

## Simulated effects of herb competition on planted *Quercus faginea* seedlings in Mediterranean abandoned cropland

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**Abstract.** We tested simulated effects of herb competition on the performance of planted seedlings of *Quercus faginea* ssp. *faginea* in Mediterranean abandoned cropland. We produced three types of environment with respect to herb competition: absence of competition (AC), below-ground competition (BGC), and total competition (TC). We assessed the performance of *Q. faginea* seedlings in each treatment in five ways: (1) seedling mortality, (2) leaf length and total plant leaf area, (3) water potential, (4) total biomass and biomass allocation, and (5) non-structural carbohydrate storage in different plant organs. We also measured (6) soil moisture at different depths and (7) biomass production of herbs. The TC treatment reduced water availability more than the BGC treatment, in agreement with the most pronounced water stress in seedlings under TC conditions. BGC and TC treatments showed a high and similar seedling mortality, which was one order of magnitude higher than that in the AC treatment. Competition treatments affected glucose concentration in both shoots and roots, and followed the rank TC > BGC > AC. *Q. faginea* seedlings might compensate a lower water availability through glucose accumulation in leaves to reduce the osmotic potential. There was a maximum starch concentration in the BGC treatment that hints that a moderate resource limitation would limit tissue growth but not carbon assimilation. We conclude that the negative effects of herbs on *Q. faginea* seedlings are mostly a result of competition for water, and that this competition is noticeable since the earliest stages of the establishment. Complete weed removal is a technique that would strongly improve seedling survivorship.

**Keywords:** Ecophysiological effect; Herb competition; Mediterranean cropland; *Quercus faginea*; Soil moisture.

**Abbreviations:** AC = Absence of competition; BGC = Below-ground herb competition; TC = Total herb competition.

### Introduction

Various social, economical and technological changes have resulted in the abandonment of extensive areas of former cropland in developed countries during the last few years (Anon. 1998). This phenomenon has created considerable quantities of 'empty' areas in some regions of these countries. These areas can be left alone so that natural secondary succession can take place (Debussche et al. 1996), or they can be planted with native shrubs and trees to reduce soil erosion, increase biological diversity and create carbon sinks (Vieira et al. 1994; Whisenant et al. 1995; Bakker et al. 1998). However, the environmental conditions of these areas largely differ from those of the sites where natural regeneration of shrubs and trees occurs. Radiation input, soil evaporation, thermal variation, and weed seed banks are much larger in recently open fields than in shrublands or forests, thus hindering the establishment of woody seedlings (Rey Benayas 1998; Aronson et al. 1998; Rey Benayas et al. 2002). These constraints require an appropriate management to guaranty the success of revegetation projects.

In patches and ecosystems dominated by herbaceous species, such as recently abandoned croplands, tree establishment is influenced by the ability of woody seedlings to survive and grow in direct competition with herbaceous vegetation (Brown et al. 1998). Many studies have demonstrated that herb competition reduces survival and growth of different woody species, either planted or naturally established (Gordon et al. 1989; Morris et al. 1993; Owens et al. 1995; Geyer & Long 1998; Holl 1998; Lemieux & Delisle 1998; Davis et al. 1999). Herbs compete with woody species for both below-ground resources (water and nutrients) and above-ground resources (light) (James 1999). Herbaceous plants allocate a larger proportion of biomass to productive tissues as compared with woody seedlings, which results in a larger growth rate (Hunt & Cornelissen 1997). Therefore, herbs may deplete both below- and above-ground resources much quicker than

woody seedlings do during their growth peak. Acclimation to cope with water and nutrient shortage takes place mainly through an increase in the root:shoot ratio (Joffre et al. 1999, 2001) and a decrease of the exposed leaf area (Sala et al. 1994; Castro-Díez et al. 1997; Rambal & Leterme 1987; Villar-Salvador 2000; Valladares & Pugnaire 1999). On the contrary, acclimation to low light occurs through the reverse trends (Valladares & Pearcy 1998; Balaguer et al. 2001). Thus, it is apparently impossible to respond to both shortages efficiently (Zavala et al. 2000; Valladares 2001). However, herbs may improve soil properties e.g. by transferring carbon from the atmosphere to the soil (Morris et al. 1993).

We assessed the performance of planted *Quercus faginea* ssp. *faginea* seedlings under simulated conditions of different levels of herb competition in abandoned cropland in a Mediterranean region. This species is a major structural component of the native plant communities in many mesic forests in Mediterranean environments. Herb competition is usually mitigated with herbicides, a practice that also eliminates the potential benefits of herbs to the soil. We ask whether the elimination of only above-ground competition could be an effective practice to reduce herb damage on extensive plantations of tree seedlings.

Water has been shown to be an important limiting factor during the establishment of tree seedlings (Canham et al. 1996; Kavanagh & Zaerr 1997; Rousset & Lepart 2000). Our hypothesis is that herb clipping might enhance seedling performance in two ways. First, by reducing herb leaf area, transpiration will decrease and so will do herb water absorption, increasing soil water availability to the seedlings. Secondly, by reducing the radiation shortage that tall herbs impose to tree seedlings, these will be more efficient to acclimate to water shortage by changing their biomass allocation pattern. To test these hypotheses, one-year old *Q. faginea* seedlings were planted under three simulated levels of herb competition: (1) no herb competition; (2) below-ground competition, where herbs were periodically clipped to a maximum height of 8 cm; (3) full competition. Soil moisture was monitored along the growing season, and the performance of the seedlings was assessed by measuring their mortality and several growth and physiological traits. If seedlings under the clipped herb treatment perform satisfactorily as compared to the total competition treatment, extensive clipping may be an environmentally-friendly technique to be considered as an alternative to herbicides in afforestation projects.

## Methods

### *Study species and site*

*Quercus faginea* ssp. *faginea* is a deciduous oak up to 20 m tall which occurs in neutral to calcareous soils from 500 to 1900 m a.s.l. in NW Africa and the Iberian peninsula. Mean annual precipitation is more than 500 mm, but the species also grows in drier areas if topographic and soil properties enhance water accumulation.

