

Influence of the ectomycorrhizas formed by *Tuber melanosporum* Vitt. on hydraulic conductance and water relations of *Quercus ilex* L. seedlings

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Abstract – The physiological impact of ectomycorrhizal infection was investigated in the association between *Tuber melanosporum* Vitt. and *Quercus ilex* L. A number of physiological parameters were investigated on 2-year-old seedlings inoculated for 22 months (I-seedlings) compared to non-inoculated plants (NI-seedlings). I-seedlings had a 100% infection rate in root tips compared to a 25% infection rate in root tips of NI-seedlings. I-seedlings had higher values of net assimilation and stomatal conductance than NI-seedlings. Root hydraulic conductance per unit root surface area of I-seedlings was much reduced to 0.44× that of NI-seedlings but had 2.5× more fine root surface area than NI-seedlings. When root conductance was scaled by leaf area, the I-seedlings had 1.27× the root conductance per unit leaf area compared to NI-seedlings. I-seedlings also had significantly higher hydraulic conductances of shoots with leaves, of shoots without leaves and lower leaf blade hydraulic resistances.

hydraulic conductance / water relations / ectomycorrhiza / *Quercus ilex* L. / HPFM

Résumé – Influence sur la conductance hydraulique et les relations hydriques des semis de *Quercus ilex* L. des ectomycorrhizes formées par *Tuber melanosporum* Vitt. L'impact physiologique dû à l'infection d'ectomycorrhizes a été étudié dans l'association *Tuber melanosporum* Vitt. et *Quercus ilex* L. Un certain nombre de paramètres physiologiques ont été mesurés sur des semis de 2 ans inoculés pendant 22 mois (semis-i) en comparaison avec des cultures saines (semis-ni). Les semis-i présentent 100 % de taux d'infection des racines, tandis que les semis-ni atteignent un taux de 25 %. Les semis-i ont des niveaux d'assimilation nette plus élevés par rapport aux semis-ni. La conductance hydraulique des racines par unité de surface des racines pour les semis-i est réduite de plus de 0,44 fois par rapport aux semis-ni, mais comporte une surface de racines 2,5 fois inférieure à celle des semis-ni. En rapportant la conductance des racines à la surface des feuilles, la conductance des racines par unité de surface des feuilles des semis-i est 1,27 fois plus élevée que celle des semis-ni. Les semis-i présentent également une conductance hydraulique des rameaux avec feuilles et sans feuilles bien plus élevée, ainsi qu'une moindre résistance hydraulique des feuilles.

conductance hydraulique / relations hydriques / ectomycorrhize / *Quercus ilex* L. / HPFM

Abbreviations

PAR: photosynthetically active radiation
 Ψ_L : leaf water potential

g_L : leaf conductance to water vapor
 P_n : net photosynthesis
 A_L : total leaf surface area
 A_R : total root surface area

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A_x : wood cross surface area
 K : hydraulic conductance
 K_R : root hydraulic conductance
 K_{RL} : root hydraulic conductance scaled by total leaf surface area
 K_{RR} : root hydraulic conductance scaled by total root surface area
 K_S : shoot hydraulic conductance
 K_{SL} : shoot hydraulic conductance scaled by total leaf surface area
 K_{SX} : shoot hydraulic conductance scaled by wood cross surface area
 LBR : leaf blade hydraulic resistance
 LSM : leaf specific mass
 h : seedling height
 \emptyset_T : stem diameter.

1. INTRODUCTION

The importance of mycorrhizal fungi for soil conservation [14], dynamics of natural ecosystems [16] and sustainable agriculture [9] is recognized worldwide from more than one century of research. Most of our understanding of mycorrhizal symbiosis comes from work on VAM (vesicular-arbuscular mycorrhizae) whose benefits to the host in terms of phosphorus uptake [1] and nutrition have recently been reviewed [10]. Considerable uncertainty still exists in the evaluation of many other aspects of the VAM-host interaction [10] among which the influence of mycorrhizae on host hydraulics and water relations which would be favoured by VAM, according to Safir et al. [32], Sands et al. [36], Huang et al. [20], Ruiz-Lozano and Azcón [31], Gemma et al. [17] or would be independent on mycorrhizae, according to Graham et al. [19], Andersen et al. [7], Steudle and Heydt [40].

