

## The response of European beech (*Fagus sylvatica* L.) seedlings from two Italian populations to drought and recovery

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**Abstract.** The response of gas exchange, leaf chlorophyll content, relative fluorescence to decreasing water potential and recovery was followed in European beech seedlings from two Italian populations, differing in their native precipitation amounts. A population from Sicily (southern Italy) was selected as representative of a xeric population while a population from central Italy, Abetone, represented a mesic one. Dry-matter partitioning, leaf area, hydraulic sufficiency and xylem embolism were evaluated in both well-watered control plants as well as in plants subjected to drought. With the onset of water stress, values of water potential, leaf relative water content, net photosynthesis, leaf conductance and leaf chlorophyll concentration decreased concurrently while relative fluorescence remained unchanged. The population from Sicily showed a delay in effects of the imposed drought. Within 5 days of rewatering, leaf conductance was not fully recovered while all of the other parameters recovered to control levels, in both populations. Total, shoot, stem and root dry weight tended to be higher in seedlings from Abetone, even though both populations had similar photosynthetic rates. The population from Sicily exhibited about 3% greater (even if not significant) allocation to roots than the population from Abetone. Seedlings from Abetone had higher, but not significant, leaf specific conductivity and per cent loss in hydraulic conductivity than seedlings from Sicily. Drought resulted in a reduction of hydraulic conductivity and hydraulic sufficiency in seedlings from both populations. Photosynthesis of water stressed plants from both populations appeared to be reduced primarily by carbon dioxide diffusion through stomata and perhaps secondarily by changes in chlorophyll concentration rather than by efficiency of photosystem II. The effect of hydraulic factors on gas exchange during drought and recovery was not clearly evident.

**Key words:** Chlorophyll content – *Fagus sylvatica* – Gas exchanges – Hydraulic conductivity – Relative fluorescence

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### Introduction

Restricted water availability causing water stress is a major environmental factor in the distribution and productivity of plants (Woodward 1987). As a consequence of genetic differentiation among populations related to differential capacity to tolerate drought (Abrams et al. 1992), forest tree species and populations within a species often segregate along moisture gradients. Investigations of the mechanisms of drought tolerance could identify differences in the physiology and productivity of forest trees adapted to xeric and mesic sites.

As drought develops assimilation rate is reduced primarily by stomatal closure, increasing resistance to CO<sub>2</sub> diffusion (Schulze 1986), further by increasing chlorophyll degradation (Michelozzi et al. 1995), and by inhibiting photosynthetic reactions (Björkman and Powles 1984). Moreover, as water potential declines as drought intensifies, a catastrophic loss of xylem function through cavitation can occur if stomatal closure is not adequate to reduce transpiration (Tyree and Ewers 1991). Avoidance of xylem embolism resulting from cavitation may be one of the most important parameters determining water stress resistance in trees. The capacity of xylem to supply water to foliage might reflect selective advantages in populations with different origins. In addition, certain stomatal sensitivity and photochemical efficiency during drought might impart moderate tolerance to water stress. Physiological and biochemical adaptations to drought could be related to higher productivity in environments subjected to a periodic dry season.

European beech is primarily confined to mountainous regions of Italy and is known to display geographical

variation in late winter xylem embolism, growth parameters, allozyme characteristics (Borghetti et al. 1993) and in its phenology and morphology (Borghetti and Giannini 1982). This might suggest potential for physiological adaptation or acclimation in European beech populations living along the moisture gradient from southern to northern Italy.

The main objective of this work was to study the response of a drought-recovery cycle on gas exchange, leaf chlorophyll content, relative fluorescence, biomass partitioning, hydraulic parameters and leaf area in *Fagus sylvatica* seedlings of different geographic origin. Information on the relationships between soil water availability and physiological response could provide a better understanding of natural distribution patterns and improvements in breeding strategies for European beech populations.

## Materials and methods

### Plant material and growth conditions

The study was conducted on 1-year-old (second growing season) seedlings of *F. sylvatica* from two wild populations, representing different locations along a rainfall gradient in Italy (see Borghetti et al. 1993). Seeds from natural stands located in Abetone (northern Tuscany, central Italy) and Sicily (southern Italy) were collected and cultured in the forest nursery of Camporgiano (northern Tuscany, central Italy). After germination, seedlings were transplanted to 2.5-l pots containing a mixture of local soil and fine gravel. In March 1993, seedlings were transferred to the nursery of the Faculty of Forestry, University of Firenze. In late June 1993, 60 seedlings selected for dimensional uniformity were moved to two growth chambers (30 seedlings in each chamber) programmed with a 14-h photoperiod of 800 PPFD  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  (as measured with a LI-190 S1 quantum sensor, LI-COR, Lincoln, Neb., USA) at the top of the foliage, 22–26 °C night-day temperature, and 65% relative humidity. In each chamber both populations were divided in two groups named S-plants (stress plants) and C-plants (control plants), and were allowed to acclimate for 2 weeks prior to the start of the experiment. During the acclimation period, the seedlings were kept well-watered and fertilized (20/20/20 N/P/K commercial fertilizer). At the end of the acclimation period, water was withheld from S-plants until preflight leaf water potentials reached  $-2 \text{ MPa}$  (8 days for Abetone and 10 days for Sicily), then seedlings were re-irrigated to avoid irreversible damage. C-plants were watered regularly. All seedlings were moved frequently in the growth chamber to minimize position effects on the bench.

