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Thinning affects water use efficiency of hardwood saplings naturally recruited in a *Pinus radiata* D. Don plantation

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Abstract

Plantations for timber production combine the productive to the nursery function for natural regeneration of native species. Thinning plays a crucial role in recruitment and establishment of native species, by modifying the irradiance regime beneath the canopy cover. It also promotes the secondary succession towards a more stable forest, which is a main goal in protected areas. The present study was carried in a timber plantation of *Pinus radiata* D. Don, located in the National Park of Cilento and Vallo di Diano (Southern Italy). The ecophysiological responses of saplings of two hardwood species, *Quercus cerris* L. and *Fraxinus ornus* L., according to two contrasting (low and high) relative irradiance (RI) regimes were analysed. Leaf and tree ring $\delta^{13}C$ values were employed as indicators of water use efficiency (WUE) to explore plant responses to light regime. A preliminary methodological comparison between bulk wood and extracted holocellulose showed no significant differences in $\delta^{13}C$ between the two materials. Results indicate lower $\delta^{13}C$ values, thus suggesting a lower WUE, at higher RI regimes (corresponding to heavy thinning) for both the studied species. Furthermore, *Q. cerris* $\delta^{13}C$ values are lower than those of *F. ornus*. These results suggest that, under the same light conditions, the mesophile *Q. cerris* exhibits a weaker stomatal control than the xerophile *F. ornus*, which keeps higher WUE. In the mesic environment studied, *Q. cerris* may overcome *F. ornus* in the long run, owing to a heavy thinning.
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1. Introduction

Non-native tree species plantations may serve as nurse crops for the re-establishment of native tree species. This is considered a potentially interesting near-to-nature silviculture to explore in forest management. Naturally recruited seedlings may experience in the understorey favourable conditions for their development, whilst in the open, unfavourable soil conditions or competition by herbaceous species may represent severe or even lethal obstacles to their establishment (Carnevale and Montagnini, 2002; Ashton et al., 1998; Fimbel and Fimbel, 1996; Guariguata et al., 1995; Geldenhuys, 1997; Jussi et al., 1995; Keenan et al., 1997; Lugo et al., 1992, 1993; Parrotta, 1995; Parrotta et al., 1997; Powers et al., 1997; Yirdaw, 2001).

In managed forests the fate of natural regeneration largely depends on the reduction of canopy cover after thinning that increases light availability in the understorey, allowing efficient resource exploitation by seedlings (Kramer, 1958; Poulson and Platt, 1989; Malcolm et al., 2001). In particular, increased light availability should positively affect carbon balance and water use by young plants (Fairbairn and Neustein, 1970; Lieffers and Stadt, 1994; Dai, 1996).

Effective tools for interpreting the time-integrated response of plants to environmental conditions are now available. Following the model proposed by Farquhar et al. (1982), evidence has arisen that carbon stable isotope composition in plant tissues ($\delta^{13}C$) is related to changes in stomatal conductance (g), driven by environmental conditions; and that carbon isotope discrimination (Δ) is related to plant water use efficiency (Ehleringer et al., 1993; Guehl et al., 1994). Also, $\delta^{13}C$ is inversely related to Δ, therefore, changes in the irradiance level in the understorey, which are the main effect of thinning, can drive changes in plant water use efficiency and net productivity that may be detectable

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through changes in plant carbon isotope composition. Pearcy and Pfitsch (1991), Pfitsch and Pearcy (1992) and Yakir and Israeli (1995) showed that a linear relationship exists between leaf $\delta^{13}C$ and irradiance level.

In woody species, tree ring carbon isotope composition can be assessed on bulk wood as well on wood biochemical components. In particular, for assessing tree ring $\delta^{13}C$, analysis of cellulose extracts is preferred because (i) cellulose does not migrate from the wood ring in which it was formed and (ii) less reactions (and fractionations) occur during its synthesis from photosynthates, in comparison with biosynthesis of lignin and other wood compounds. On this topic Borella et al. (1998) discussed the differences measured in tree ring $\delta^{13}C$ of different wood components and the uncertainties (pooling, milling and cellulose extraction) arising when measuring. In the present work, a preliminary methodological issue involved a comparison of $\delta^{13}C$ values of bulk wood and holocellulose extracts obtained from annual woody rings in the studied hardwood species.

