

Effect of controlled inoculation with specific mycorrhizal fungi from the urban environment on growth and physiology of containerized shade tree species growing under different water regimes

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Abstract The aim of this work was to evaluate the effects of selected mycorrhiza obtained in the urban environment on growth, leaf gas exchange, and drought tolerance of containerized plants growing in the nursery. Two-year-old uniform *Acer campestre* L., *Tilia cordata* Mill., and *Quercus robur* L. were inoculated with a mixture of infected roots and mycelium of selected arbuscular (maple, linden) and/or ectomycorrhiza (linden, oak) fungi and grown in well-watered or water shortage conditions. Plant biomass and leaf area were measured 1 and 2 years after inoculation. Leaf gas exchange, chlorophyll fluorescence, and water relations were measured during the first and second growing seasons after inoculation. Our data suggest that the mycelium-based inoculum used in this experiment was able to colonize the roots of the tree species growing in the nursery. Plant biomass was affected by water shortage, but not by inoculation. Leaf area was affected by water regime and, in oak and linden, by inoculation. Leaf gas exchange was affected by inoculation and water stress. V_{cmax} and J_{max} were increased by inoculation and decreased by water shortage in all species. F_v/F_m was also generally higher in inoculated plants than in control. Changes in PSII photochemistry and photosynthesis may be related to the capacity of inoculated

plants to maintain less negative leaf water potential under drought conditions. The overall data suggest that inoculated plants were better able to maintain physiological activity during water stress in comparison to non-inoculated plants.

Keywords *Acer campestre* · Leaf gas exchange · OJIP test · PSII photochemistry · *Tilia cordata* · *Quercus robur* · Water stress

Introduction

Mycorrhizal fungi are beneficial soil-inhabiting fungi that establish natural symbiotic associations with roots of native plants (Klingeman et al. 2002). Mycorrhizae affect the morphology of infected roots and induce changes to rhizosphere functioning (Kothari et al. 1990; Barea et al. 2002; Habte 2006), and they may play a role in enhancing plant tolerance to some natural and anthropogenic stressors, including water stress (Entry et al. 2002).

Perennial plants cope with water stress either by drought avoidance (maintaining high internal water potential at low external water potential) or by drought tolerance (survival at low internal water potential) or by both strategies (Levitt 1980). A series of studies, reviewed in Augé (2001), have demonstrated that mycorrhizae can increase tolerance to water stress in both drought-avoider and drought-tolerant species. Mechanisms proposed to explain the increased tolerance include greater root growth (Osonubi et al. 1992), increased soil water extraction (Graham and Syvertsen 1984), less negative water status during drought (Newman and Davies 1988), quicker recovery of water potentials after drought (Gemma et al. 1997), and higher osmotic adjustment

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and activity of the antioxidant system during drought (Davies et al. 1993; Kalafallah and Abo-Ghalia 2008).

Mycorrhizae are abundant in the natural, undisturbed environment, but any soil disturbance can alter fungal populations and reduce or change mycorrhizal colonization of tree roots (Giovannetti and Gianinazzi-Pearson 1994). Urbanization causes a heavy disturbance which reduces and/or alters vesicular–arbuscular and ectomycorrhizal populations and root colonization of shade tree species (Stabler et al. 2001; Timonen and Kauppinen 2008; Bainard et al. 2011). Moreover, the built environment is often characterized by drought, high soil and air temperature, low soil fertility, and soil compaction, which may result in various degrees of stress on urban trees (Ferrini et al. 2008). For these reasons, newly transplanted trees from the nursery may face a particularly difficult situation because they are moved from the optimal conditions of the nursery to the suboptimal or stressful conditions of the urban environment (Franco et al. 2006). Moreover, most of the naturally occurring mycorrhiza in the nursery are not capable of surviving in urban soils (Weber and Claus 2000), and this may further predispose trees to stress.

To overcome this problem, many commercial mycorrhizal inoculants have been developed and tested in both nursery and urban environments (Stabler et al. 2001; Appleton et al. 2003; Corkidi et al. 2004, 2005; Wiseman and Wells 2009). Reduced viability of commercial products is often a constraint to the success of the inocula. Mycorrhizae not native to a site may not function at that site because they are not adapted to grow in that location. Moreover, not all combinations of plant host and mycorrhiza-forming fungi are functionally compatible (Sylvia et al. 2003; Linderman and Davies 2004; Allen et al. 2005).