The study was conducted in the Juan Carlos I Botanical Garden at the Alcalá de Henares University, central Spain (40° 35' N, 3° 25' W). Mean annual temperature is 13.5 °C and mean annual precipitation 450 mm, with a pronounced summer drought. Total precipitation during the experiment (February to June 2000) was 179 mm and mean temperature was 14.6 °C.

### *Experimental design*

*Q. faginea* seedlings (1 yr old;  $n = 60$ ) were planted in early February 2000 in polyethylene containers (40 cm  $\varnothing$  and 80 cm deep) filled with a local haploxeralf typic soil (Anon. 1995) of soil texture class clayey loam (Bienes & Nieves 2000) consisting of: 5.4% coarse sand, 39.3% fine sand, 37.6% silt and 17.8% clay. The top soil layer (4-5 cm) was excluded to avoid the natural weed seed bank. The seedlings were planted with 20-cm deep plugs. All containers were left outside and exposed to full sunshine. We added 3 L of water per container right after plantation to eliminate soil cavities.

The experiment consisted of three treatments, each with 20 replicates of planted *Q. faginea* seedlings: (1) absence of herb competition (AC); (2) below-ground herb competition (BGC); and (3) total herb competition (TC). The herbaceous community was simulated by sowing a seed mixture in mid-February that consisted of 15 g of *Lolium rigidum* seeds and 5 g of *Medicago sativa* seeds per container. The mixture we used guarantees a strong competition effect and a similar herb water extraction in all containers (Kollmann & Reiner 1996; Peñuelas et al. 1996). Immediately after sowing, we added 1 L of water per container to foster seed germination, and another liter of water per container was added one week later. Besides the irrigation right after planting and sowing, we added 0.75, 4, 0.5, and 3 L of water per container at different times during the experiment to avoid excessive soil desiccation in the containers and support plant growth. In the BGC treatment, herbs were clipped every two weeks since early April (6  $\times$  in total) in order to maintain them below 8 cm above ground, a lower height than the mean height of the terminal buds of the *Q. faginea* seedlings when they were planted. For the TC treatment, herbs were left to grow freely.

### Measurements

We examined the performance of *Q. faginea* seedlings in the different treatments in five ways: (1) seedling mortality, (2) leaf length and total leaf area, (3) water potential, (4) total biomass and biomass allocation to roots, stems and leaves, and (5) non-structural carbohydrate storage in roots and shoots. We also measured (6) soil moisture at different depths and (7) biomass production of herbs.

Monitoring occurred from mid-February through late June when seedlings were harvested. The first leaves were unfolded at mid-March in the AC treatment, and they were delayed in the competition treatments.

Seedling shoot mortality was assessed at the beginning of the dry season (mid-May) and after a few weeks of summer drought (mid-June). We deemed a seedling as dead if all their aerial shoots were clearly dry, though we are aware that some of these seedlings might be capable of resprouting later under less stressful conditions. Leaf length was measured in all leaves  $\geq 5$  mm in all seedlings at the beginning of April, before the summer drought. For semi-destructive measurement (3) and destructive measurements (2), (4) and (5), five randomly selected alive seedlings per treatment were sacrificed at mid-June. Before that, pre-dawn and mid-day leaf water potential were measured using a Scholander chamber. Then the seedlings were taken to the laboratory and fractioned into leaves, stems and roots. The total fresh leaf area was measured with a delta-T leaf area meter. Roots were gently washed and the three plant fractions were kept in an oven at 80 °C for 48 hr before weighing. The roots were also incinerated at 550 °C and the ash weight was subtracted to eliminate the weight of mineral residuals. Then, we measured total biomass and the proportion of biomass allocated to leaves, stems, and roots.

Glucose and starch concentration in root and shoots was also determined (pooled stems and leaves). We could not differentiate stem and leaf tissues because leaf samples were too small to assess carbohydrate concentration. Plant material was collected in the field at dawn and transported to the laboratory in a refrigerated box. There it was oven-dried at 60 °C for three days and then ground with a Culatti mill through a 0.5 mm-mesh screen. Monosaccharids and disaccharids were extracted with hot ethanol. Approximately 7 ml of 95% ethanol were added to 50 g of the sample in a Pyrex tube and warmed to 80 °C for 90 minutes, shaking periodically. Then the extracts were separated by centrifugation and the residue re-extracted. The ethanol was removed from the extract by evaporation. The rest of the non-structural carbohydrates contained in the residue (oligosaccharids and starch) were broken to monosaccharids through an

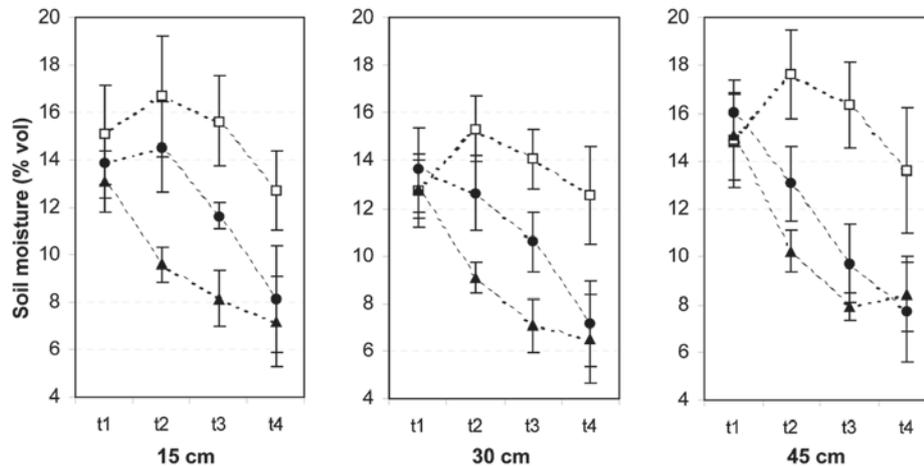
enzymatic procedure; 6 mL of acetate buffer (pH = 4.5) containing 5.6 units of  $\alpha$ -amylase and 0.4 of amyloglucosidase were added to the dry residue and left at 45 °C for 24 hr. The liquid extract containing the sugars was then separated by centrifugation. The monosaccharide content of both extracts was colorimetrically assessed using the DuBois et al. (1956) method. The dry extract resulting after ethanol evaporation was dissolved on 2 ml of acetate buffer. Two aliquots of both solutions (from the ethanol and the enzymatic extractions) were dissolved in distilled water to a final volume of 1 mL. Then 1 mL of 5% phenol and 5 mL of concentrated sulfuric acid were added to each tube. In presence of the acid, phenol reacts with the monosaccharides producing a coloured product whose absorbance at 483 nm is proportional to the sugar concentration.