Water use efficiency (WUE), the ratio between photosynthesis and transpiration, can be viewed as a key functional trait under Mediterranean conditions (Dewar, 1997). Differences in WUE could affect species composition and success of natural regeneration of native species, by promoting the species, which better respond to site conditions. In particular, species with lower WUE could take advantage in wet sites, whilst species with higher WUE could prevail in dry sites. Although very few information is available on the relationship between thinning intensity and WUE (Fotelli et al., 2003), the link between different site resources and WUE is well documented (see, among others, Ehleringer et al., 1993).

The main objective of this study was to assess, based on measurements of leaf and tree ring carbon isotope composition and leaf gas exchanges, if differences in irradiance level in the understorey determined by thinning, translate into differences in water use efficiency (WUE) of *Quercus cerris* L. and *Fraxinus ornus* L. saplings, naturally recruited in a *Pinus radiata* D. Don plantation growing in Southern Italy.

2. Materials and methods

2.1. Study site and stand characterization

The study was carried out, during 2001 and 2002, in a 23 year old *P. radiata* D. Don plantation (2.0 m × 2.0 m spacing) growing in Southern Italy (Stio, province of Salerno,

$40°18'32''$N, $15°15'07''$E, 700 m a.s.l.). Climate is Mediterranean, humid type; mean annual temperature is 13 °C and annual precipitation is 1200 mm. Rainfall peaks in autumn and winter, with about 15% of total precipitation occurring during summer. Bedrock is flysch and soil profile, lacking in horizons in the first 0.8–1.0 m due to deep ploughing, is represented by sand (33%), clay (29%), and silt (38%).

Measurements have been performed in three pine stands experiencing the same environmental conditions: the first one was unthinned (T_{con}), whilst the second (T_1) and the third (T_2) at the age of 10 years underwent systematic thinning at different intensities: one third, corresponding to one row every third one, and a half, corresponding to one row every other one, of trees were felled in T_1 and T_2, respectively. Based on measurements performed in four 1 m × 10 m transects in each stand, *Q. cerris* saplings were frequent in all stands; in contrast, *F. ornus* saplings were frequent only in T_2. However, during the sampling for carbon isotope analysis two *F. ornus* saplings were found in T_1 and collected (Table 1).

Pine stand leaf area index (LAI) was estimated using a LAI-2000 canopy analyzer (LI-COR, Lincoln, NE, USA); instrument readings were multiplied by 1.5 for taking account of mutual shading of clumped needles (Gower and Norman, 1991). Photosynthetic active radiation (PAR) was measured as the integral of photosynthetic photon flux density (PPFD), by means of a LI-1800 spectroradiometer (LI-COR, Lincoln, NE, USA). For each stand, below-canopy relative irradiance (RI) was computed as the ratio of PAR readings taken within each stand and in the open, in the morning of clear summer days, 1 m above ground. In each stand two 1 m × 10 m transects were obtained along the diagonal and the side of a quadrat with vertices represented by four trees; 12 readings were taken 1 m apart each other in each transect for both LAI and RI.

Measurements indicate that, 13 years after thinning, significant differences in RI can be found between T_2, on one side, and T_{con} and T_1, on the other side (Table 1). For all successive comparisons, T_{con} and T_1 have been merged into one treatment, characterised by low relative irradiance conditions. Therefore, two contrasting light conditions were considered: low ($RI_{low} = T_{con} + T_1$) and high ($RI_{high} = T_2$) irradiance.

2.2. Carbon isotope analyses

In December 2001, before leaf abscission, *Q. cerris* and *F. ornus* saplings, growing in RI_{low} ($n = 30$ for *Q. cerris*, $n = 2$ for *F. ornus*) and RI_{high} ($n = 15$ for both *Q. cerris* and *F. ornus*)

Table 1
Stand characteristics; LAI is leaf area index of the pine plantation, RI relative irradiance (mean ± S.E., $n = 12$) and regeneration index (density × mean height, according to Magini, 1967) of native species natural regeneration