### Physiological measurements

Measurements were conducted in the growth chambers during the soil drying-recovery cycle. On each sampling day, 10 seedlings per population, 6 S-plants and 4 C-plants were measured. Xylem water potential ( $\Psi$ ) was measured with a pressure chamber on a minor twig from sample plant, one hour before the beginning of the light period (simulating "predawn" conditions). Branches were randomly selected among those of the same development stage.

Leaf relative water content was calculated as  $\text{RWC} = (\text{fresh weight} - \text{dry weight}) / (\text{saturated weight} - \text{dry weight})$ . Leaves of the same age-group were harvested immediately after  $\Psi$  measurements had been made and fresh weight determined. Then, each leaf was placed in a vial with the cut end immersed in distilled water, covered with parafilm and moved to a growth chamber in the dark for 24 h.

Saturated weights were then measured. Finally, leaves were oven-dried at 70 °C for 48 h and their dry weights were determined.

Net photosynthesis ( $A$ ) was measured 1 h after the onset of the light period on the same plants selected for  $\Psi$  and RWC measurements with a portable gas exchange open system (LCA-2, Analytical Development Company Ltd., Hoddesdon, UK). For each measurement,  $A$  was calculated from the inlet and outlet  $\text{CO}_2$  flux and the flow rate of the air stream. One leaf per plant was measured twice.

Leaf conductance to water vapour diffusion ( $g$ ) was determined immediately on the same leaf after measuring  $A$  with a steady-state porometer (LI-1600, LI-COR, Lincoln, Neb., USA) and was then followed by chlorophyll a fluorescence induction kinetics measurements (CF-1000, P. K. Morgan Instruments, Andover, Mass., USA). Leaves were dark acclimated for 15 min in supplied cuvettes and were irradiated with 600  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  PPFD actinic light. Fluorescence kinetics were recorded over a 150 s run-time.

Chlorophyll content (Chl a, b, and total) was measured on leaf disks ( $2.4 \times 10^{-5} \text{ m}^2$ , 5–15 per leaf), taken with a cork borer from the same leaves sampled above. Avoiding direct light, leaf disks (about 0.025 g FW) were homogenized in cold 80% acetone (v/v) and then centrifuged at 10000 rpm for 7 min at 0 °C. Chlorophyll concentrations were determined with a spectrophotometer (DU-65, Beckman, Fullerton, Calif., USA) following the procedure of Arnon (1949).

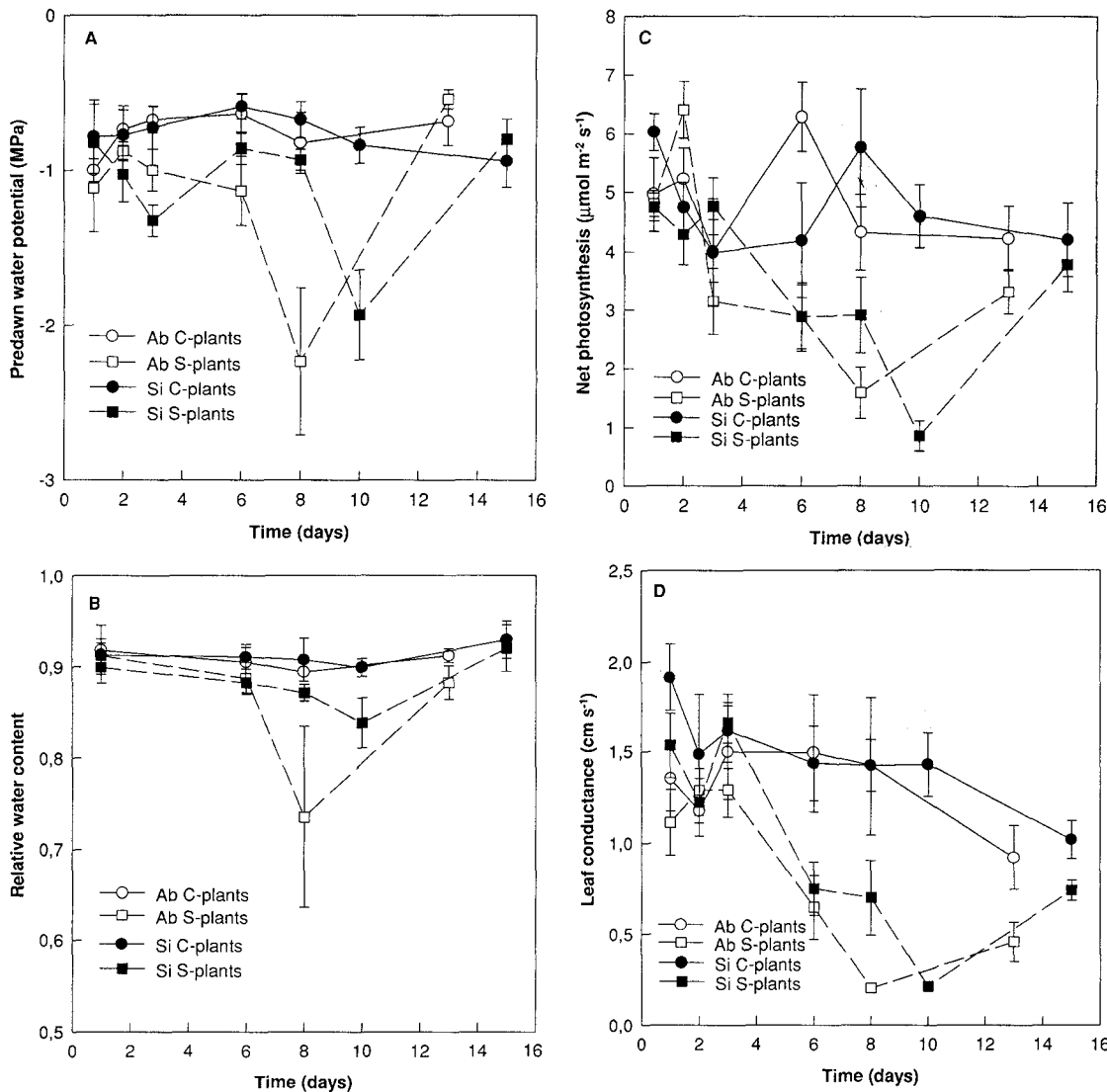
### Stem hydraulic properties and biomass measurements

