGR);

15   relative growth rate of total plant, shoot and root masses were higher under reduced

16   water in *Genista* and *Lycium*, whereas differences in *Retama* were less patent (Table 3).

17   This mirrored in water use efficiency of productivity and shoot plasticity. Plants

18   supplied with lower water quantity produced significantly more biomass per water

19   received than those in control in *Lycium* (M-W U$_{water}$=5, P<0.01), and marginally in

20   *Genista* (M-W U$_{water}$=8, P=0.06). In *Retama*, however, biomass gain was independent

21   of water provided (M-W U$_{water}$=27, P=0.91, Fig. 3c).

22

23   **Discussion**

24   A small reduction in water supply enhanced root elongation in all our species at

25   very early stages of development, when cotyledons were still attached. This could be an

1   analogous response to etiolation of shoots under shaded conditions (Leishman and

2   Westoby 1994b). Despite the contrast in seed mass and drought tolerance among

3   *Retama*, *Lycium* and *Genista*, all three species, either drought-tolerant or sensitive, large

4   or small-seeded, responded equally to reduced watering. These data evidence the

5   importance of water availability for seedling development during such early stage. The

6   increase in root length and area in plants under reduced watering can be interpreted as

7   an adjustment of absorptive surfaces to find water resources (Hutchings and de Kroon

8   1994). By increasing root length, plants exploit a larger soil volume tapping otherwise

9   unexplored areas and increase their resource uptake capacity, which depends on root

10   surface area (Lambers et al. 1998b).

11   Our findings agree with reports showing root elongation in response to low soil

12   moisture (Evans and Etherington 1991). Reader et al. (1993) found that rooting depth of

13   seedlings of wild species increased in response to drought due to higher elongation

14   rates, particularly in species that regenerate mainly from seeds after disturbance

15   (seeders), suggesting that selective pressures favour plasticity in root growth, affecting

16   traits that promote seedling survival. Although we do not report significant differences

17   in rooting depth between control and reduced water (P=0.13), most likely because of the

18   short time period considered, our data are consistent with this explanation. Thus, early

19   root growth shows an adaptive strategy to deal with water stress at the seedling stage

20   (Fitter 1991). Root elongation and deeper rooting depth in response to water stress is

21   presumably also an adaptation that allows exploitation of declining soil moisture

22   (Lambers et al. 1998a) and in fact, the ability to develop roots accessing deep soil

23   moisture has proved decisive for survival of seedlings during summer months in a

24   Mediterranean semiarid environment (Padilla and Pugnaire 2007). Our hypothesis that

25   root growth response would be stronger in the drought-sensitive and large-seeded

1   *Retama* because of its sensitivity to dehydration and larger seed reserves could be

2   rejected since a drought-tolerant and small-seeded species (*Genista*) showed a distinctly

3   plastic response. Developing seedlings of large-seeded species acquire most resources

4   from seed reserves (Fenner and Kitajima 1999), and then they are relatively more

5   independent from external resources than small-seeded species. However, the weak

6   response we found may not involve a disadvantage in the field, since germination

7   timing and seedling size may offset low root growth capacity. Interestingly, there are

8   reports of greater root elongation rate in drought-tolerant turfgrass (Huang 1999) and

9   phreatophyte seedlings (Horton and Clark 2001), and in seedlings of species restricted

10   to dry sites (Evans and Etherington 1991) when subjected to lower water availability. It

11   is clear that root plasticity is under genetic control (Sydes and Grime 1984; Sharp et al.

12   2004) and species do not show the same ability to elongate; however, whether root

13   plasticity is linked to the species' drought tolerance, and the underlying mechanisms,

14   still remains unclear.

15   Surprisingly, we found larger shoot mass and higher RGR in *Lycium* and

16   *Genista* seedlings supplied with less water, whereas differences were negligible in

17   *Retama*. It is improbable that this was due to greater root biomass allocation or root

18   length exploiting potentially more soil volume of *Retama*, since it allocated the least to

19   roots (lowest R:S ratio) and showed one of the shortest root lengths at transplant.