Soil moisture was measured four times (mid-March, twice in May and mid-June) at 15, 30, and 45 cm depth using the TRIME method (Dirksen & Hilhorst 1994), a specially designed TDR technique (IMKO, Micromodul-technik company). Clipping was performed on the 20 BGC containers every ca. two weeks since the beginning of April, up to a total of six clippings. The clipped herb biomass was dried at 80 °C and weighed to estimate the accumulated aerial herb production.

### Data analysis

Because a major difficulty in experiments with young plants is the variability in plant size, we first tested if there were differences among treatments in seedling height and stem diameter right after plantation took place. The ANOVA indicated that both measurements were not different among treatments ( $F_{2,57} = 1.297$ ;  $p = 0.29$  and  $F_{2,57} = 0.926$ ;  $p = 0.4$ , respectively). The initial size of the seedlings was the following: height (cm) =  $21.22 \pm 11.36$  (SD), and stem diameter (mm) =  $6.04 \pm 12.36$ . In order to obtain a reference value of the initial seedling weight, 10 non-planted seedlings were weighed prior to the experiment and they averaged  $8.4 \text{ g} \pm 1.07$ .

Statistical analyses were  $\chi^2$  based to test the effects of treatments on seedling mortality, and ANOVA and Kruskal-Wallis to test the effects of treatments on the rest of the above mentioned measures, including a repeated measure ANOVA for testing differences in soil moisture. We also correlated the amount of accumulated herb biomass production with both the soil moisture at different depths and with the average profile moisture for the BGC treatment.



**Fig. 1.** Variation of soil moisture with herb treatment, soil depth, and time. Symbols for treatments: □ = absence of competition; ● = below-ground competition; ▲ = total competition. Time notation: t1 = 15.03.00, t2 = 05.05.00, t3 = 22.05.00, t4 = 29.06.00. Every point in the graph represents the mean of 20 measurements.

## Results

### Soil moisture

A repeated-measure analysis of variance indicated significant effects of the competition treatment, soil depth, and time on the soil moisture (Table 1). Soil moisture progressively decreased along the growing period, and this decrease was more pronounced in the presence of herbs (Fig. 1). The differences in soil moisture between the BGC and TC treatments were usually smaller than those found between the AC and BGC treatments, these differences decreasing with depth across the soil profile (Fig. 1).

The relationship between the accumulated amount of herb biomass production and the average moisture across the soil profile in the BGC treatment at the end of the experiment (Pearson's  $r = -0.58$ ;  $P = 0.03$ ;  $n = 20$ ) suggests that herb production reduced water availability to *Q. faginea* seedlings. This reduction was most pronounced at 45-cm depth (Pearson's  $r = -0.64$ ;  $P = 0.01$ ;  $n = 20$ ) and least at 15 cm depth (Pearson's  $r = -0.4$ ;  $P = 0.1$ ;  $n = 20$ ).

### Seedling mortality

The results of the two mortality counts indicated significant effects of competition treatments. Right after the start of the dry season, all seedlings in the control treatment were alive, while 7 out of 20 seedlings presented their aerial parts dry in both the BGC and TC treatments ( $\chi^2 = 22.9$ ;  $P = 0.001$ ;  $df = 5$ ). At that time herb clipping had been practiced four times in the BGC treatment. At the end of the experiment, all seedlings but one in the AC treatment were alive, while 13 out of 15 seedlings died in the BGC and TC treatments ( $\chi^2 = 26.7$ ;  $P = 0.0001$ ;  $df = 5$ ).

### Growth, biomass partitioning, and physiological traits

One of the earliest observations in our experiment was that the new leaves of the seedlings in the AC treatment were significantly larger than those under competition from herbs (mean leaf length in early April in AC was  $18.7 \text{ mm} \pm 9.22$ , and it averaged  $15.4 \text{ mm} \pm 7.27$  in the competition treatments;  $F_{1,37} = 5.9$ ;  $P = 0.01$ ). At that time clipping had not been performed

**Table 1.** Results of a repeated-measure analysis of variance used to test the effects of the herb treatment, soil depth, and time on soil moisture.