Controlled mycorrhization is a technique which consists of inoculating seedlings in the nursery with mycelia of mycorrhizal fungi selected for their better performance among the resident symbionts at the outplanting site (LeTacon et al. 1992; Garbaye and Churin 1996), and it has been the most practical and cost-effective way of utilizing mycorrhizal technology (Johnson and Pflieger 1992). This technique can be applicable to landscaping and urban forestry if: (1) it is possible to find effective native symbionts in the outplanting site (i.e., urban areas); (2) the selected strains are able to survive in nursery conditions; and (3) mycorrhiza survives transplanting in the landscape. Timonen and Kauppinen (2008) demonstrated that despite similar frequency of colonization, healthy lindens growing in the Helsinki city area were associated with different mycorrhizal fungi when compared to unhealthy lindens growing in the same environment. Therefore, harvesting proper inocula in the urban environment is possible from roots of healthy mature trees.

Little evidence was found about the survival, the inoculum potential, and benefits for plant growth and physiology of native mycorrhizal fungi harvested in a city on containerized plants growing in a standard nursery substrate. Inoculating young plants (1 or 2 years old) in the nursery is far cheaper than inoculating older trees because a lower quantity of inoculum is required. A successful inoculation in the nursery may result in more stress-tolerant plants during production, which leads to reduced fertilizers and water requirements and increased water use efficiency during production (Davies 2000; Marin 2006; Quoreshi 2008). A recent study found that mycorrhizal symbiosis had a beneficial effect on plant water status and photosynthetic activity in *Arbutus unedo* and that the combined effect of mycorrhiza and deficit irrigation in the nursery produced a hardening process of strawberry tree (Navarro-Garcia et al. 2011). If the value-added benefits of mycorrhiza in producing superior plants are demonstrated, the nursery industry can market and command a higher price for more stress-resistant mycorrhizal nursery crops (Davies et al. 2000).

The aim of this work was to evaluate whether inoculation with specific mycorrhiza obtained in the urban environment can increase mycorrhizal frequency, growth, leaf gas exchange, and drought tolerance of containerized plants growing in the nursery. Leaf gas exchange as affected by mycorrhizae has been widely studied, but most comparative studies of photosynthesis in mycorrhizal and non-mycorrhizal plants report net carbon exchange rates, and only a few studies offer more details (Allen et al. 1981; Augé 2001). To provide further insight on the effect of mycorrhizae on gas exchange, we investigated the kinetic parameters of Rubisco, Ribulose regeneration, stomatal vs. metabolic limitations to photosynthesis, and the kinetics of the Chl-*a* (OJIP) transient in plants inoculated or non-inoculated with selected mycorrhizal inoculum and either grown in well-watered or water shortage conditions.

Materials and methods

Root sampling, morphotyping, and selection of mycorrhizal strains

To produce the mycorrhizal inoculum for controlled inoculation with specific fungal strains from the urban environment, in May 2007, five to seven healthy mature hedge maples (*Acer campestre* L.), littleleaf lindens (*Tilia cordata* Mill.), and pedunculate oaks (*Quercus robur* L.) were selected in Milan (Italy, 45°28' N, 9°12' E). Selected plants were grown on soils with pH 6.5–7, low to normal (17–32 ppm) extractable P, and 2% organic matter.

The sampling operation was done in cooperation with Floricoltura San Donato—Grandi Trapianti Italiani. The root sampling was conducted as in Requena et al. (1996). Briefly, from each tree, five samples, which contained fine roots and moist soil (approx. 500 g), were harvested at approximately 1 m from the trunk, sealed in a plastic bag, and immediately carried to MicoMax laboratory (Wuppertal, Germany). Fresh roots were gently separated from soil. Colonization was vesicular–arbuscular in maple and ectomycorrhizal in oak. On linden roots, both vesicular–arbuscular (VAM) and ectomycorrhizae (ECM) were found.