Treatment	Tree (ha^{-1})	LAI (m^2 m^{-2})	RI (%)	Regeneration index	
				Quercus cerris	Fraxinus ornus
Unthinned forest, T_{con}	2500	4.2 ± 0.1a	4.4 ± 0.3a	27.6	Absent
Thinned stand, T_1	1600	3.5 ± 0.4a	10.1 ± 1.2a	28.2	Absent
Thinned stand, T_2	1250	2.6 ± 0.1b	50.2 ± 11.0b	32.5	33.0

Means followed by different letters differ at $P < 0.05$ according to S–N–K multiple comparison test.

were randomly selected, discarding plants with root collar diameter < 4 cm. This sampling strategy allows to obtain an integration of the whole season, since $\delta^{13}C$ time-integration potential can differ between tree-rings and leaves due to leaf metabolism and age (Fotelli et al., 2003). For each sapling, a wood disc was removed from the lowermost stem portion and one leaf was collected from the top of the crown. Samples were stored in sealed plastic bags, taken immediately to the laboratory and oven dried at 60 °C for 48 h.

Thin wood shavings were removed with a razor blade from each of the last three annual growth rings (1999, 2000 and 2001). Wood material was further reduced to 0.2 mm particles using a micro-gear miller and a ceramic mortar, whilst leaves were ground in liquid nitrogen. Each wood sample underwent cellulose extraction according to the Jaime–Wise method, as modified by Leavitt and Danzer (1993) (Table 2). Leaf and cellulose samples were then analysed for carbon stable isotope composition ($\delta^{13}C$) in a Finnigan Delta Plus mass spectrometer (Finnigan MAT GmbH, Bremen, Germany).

Tree ring carbon isotope composition $\delta^{13}C$ did not differ among the annual growth rings in any of the studied tree species (D'Alessandro et al., 2004), so that $\delta^{13}C$ values of 1999, 2000 and 2001 were pooled into a single data set for the present purposes.

2.3. Gas exchange measurements

During a clear day in July 2002, leaf gas exchange measurements were performed on 10 Q. cerris saplings (five growing at RI_{low} and five growing at RI_{high}) and two F. ornus plants, growing at RI_{high}.

The day before, all plants had been copiously watered to exclude any effect of soil water availability on saplings. Predawn water potential was subsequently measured on one leaf per plant using a pressure chamber (PMS Ins., Corvallis, OR): no significant difference was found between plants growing under different RI conditions nor between species. Leaf gas exchange measurements (PAR, A and g) have been carried out in an attempt to assess intrinsic water use efficiency (iWUE, i.e. the ratio A/g) under contrasting RI conditions.

At regular intervals from dawn to sunset, net carbon assimilation (A) and stomatal conductance to water vapour (g) were measured on three leaves from each plant using a portable infrared gas analyzer and broadleaf cuvette (CIRAS 1 and PLC,

respectively, PP Systems, Hitchin, UK); values were recorded after steady state conditions had been attained for at least 5 min.

2.4. Statistics

Main effects (differences in carbon isotope composition between annual growth rings, between contrasting light conditions and between species), were assessed by one-way ANOVA and independent samples t-tests. All statistics were performed with the SPSS statistical package version 10.0 (SPSS Inc. 1989–1999).

3. Results

3.1. Wood and cellulose $\delta^{13}C$

Carbon stable isotope composition did not significantly differ between annual growth rings in either tree species, for cellulose (CS) and bulk wood (WS) samples (data not shown). For all comparisons, samples from adjacent tree rings were pooled. Similarly, no significant differences in average $\delta^{13}C$ values were found between CS and WS samples (Table 3). Significant linear regressions were obtained between individual $\delta^{13}C$ values of CS versus WS samples in both species (Fig. 1, left panels). The points describing the relationship between predicted and actual $\delta^{13}C$ values of CS samples (Fig. 1, right panels) had an uneven distribution, particularly in Q. cerris, reflecting a rather high variability of the residuals.

$\delta^{13}C$ values from cellulose extracts were used for all successive comparisons.

The positive linear relationship between tree ring and leaf $\delta^{13}C$, widely documented in the literature, was confirmed by our results for both species (data not shown) and will not be further discussed.