Effect	df effect	df error	F	p-level
Herb competition	2	60	127.4	0.0001
Soil depth	2	60	13.2	0.0001
Time	3	180	133.7	0.0001
Herb competition*soil depth	4	60	2.2	0.08
Herb competition*time	6	180	58.8	0.0001
Soil depth*time	6	180	3.1	0.006
Herb competition*soil depth*time	12	180	3.9	0.0001

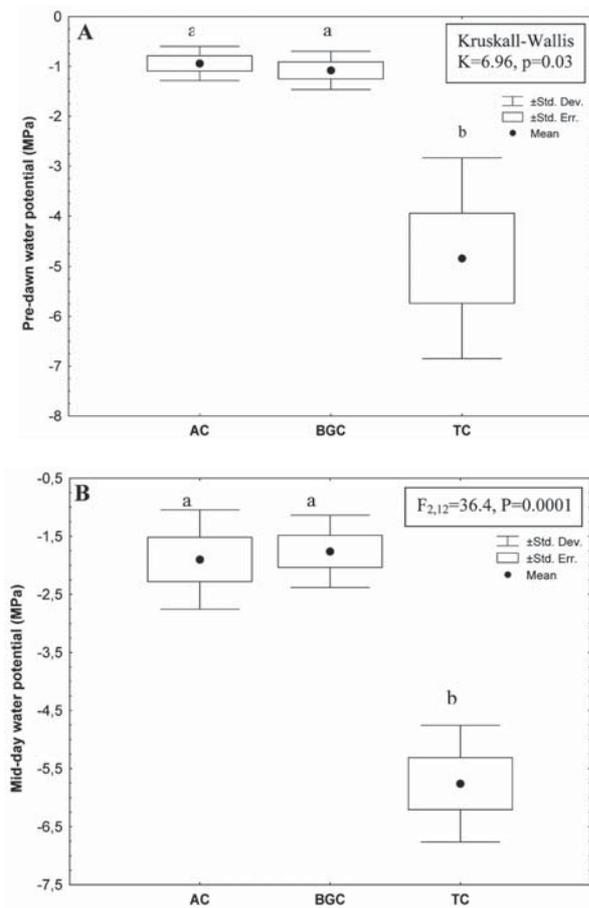
yet in the BGC treatment because herbs did not attain 8 cm in height; thus, this observation was an effect of below-ground competition. The differences in average total plant leaf area at the end of the experiment were not significant (Table 2a; Kruskal-Wallis  $K = 2.14$ ;  $p = 0.34$ ).

Pre-dawn and mid-day water potential were different among competition treatments. Seedlings in the TC treatment were more water-stressed than those in the BGC or AC treatments according to *post-hoc* tests (Fig. 2a, b).

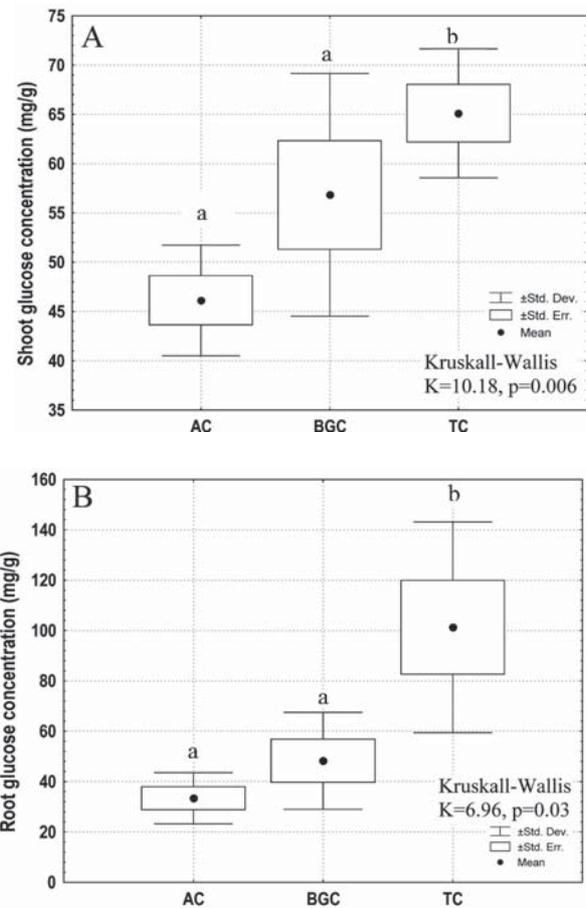
Total seedling biomass was not different among treatments (Table 2b;  $F_{2,12} = 0.74$ ;  $p = 0.5$ ). Dry matter of survivors at the end of the experiment averaged  $8.48 \pm 2.53$  g, and it represented an increment of 0.08 g as compared to the reference value at the beginning of the experiment (see the section on Data analysis). The

proportion of biomass allocated to leaves, stems, and roots among treatments resulted in marginal differences ( $F_{2,12} = 3.1$ ;  $p = 0.08$ ) only in biomass allocated to leaves, and it followed the order  $TC < BGC < AC$  (Table 2c). The allocation to stems and roots resulted in non-significant differences among treatments (Table 2d, e,  $F_{2,12} = 2.53$ ;  $P = 0.11$ ;  $F_{2,12} = 1.62$ ;  $P = 0.24$ , respectively).

Competition treatments significantly affected the glucose concentration in both shoots and roots, following the order  $TC > AGC > AC$  in both plant compartments (Fig. 3a, b). Differences in starch concentration among treatments did not appear for shoots (Table 2f, Kruskal-Wallis  $K = 2.14$ ;  $p = 0.34$ ) and marginally appeared for roots (Table 2g,  $F_{2,12} = 2.72$ ;  $P = 0.106$ ), and in both cases it tended to be larger for the BGC treatment.



**Fig. 2.** Variation of (A) pre-dawn and (B) mid-day water potential with herb treatment. Pre-dawn comparisons are between treatments based on Mann-Whitney's *U* post-hoc test (nominal  $P$ -value  $< 0.05$ ); mid-day comparisons based on Tukey's *post-hoc* test (nominal  $P$ -value  $< 0.05$ ).



**Fig. 3.** Variation of glucose concentration in (A) shoots and (B) roots with herb treatment. Comparisons between treatments are based on Mann-Whitney's *U* post-hoc test (nominal  $P$ -value  $< 0.045$ ).

**Table 2.** Values (mean  $\pm$  SD) in the three competition treatments of (a) Average total leaf area; (b) Total seedling biomass; (c-e) Proportion of biomass allocated to leaves, stems, and roots, respectively; (f,g) Starch concentration for shoots and roots, respectively. Differences for these values among treatments are never different at  $P < 0.05$ .