ECM in oak and linden were characterized according to their exterior features (Agerer 1987–1998; Timonen and Kauppinen 2008). To select the best performing ECM strains, roots were observed with a stereomicroscope (Zeiss, Milan, Italy) and root colonization of each fungal species/strain was measured on harvested roots as the frequency of mycorrhizal root tips (Newton and Pigott 1991). Sections were made of the root tips using a razorblade and fungus–host compatibility was evaluated on the basis of the structure of the Hartig net (Theodorou and Reddel 1991; Bundrett et al. 1996; Menkis et al. 2005).

To reveal AM fungal structures in maple and linden, every root harvested from mature trees was cut into 20-mm-long segments, taking care not to mix segments of different roots and samples. A subset of root segments was stained with 0.05% Trypan blue in lactoglycerol (Koske and Gemma 1989; Klingeman et al. 2002), while the corresponding segments in the other subsets were used to determine the vitality of the mycelium and phosphatase activity and, eventually, to start cultures. Criteria for morphological AMF characterization were based on spore size and color, wall structure, and hyphal attachment (Walker 1983; Shenk and Perez 1990; Calvente et al. 2004). To select the best AMF strains, the following parameters were measured: (1) The percentage of colonization was determined by the magnified intersection method (McGonigle et al. 1990); (2) vitality of the mycelium was determined by the succinate dehydrogenase reaction (Gianinazzi and Gianinazzi-Pearson 1992); and (3) alkaline phosphatase (ALP) activity in the intraradical mycelium was quantified. To determine ALP activity, roots were incubated for 3 h in an enzyme solution (0.05 M Tris–HCl, pH 9.2, 0.05% sorbitol, 15 U/mL cellulase and 15 U/mL pectinase) and stained with an ALP staining solution (0.05 M Tris–HCl, pH 9.2, 1 mg/mL Fast Blue RR salt, 1 mg/mL α -naphthyl acid phosphate monopotassium salt, 0.5 mg/mL $MgCl_2$, and 0.05 mg/l $MnCl_2$; Tisserant et al. 1993; Janoušková et al. 2009).

According to the selection criteria mentioned above for ECM (frequency of colonization and fungus–host compatibility) and AMF (root colonization, vitality of the mycelium, phosphatase activity), the fungal strains which

showed the best performances were: (1) *Glomus geosporum*, *Glomus mossae*, and *Glomus clarum* on *A. campestre*; (2) *Scleroderma* spp. on *Q. robur*; and (3) *Boletus edulis* and *G. mossae* on *T. cordata*.

Isolation of ECM and AMF cultures

To start cultures of the selected ectomycorrhizal strains, root tips where selected fungi were found were minced to small pieces and plated in Petri dishes containing one half-strength modified Melin–Norkrans medium containing 30 mg/l chlor-tetracycline and 1 mg/l Benomyl (Erland and Söderström 1990). Dishes were checked daily and emerging culture was transferred to agar medium (Menkis et al. 2005). Fungal mycelia were examined under a microscope (Zeiss), equipped with $\times 25$, $\times 100$ and $\times 1,000$ magnifications to check morphological characteristics (Menkis et al. 2005). After 1 month of culture at 25°C, ten plugs (0.5-cm diameter) of mycelium were aseptically removed from the margin of the colony and were put in direct contact with seedling roots for inoculating lindens and oaks. Oak and linden seeds were surface-sterilized with a 10% sodium hypochlorite solution, washed, pre-germinated, and transplanted in a peat/pumice (3:1) substrate previously sterilized by autoclaving (100°C for 1 h day⁻¹ for three consecutive days; Porcel and Ruiz-Lozano 2004).

To start cultures of the selected AMF strains, spores of the selected fungal strains were extracted from selected samples by wet sieving and decanting followed by sucrose centrifugation (Sieverding 1991). Maple and linden seeds were surface-sterilized with a 10% sodium hypochlorite solution, washed, pre-germinated, and transplanted in a sterilized peat/pumice (3:1) substrate. On average, 50 spores obtained from the selected root samples were used to inoculate each seedling (Calvente et al. 2004).