As  $\Psi$  recovered in both populations, all 30 seedlings (9 S-plants plus 6 C-plants per population) from one growth chamber were harvested and dry weights of the plant components determined. Each seedling was separated into foliage, stem and roots, then dried for 48 h at 70 °C in an oven.

The remaining 30 seedlings (9 S-plants plus 6 C-plants per population) in the other growth chamber were maintained, for an additional 20 day period, like the control seedlings and then measured for leaf area and hydraulic parameters. Total leaf area ( $L$ ) of each seedlings was measured with a portable leaf area meter (LI-3000, LI-COR Lincoln, Neb., USA), and the number of leaves per seedling was counted. It must be pointed out that no leaves were added during this period; beech shows determined growth and the number of leaves produced by a bud is limited (Eschrich et al. 1989).

Hydraulic conductivity, considered as  $k_h = \text{mass flow rate/pressure gradient}$ , was measured before and after removing air embolisms with an apparatus similar to that described by Sperry et al. (1988a). The stem of each seedling was cut under water and, then while still submerged, recut to avoid further embolism, in order to obtain a central stem segment of  $0.23 \pm 0.01 \text{ m}$ . Both ends of the stem were fitted with rubber gaskets and trimmed in distilled water. The perfusing solution was 10 mM ( $10 \text{ mol m}^{-3}$ ) oxalic acid in distilled water (pH 1.8) to minimize microbial occlusions of xylem vessels. The perfusing solution was degassed by agitating under vacuum for 30–45 min and then introduced into an air-free plastic ball enclosed in a compressed gas tank. The solution, passing through a 0.2  $\mu\text{m}$  inline filter (POLY-CAP-36AS, Arbor Technologies, Ann Arbor, Mich., USA), perfused the samples under a constant pressure gradient maintained by a pressure regulator. Stopcocks allowed selective influx for measuring the initial conductivity ( $k_i$ ) on one sample at a time under a pressure gradient of 10 KPa. The maximum conductivity ( $k_m$ ) was determined by pressurizing the solution through all the segments at 180 KPa for 60 min. The permeating solution flowed from the pressurizing reservoir across the samples and into a container on an analytical balance joined to a computer that automatically made the calculations. Measurements of  $k_h$  were recorded every 30 s and calculated by averaging ten readings after steady-state had been achieved. Embolism causing per cent loss of hydraulic conductivity (LOSK) was assessed from  $100(k_m - k_i)/k_m$ . Leaf specific conductivity (LSC) was determined as  $k_h/L$ .

To investigate the relationship between  $\Psi$  and LOSK and to determine an in vivo vulnerability curve for beech, a preliminary drought-recovery cycle was followed in 3-year-old *F. sylvatica* seed-



**Fig. 1A–D.** Patterns of: **A** predawn water potential ( $\Psi$ ), **B** relative water content (RWC), **C** net photosynthesis ( $A$ ), and **D** leaf conductance ( $g_l$ ), during the course of a water stress-recovery cycle in seedlings of *Fagus sylvatica* from Abetone (open symbols) and Sicily

(closed symbols) populations. Continuous lines and circles indicate C-plants, broken lines and squares indicate S-plants. Bars represent  $\pm$  SE of the mean; bars not visible indicate SE smaller than the symbol

lings from Capracotta (central Italy) (see Borghetti et al. 1993; Tognetti et al. 1994); seedlings were cultured, treated and measured as described above. Leaf water potential ( $\Psi$ ) and LOSK were sampled on seedlings periodically over a 10 day drought and 20 day recovery period.

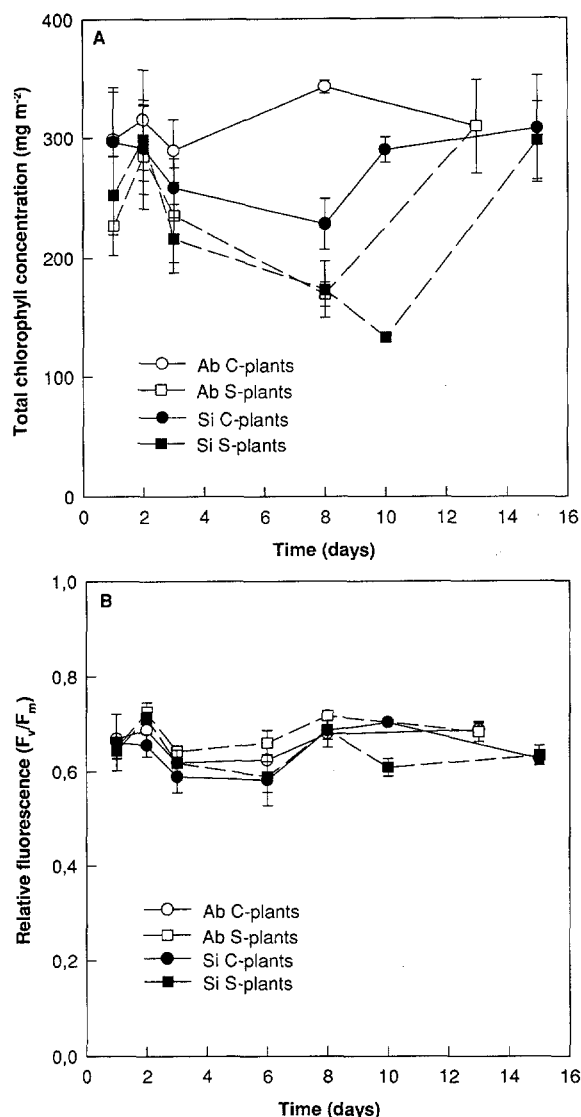
Statistical analyses of data were performed using analysis of variance methods followed by Duncan's Multiple Range Test with  $P < 0.05$ .