Table 2
Jaime–Wise method modified by Leavitt and Danzer (1993) for holocellulose extraction

Step	Procedure	Time needed	Goal
1	Toluene and ethanol 2:1 solution	48 h	Removing sugars and lipids
2	Pure ethanol solution	48 h	Removing sugars and lipids
3	Sodium chlorite and glacial acetic acid at 70 °C	4 days	Removing lignin

Table 3
Independent samples t-test for differences in $\delta^{13}C$ between sample material, contrasting light conditions and species

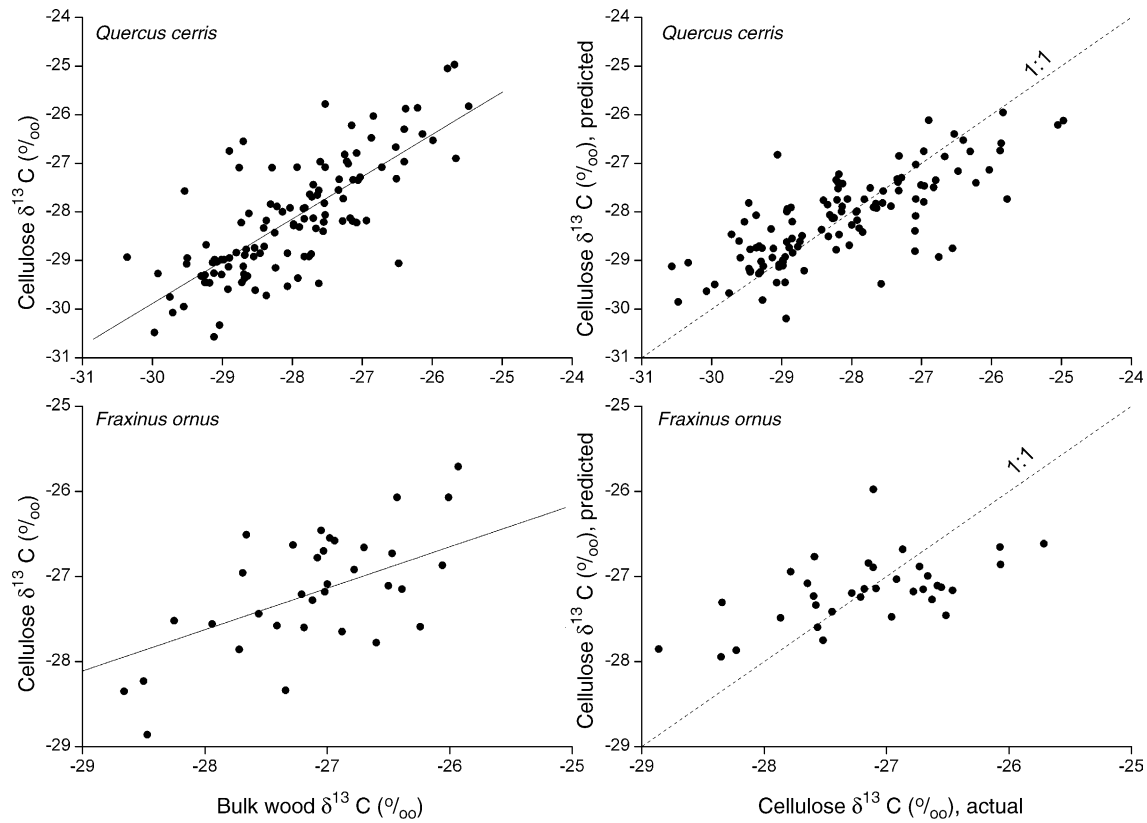| Comparison	t	d.f.	P
CS vs. WS			
Q. cerris	1.85	115	0.06
F. ornus	1.05	39	0.30
RI_{low} vs. RI_{high}			
Q. cerris			
Leaf	4.19	88	0.0001
Tree ring	4.92	115	0.001
F. ornus			
Leaf	2.79	31	0.009
Tree ring	3.08	39	0.004
F. ornus vs. Q. cerris			
RI_{low}	−3.11	84	0.003
RI_{high}	−8.58	68	0.0001

Fig. 1. Left panels: linear regression between cellulose and bulk wood δ^{13}C (solid circles, *Quercus cerris*: $y = -3.80 + 0.87x$, $R^2 = 0.58$, $n = 116$, $P < 0.0001$; open circles, *Fraxinus ornus*: $y = -13.99 + 0.49x$, $R^2 = 0.31$, $n = 35$, $P < 0.000$); right panels: predicted vs. actual δ^{13}C in cellulose extracts; 1:1 line shown.