Multiplication of the inocula

After inoculation, all the seedlings were grown in a greenhouse under natural light and ambient temperature. Non-inoculated seedlings of maple, linden, and oak were also grown in the greenhouse on separate benches. Seedlings were irrigated two or three times a week, according to their needs. Irrigation was performed to recover water content of the substrate to $\approx 70\%$ of water holding capacity of the substrate to assure that plants had access to sufficient water, at the same time avoiding water excess and oxygen deprivation (Ijdo et al. 2011). Seedlings were fertilized with a low concentration (one third of the label dose, 1 kg/m³) of a controlled release fertilizer (Ficote® 15-8-12, 8–9 months, Scott International B.V., Geldermasel, the Netherlands). Substrate samples were periodically harvested from inoculated plants to confirm the development of selected fungal cultures. To do this, roots

were washed free from substrate with a flush of air, observed with a Zeiss microscope, and compared to known morphotypes.

After 8 months, fine roots were harvested from inoculated seedlings, cut into small pieces, and used to produce the inoculum. The inoculum was composed of infected root pieces, fungal mycelium, montmorillonite and a hydrogel to avoid dehydration. Four inocula were made in total: one for maple, one for oak, and two for linden (one containing ECM and one containing AMF, which were kept separate). A similar procedure was followed on non-inoculated plants, and the material obtained was mixed with montmorillonite and hydrogel.

Plant material and experimental conditions

In March 2008, 80 uniform 2-year-old hedge maples (*A. campestre* L.), 80 uniform littleleaf lindens (*T. cordata* Mill.), and 80 uniform pedunculate oaks (*Q. robur* L.) were obtained from a local commercial nursery located a few kilometers apart from the experimental field. To obtain the seedlings, seeds were seeded in trays in a greenhouse of the nursery and, after emergence, were transplanted in 0.4-L containers in a peat/perlite substrate (3:1) fertilized with 4 kg/m³ of a controlled-release fertilizer (Osmocote, 10-10-17, 6 months formulation, Scotts, Marysville, OH, USA). When 40–60 cm tall, seedlings were moved outdoors and irrigated as required with a sprinkler system, as in standard nursery practices.

After two growing seasons in the commercial nursery, plants were delivered and immediately re-potted in 3-L (0.792-gal) containers using a peat/pumice (3:1) substrate. Substrate was amended with 4 kg/m³ (5.057 lb/yard³) dolomite to neutralize pH (Corkidi et al. 2004). One kilogram per cubic meter of a controlled release fertilizer (Ficote® 15-8-12, 8–9 months, Scott International B.V.) was added to the medium. A reduced dose of fertilizer (1 kg/m³, 1.69 lb/yard³), if compared to the label dose (4 kg/m³, 6.74 lb/yard³), was used in this experiment to avoid an excessive soil chemical fertility which may decrease mycorrhizal colonization. Substrate chemical analysis confirmed that Melich-1 extractable P concentration was low (10–15 ppm). A similar amount of P was used in other works (Nadian et al. 1997; Corkidi et al. 2004, 2005) and was found to be close to the P concentration that promoted the maximum mycorrhizal colonization in clover (Nadian et al. 1996).

At the time of potting, 25 mL of species-specific inoculum was added to half of the plants (+I, one third on the bottom and two thirds along the sides of the container). The inoculum was carefully spread in direct contact with absorbing roots. A mixture of non-mycorrhizal roots, montmorillonite and hydrogel, was added to the remaining half of

the plants (–I). In the case of linden, plants were inoculated with both ECM and AMF, taking care not to mix the two products. +I and –I plants were randomly selected from plant population and were of similar size at the time of inoculation.

Container capacity, wilting point, and effective water holding capacity of the substrate were determined with a gravimetric method as described by Sammons and Struve (2008). Before the beginning of the experiment, 18 containers were lined with a plastic bag and weighed. Then, containers were filled to within 2.5 cm of the rim with water, the water height marked, and the weight recorded, which yielded the container volume. The containers were emptied and filled with oven-dried substrate (72 h at 70°C) to the volume mark and weighed. Six lindens, six oaks, and six maples were planted in the containers before weighing. Then, the substrate was saturated with water and weighed. The weight of the water added represented the total pore space of the substrate. Holes were made in the plastic bag and substrate was allowed to drain for 1 h, after which containers were weighed again. The difference between drained weight and the oven-dried weight represents substrate water holding capacity. The difference in weight between saturation and the drained weights represents the air-filled pore space. On average, water holding capacity of the containers was about 1,200 mL, which is consistent with that reported in other studies with similar substrates (Fini et al. 2008).