Even less is known of the influence of the ectomycorrhizal symbiosis on nutrient uptake and allocation [11, 13] and water relations [12] of forest trees. For example, ectomycorrhizal symbiosis has been reported to have negative or no effects on root hydraulic conductance (K_R) of Douglas fir seedlings [12]. This finding is in contrast with the classical interpretation suggesting that ectomycorrhizal infection of tree roots enhances root water uptake [24, 32].

Equal uncertainty appears to exist in the literature regarding the effects of VAM and ectomycorrhizae on the host leaf water status, stomatal conductance and drought recovery [2–5, 21]. The disagreement among studies regarding effects of mycorrhizae on the host root hydraulic conductance has been suggested to be due to changes in the root cortex anatomy caused by VAM but

not by ectomycorrhizae [40] rather than to differences in the hydraulic conductance of the extraradical hyphae [6].

Most studies of the hydraulic conductance of mycorrhizal roots have been performed in young seedlings, two to ten months of age and one to nine months after mycorrhizal inoculation [7, 8, 13, 19]. In turn, ectomycorrhizae have been mainly studied in high altitude and/or latitude forest trees [12, 29, 35].

In the present study, we report the effects of an ectomycorrhizal fungus (*Tuber melanosporum* Vitt.) on some water relations parameters and especially on hydraulic conductance of roots, stems and leaves of *Quercus ilex* L. (Holm oak), a typical Mediterranean sclerophyllous tree. This mycorrhizal association is of importance to agricultural and silvicultural activity in abandoned areas of central Italy because of the high commercial value of truffle produced in northern and central Italy. The field plantations are usually made with seedlings, two years of age and 22 months after mycorrhizal inoculation, i.e. after sufficient time to allow the mycorrhizal symbiosis to produce its supposedly beneficial effects on the host. Perhaps some of the uncertainty concerning the influence of ectomycorrhizae on host hydraulics and water relations might be caused by studies made too soon after fungal inoculation, i.e., not allowing sufficient time for differences to develop in host biomass and/or significant changes in plant anatomy and morphology.

2. MATERIALS AND METHODS

2.1. Plant material

Experiments were conducted on 2-year-old seedlings of *Quercus ilex* L. provided by a private nursery specialized in the production of forest seedlings infected with different *Tuber* species (MICOPLANT, Asti, Piemonte, Italy). All seedlings had been grown in pots containing a clayey-calcareous soil collected from the hills surrounding Asti. Soil was carefully disinfected before planting seedlings and inoculation with *Tuber melanosporum* Vitt. Twenty inoculated seedlings were studied, 22 months after inoculation (I-seedlings) and 12 seedlings of the same age not inoculated with the ectomycorrhiza (NI-seedlings). I- and NI-seedlings were grown under identical greenhouse conditions in a manner designed to minimize the risk of accidental infection of NI-seedlings with mycorrhizae. The degree of infection of both I- and NI-seedlings was measured at the end of experiments (see below) so as to check any eventual contamination of the control (NI) seedlings.

Twenty days before measurements, all seedlings were transferred to a room where the air temperature was

adjusted to vary between 18 and $24 \pm 1^\circ\text{C}$, relative humidity was set at $40 \pm 5\%$ and light was provided by iodine-vapor lamps (OSRAM HQI-T, 1000 W/D) with a photosynthetic photon flux density (PAR) of about $260 \mu\text{mol m}^{-2} \text{s}^{-1}$ measured at the leaf surface using a quantum sensor (LI-COR model LI-190S1) connected to a LI-COR model LI-1600 porometer. The photoperiod was 12 h. Seedlings received irrigation with tap water filtered to $0.2 \mu\text{m}$ to prevent contamination with fungal spores and soil was maintained at field capacity. Seedling height and stem diameter 30 mm above the soil was measured using a digital caliper (MITUTOYO model Digimatic, accuracy $\pm 0.01 \text{ mm}$) immediately prior to the experiments.