20   Rather, seed size and cotyledon reserves can explain such response, since they strongly

21   affect seedling growth (Leishman and Westoby 1994b; Cornelissen et al. 1996; Bonfil

22   1998; Hanley et al. 2004; Hanley and May 2006). Large-seeded species, indeed, have

23   storage cotyledons characterized by a slow, prolonged mobilization of reserves (Kidson

24   and Westoby 2000), relying to a greater extent on cotyledons than on soil resources and

25   light (Milberg and Lamont 1997), whereas small-seeded species are more dependent on

1   light and soil resources (Leishman and Westoby 1994b; Fenner and Kitajima 1999). In

2   our experiment, all three species retained green cotyledons until harvest, but cotyledon

3   reserves lasted longer in *Retama* than in *Lycium* and *Genista* because of its differences

4   in seed size (up to two orders of magnitude) and cotyledon mass. All three species

5   increased root absorptive surface with lower water availability as a strategy to maximize

6   water uptake, allowing secondarily greater nutrient uptake; *Retama*, however, did not

7   show changes in shoot growth due to its greater dependency on cotyledons. In this

8   sense, Jurado and Westoby (1992) found that seedlings from large-seeded species

9   thrived better under nutrient stress than small-seeded species, since their growth

10   remained independent from external resources, and similar results were reported by

11   Milberg and Lamont (1997). In conclusion, increased root absorptive surface caused by

12   low water availability was a response of all three species to maximize water uptake,

13   which also allowed for greater nutrient uptake. In fact, Wan *et al.* (2002) also found that

14   drought induced root production and enabled droughted plants to produce above-ground

15   biomass similar to that of plants receiving full watering. However, in our experiment,

16   growth depended on cotyledon reserves. Shoot growth and RGR was higher under

17   reduced watering in *Genista* and *Lycium* because of greater root exploitation and

18   resource uptake, while *Retama* depended more on cotyledon reserves and shoot growth

19   was relatively unaffected by nutrient uptake.

20   Having small-diameter roots (i.e., higher SRL) favours greater rates of water and

21   nutrient uptake (Eissenstat 1992; Cornelissen et al. 2003), therefore larger SRL under

22   reduced water availability could be expected as a strategy to maximize absorptive

23   surfaces (Reich et al.1998; Wright and Westoby 1999). All species showed increased

24   root length under reduced water availability, evidencing changes in root morphology

25   with water quantity, but SRL did not differ between watering treatments. This

1 inconsistency can be due to the fact that we used the whole root systems to obtain this

2 measurement, and Nicotra et al. (2002) showed that SRL of the entire root systems can

3 differ from that measured on the main axis or secondary roots. Similarly, large biomass

4 allocation to roots relative to shoots (i.e., higher R: S ratio) also favours water and

5 nutrient uptake (Chapin et al. 1987; Lambers et al. 1998a), and therefore we expected

6 larger R: S ratios under reduced water availability. However, plants did not respond to

7 water stress by shifting allocation patterns, and the R:S ratio did not change. Although

8 the allocation model is widely accepted (see e.g., Chapin et al. 1987; Kozlowski and

9 Pallardy 2002), other factors do impact upon R:S partitioning. Evidence suggests that

10 plasticity in R:S ratio may be highly species-specific (Joslin et al. 2000) and that in

11 some species R:S ratio is remarkably stable (Klepper 1991) or subjected to

12 developmental constraints (Gedroc et al. 1996; McConnaughay and Coleman 1999).

13 Additionally, root demography and the ability to alter rates and place of root

14 proliferation may have greater importance for plants than changes in mass allocation

15 between roots and shoots (Reynolds and D'Antonio 1996).

16   Overall, we showed that very young seedlings responded to reduced water

17 availability by elongating roots, whereas no significant changes in R:S ratio were

18 detected. Greater absorptive root surface likely allowed seedlings to increase growth

19 rate in the small-seeded species, whereas growth of the large-seeded species seemed

20 independent from external resources. Root growth may be considered an important

21 factor in early seedling development, since rapid extension of roots enables seedlings to

22 tap water from previously unexplored areas of soil (Schütz et al. 2002). Regardless of

23 seed size and drought tolerance strategy, root elongation in our three species is a

24 common adaptive trait to cope with soil dryness at early stages. However, further

25 research is needed to link root plasticity to species-specific drought tolerance.

1

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13

1   **Table 1.** Initial plant size (mg). Values are means ± 1SE. n=6 for each species, except

2   seed mass (n=10).

3

	Genista	*Lycium*	*Retama*
Seed mass	4.61±0.36	3.46±0.21	110.75±5.02
Shoot mass	3.17±0.75	4.40±0.78	24.43±1.85
Cotyledon mass	2.18±0.98	2.93±0.47	21.65±1.58
Root mass	1.53±0.35	1.58±0.27	4.32±1.09
R:S ratio	0.50±0.05	0.37±0.03	0.18±0.05

1   **Table 2.** F-values of factorial ANOVA at harvest. RER, root elongation rate between harvest and the beginning of altering watering. SRL,

2   specific root length. Superscripts show significance P-values.