Plants were placed outside, in a tunnel covered with Nowoflon (ET6235–Z; Nowofol, Siegsdorf, Germany) to exclude natural rainfall. Plants were grown under natural sunlight (photosynthetic active radiation (PAR) during the growing season ranged from 50 to 300 W m^{–2}) and ambient temperature, and were either irrigated daily in order to restore container capacity (WW) or irrigated daily to the 30% of container water capacity (WS). Irrigation to the 25–40% of container capacity is generally used to induce water stress and promote hardening of shade and fruit trees and other ornamental plants (Ruiz-Sanchez et al. 2000; Gu et al. 2007; Van Iersel et al. 2010). Irrigation was performed daily, except on days when water relations were measured.

The experiment was a randomized block design with six blocks and five plants per species and treatment in each block. The entire experiment was conducted under non-sterile conditions.

Measurements

In September 2008 and 2009, 72 plants (one plant per block, treatment, and species) were harvested for biomass measurement. Plants were cut at the root flare and roots were cleaned from the medium with a flush of air. Leaves were separated from stems and scanned with an A3 scanner. An image analysis software (Image Tool 1.3, UTHSCSA)

was used to measure leaf area. To determine dry weight leaves, stems and roots were oven-dried at 70°C until constant weight was reached (≈ 72 h). Root-to-shoot ratio was calculated as the ratio between root dry weight and leaves + stem dry weight.

In September 2008 (7 months after inoculation), a sample of fine roots + soil was harvested from two plants per block, treatment, and species (144 plants). Roots were carefully separated from the soil and cut into 1-cm-long pieces. For ECM (*Scleroderma* on oak and *Boletus* on linden), frequency of mycorrhizal roots was measured on 200 root tips as the ratio of mycorrhizal root tips to total root tips (Newton and Pigott 1991). To evaluate colonization of AMF (*Glomus* spp. on maple and linden), 200 root segments were cleared with 10% KOH solution, followed by a 3% solution of hydrogen peroxide and acidified with 5 N HCl solution. Clean roots were stained using 0.05% Trypan blue in lactoglycerol (Koske and Gemma 1989; Klingeman et al. 2002). Percentage of root colonization was measured by counting cross-hair intersections with a stereomicroscope (McGonigle et al. 1990).

Leaf gas exchange was measured in June 2008, July 2008, September 2008, June 2009, and July 2009, respectively 4, 5, 7, 17, and 18 months after inoculation. Gas exchange was measured with an infrared gas analyser (Ciras-2, PP-System, Hertfordshire, UK) on three leaves per treatment, species, and replicate (216 leaves in total per sampling date). Measurements were taken on the first fully expanded leaf of the shoot, at saturating ($1,300 \mu\text{mol m}^{-2} \text{s}^{-1}$) light intensity, ambient temperature, and 360 ppm CO_2 . Water use efficiency was calculated as the *A*-to-*E* ratio (Jifon and Syvertsen 2003; Fini et al. 2009). Assimilation (*A*) to internal CO_2 concentration (C_i) response curves were drawn in July 2009 (17 months after inoculation) by varying the external CO_2 concentration (C_a) from 0 to 1,800 ppm (Guidi et al. 2008).