2.2. Pressure-Volume (P - V) curve and gas exchange measurements

Effects of the ectomycorrhiza on solute accumulation (osmotic pressure at full turgor) in the leaves of I- and NI-seedlings were estimated by measuring four P - V curves of leaves from both groups of seedlings using the pressure chamber technique [37, 42]. One-year-old leaves were detached while in plastic bags to minimize evaporation and rehydrated to near full turgor by immersing their petioles in distilled filtered water. Leaves remained in the dark and in contact with water for about 30 min. This time interval was sufficient for leaf water potential (Ψ_L) to increase to about -0.15 MPa . P - V curves were measured in the usual way [33, 42] and recorded as the inverse of the balancing pressure versus the weight of water expressed.

Leaf conductance to water vapor (g_L) was measured on at least one leaf per I-seedling and at least five leaves per NI-seedling. Measurements were repeated on two different days. The larger number of g_L measurements per plant in NI-seedlings was needed to compensate the higher scatter of g_L data in the control group. All g_L measurements were performed between 11.30 and 12.30 h i.e. in the middle of the light period, using a steady-state porometer (LI-COR model LI-1600). Each measurement was completed in about 30 s and the relative humidity inside the chamber was kept near the ambient to reproduce external conditions.

Net photosynthesis (P_n) was measured on attached leaves using an infrared gas analyzer (IRGA, model LCA-4, Analytical Development Company Ltd.) equipped with a broad chamber (model PLC 4B), 625 mm^2 surface area. Forty minutes were required for equilibration of the instrument with the external conditions. P_n measurements were recorded at 10 min intervals on one to two leaves per seedling of both I- and NI-seedlings.

2.3. Hydraulic measurements

All hydraulic measurements were performed using the High Pressure Flow Meter (HPFM) technique introduced by Tyree et al. [44, 45] and described in detail by some of us [26, 46]. The HPFM was used in the "transient mode" i.e. by rapidly changing the pressure (P) applied to roots or stems (see below) and simultaneously measuring the corresponding flow (F). This procedure allows quite rapid measurements of F and P (of the order of seconds) and calculation of hydraulic conductance (K) from the slope of the linear regression of F to P . Both root systems and stems were perfused under pressure with distilled water filtered to $0.1 \mu\text{m}$ to prevent xylem clogging by bacterial or debris particles.

After cleaning the surface of the pot under a water stream, pots were enclosed in plastic bags fitted tightly to the seedling stem and immersed in distilled filtered water. The shoot was then cut off under water, at about 30 mm above the soil. The excised root system was immediately connected to the HPFM and P was continually increased from 0.03 to 0.42 MPa at a rate of 4 to 5 kPa s^{-1} while recording F and P every 3 s.

During measurements of the root hydraulic conductance (K_R), the cut shoot remained in contact with water while covered with plastic film to minimize transpiration. The shoot was then connected to the HPFM and perfused with distilled filtered water at a pressure of 0.3 MPa to induce full hydration of the leaves as revealed by the leaf surface becoming wet. The pressure was then released to 0.03 MPa and maintained constant for 10 min to allow internal pressures to equilibrate. At least three transient F versus P measurements were made of each leafy stem, and hydraulic conductance (K_S) was calculated as reported above.