3.2. Water use efficiency in two contrasting RI conditions, estimated by means of δ^{13}C

In both species δ^{13}C was found to differ significantly between contrasting light conditions (Table 3), as well as leaf (data not shown) or tree ring δ^{13}C (Fig. 2) is considered. In particular, tree ring δ^{13}C was significantly higher at RI_{low} in both species.

As for differences between species, *F. ornus* is characterized by higher δ^{13}C values in both irradiance conditions (Fig. 2 and Table 3).

The ratio between net assimilation (A) and stomatal conductance (g) from gas exchange measurements can be used as an estimate of intrinsic water use efficiency (iWUE = A/g). In this case, a different picture emerged, as in *Q. cerris* daily averages of iWUE were significantly lower at RI_{low}, as reflected by well differentiated A to g relationships (Fig. 3 and Table 4).
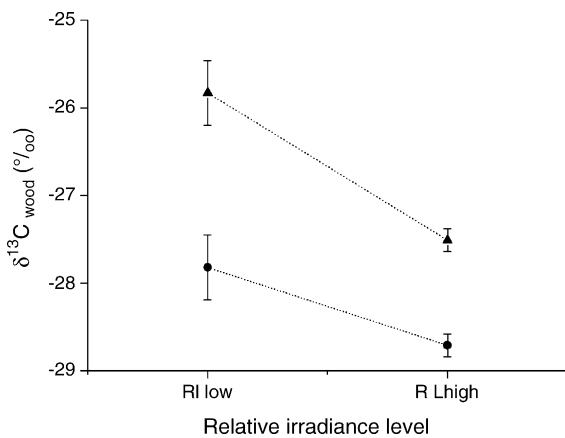

Fig. 2. Tree ring carbon isotope composition (δ^{13}C) as a function of contrasting RI conditions (see Table 1). Triangles and circles indicate *Q. cerris* and *F. ornus*, respectively; bars are standard errors. Means are significantly different ($P < 0.05$) between RI levels and between species, according to independent samples *t*-test.

Table 4
Independent samples *t*-test for differences in iWUE of *Q. cerris* between contrasting light regimes, as reflected by A to g relationships and in δ^{13}C between species at RI_{low} and RI_{high}

	t	d.f.	P
RI_{low} vs. RI_{high}	−19.3	123	<0.0001
F. ornus vs. *Q. cerris*			
RI_{low}	−3.11	84	0.003
RI_{high}	−8.58	68	<0.0001

Table 5
Relationship existing between iWUE and PAR

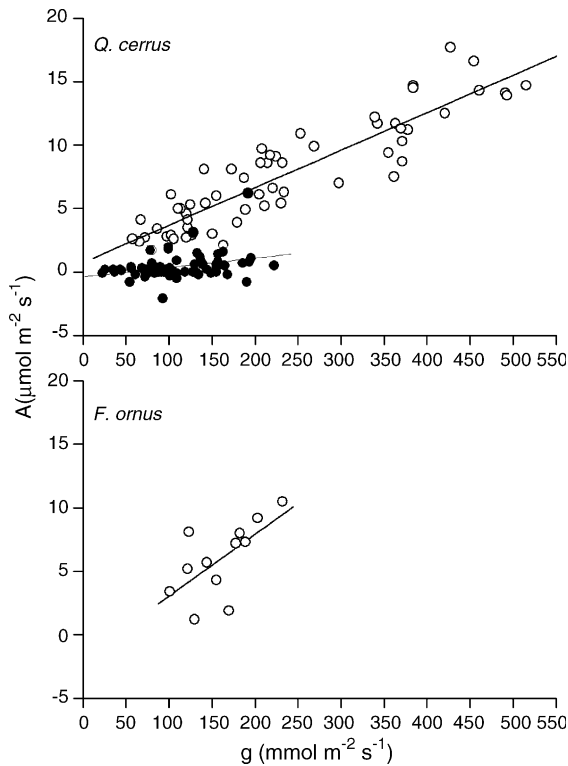| Species	r	N	P
Q. cerris	0.77	125	<0.0001
F. ornus	0.83	12	0.0001