## Results

Prelight water potential began declining on day 3 in seedlings from both populations (Fig. 1A) with the Abetone seedlings showing a consistent decrease to a minimum of  $-2.3$  MPa on day 8, at which time the seedlings were rewatered. In contrast, the seedlings from Sicily showed a

general maintenance of water potential of about  $-1.2$  MPa through day 8 and then a rapid drop to  $-2.0$  MPa on day 10. Upon rewatering,  $\Psi$  in both populations increased within 5 days to control levels. Population differences in RWC were also observed (Fig. 1B). The population from Abetone exhibited a decrease in RWC along with  $\Psi$ , while the other seed source showed only a slight decrease in RWC. The differences in RWC between populations and between C-plants (0.89 Abetone, 0.90 Sicily) and S-plants (0.74 Abetone, 0.84 Sicily) on peak-stress days were not significant. After pooling C-plants and S-plants data,  $\Psi$  and RWC were found to be significantly ( $P < 0.00001$ ) and linearly correlated in both Abetone and Sicily populations ( $r^2 = 0.70$  and  $r^2 = 0.58$ , respectively).

Maximum values of  $A$  and  $g_l$  were maintained for the first 3 days after withholding irrigation in both populations (Fig. 1C, D). The reduction of  $\Psi$  resulted in a rapid decline



**Fig. 2A, B.** Patterns of: **A** total chlorophyll concentration (Chl) on a leaf area basis and **B** relative fluorescence ( $F_v/F_m$ ), during the course of the water stress-recovery cycle in seedlings of *F. sylvatica* from Abetone (open symbols) and Sicily (closed symbols) populations. Continuous lines and circles indicate C-plants, broken lines and squares indicate S-plants. Bars represent  $\pm$ SE of the mean; bars not visible indicate SE smaller than the symbol

of both  $A$  and  $g_1$  (under  $2 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  and  $0.25 \text{ cm s}^{-1}$ , respectively) in S-plants, but the population from Abetone reached its minimum 2 days before the population from Sicily. On day 8 (the peak-stress period for Abetone S-plants) the two populations showed significant differences ( $P < 0.05$ ) in  $\Psi$ ,  $A$  and  $g_1$ . Five days after rewatering,  $\Psi$  completely recovered in S-plants; however there was only partial recovery of  $g_1$ . At this date substantial differences (Abetone  $P < 0.05$  and Sicily  $P = 0.06$ ) remained between S-plants and C-plants (Fig. 1 A, D). No significant differences in  $A$  were observed between treatments and populations at the end of the experiment. After pooling C-plants and S-plants data, a significant ( $P < 0.00001$ ) and exponential correlation was found between  $A$  and  $g_1$  in both the populations (Abetone:  $r^2 = 0.62$  and Sicily:  $r^2 = 0.70$ );

**Table 1.** Mean values ( $\pm$ SE) of stem, root, foliage and total dry weight (DW); shoot and root to total dry weight ratio; leaf area (L) per leaf and per plant; leaf specific conductivity on maximum hydraulic conductivity basis [ $\text{LSC}(k_m)$ ] in seedlings of *F. sylvatica* from Abetone ( $n = 15$ ) and Sicily ( $n = 15$ ) populations. Means followed by the same letter within a row are not different at  $P < 0.05$