At the end of K_S measurements, all the leaves of each shoot were removed by cutting off the leaf blade at the junction with petioles. Hydraulic measurements were then repeated of the leafless stem so that the hydraulic resistance of the leaf blade was obtained by difference of resistances:

$$\begin{aligned} R_{\text{leaf blade}} &= R_{\text{shoot}} - R_{\text{shoot minus leaves}} \\ &= 1/K_{\text{shoot}} - 1/K_{\text{shoot minus leaves}} \end{aligned}$$

Total leaf surface area (A_L , one side only) of each seedling was measured using a leaf area meter (LI-COR model LI-3000A). Leaf dry weight was obtained after leaves had remained in oven at 70°C for 3 d. The total surface area of fine roots ($< 2 \text{ mm}$ in diameter as measured using a digital caliper, accuracy $\pm 0.01 \text{ mm}$) of each seedling (A_R) was estimated as follows. The soil was washed from the root system under a gentle jet of water. Then, fine roots were cut off in segments up to 50 mm in

length. About 50 root segments per plant were put into a glass box and covered with a white plastic sheet to keep them in a fixed position and improve the contrast of root image. The box was placed on a scanner (EPSON model GT-9000) connected to a computer. Customized software written in Trieste was used to read bit-map images and calculate the surface area of the roots. Root images were processed by the software and root surface area (A_R) was obtained by assuming cylindrical geometry. Other representative root samples of I- and NI-seedlings were shipped to Vermont where an image analysis system (DT-Scan, Delta-T devices, Cambridge England) was used to determine the surface area distribution by diameter size class and root length. Since the diameter resolution of the image analysis system was ± 0.05 mm a subset of root tips were measured at $50\times$ in a binocular microscope using an ocular micrometer to estimate the mean diameter of roots in the infected zone (hyphal sheaths) of I-seedlings compared to the same region in NI-seedlings.

K_R was normalized by dividing it by both A_R and A_L , thus obtaining the root hydraulic conductance per root (K_{RR}) and per leaf (K_{RL}) unit surface area. Although K_{RR} provides a physiologically correct estimate of the efficiency of an individual root [39], it provides no overview of the total ability of the entire root system to provide water to the shoot. A root system with low K_{RR} may be able to compensate by having more root area, but knowing only K_{RR} and total root area is not sufficient information without scaling root size (or conductance) to shoot size. Leaf area is an ecologically meaningful measure of shoot size because it is a measure of photosynthetic surface area. Hence, root conductance scaled to leaf surface area, K_{RL} , is useful for estimating the sufficiency of roots to supply a unit area of leaves with water and nutrients [22, 47]. K_S measured in whole shoots was normalized by dividing it by A_L (K_{SL}). K_S as measured in shoots without leaf blades was normalized by dividing it by the wood cross surface area (A_X) at the stem cut surface (K_{SX}). A_X was measured on stems with bark removed using a digital caliper (see above) and the pith cross surface area was subtracted after measuring it under binocular microscope. In other words, K_{SL} represents the hydraulic conductance of the epigeal organs as a whole while K_{SX} is the physical hydraulic conductance of the stem.

2.4. Mycorrhizal infection

The degree of infection of *T. melanosporum* in I-seedlings and NI-seedlings was measured on the root systems of all the seedlings under study. The percentage of mycorrhizal infection was estimated by visual observation of hyphal sheaths covering unstained root tips. To this purpose, at least 80% of the roots of each seedling

was observed and quantification of mycorrhizal infection was performed using the grid-line intersect method [18] slightly modified to take into account the non-linearity of the infection.

3. RESULTS

Mycorrhizal infection was 100% in the purposefully inoculated seedlings of *Q. ilex* (table I) but some contamination of root tips (about 25%) with this ectomycorrhiza was observed in the controls (NI-seedlings). No different fungal species were detected in the seedlings under study. The conversion factor between root surface area and dry weight was measured on 14 samples each for I- and NI-roots and was not significantly different. The pooled mean and SEM was $74 \pm 7 \times 10^{-4} \text{ m}^2 \text{ g}^{-1}$ ($n = 28$). The diameter of roots including hyphal sheaths was significantly different from NI-roots measured in the same region, i.e., 0.193 ± 0.010 versus 0.167 ± 0.009 mm ($n = 10$, $p = 0.026$), respectively. Roots of I-seedlings had significantly higher dry weights and hence root surface area by a factor of 2.5 times than NI-seedlings ($p = 0.01$, see table I). Since root length was correlated with surface area ($r^2 = 0.82$, data not shown), roots of I-seedlings were also significantly longer than NI-seedlings. An analysis of root surface area versus diameter size classes revealed that about 75% of the root surface area was in roots ≤ 1