3

Effect (df)	F-values								
	RER	Root Mass	Shoot mass	Plant mass	R:S ratio	Root length	Root area	SRL	Rooting depth
Species (2)	$29.15^{<0.001}$	$33.77^{<0.001}$	$143.44^{<0.001}$	$134.40^{<0.001}$	$8.40^{<0.001}$	$27.53^{<0.001}$	$22.94^{<0.001}$	$16.82^{<0.001}$	$37.97^{<0.001}$
Water (1)	$6.71^{0.013}$	$8.20^{0.007}$	$6.49^{0.015}$	$9.37^{0.004}$	$0.66^{0.421}$	$7.79^{0.008}$	$8.02^{0.007}$	$0.03^{0.858}$	$2.36^{0.133}$
Species x Water (2)	$0.01^{0.993}$	$3.87^{0.029}$	$3.90^{0.028}$	$4.91^{0.012}$	$1.43^{0.254}$	$0.10^{0.909}$	$0.47^{0.627}$	$1.20^{0.312}$	$0.19^{0.826}$

1 **Table 3.** Plant growth and root traits. Plant, shoot and root mass (mg), root-to-shoot

2 ratio, relative growth rate (RGR, mg g^{-1} wk^{-1}) of total plant, shoot, and roots between

3 the transplant and harvest dates, maximum rooting depth (cm) and specific root length

4 (SRL, cm mg^{-1}) in the control and reduced treatment. Different letters in a row show

5 significant differences (P<0.05) after Tukey test. Values are means ± 1SE. *n*=6-9.

6

	Genista		*Lycium*		*Retama*	
	Control	Reduced	Control	Reduced	Control	Reduced
Plant mass	7.0±0.9^a	10.5±0.7^b	12.9±0.8^{bc}	17.9±1.3^c	38.4±3.0^d	35.1±3.5^d
Shoot mass	4.9±0.7^a	7.2±0.5^b	9.6±0.6^{bc}	12.3±1.0^c	30.4±2.8^d	27.5±2.6^d
Root mass	2.2±0.3^a	3.3±0.4^{ab}	3.3±0.4^a	5.6±0.5^{bc}	8.0±0.5^c	7.6±1.1^c
R:S ratio	0.48±0.08^{ac}	0.46±0.05^{abc}	0.35±0.04^{abc}	0.47±0.04^a	0.28±0.03^{bc}	0.27±0.02^c
Plant RGR	84.1±30.0	147.8±28.3	143.5±22.4	197.8±23.0	47.9±13.7	31.6±14.9
Shoot RGR	87.4±31.8	161.2±28.0	146.2±22.7	186.0±23.7	34.4±15.9	18.7±14.1
Root RGR	70.9±33.7	146.2±33.5	129.0±27.5	223.3±24.8	123.9±25.1	106.7±29.6
Rooting depth	13.1±1.6^a	14.6±1.0^{ab}	29.2±1.2^{cd}	33±2.1^c	23.2±2.5^{bc}	25.4±2.9^{cd}
SRL	9.1±1.0^{ac}	8.1±1.4^{abc}	13.8±2.2^a	9.3±0.7^{ac}	4.2±0.5^{bd}	5.2±0.7^{cd}

7
8

9

1   **Figure captions**

2   Figure 1. Experimental glass cases design and size (in cm). New root segments were

3   traced on the glass weekly. Fifteen very young seedlings were placed at random in each