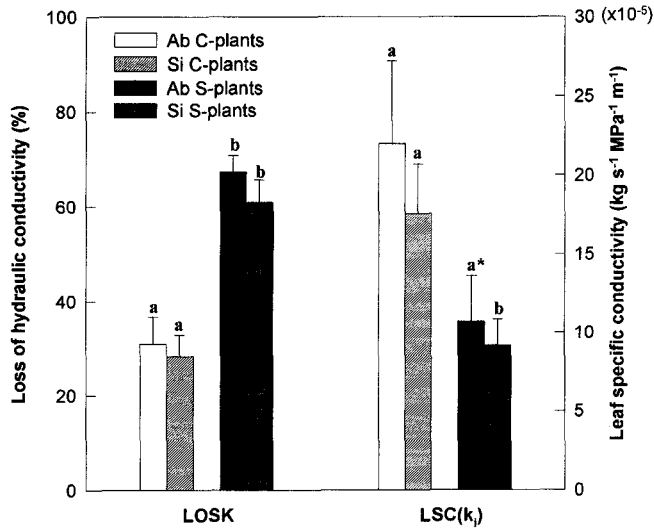
Parameter	Population	
	Abetone	Sicily
Stem DW (g)	$4.97 \pm 0.67a$	$2.74 \pm 0.18b$
Root DW (g)	$6.01 \pm 0.77a$	$4.28 \pm 0.23b$
Foliage DW (g)	$1.93 \pm 0.20a$	$1.61 \pm 0.12a$
Total DW (g)	$12.91 \pm 1.56a$	$8.62 \pm 0.45b$
Shoot/total DW ratio	$0.53 \pm 0.01a$	$0.50 \pm 0.01a$
Root/total DW ratio	$0.47 \pm 0.01a$	$0.50 \pm 0.01a$
$L \times \text{leaf}$ ( $\times 10^{-3} \text{ m}^2$ )	$1.00 \pm 0.14a$	$1.15 \pm 0.28a$
$L \times \text{plant}$ ( $\times 10^{-2} \text{ m}^2$ )	$2.46 \pm 0.30a$	$2.61 \pm 0.20a$
$\text{LSC}(k_m)$ ( $\times 10^{-5} \text{ kg s}^{-1} \text{ MPa}^{-1} \text{ m}^{-1}$ )	$33.27 \pm 5.68a$	$25.29 \pm 2.79a$

similarly, between  $A$  and  $\Psi$  ( $r^2 = 0.57$ ,  $P < 0.00001$  in both Abetone and Sicily population), and between  $g_1$  and  $\Psi$  (Abetone:  $r^2 = 0.44$ ,  $P < 0.001$  and Sicily:  $r^2 = 0.35$ ,  $P < 0.01$ ). Again, a significant and linear relationship existed between  $A$  and RWC (Abetone:  $r^2 = 0.53$ ,  $P < 0.001$  and Sicily:  $r^2 = 0.32$ ,  $P < 0.05$ ), and between  $g_1$  and RWC in the population from Abetone ( $r^2 = 0.33$ ,  $P < 0.05$ ) but not in the population from Sicily ( $r^2 = 0.23$ ,  $P = 0.1$ ).

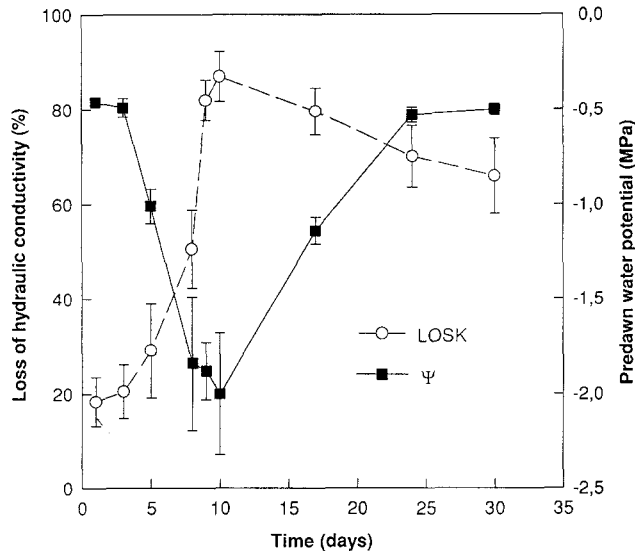
Total Chl concentration on a leaf area basis dropped in S-plants with decreasing  $\Psi$ , reaching a minimum ( $169.53 \text{ mg m}^{-2}$ ,  $P = 0.07$  and  $132.82 \text{ mg m}^{-2}$ ,  $P < 0.001$ ) on days 8 and 10, respectively, in the populations from Abetone and Sicily (Fig. 2 A). After rewatering, Chl concentrations recovered substantially to control level (Fig. 2 A). Total Chl concentrations in C-plants remained constant during the course of the experiment without significant differences between populations ( $278,70 \text{ mg m}^{-2}$  and  $311.34 \text{ mg m}^{-2}$ , respectively, for Sicily and Abetone). After pooling C-plants and S-plants data, total Chl concentration on a leaf area basis was significantly and exponentially correlated to  $A$  in both the populations (Sicily:  $r^2 = 0.72$ ,  $P < 0.01$ ; Abetone:  $r^2 = 0.66$ ,  $P < 0.05$ ).

The chlorophyll a fluorescence parameter of relative fluorescence ( $F_v/F_m$ ) exhibited only small fluctuations during the water stress and recovery period (Fig. 2 B). The seedlings from the Sicily population showed significant differences ( $P < 0.05$ ) between C-plants and S-plants on day 10 (peak-stress) for  $F_v/F_m$  and the decrease corresponded to the large drop in total chlorophyll concentration (Fig. 2 A). In contrast, there was little relationship between chlorophyll concentration and  $F_v/F_m$  in the Abetone seedlings with fluorescence in the S-plants always being slightly higher than the C-plants. It is noteworthy, however, that the population from Sicily had consistently lower  $F_v/F_m$  throughout the study which was observed in another study of fluorescence parameters between these two populations (J. D. Johnson, R. Tognetti, M. Michelozzi, unpublished data).