Table I. Comparison of not-inoculated (NI, see text) to inoculated (I) seedlings of *Quercus ilex* L. in terms of effective percentage of mycorrhizal roots, seedling height above the soil (h), stem diameter (\varnothing_T), total leaf (A_L) and root (A_R) surface area and leaf specific mass (LSM). Values are means \pm SEM. The number of asterisks corresponds to significance of recorded differences, $P = 0.05$ (*), 0.01 (**), and 0.001 (***), as calculated using the Student's t-test.

	NI-seedlings	I-seedlings	Significance
Mycorrhizal Infection %	25.7 ± 2.3 ($n = 9$)	100 ($n = 16$)	***
Seedling height m	0.42 ± 0.16 ($n = 10$)	0.35 ± 0.13 ($n = 16$)	**
Stem diameter mm	2.69 ± 0.09 ($n = 10$)	3.04 ± 0.12 ($n = 16$)	***
Leaf surface area $\text{m}^2 \times 10^{-4}$	181 ± 17 ($n = 10$)	194 ± 14 ($n = 16$)	
Leaf specific mass g dm^{-2}	0.954 ± 0.037 ($n = 10$)	1.075 ± 0.067 ($n = 16$)	*
Root surface area $\text{m}^2 \times 10^{-4}$	36.8 ± 8.3 ($n = 10$)	92.3 ± 11.6 ($n = 16$)	**
Total root dry weight g	0.50 ± 0.11 ($n = 10$)	1.25 ± 0.16 ($n = 16$)	***

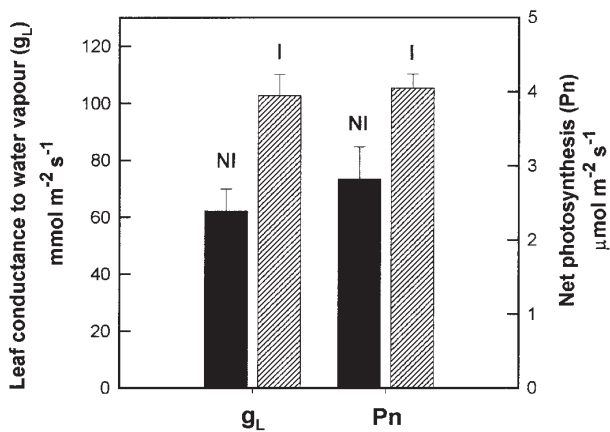


Figure 1. Leaf conductance to water vapor (g_L) and net photosynthesis (P_n) measured in not-inoculated (NI, black columns) and inoculated (I, dashed columns) seedlings of *Quercus ilex* L. Vertical bars represent the standard error of the mean ($n = 113$ and 54 for g_L measurements of NI- and I-seedlings, respectively; $n = 18$ and 26 for P_n measurements of NI- and I-seedlings, respectively). 20 I-seedlings and 12 NI-seedlings were sampled. Differences in g_L and P_n were both significant, $P = 0.01$, Student's t -test used.

mm diameter with a modal diameter of about 0.24 mm with no significant difference between I- and NI-seedlings (data not shown).

About two years after inoculation I-seedlings appeared to be shorter than NI-seedling (Col. 2, table 1) but with thicker stems while the total leaf surface area was not significantly different in the two groups (Cols. 3 and 4, table 1). The leaf specific mass i.e. the ratio of leaf dry weight to surface area (LSM) was about 13% higher in I-seedlings than in NI-ones with a weak statistical significance of the difference ($P = 0.05$).

Pressure-volume curves did not reveal any significant difference in the leaf osmotic pressure at full turgor, 2.06 and 1.98 MPa in I- and NI-seedlings, respectively.