Fig. 3. Net assimilation (A) vs. stomatal conductance to water vapour (g) measured in *Q. cerris* and *F. ornus* saplings at RI_{high} (open circles, $y = 0.74 + 0.030x$, $r = 0.91$, $n = 60$, $P < 0.0001$ and $y = -1.80 + 0.049x$, $r = 0.65$, $n = 12$, $P = 0.023$, respectively) and at RI_{low} (solid circles, $y = -0.39 + 0.007x$, $r = 0.33$, $n = 65$, $P = 0.008$). For *Q. cerris*, the slope of the linear regression is significantly higher at RI_{high} ($t = 16.47$, d.f. = 121, $P = 0.05$) than at RI_{low}, according to Student's *t*-test. At RI_{high}, the slope of the linear regression is significantly higher for *F. ornus* ($t = 1.71$, d.f. = 68, $P = 0.05$) than for *Q. cerris*, according to Student's *t*-test.

Our results did not show any significant difference in daily averages of iWUE between the studied species growing at RI_{high}, as reflected by A to g relationships (Fig. 3). Also, a positive significant relationship was found between iWUE and PAR in both species (Table 5). According to Student's *t*-test (Zar, 1984) the slopes of the lines do not significantly differ between the two species ($t = 5.0$, d.f. = 1, $P = 0.13$), suggesting the same pattern of iWUE in both species.

Different behaviours relative to water use efficiency between the studied species are suggested by differences in $\delta^{13}C$ values (see also Fig. 2); namely *F. ornus* is characterized by higher $\delta^{13}C$ values, suggesting higher water use efficiency, in both irradiance conditions (Table 4).

4. Discussion

Climatic conditions in the study site did not vary significantly during the study period (data not shown): mean annual temperature and precipitation were slightly above the average; in addition, the two treatments (RI_{low} and RI_{high}) were characterized by strongly different light conditions. Under such conditions, adjacent tree rings can be pooled for physiological studies at the short term, as suggested by Borella et al. (1998).

With respect to the linear regression reported by Borella et al. (1998 – Fig. 3b) for oak wood, we observed a lower correlation coefficient between $\delta^{13}C$ of CS and WS, due to a larger variability in our data, but a higher slope of the linear relationship. In the studied species wood $\delta^{13}C$ seems mainly affected by cellulose carbon isotope composition (Fig. 1) and, as far as $\delta^{13}C$ is used as 'climate' proxy and as ecophysiological trait, bulk wood seems a suitable material to work with. This agrees with previous findings (Borella et al., 1998) and only under conditions which may favour rapid polysaccharides degradation, as may occur with wood material used for paleoclimatic studies, extraction of lignin may be recommended in hardwood species (Schleser et al., 1999). On the other hand, the wide scatter of predicted vs. actual cellulose $\delta^{13}C$ data relative to the 1:1 relationship in *Q. cerris* (much larger than in *F. ornus*, sampled only in RI_{high}) could depend on the fact that saplings were sampled in stands with contrasting light conditions. Therefore, linear models relating $\delta^{13}C$ in cellulose and bulk wood samples should be applied with some caution.

The model proposed by Farquhar et al. (1982) suggests that carbon isotope discrimination (Δ) on plant dry matter can provide a long-term assimilation weighed estimate of plant WUE. The relationship between WUE and $\delta^{13}C$ is basically due to their independent linkages to the ratio of internal to ambient CO_2 concentrations (c_i/c_a). Experimental evidence (e.g. Guehl et al., 1994) shows that Δ is negatively related to WUE, with a linear relationship.