Total, shoot, stem and root dry-matter was significantly higher ( $P < 0.05$ ) in the population from Abetone than in the population from Sicily (Table 1). Foliage dry biomass



**Fig. 3.** Percentage loss of hydraulic conductivity (LOSSK) and leaf specific conductivity on initial conductivity basis [LSC(k<sub>i</sub>)] in seedlings of *F. sylvatica* from Abetone and Sicily populations. Bars represent ±SE of the mean. Error bars that are not designated by a common letter are significantly different at *P* < 0.05 using Duncan test; a\* = significant at *P* = 0.06



**Fig. 4.** Patterns of predawn water potential (Ψ) (closed squares and continuous line) and per cent loss of hydraulic conductivity (LOSSK) (open circles and broken line), during the course of the water stress-recovery cycle in seedlings of *F. sylvatica* from the Capracotta population. Bars represent ±SE of the mean; bars not visible indicate SE smaller than the symbol

was also lower, but not significantly so, in the population from Sicily. The relative partitioning of dry-matter between above and below ground organs was similar in both seed sources (Table 1), though, Abetone seedlings tended to have a greater proportion in shoots and less in roots than Sicily seedlings, 53% versus 50% and 47% versus 50%, respectively. As expected, biomass production did not differ significantly between treatments because stem growth was negligible at the time of biomass measurements

(early August), the seedlings had set bud (late July), and the study was of short duration.

Measurements of L per leaf (an average of  $1.0 \times 10^{-3} \text{ m}^2$ ) and per seedling (an average of  $2.5 \times 10^{-2} \text{ m}^2$ ) did not differ significantly between the two populations (Table 1) nor treatments (data not shown).

LSC based on  $k_m$  was higher in the population from Abetone ( $33 \times 10^{-5} \text{ kg s}^{-1} \text{ MPa}^{-1} \text{ m}^{-1}$ ) than in the population from Sicily ( $25 \times 10^{-5} \text{ kg s}^{-1} \text{ MPa}^{-1} \text{ m}^{-1}$ ), but without significant differences (Table 1). LSC calculated on a  $k_i$  basis was higher in C-plants than S-plants in both Sicily (*P* < 0.05) and Abetone (*P* = 0.06), even 20 days from rewatering. Abetone seedlings showed higher (but not significant) values than Sicily in both treatments (Fig. 3).

Measurements of LOSK showed a considerable amount of xylem embolism in seedlings subjected to water stress (Fig. 3). After nearly 3 weeks from rewatering with Ψ at pre-stress values, significant differences between treatments (*P* < 0.05) were observed. The degree of xylem embolism, as measured by LOSK, was consistently higher in the population from Abetone than in the population from Sicily for both S-plants (67% as opposed to 61%) and C-plants (31% as opposed to 28%). There were, however, no significant differences between the two populations (Fig. 3).

The seedlings from Capracotta showed good correspondence between changes in Ψ and LOSSK (Fig. 4). A sharp increase in LOSSK occurred as Ψ fell below -1 MPa. The minimum LOSSK averaged 18.4% on day 1, about 50% after 8 days without watering (Ψ was -1.84 MPa), and the maximum of 87% occurred on day 10 just before rewatering (Ψ was -2 MPa). After rewatering, LOSSK only partially recovered to about 65% by day 30 even after Ψ returned to pre-stress values (-0.5 MPa). This partial recovery of LOSSK was similar to that observed in the seedlings from Abetone and Sicily (Fig. 3), though Ψ recovered more quickly, within 5 days (Fig. 1A), which is attributable to the smaller seedling size. It must be pointed out that the xylem anatomy (being of great importance in vulnerability to embolism) was found not to be significantly different in the three populations examined (see Borghetti et al. 1993).

**Discussion**

There was a temporal lag in the response to water stress between the two populations. Compared to the population from Sicily, the Abetone population reached peak-stress 2 days earlier. As Ψ and RWC decreased, the decline in *A* of both populations was evident. Whereas, *g<sub>i</sub>* was slightly less responsive to leaf water status parameters, particularly in the population from Sicily. Indeed, open stomata seemed to be maintained longer in the more xeric population, resulting in a maintenance of photosynthetic efficiency without much change in Ψ or RWC. A high correlation between *g<sub>i</sub>* and *A* was observed, indicating a good coordination between the functioning of stomata and the photosynthetic performance in both populations (Weber and Gates 1990; Ni and Pallardy 1991; Parker and Pallardy 1991; Tognetti et al. 1994). The more rapid decline in *g<sub>i</sub>* as opposed to Ψ and RWC could be explained by the antici-

pated sensitivity to non-hydraulic signals during the initial stages of soil drying. Recently, many authors have suggested that the biochemical response might be mediated by plant hormones originated in dehydrating roots and translocated to the shoot by the transpiration stream (Zhang and Davies 1989; Trejo and Davies 1991; Khalil and Grace 1992). The relationship between RWC and  $\Psi$  might represent an advantage during drought in the Mediterranean environment, particularly for the more xeric population (Sicily). The small change in RWC as  $\Psi$  decreased could allow more complete recovery of the equilibrium between plant and soil water potentials during the night (Abrams 1988).

Total Chl concentrations on leaf area basis and  $A$  were well correlated and similarly affected by water stress in the population from Sicily but less in Abetone. On the other hand, variation in Chl  $a/b$  ratio (data not reported) during drought was not significant, showing a lack of significant reduction in the membrane-dependent (electron transport) photosynthetic efficiency in the two populations. It is known that Chl  $a/b$  ratio and thylakoid membrane stacking, containing predominantly light harvesting units, are inversely correlated (Virzo De Santo et al. 1984). In fact, relative fluorescence (and the other chlorophyll  $a$  fluorescence parameters, data not shown) remained almost constant in both populations and treatments. The two European beech populations, apparently, exhibited no photoinhibition effect on photosynthesis in seedlings subjected to water stress (Epron and Dreyer 1993). The lack of drought effect on photochemical efficiency of photosystem II might be indicative of an adaptative resistance mechanism to leaf water deficit caused by periodic drought conditions (common in Italy). Moreover, the rate of regreening was rapid and total Chl concentration recovered completely within 5 days from rewatering.