The stomatal limitation (L_s) and the relative mesophyll limitation (L_m) were calculated from *A*/ C_i curves as described in previous works (Lawlor 2002; Long and Bernacchi 2003). Estimates of the apparent maximum rate of carboxylation by Rubisco (V_{cmax}) and the apparent maximum electron transport rate contributing to ribulose 1,5-BP regeneration (J_{max}) were made using the equations found by Sharkey et al. (2007):

$$A = V_{\text{cmax}} \left[\frac{C_i - \Gamma^*}{C_i + K_C(1 + O/K_O)} \right] - R_d \quad (1)$$

$$A = J \left[\frac{C_i - \Gamma^*}{4C_i + 8\Gamma^*} \right] - R_d \quad (2)$$

where *A* is the Rubisco-limited (Eq. 1) or the RuBP-limited (Eq. 2) carbon assimilation, C_i is the internal CO_2 concentration, K_C is the Michaelis constant of Rubisco for

carbon dioxide, O is the internal O_2 concentration, K_O is the inhibition constant, Γ^* is the photorespiratory compensation point, and R_d is the day respiration. Equation 1 lends itself to a linear regression approach to estimating V_{cmax} as the slope and $-R_d$ as the intercept (Long and Bernacchi 2003).

Chlorophyll fluorescence was measured on the same dates and plants as leaf gas exchange by using a HandyPEA portable fluorescence spectrometer (Hansatech Instruments Ltd., King's Lynn, UK). Fluorescence values were obtained after adapting leaves to darkness for 40 min by attaching light exclusion clips to the leaf surface of whole trees. Upon the application of a saturating flash of actinic light ($3,000 \mu\text{mol m}^{-2} \text{s}^{-1}$ for 1 s), fluorescence raises from the ground state value (F_o) to its maximum value, F_m . This allows the determination of the maximal quantum yield of PSII (F_v/F_m ; Pinior et al. 2005). F_v/F_m data are presented as the average of measurements taken 4, 5, and 7 months after inoculation (2008) and as the average of measurements taken 17 and 18 months after inoculation (2009). The fluorescence transient was measured at 10 μs , 100 μs , 1 ms, and 100 ms after illumination and analyzed following the equations of the OJIP test (Strasser et al. 2000).

Leaf pre-dawn (Ψ_{pd}), xylem (Ψ_{xi}), and midday (Ψ_{m}) water potentials were measured 18 months after inoculation with a pressure bomb (PMS Instruments, Albany, OR, USA; Scholander et al. 1964). Pre-dawn water potential was measured between 0500 and 0630 hours. Midday water potential was measured between 1300 and 1430 hours (Ψ_{m}). Xylem water potential was measured between 1300 and 1430 hours on leaves previously adapted to dark for one and half hour with a plastic bag covered with aluminum foil (Jones 1992). Measurement of leaf water relations were not performed on oak because of the short petiole which did not fit in the chamber.

Soil-to-plant (K_{sp} , $\text{mm s}^{-1} \text{MPa}^{-1}$), soil-to-xylem conductivity (K_{sx} , $\text{mm s}^{-1} \text{MPa}^{-1}$), and leaf conductance (K_l , $\text{mm s}^{-1} \text{MPa}^{-1}$) were calculated on a leaf area basis, assuming Ψ_{pd} is an estimate of soil water potential (Costa e Silva et al. 2004):

$$K_{\text{sp}} = \frac{E}{\Psi_{\text{pd}} - \Psi_{\text{md}}} \quad (3)$$

$$K_l = \frac{E}{\Psi_x - \Psi_{\text{md}}} \quad (4)$$

$$K_{\text{sx}} = \frac{E}{\Psi_{\text{pd}} - \Psi_x} \quad (5)$$

where Ψ_{pd} , Ψ_{m} , and Ψ_x are leaf pre-dawn, leaf midday, and xylem water potentials, respectively, and *E* is the transpi-

ration rate ($\text{m}^3 \text{m}^{-2} \text{s}^{-1}$) through the system measured between predawn and midday.

Statistics

Quantitative data were analyzed with two-way generalized linear model (GLM; SPSS 16.0, SPSS Inc., Chicago, IL, USA). Percentages of mycorrhizal colonization data were arcsin-transformed prior to analysis. Parameters which showed significant interaction between factors were analyzed with one-way GLM model. Gas exchange data were analyzed with repeated measures ANOVA, then within each sampling date, differences between treatments were analyzed using one-way ANOVA and means were separated with Duncan's multiple range test. The three species were analyzed independently because of the different inocula tested.