	Absence of competition	Below-ground competition	Total competition
a. Average total leaf area (cm <sup>2</sup> )	65.33 $\pm$ 52.47	44.72 $\pm$ 19.32	23.0 $\pm$ 28.55
b. Total seedling biomass (g)	9.48 $\pm$ 2.76	8.47 $\pm$ 2.05	7.49 $\pm$ 2.86
c. Proportion of biomass allocated to leaves	8.11 $\pm$ 3.62	6.72 $\pm$ 2.33	3.62 $\pm$ 2.65
d. Proportion of biomass allocated to stems	10.06 $\pm$ 2.54	16.12 $\pm$ 5.33	13.4 $\pm$ 4.44
e. Proportion of biomass allocated to roots	81.83 $\pm$ 5.45	77.16 $\pm$ 5.35	82.99 $\pm$ 5.44
f. Starch concentration for shoots (mg.g <sup>-1</sup> )	59.79 $\pm$ 6.04	61.23 $\pm$ 25.75	51.74 $\pm$ 9.29
g. Starch concentration for roots (mg.g <sup>-1</sup> )	174.06 $\pm$ 54.36	223.52 $\pm$ 48.4	147.71 $\pm$ 53.65

## Discussion

We found significant differences among treatments related to soil conditions and seedling performance evaluated as mortality, growth, and physiological responses. Overall, our data indicate that, for the conditions of our experiment, which are representative of fertile and recently abandoned cropland in Mediterranean environments, herbs strongly compete with *Q. faginea* seedlings. Nevertheless, plant interactions are dynamic relationships and positive and negative interactions act simultaneously, the outcome depending on abiotic conditions (Berkowitz et al. 1995; Holzappel & Mahall 1999; Pugnaire & Luque 2001). Actually, other studies have shown facilitation or neutral processes of herbs in the recruitment of woody species in environments under more severe abiotic conditions (e.g. less water availability and higher radiation inputs) and less herb density (Rejmánek & Lepš 1996; Brown & Archer 1999; Paez & Marco 2000).

### Soil moisture

Herbs reduced soil moisture, mainly in the TC treatment. In addition, the amount of herb biomass produced in the BGC treatment was negatively correlated with soil moisture. These patterns suggest that a larger amount of standing crop transpired more water and hence provoked the reduction in soil moisture. This moisture reduction increased along the growth period, when shortage of water and temperatures are higher. The profile of soil moisture indicates that increased transpiration from herbs outweighed the reduction of evaporation from soils due to shading by herbs (Rey Benayas et al. 2002).

Differences in soil moisture between the BGC and TC treatments were very small one month after the beginning of the experiment, when much of the soil water might have been invested in germination of herb seeds and their immediate growth (López-Pintor et al. 2000; Rebollo et al. 2001), and at the end of it, when soil water was already so low that provoked a severe herb

and seedling mortality (Fig. 1). This desiccation effect has been widely observed in numerous studies on the influence of herb competition on the establishment of woody seedlings (Knoop & Walker 1985; Zutter et al. 1986; Gordon et al. 1989; Kolb & Robberecht 1996; Harrington 1991). Henkin et al. (1998) also found that soil water depletion during the spring-summer transition period left very little water availability in the rooting zone of the herbaceous vegetation to sustain the demands of woody seedlings throughout the summer.

### Seedling response to the competition environment

The pattern of soil moisture in the competition treatments may explain in part the differences in water potential found in *Q. faginea* seedlings (Fig. 3a, b). Both the pre-dawn and mid-day water potential of the seedlings subjected to the AC and BGC treatments were substantially higher than those of the seedlings in the TC treatment, where water availability was the lowest. This means that the clipping treatment ameliorated the water stress of the seedlings.

However, the BGC and TC treatments did not differ in seedling mortality. In other words, the elimination of aerial competition from herbs did not translate into more survival of planted *Q. faginea* seedlings at the beginning of the dry season. Similarly, Brown et al. (1998) imposed different levels of grass clipping intensity (no clipping, clipped to 5 cm and clipped to 25 cm) and did not find any effect on shrub seedling survival. In our case it is more likely that competition is mostly for water since the plantation substrate is not infertile. Anderson et al. (2001) found that competition for water was a key mechanism in oak-understorey interactions. Henkin et al. (1998) suggested that the success of woody seedling establishment mainly reflected the accessibility of water below the rooting zone of the herbaceous vegetation. Rey Benayas et al. (2002) obtained the same result for planted *Retama sphaerocarpa* seedlings in the same type of habitat than the one studied here. However, these authors found a positive effect of weed clipping on

seedling survival in early autumn. The subterranean parts of both plant species easily resprout when their aerial biomass is removed and conditions are not extremely severe. Hill et al. (1995) found that the duration of competition was more important than the initial intensity of competition in determining tree establishment. Some differences between the BGC and TC treatments might have appeared in a longer-lasting experiment if seedling roots in the BGC treatment were vigorous enough to sprout. Other studies in different parts of the world have highlighted the importance of weed clipping and herbicide treatments for the success of afforestation practices (Raza 1993; Flemming & Wood 1996; Peñuelas et al. 1996; Holl 1998; Imo & Timmer 1998, 1999).

The early observation of reduced leaf length in the BGC and TC treatments was an effect of below-ground competition. We also found consistencies in the competition treatment ranking for total plant leaf area and biomass allocated to leaves. Both the lower size of the new leaves and the lower proportion of biomass allocated to leaves are mechanisms to reduce transpiration. One would expect an inverse relationship between biomass allocated to leaves and roots that may constitute further evidence of the trade-off in the development of plant tissues aimed at capturing different limiting resources (Tilman 1988; Lewis & Tanner 2000; Zavala et al. 2000; Valladares 2001). However, neither the differences in total plant leaf area nor biomass allocated to roots between competition treatments were significant, may be due to the short duration of our experiment or because *Q. faginea* has a low response to water stress at the root level as it has been observed for *Q. ilex* (Villar pers. comm.). Sack & Grubb (2002) found that watering frequency, i.e. drought intensity, did not significantly alter biomass allocation across three irradiance treatments in seedlings of four Mediterranean species. They claim that “such orthogonal impacts of deep shade and drought on seedling growth and biomass allocation indicate a large potential for niche differentiation at combinations of irradiance and water supply for species of forest seedlings”.