Leaf conductance to water vapor (g_L) was 1.65 times higher in I- than in NI-seedlings (103 versus 62 $\text{mmol m}^{-2} \text{s}^{-1}$, respectively, figure 1). Similar differences were recorded of net photosynthesis (P_n): 4.1 versus 2.8 $\mu\text{mol [CO}_2\text{] m}^{-2} \text{s}^{-1}$ in I- and NI-seedlings, respectively (figure 1).

3.1. Hydraulic measurements

Root hydraulic conductance normalized by total root surface area (K_{RR}) in I-seedlings was less than half that of NI-seedlings i.e. 9.4 versus $21.6 \times 10^{-5} \text{ kg s}^{-1} \text{ m}^{-2} \text{ MPa}^{-1}$, respectively (figure 2). This means that the unit surface

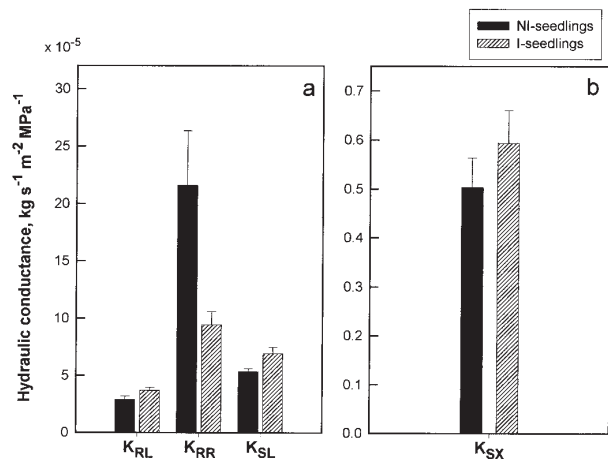


Figure 2. Comparison between not-inoculated (NI, black columns) and inoculated (I, dashed columns) seedlings of *Quercus ilex* L. in terms of: root hydraulic conductance (K_R) normalized by total root (K_{RR}) and total leaf surface area (K_{RL}); hydraulic conductance of whole shoots normalized by total leaf surface area (K_{SL}) and of leafless shoots normalized by wood cross surface area (K_{SX}). Vertical bars represent the standard error of the mean ($n = 16$ for I-seedlings and $n = 10$ for NI-seedlings). All differences were significant, $P = 0.001$ for K_{RL} and $P = 0.01$ for the other parameters, Student's t -test used.

area of highly infected roots would conduct water much less efficiently than moderately infected roots. When K_R was normalized by total leaf surface area (K_{RL} , figure 2), the K_{RL} of I-seedlings was $3.7 \times 10^{-5} \text{ kg s}^{-1} \text{ m}^{-2} \text{ MPa}^{-1}$ versus $2.9 \times 10^{-5} \text{ kg s}^{-1} \text{ m}^{-2} \text{ MPa}^{-1}$ in NI-seedlings. Hence a unit leaf surface area of I-seedlings was supplied with water about 27% better than NI-seedlings. Differences between I- and NI-seedlings in terms of both K_{RR} and K_{RL} were highly significant ($P = 0.01$ and $P = 0.001$, respectively).

The hydraulic conductance of shoots with leaves, normalized by leaf surface area (K_{SL}) was significantly larger (by 30%) in I- than in NI-seedlings (figure 2). Also K_{SX} (= the hydraulic conductance of the stem) of I-seedlings was about 18% higher than that measured in NI-seedlings (0.59 versus $0.50 \text{ kg s}^{-1} \text{ m}^{-2} \text{ MPa}^{-1}$, respectively). In other words, stems of *Q. ilex* seedlings appeared to have significantly higher hydraulic conductance when their root systems were fully associated with *T. melanosporum*.

The resistance to water flow of the leaf blade (LBR) was lower in I-seedlings than in NI-seedlings (figure 3). Although differences between the two groups were not very much (1.18 versus $0.99 \times 10^4 \text{ MPa m}^2 \text{ s kg}^{-1}$ i.e. only about 15%) they were highly significant ($P = 0.001$).