4   case. Only five plants have been drawn for clarity.
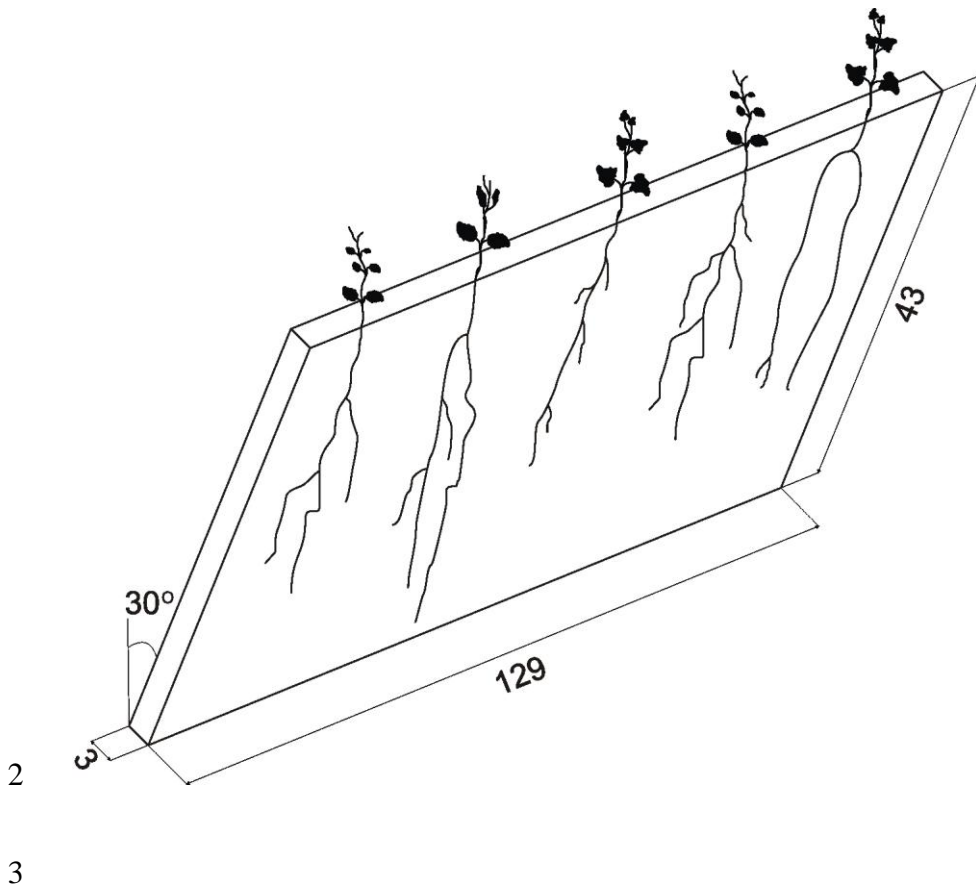
5

6   Figure 2. Root elongation curves. Cumulative root length over time in control (solid

7   symbols) and reduced watering (white symbols), and fitted linear functions (lines) with

8   r^2 and P-values of regression. Growth curves of control and reduced treatments are

9   statistically different (ANOVA$_{water}$ $F_{1,39}$=6.589, P=0.014), regardless of species

10   (ANOVA$_{species\ x\ water}$ $F_{2,39}$=0.062, P=0.940).

11

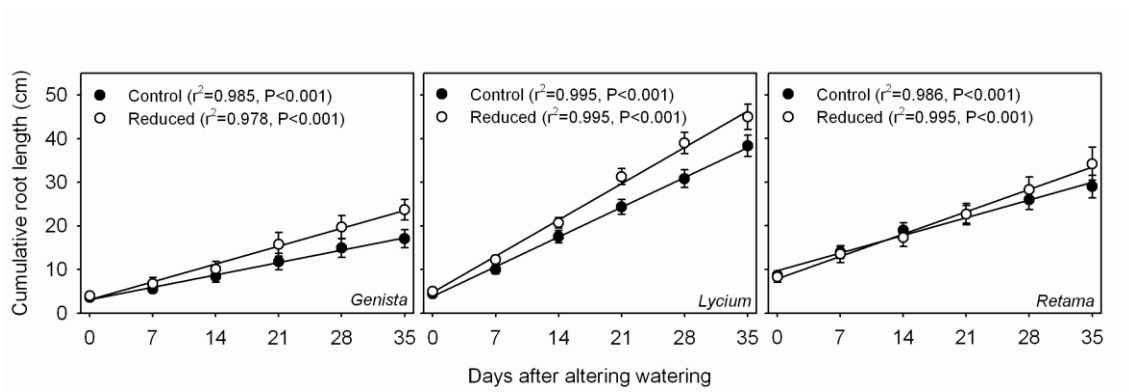12   Figure 3. Plant growth at harvest. a) Mean root elongation rate (mm day^{-1}) in the control

13   (solid bars) and reduced watering treatment (white bars), ANOVA$_{water}$ P=0.013; b) root

14   area (cm^2) at harvest, ANOVA$_{water}$ P=0.007; c) water use efficiency (mg L^{-1}), Mann-