Total, shoot, stem and root dry weight were significantly higher in seedlings from Abetone although  $A$  was similar in the two populations. European beech productivity has been shown to be only indirectly related to assimilation (Stickan and Zhang 1992). Moreover, in another experiment, the population from Abetone showed significantly higher height increment in spring than the population from Sicily (Borghetti et al. 1993). Plants of more xeric origin tend to have a greater proportion of roots (and a smaller proportion of shoots) than those of more mesic origin. Seedlings from Sicily exhibited about 3% more partitioning to the root system (and a similar amount less to the shoot) than seedlings from Abetone. This difference could account for the more rapid development of water stress in the population from Abetone. Leaf area did not vary significantly between treatments or populations. Both  $L$  per seedling and  $L$  per leaf were not significantly different in the two seed sources, probably because of the same growth conditions and watering regime in the nursery, where they were grown until the experiment started. The variation between populations in total dry weight was mainly due to stem and root components, while leaf investment (on both area and dry weight basis) was similar in both populations, confirming the hypothesis that growth is poorly related to  $A$  in *F. sylvatica*.

Higher  $LSC(k_m)$  and  $LSC(k_i)$  in seedlings from Abetone than from Sicily showed a tendency for higher, though not significant, hydraulic sufficiency and xylem "redundancy" (due to the higher woody tissue investment) in the former population. This might represent an advantage in xeric conditions, but a cost to growth and maintenance respiration for the population from Abetone which is confined to more mesic sites.  $LSC(k_i)$  declined, in the two populations, as a consequence of drought-caused xylem embolism (Shumway et al. 1993). Leaf area did not match the reduction in hydraulic conductivity. The absence of a complete  $LSC(k_i)$  (and LOSK) recovery in S-plants of both populations might also be responsible for the incomplete recovery of  $g_l$  observed after rewatering. The mechanism of the stomatal response to xylem embolism, however, is not completely understood (Sperry and Pockman 1993). Although well watered, C-plants showed a certain degree of embolism, averaging about 30% of LOSK with no significant differences between populations. Such "native" xylem embolism during the growing season was also found in other diffuse-porous tree species (Sperry et al. 1988b; Tyree and Ewers 1991; Tognetti and Borghetti 1994) and also in a natural stand of mature *F. sylvatica* (R. Tognetti, unpublished data). S-plants of the populations studied experienced an increase in LOSK as a consequence of water stress and decreasing  $\Psi$ . This high vulnerability of European beech to embolism was found in another experiment on branch segments collected periodically in the forest and on large branches dehydrating in the air in the laboratory (R. Tognetti, unpublished data). Tyree and Yang (1992) found that LOSK in *Acer saccharum* Marsh. did not recover when  $\Psi$  remained below  $-6$  KPa. A similar vulnerability to embolism, measured on both branch segments excised from mature trees and seedlings dehydrated in pots, has been already highlighted in *Populus deltoides* Bartr., a diffuse-porous tree species living in moist environments (Tyree et al. 1992). In the present study no substantial recovery of hydraulic conductivity occurred in either population after rewatering. New vessels production in our seedlings was probably ending at the time of hydraulic recovery (late August), thus, no adequate additional conduits were produced to replace those impaired by water stress-induced xylem embolism. Moreover, in controlled environments the possible role of rainfall in embolism recovery (Zimmermann and Milburn 1982) is obscured. On the other hand, it has been hypothesized that *Fagus* lacks the refilling mechanisms to recover completely the hydraulic conductivity in previously embolized xylem vessels within a growing season (Sperry 1993).

The populations from Abetone and Sicily were studied in another experiment on winter embolism and showed no significant differences in vessel density and diameter, ultrasound acoustic emissions or LOSK, though the populations were differentiated by isoenzyme markers (Borghetti et al. 1993). Seedlings from Sicily were found to be less embolized due to water stress (this experiment) and more embolized because of freezing-thawing (Borghetti et al. 1993) than seedlings from Abetone.

In conclusion, our data show that in S-plants  $A$  appeared to be primarily affected by  $CO_2$  diffusion through the stomata and perhaps secondarily by changes in Chl con-

centration, rather than by the light reactions of photosynthesis (photosystem II) in both European beech populations. A role of hydraulic factors affecting gas exchange during stress and recovery cannot be ruled out. However, because LSC(k<sub>m</sub>) (free of emboli) did not differ between the two populations, hydraulic architecture cannot be a principal factor in their geographical occurrence. Seedlings from Abetone displayed higher below- and above-ground productivity than seedlings from Sicily, but the two populations had similar leaf area, biomass and gas exchanges. On the other hand, S-plants of Sicily showed a delay in response to drought. A population of trees, such as Sicily, with decreased stomatal sensitivity during drought might continue to grow longer under water stress conditions. Despite that European beech is subjected to more xeric conditions in Sicily than in Abetone, it is apparent from this study that regional climatic features of these populations are only moderately reflected in the seedling water stress response in controlled environments.