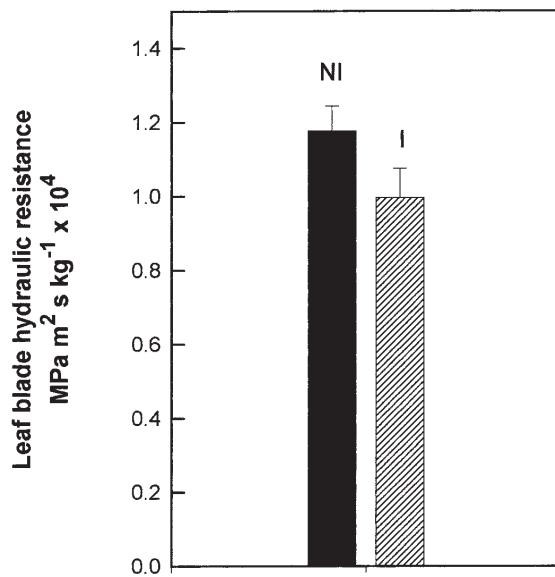


Figure 3. Leaf blade resistance (*LBR*) measured in not-inoculated (NI, black columns) and inoculated (I, dashed columns) seedlings of *Quercus ilex* L. Vertical bars represent the standard error of the mean ($n = 16$ for I-seedlings and $n = 10$ for NI-seedlings). Differences were significant, $P = 0.001$, Student's *t*-test used.

4. DISCUSSION

Seedlings of *Q. ilex*, 22 months after inoculation with *T. melanosporum* i.e. when they were considered ready for planting in the field, showed a significant advantage of mycorrhizal symbiosis in some physiological traits but not in others (e.g., low root conductance per unit root area). What we call NI- (not-inoculated) seedlings were contaminated by the ectomycorrhiza in about 25% of the roots. This, however, was not sufficient to improve seedling hydraulics and water relations compared to seedlings with 100% infected roots. Visually, I- and NI-seedlings were very similar to each other because they showed equal A_L 's and differences in seedling height and stem diameter were too small to be evident at first sight. The leaf specific mass (*LSM*) of I-seedlings, however, was slightly but significantly higher (by about 13%) than that of NI-seedlings. This finding is in agreement with the higher g_L and P_n recorded in I- with respect to NI-seedlings (figure 1). In other words, I-seedlings appeared to maintain higher stomatal aperture than NI-seedlings, which favoured higher CO_2 fixation with consequent higher production of leaf mass per unit surface area.

The measurements of g_L and P_n were not extensive enough to draw the general conclusion that net assimilation integrated over a whole growth season is higher in I- and in NI-seedlings. Nevertheless, g_L and P_n were measured under identical conditions of light, temperature and humidity so differences observed do show the potential impact of *T. melanosporum* under these specific environmental conditions. The higher dry matter investment in leaves and roots (table 1) in I- versus NI-seedlings do suggest an improved net assimilation rate over the entire life of the seedlings.

Leaf dry weight is mainly the expression of the weight of tissues with thick and/or lignified cell walls like in epidermal cells, sclerenchymatous sheaths around the bundles and isolated sclereids in the mesophyll [15]. All these anatomical features make the leaf blade coriaceous to the touch and lead to sclerophylly. The higher *LSM* recorded in I- versus NI-seedlings suggests that plants invested a larger amount of photosynthetic products in the accumulation of cell wall materials in the leaf rather than in seedling growth. Although the functional significance of sclerophylly is still matter of debate [23, 25, 28, 34, 41], the sclerophyllous habit is typical of all Mediterranean evergreens and has been recently interpreted as a factor improving leaf rehydration after water stress release [34]. In this respect, mycorrhizal symbiosis might improve the drought recovery of *Q. ilex* after the scarce summer rainfalls typical of the Mediterranean areas.