15   Whitney test. A cross indicates marginal differences between water quantities (P<0.1)

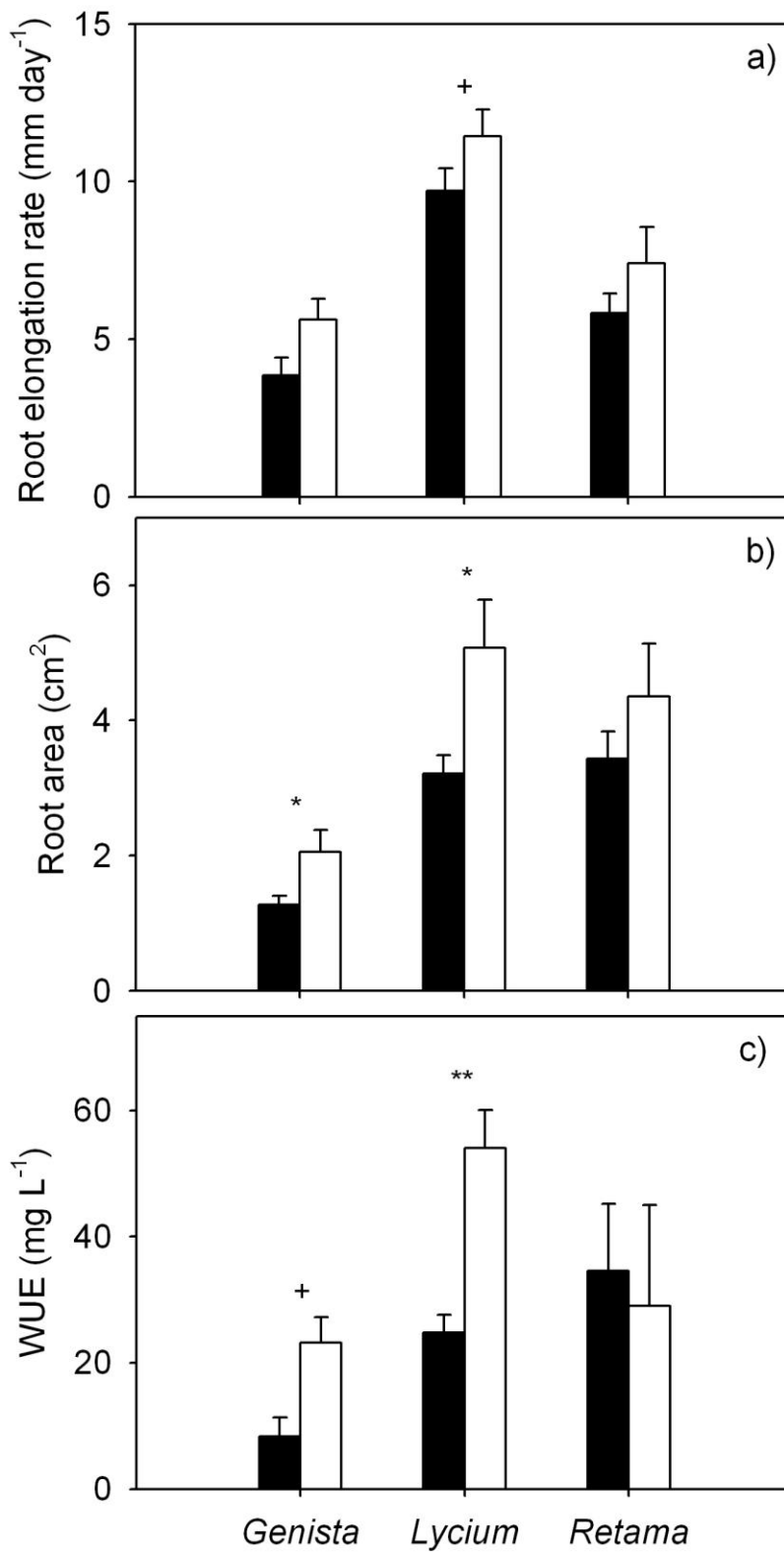16   and asterisks significant differences (*, P<0.05; **, P<0.01). Values are means ± 1SE.

17   n=6-9.

18

1   Figure 1

2

3

1   Figure 2

Cumulative root length (cm)

Control (r²=0.985, P<0.001)
Reduced (r²=0.978, P<0.001)

Control (r²=0.995, P<0.001)
Reduced (r²=0.995, P<0.001)

Control (r²=0.986, P<0.001)
Reduced (r²=0.995, P<0.001)

Genista

Lycium

Retama

Days after altering watering

2

3

1   Figure 3

2