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## References

- Abrams MD (1988) Comparative water relations of three successional hardwood species in central Wisconsin. *Tree Physiol* 4: 263–273
- Abrams MD, Kloppel BD, Kubiske ME (1992) Ecophysiological and morphological responses to shade and drought in two contrasting ecotypes of *Prunus serotina*. *Tree Physiol* 10: 343–355
- Arnon DI (1949) Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiol* 24: 1–15
- Björkman O, Powles SB (1984) Inhibition of photosynthetic reactions under water stress: interaction with light level. *Planta* 161: 490–504
- Borghetti M, Giannini R (1982) Indagini preliminari sulla variazione di alcuni caratteri in piantine di faggio di provenienza diversa. *Ann Acc It Sci For* XXXI: 119–134
- Borghetti M, Leonardi S, Raschi A, Snyderman D, Tognetti R (1993) Ecotypic variation of xylem embolism, phenological traits, growth parameters and allozyme characteristics in *Fagus sylvatica*. *Funct Ecol* 7: 71–720
- Eschrich W, Burchardt R, Essiamah S (1989) The induction of sun and shade leaves of European beech (*Fagus sylvatica* L.): anatomical studies. *Trees* 3: 1–10
- Epron D, Dreyer E (1993) Photosynthesis of oak leaves under water stress: maintenance of high photochemical efficiency of photosystem II and occurrence of non-uniform CO<sub>2</sub> assimilation. *Tree Physiol* 13: 107–117
- Khalil AAM, Grace J (1992) Acclimation to drought in *Acer pseudo-platanus* L. (Sycamore) seedlings. *J Exp Bot* 43: 1591–1602
- Michelozzi M, Johnson JD, Warrag EI (1995) Response of ethylene and chlorophyll in two *Eucalyptus* clones during drought. *New For* (in press)
- Ni BR, Pallardy SG (1991) Response of gas exchange to water stress in seedlings of woody angiosperms. *Tree Physiol* 8: 1–9
- Parker WC, Pallardy SG (1991) Gas exchange during a soil drying cycle in seedlings of four black walnut (*Juglans nigra* L.) families. *Tree Physiol* 9: 339–348
- Schulze E-D (1986) Carbon dioxide and water vapor exchange in response to drought in the atmosphere and in the soil. *Annu Rev Plant Physiol* 37: 247–274
- Shuway DL, Steiner KC, Kolb TE (1993) Variation in seedlings hydraulic architecture as a function of species and environment. *Tree Physiol* 12: 41–54
- Sperry JS (1993) Winter xylem embolism and spring recovery in *Betula cordifolia*, *Fagus grandifolia*, *Abies balsamea* and *Picea rubens*. In: Borghetti M, Grace J, Raschi A (eds) Water transport in plants under climatic stress. Cambridge University Press, Cambridge, pp 86–98
- Sperry JS, Pockman WT (1993) Limitation to transpiration by hydraulic conductance and xylem cavitation in *Betula occidentalis*. *Plant Cell Environ* 16: 279–287
- Sperry JS, Donnelly JR, Tyree MT (1988a) A method for measuring hydraulic conductivity and embolism in xylem. *Plant Cell Environ* 11: 35–40
- Sperry JS, Tyree MT, Donnelly JR (1988b) Seasonal occurrence of xylem embolism in sugar maple (*Acer saccharum*). *Am J Bot* 75: 1212–1218
- Stickan W, Zhang X (1992) Seasonal changes in CO<sub>2</sub> and H<sub>2</sub>O gas exchange of young European beech (*Fagus sylvatica* L.). *Trees* 6: 96–102
- Tognetti R, Borghetti M (1994) Formation and seasonal occurrence of xylem embolism in *Alnus cordata*. *Tree Physiol* 14: 241–250
- Tognetti R, Michelozzi M, Borghetti M (1994) Response to light of shade-grown beech seedlings objected to different watering regimes. *Tree Physiol* 14: 751–758
- Trejo CL, Davies WJ (1991) Drought-induced closure of *Phaseolus vulgaris* L. stomata precedes leaf water deficits and any increase in xylem ABA concentration. *J Exp Bot* 42: 1507–1515
- Tyree MT, Ewers FW (1991) The hydraulic architecture of trees and other woody plants. *New Phytol* 119: 345–360
- Tyree MT, Yang S (1992) Hydraulic conductivity recovery versus water pressure in xylem of *Acer saccharum*. *Plant Physiol* 100: 669–676
- Tyree MT, Alexander J, Machado J-L (1992) Loss of hydraulic conductivity due to water stress in intact juveniles of *Quercus rubra* and *Populus deltoides*. *Tree Physiol* 10: 411–415
- Virzo De Santo A, Ligrone R, Alfani A, Fioretto A, Russo G (1984) CAM activity and day/night changes in the ultrastructure of stem chlorenchyma of *Cissus quadrangularis* L. as influenced by drought. *Plant Cell Environ* 7: 105–112
- Weber JA, Gates DM (1990) Gas exchange in *Quercus rubra* (northern red oak) during a drought: analysis of relations among photosynthesis, transpiration, and leaf conductance. *Tree Physiol* 7: 215–225
- Woodward FI (1987) Climate and plant distribution. Cambridge University Press, Cambridge
- Zhang J, Davies WJ (1989) Abscisic acid produced in dehydrating roots may enable the plant to measure the water status of the soil. *Plant Cell Environ* 12: 73–81
- Zimmermann MH, Milburn JA (1982) Transport and storage of water. In: Lare OL, Nobel PS, Osmond CB, Ziegler H (eds) Physiological plant ecology, II; Encyclopedia of plant physiology new series, vol 12B. Springer, Berlin Heidelberg New York, pp 135–151