The pattern of glucose concentration is in agreement with the patterns of soil moisture and water potential (Fig. 3a, b). The accumulation of monosaccharids in leaf vacuoles during water-stress periods may contribute in some plant species to decrease their leaf osmotic potential, enhancing water extraction from dry soils (Gebre et al. 1994; Epron & Dreyer 1996; Clifford et al. 1998). Thus, the observed increase in shoot glucose concentration in the BGC and TC treatments may reflect that *Q. faginea* seedlings compensate a lower water availability with osmotic adjustment.

The allocation of carbohydrates to starch is expected

to be enhanced by environmental limitations to tissue growth (Bloom et al. 1985; Herms & Mattson 1992) such as resource depletion by herb competition. All seedlings exhibited about three times more starch concentrations in roots as compared with their shoots (Table 2d). The smaller differences in starch concentration among treatments as compared to the glucose content have two explanations. First, it may be due to the slower response of starch biosynthesis to stress, so that more than one growing season is necessary to find divergence among treatments. And secondly, a fraction of the original starch concentration may have been excised into glucose as competition intensity increases (Epron & Dreyer 1996). The maximum starch concentration in the BGC treatment can be explained as follows. The most favourable environment (AC) would have favoured the allocation of current assimilation to present growth, while a moderate resource limitation (BGC treatment) would limit tissue growth, but not carbon assimilation, the excess of carbon being invested in carbon-rich compounds such as starch (Herms & Mattson 1992). On the other hand, the strong above- and below-ground resource limitation imposed by the TC treatment severely limits carbon assimilation through both water and light shortages, so that plants cannot accumulate starch.

### Conclusions and implications for plantation management

We conclude that the negative effects of herbs in the establishment of *Q. faginea* are primarily a result of competition for water, as indicated by the soil water availability and the same seedling mortality in the BGC and TC treatments. This competition effect is noticeable since the earliest stages of the establishment, right after planting, as pointed out by the smaller leaves of the seedlings under below-ground competition. Some remarkable responses in seedlings under below-ground competition conditions are a water potential similar to that of the seedlings grown in absence of herb competition, an increase in glucose concentration that may favour water extraction from soils, and an accumulation of starch that may provide them a reserve for future harsh conditions. However, in our experiment these features did not translate into fewer dead seedlings in the BGC treatment as compared to the TC one. These apparently dead seedlings may resprout in the following favourable growth period, in agreement with the observations of Rey Benayas (1998) on *Q. ilex* seedlings.

For management of *Q. faginea* plantations in abandoned Mediterranean cropland, we conclude that it is necessary that the introduced seedlings of this species take advantage of a low competition environment from

herbs during the favourable period before the dry season (Paez & Marco 2000). In response to our question, clipping of weeds around the seedlings is not a technique that improves seedling survivorship in the short term (first growth season), and herbicide application before weed proliferation would provide better results. Consequently, further research is necessary to analyse the trade-off between the pursued environmental benefit (seedling establishment) and the potential risk of soil and water pollution, and alternatives such as weed hoeing must be tested.