In our opinion, the differences in growth and leaf morphology of I- and NI-seedlings (about 12 to 13% for Φ_T , h and *LSM*, table 1, measured after 22 months) may have been too small to be noticed in seedlings less than one year of age or receiving inoculation too recently (see above) and this might be the reason why such effects of mycorrhizae have been reported as dubious in other studies [11].

Root conductance on a root surface area basis (K_{RR}) was 2.5 times lower in I- and NI-seedlings. Although K_{RR} is the "standard" way of expressing root conductance, it is essential to realize that normalization by root surface area can be quite misleading because we rarely know what surface area to use. Ideally K_R (the unscaled root conductance) should be divided by the surface area of roots responsible for most (say > 90%) of the water absorption. It is generally assumed that smaller diameter roots absorb most of the water. The root image analysis system we used gave root surface area as a function of diameter, but we have no way of knowing what root diameters to exclude from the "active" root area. A further complicating factor is that ectomycorrhizae may actually alter the regions of roots involved in water absorption. The hyphal sheaths occupy only the first 1 to

3 mm of root tip and the sheaths make the roots 15% bigger in diameter than the NI-seedlings. If we assume that all water absorption is in the region of the hyphal sheaths in I-seedlings and over a similar length of root in NI-seedling then I-seedlings will have 15% more "active" surface area than NI-seedlings, but this alone could not account for the 250% lower K_{RR} in I- versus NI-seedlings. Apparently the I-seedlings compensated for low K_R per unit root surface area by investing more carbon in more root mass. This, in turn, would produce the beneficial effect of increasing the water supply to a unit surface area of leaf (i.e., a 27% higher K_{RL} of I- than NI-seedlings, *figure 2*), thus allowing higher stomatal aperture [38] and, consequently, increasing CO_2 fixation (higher g_L and P_n in I-seedlings, *figure 1*). The lower hydraulic resistance of the leaf blade recorded in I- versus the NI-seedlings would further favor the water transport within the leaf.

Perhaps the most convincing evidence of the negative impact of *T. melanosporum* on root hydraulic conductance can be gained by looking at the dry matter cost of the roots to produce a unit of hydraulic conductance. Our unit of hydraulic conductance, K_R , is $1 \text{ kg water s}^{-1} \text{ MPa}^{-1}$. I-seedlings 2-years old have to invest 2.5 times as much carbon to achieve a unit of K_R than NI-seedlings of the same age. This follows because I- and NI- roots had the same surface area per unit dry weight and the surface area of I-seedlings was 2.5 time that of NI-seedlings.

Generally, higher stem hydraulic conductance per unit stem cross section is mainly dependent on the xylem conduit radii [43]. Therefore, the higher K_{SX} recorded in I-seedlings suggests that these had more efficient xylem than NI-seedlings.

Mycorrhizal seedlings clearly suffer a disadvantage of lower root conductance. This is compensated at a cost of more carbon investment in fine roots to provide a more sufficient water supply to shoots. Hence, if there is an advantage of mycorrhizal infections by *T. melanosporum* in *Q. ilex* we must look at the additional advantages gained by improved nutrient balance and the effect of improved nutrition on enhanced carbon gain.

In conclusion, a general view of the hydraulics of *Q. ilex* seedlings under study shows that mycorrhizal infection had induced: a) lower hydraulic conductance of roots per unit root surface area, but this was compensated by the increase in the amount of root (mass of fine roots and surface area) which would, in turn, improve the total nutrient uptake; b) more efficient vertical water transport to (higher K_{SX}) and within leaves (lower LBR); c) higher CO_2 fixation; d) higher leaf specific mass.

We feel that more studies on field-growing plants are needed to clarify the possible role of the mycorrhizal

symbiosis in the drought tolerance of plants. The HPFM is, in this respect, a useful instrument because it allows easy measurement of root and stem hydraulic conductance of plants. The HPFM method may allow evaluation of mycorrhizal associations of older plants [27, 46] without the artifact caused by restricted root growth in pots [30].

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