Carbon isotope composition ($\delta^{13}C$) is inversely related to Δ, so that both *Q. cerris* and *F. ornus* saplings seem to be characterized by a higher long-term water use efficiency (water conservative strategy) under low irradiance levels R_{low} (Fig. 2). Indeed, reduction of stand leaf area by thinning can have significant effects even on soil moisture (increase due to reduced transpiration of the overstorey as well as reduction due to higher evaporation) and soil moisture can certainly affect isotopic composition of plant dry matter through changes in stomatal conductance. No data of soil moisture in the different treatments were available at the study site; however, during daily gas exchange measurements on previously watered *Q. cerris* saplings, higher light availability (RI_{high}) determined a significant increase in maximum stomatal conductance (230 mmol m^{-2} s^{-1} versus 60 mmol m^{-2} s^{-1} at RI_{high} and RI_{low}, respectively) and a drop of minimum xylem water pressure relative to RI_{low} (D'Alessandro et al., 2005). Consequently, lower $\delta^{13}C$ values measured at RI_{high} could be explained by a higher stomatal conductance due to higher irradiance. In our study minimum xylem water pressure did not significantly differ between species; yet *F. ornus* drops earlier (10:00 a.m. solar time) than *Q. cerris* (13:30 p.m. solar time). In addition, maximum stomatal conductance was significantly ($P < 0.05$) higher in *Q. cerris* relative to *F. ornus* (D'Alessandro et al., 2005).

As for the different WUE pattern emerged from gas exchange measurements, it is worth remembering that carbon isotope composition $\delta^{13}C$ on plant dry matter represents a long-term assimilation weighed integration of c_i/c_a, which cannot be simply related to iWUE. In particular, under variable environmental

conditions short-term gas exchange measurements may not be very effective in integrating long-term transpiration efficiency (Ebdon et al., 1998; Ripullone et al., 2004).

In contrast with our findings based on $\delta^{13}C$, an increase of water use efficiency has been observed after thinning in some previous studies (e.g. Yakir and Israeli, 1995; Hanba et al., 1997; Welander and Ottosson, 2000; Warren et al., 2001), often interpreted as an effect of modified environmental conditions which promote stomatal closure and reduce transpiration. Under conditions of relatively low irradiance, spots of high irradiance (sunflecks) may occur frequently, promoting photosynthetic activity (Chazdon, 1988); yet, when sunflecks have a short duration, stomata may not react as quickly as the photosynthetic apparatus to sudden changes of irradiance (Nobel, 1991), so we suggest that this could imply lower stomatal conductance to water vapour and increased water use efficiency.

Furthermore, in this study saplings, which underwent $\delta^{13}C$ evaluation experienced a Mediterranean climate with a summer high vapour pressure deficit. Under such conditions plants may be expected to regulate stomatal behaviour in a manner that minimizes water loss. In contrast, saplings, which underwent gas exchange measurements were kept well watered; under such conditions plants may be expected to exhibit stomatal behaviour that maximizes carbon gain even at the possible expense of larger water loss (Allen and Pearcy, 2000). Differences in $\delta^{13}C$, and therefore in WUE, between the studied species could be due to different responses to water availability variations during the growing season: *F. ornus* is a moderately drought tolerant species (Tretiach, 1993), with a strong stomatal control and high hydraulic resistance at root level (Nardini et al., 2003), whilst *Q. cerris*, characterised by moderate hairiness and raised elliptical stomata (Bussotti and Grossoni, 1997), is reported as a less water conservative species, with scarce stomatal control (Nardini et al., 1999; Nardini et al., 2003) and with lower hydraulic resistance (Nardini and Tyree, 1999). This means that the latter species tends to maximize carbon assimilation, even in severe drought stress conditions, by keeping stomata open.

5. Conclusion

In view of the close physiological link between light and $\delta^{13}C$, the results of this study suggest that if timber plantations are to play a nurse effect for native species, it is necessary to consider light environment in the understorey, as a main tool to promote and orienteer the secondary succession. In forest understorey, light is a more important limiting factor than water stress for plant growth (Chazdon, 1988). The optimal stand density for timber plantations must be regulated according to light needs of the species one wants to enter the understorey. In the mesic study site, saplings of *Q. cerris* may take advantage over those of *F. ornus* in the medium and long term, as it happens for plants from mesic or wet habitat (Ward et al., 2002). Yet, further research is needed to understand plant physiological responses in the understorey during a longer period and consider the effect of subsequent thinning

operations on the assimilation/transpiration balance of each species of the succession.

As for the use of carbon isotope discrimination as an indicator of WUE, one must be very cautious when trying to infer plant responses on a daily basis, which strongly depend on changing micro-environmental conditions during the day. On the contrary, tree ring $\delta^{13}C$ is affected by seasonal environmental patterns experienced by the sapling in its growth site.

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