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## References

- Anon. 1995. *Soil taxonomy*. Agriculture Handbook 436. USDA, Washington, DC, US.
- Anon. 1998. *The state of food and agriculture*. FAO, Rome, IT.
- Anderson, L.J., Brumbaugh, M.S. & Jackson, R.B. 2001. Water and tree-understory interactions: A natural experiment in a savanna with oak wilt. *Ecology* 82: 33-49.
- Aronson, J., Floc'h, E.L., David, J.F., Dhillon, S., Abrams, M., Guillerm, J.L. & Grossmann, A. 1998. Restoration ecology studies at Cazarils (southern France): Biodiversity and ecosystem trajectories in a Mediterranean landscape. *Landscape Urban Plann.* 41: 273-283.
- Balaguer, L., Martínez-Ferri, E., Valladares, F., Pérez-Corona, M.E., Baquedano, F.J., Castillo F.J. & Manrique, E. 2001. Population divergence in the plasticity of the response of *Quercus coccifera* to the light environment. *Funct. Ecol.* 15: 124-135.
- Bakker, J.P., van Andel, J. & van der Maarel, E. 1998. Plant species diversity and restoration ecology: Introduction. *Appl. Veg. Sci.* 1: 3-8.
- Berkowitz, A.R., Canham, C.D. & Kelly, V.R. 1995. Competition vs. facilitation of tree seedling growth and survival in early successional communities. *Ecology* 76: 1156-1168.
- Bienes, R. & Nieves, M. 2000. Edafología. In: Mauri, P.V. (ed.) *El Encín: Clima, suelo y vegetación*, pp. 91-114. Consejería de Medio de la Comunidad de Madrid, Madrid, ES.
- Bloom, A.J., Chapin III, F.S. & Mooney, H.A. 1985. Resource limitation in plants – an economic analogy. *Annu. Rev. Ecol. Syst.* 16: 363-392.
- Brown, J.R. & Archer, S. 1999. Shrub invasion of grassland: recruitment is continuous and not regulated by herbaceous biomass or density. *Ecology* 80: 2385-2396.
- Brown, J.R., Scanlan, J.C. & McIvor, J.G. 1998. Competition by herbs as a limiting factor in shrub invasion in grassland: A test with different growth forms. *J. Veg. Sci.* 9: 829-836.
- Canham, C.D., Berkowitz, A.R., Kelly, V.R., Lovett, G.M., Ollinger, S.V. & Schnurr, J. 1996. Biomass allocation and multiple resource limitation in tree seedlings. *Can. J. For. Res.* 26: 1521-1530.
- Castro-Díez, P., Villar-Salvador, P., Pérez Rontomé, C., Maestro Martínez, M. & Montserrat Martí, G. 1997. Leaf morphology and leaf chemical composition in three *Quercus* (*Fagaceae*) species along a precipitation gradient in NE Spain. *Trees* 11: 127-134.
- Clifford, S.C., Arndt, S.K., Corlett, J.E., Joshi, S., Sankhla, N., Popp, M. & Jones, H.G. 1998. The role of solute accumulation, osmotic adjustment and changes in cell wall elasticity in drought tolerance in *Ziziphus mauritiana* (Lamk.). *J. Exp. Bot.* 49: 967-977.
- Davis, M.A., Wrage, K.J., Reich, P.B., Tjoelker, M.G., Schaeffer, T. & Muermann, C. 1999. Survival, growth, and photosynthesis of tree seedlings competing with herbaceous vegetation along a water-light-nitrogen gradient. *Plant Ecol.* 145: 341-350.
- Debussche, M., Escarré, J., Lepart, J., Houssard, C. & Lavorel, S. 1996. Mediterranean plant succession: Old-fields revisited. *J. Veg. Sci.* 7: 519-526.
- Dirksen, C. & Hilhorst, M.A. 1994. *Calibration of a new frequency domain sensor for soil water content and bulk electrical conductivity*. Symp. on TDR in environmental, infrastructure, and mining applications, 07.-09.09.1994, Evanston, Ill., U.S. Bureau of Mines Spec. Publ. SP 19-94: 143-153.
- DuBois, M., Gilles, A., Hamilton, J.K., Rebers, P.A. & Smith, F. 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* 28: 350-356.
- Epron, D. & Dreyer, E. 1996. Starch and soluble carbohydrates in leaves of water-stressed oak saplings. *Ann. Sci. For.* 53: 263-268.
- Fleming, R.A. & Wood, J.E. 1996. Modeling the effects of herbicide release on early growth and survival of *Picea mariana*. *N. Z. J. For. Sci.* 26: 202-221.
- Gebre, G.M., Kuhns, M.R. & Brandle, J.R. 1994. Organic solute accumulation and dehydration tolerance in three water stressed *Populus deltoides* clones. *Tree Physiol.* 14: 575-587.
- Geyer, W.A. & Long, C.E. 1998. Weed management in plantings of tree and shrub seedlings with sulfometuron methyl (Oust). *Trans. Kansas Acad. Sci.* 101: 120-124.
- Gordon, D.R., Welker, J.M., Menke, J.W. & Rice, K.J. 1989. Competition for soil water between annual plants and blue oak (*Quercus douglasii*) seedlings. *Oecologia* 79: 533-541.
- Harrington, G.N. 1991. Effects of soil moisture on shrub seedling survival in a semi-arid grassland. *Ecology* 72:

- 1138-1149.
- Henkin, Z., Seligman, N.G., Kafkafi, U. & Prinz, D. 1998. End-of-season soil water depletion in relation to growth of herbaceous vegetation in a sub-humid Mediterranean dwarf-shrub community on two contrasting soils. *Plant Soil* 202: 317-326.
- Herns, D.A. & Mattson, W.J. 1992. The dilemma of plants: to grow or defend. *Q. Rev. Biol.* 67: 283-335.
- Hill, J.D., Canham, C.D. & Wood, D.M. 1995. Patterns and causes of resistance to tree invasion in rights-of-way. *Ecol. Appl.* 5: 459-470.
- Holl, K.D. 1998. Effects of above- and below-ground competition of shrubs and grass on *Calophyllum brasiliense* (Camb.) seedling growth in abandoned tropical pasture. *For. Ecol. Manage.* 10: 187-195.
- Holzappel, C. & Mahall, B.E. 1999. Bidirectional facilitation and interference between shrubs and annuals in the Mojave desert. *Ecology* 80: 1747-1761.
- Hunt, R. & Cornelissen, J.H.C. 1997. Physiology, allocation and growth rate: a reexamination of the Tilman model. *Am. Nat.* 150: 122-130.
- Imo, M. & Timmer, V.R. 1998. Vector competition analysis: A new approach for evaluating vegetation control methods in young black spruce plantations. *Can. J. Soil Sci.* 78: 3-15.
- Imo, M. & Timmer, V.R. 1999. Vector competition analysis of black spruce seedling responses to nutrient loading and vegetation control. *Can. J. For. Res.* 29: 474-486.
- James, F., Jr. 1999. Fertilization effects on interactions between above- and belowground competition in an old field. *Ecology* 80: 466-480.
- Joffre, R., Rambal, S. & Damesin, C. 1999. Functional attributes in Mediterranean-type ecosystems. In: Pugnaire, F.I. & Valladares, F. (eds.) *Handbook of functional plant ecology*, pp. 347-379. Marcel Dekker Inc., New York, NY, US.
- Joffre, R., Rambla, S. & Winkel, T. 2001. Respuesta de las plantas mediterráneas a la limitación de agua: desde la hoja hasta el dosel. In: Zamora, R. & Pugnaire, F.I. (eds.) *Ecosistemas mediterráneos. Análisis funcional*, pp. 37-65. CSIC-AEET, Granada, ES.
- Kavanagh, K.L. & Zaerr, J.B. 1997. Xylem cavitation and loss of hydraulic conductance in western hemlock following planting. *Tree Phys.* 17: 59-63.
- Knoop, W.T. & Walker, B.H. 1985. Interactions of woody and herbaceous vegetation in a southern African savanna. *J. Ecol.* 73: 235-253.
- Kolb, P.F. & Robberecht, R. 1996. *Pinus ponderosa* seedling establishment and the influence of competition with the bunchgrass *Agropyrum spicatum*. *Int. J. Plant Sci.* 157: 509-515.
- Kollmann, J. & Reiner, S.A. 1996. Light demands of shrub seedlings and their establishment within scrublands. *Flora* 191: 191-200.
- Lemieux, C. & Delisle, C. 1998. Using cover crops to establish white and black spruce on abandoned agricultural lands. *Phytoprotection* 79: 21-33.
- Lewis, S.L. & Tanner, E.V.J. 2000. Effects of above- and belowground competition on growth and survival of rain forest tree seedlings. *Ecology* 81: 2525-2538.
- López-Pintor, A., Espigares, T., Rey Benayas, J.M. & Gómez Sal, A. 2000. Effect of simulated parent-created microenvironmental conditions on germination of *Retama sphaerocarpa* (L.) Bois. seeds. *J. Med. Ecol.* 1: 219-226.
- Morris, L.A., Moss, S.A. & Garbett, W.S. 1993. Competitive interference between selected herbaceous and woody plants and *Pinus taeda* L. during two growing seasons following planting. *For. Sci.* 39: 166-187.
- Owens, M.K., Wallace, R.B. & Archer, S.R. 1995. Landscape and microsite influences on shrub recruitment in a disturbed semi-arid *Quercus-Juniperus* woodland. *Oikos* 74: 493-502.
- Paez, S.A. & Marco, D.E. 2000. Seedling habitat structure in dry Chaco forest (Argentina). *J. Arid Envir.* 46: 57-68.
- Peñuelas, J.L., Ocaña, L., Domínguez Lerena, S. & Renilla, I. 1996. Experiencias sobre el control de la competencia herbácea en repoblaciones de terrenos agrícolas abandonados. *Montes* 45: 30-36.
- Pugnaire, F.I. & Luque, M.T. 2001. Changes in plant interactions along a gradient of environmental stress. *Oikos* 93: 42-49.
- Rambal, S. & Leterme, J. 1987. Changes in aboveground structure and resistances to water uptake in *Quercus coccifera* along a rainfall gradient. In: Tenhunen, J.D., Catarino, F.M., Lange, O.L. & Oechel, W.C. (eds.) *Plant response to stress. Functional analysis in Mediterranean ecosystems*, pp. 191-200. NATO ASI Series, Springer-Verlag, Berlin, DE.
- Raza, U.H. 1993. Response of planted seedlings of silver fir (*Abies pindrow*) to site preparation and weed control in the moist temperature forests of Pakistan. *Pakistan J. For.* 43: 90-99.
- Rebollo, S., Pérez, L., García, M.T., Rey Benayas, J.M. & Gómez Sal, A. 2001. Recruitment in a Mediterranean annual plant community: seed bank, emergence, litter, and intra- and interspecific interactions. *Oikos* 95: 485-495.
- Rejmánek, M. & Lepš, J. 1996. Negative associations can reveal interspecific competition and reversal of competitive hierarchies during succession. *Oikos* 76: 161-168.
- Rey Benayas, J.M. 1998. Growth and mortality in *Quercus ilex* L. seedlings after irrigation and artificial shading in Mediterranean set-aside agricultural lands. *Ann. Sci. For.* 55: 801-807.
- Rey Benayas, J.M., López-Pintor, A., García, C., de la Cámara, N., Strasser, R. & Gómez Sal A. 2002. Early establishment of planted *Retama sphaerocarpa* seedlings under different levels of light, water and weed competition. *Plant Ecol.* 159: 201-209.
- Rousset, O. & Lepart, J. 2000. Positive and negative interactions at different life stages of a colonizing species (*Quercus humilis*). *J. Ecol.* 88: 401-412.
- Sack, L. & Grubb, P.J. 2002. The combined impacts of deep shade and drought on the growth and biomass allocation of shade-tolerant woody seedlings. *Oecologia* 131: 175-185.
- Sala, A., Sabaté, S., Gracia, C. & Tenhunen, J.D. 1994. Canopy structure within a *Quercus ilex* forested watershed: variations due to location, phenological development, and wa-

- ter availability. *Trees* 8: 254-261.
- Tilman, D. 1988. *Plant strategies and the dynamics and structure of plant communities*. Princeton University Press, Princeton, NJ, US.
- Valladares, F. 2001. Características mediterráneas de la conversión fotosintética de la luz en biomasa: de órgano a organismo. In: Pugnaire, F. & Zamora, R. (eds.) *Ecosistemas mediterráneos. Análisis funcional*, pp. 67-93. AEET/CSIC, Madrid, ES.
- Valladares, F. & Pearcy, R.W. 1998. The functional ecology of shoot architecture in sun and shade plants of *Heteromeles arbutifolia* M. Roem., a Californian chaparral shrub. *Oecologia* 114: 1-10.
- Valladares, F. & Pugnaire, F. 1999. Tradeoffs between irradiance capture and avoidance in semi-arid environments assessed with a crown architecture model. *Ann. Bot.* 83: 459-469.
- Vieira, I.C.G., Uhl, C. & Nepstad, D. 1994. The role of the shrub *Cordia multispicata* Cham. as a 'succession facilitator' in an abandoned pasture, Paragominas, Amazonia. *Vegetatio* 115: 91-99.
- Villar-Salvador, P. 2000. *Estrategias ecológicas y funcionales del xilema en plantas leñosas mediterráneas*. Ph.D. Dissertation, Universitat de València, València, ES.
- Whisenant, S.G., Thurow, T.L. & Maranz, S.J. 1995. Initiating autogenic restoration on shallow semiarid sites. *Rest. Ecol.* 3: 61-67.
- Zavala, M.A., Espelta, J.M. & Retana, J. 2000. Constraints and trade-offs in Mediterranean plant communities: the case of mixed holm oak (*Quercus ilex* L)-Aleppo pine (*Pinus halepensis* Mill.) forests. *Bot. Rev.* 66: 119-149.
- Zutter, B.R., Glover, G.R. & Gjerstad, D.H. 1986. Effects of herbaceous weed control on a young loblolly pine plantation. *For. Sci.* 32: 882-899.

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