Results

Root colonization

Inoculation increased the percentage of root colonization in all species (Table 1). Seven months after inoculation with AM fungi, +I maple and +I linden had higher percentage of colonized roots in comparison to -I plants. Inoculation with ECM increased the frequency of colonized root tips to 81% in linden and to 80% in oak. -I lindens and oaks had significantly lower levels of colonization. Moreover, mycorrhiza of non-inoculated plants was characterized as simply ramified, contact, or short-distance exploration types, whereas the observation of inoculated roots revealed the presence of long-distance exploration type structures, such as type F rhizomorphs, typical of *Boletus* and *Scleroderma*. Water shortage increased root colonization by AMF and ECM in maple and oak, respectively. No effect of water shortage on root colonization was found in

linden. No interaction between inoculation and water shortage was found.

Biomass and leaf area

Inoculation had no effect on total plant biomass, root-to-shoot ratio, and leaf area in maple (Table 2). Water shortage had no effect on plant dry weight, root-to-shoot ratio, and leaf area of maple in 2008. In 2009, plant dry biomass and leaf area were reduced by water shortage. Root-to-shoot ratio was not affected by water regime in maple.

Inoculation did not affect dry biomass and root/shoot ratio in linden. Inoculation significantly increased leaf area in linden in 2009 (Table 2). Water shortage reduced plant biomass and leaf area of linden, but had no effect on root-to-shoot ratio.

In oak, inoculation had no effect on plant biomass and root-to-shoot ratio. Inoculated oaks had larger leaf area in 2008 and 2009. Water shortage had no effect on plant biomass in 2008, but reduced biomass in 2009. In 2008 and 2009, leaf area was lower in the WS treatment and root-to-shoot ratio increased under water shortage.

No interaction between factors was found in any of the investigated species.

Leaf gas exchange

Carbon assimilation and transpiration were significantly affected by treatments (Fig. 1). In maple, WW plants had higher A and E with respect to WS plants throughout the experiment (Fig. 1a, b). No difference between +I WS and -I WS maples were found for A 4, 5, and 7 months after inoculation. Seventeen and 18 months after inoculation, +I WS maples had higher A compared to -I WS plants. E was similar in +I WW and -I WW maples, and in WS maples, inoculation increased E only 18 months after inoculation.

Table 1 Effect of mycorrhizal inoculation, water regime, and their interaction on mycorrhizal colonization (measured 7 months after inoculation) of hedge maple, littleleaf linden, and pedunculata oak

Species	Inoculation (I)		Water regime (W)		Significance		
	+I (%)	-I (%)	WW (%)	WS (%)	I	W	I × W
Maple	53 a	24 b	33 b	44 a	**	**	ns
Linden (ECM)	81 a	59 b	68 a	72 a	**	ns	ns
Linden (AMF)	17 a	10 b	14 a	14 a	*	ns	ns
Oak	80 a	41 b	54 b	61 a	**	**	ns

Different letters within the same line and factor indicate significant differences between +I and -I plants and between WW and WS plants +I inoculated plants, -I non-inoculated plants, WW well-watered conditions, WS water shortage conditions

* $P \leq 0.05$; ** $P \leq 0.01$ (differences between treatments)

Table 2 Effect of mycorrhizal inoculation, water regime, and their interaction on total plant dry weight, root-to-shoot ratio, and leaf area in hedge maple, littleleaf linden, and pedunculate oak

Species	Parameter	Inoculation (I)		Water regime (W)		Significance		
		+I	-I	WW	WS	I	W	I × W
Maple	Plant DW 2008 (g)	94.3 a	101.1 a	105.3 a	90.1 a	ns	ns	ns
	Plant DW 2009 (g)	248.2 a	238.2 a	292.2 a	195.2 b	ns	**	ns
	Root/shoot 2008	1.0 a	1.1 a	1.0 a	1.1 a	ns	ns	ns
	Root/shoot 2009	0.9 a	1.0 a	1.0 a	0.9 a	ns	ns	ns
	Leaf area 2008 (cm ²)	1437.7 a	1494.8 a	1514.1 a	1414.8 a	ns	ns	ns
	Leaf area 2009 (cm ²)	5398.3 a	4964.9 a	5859.5 a	4503.7 b	ns	*	ns
Linden	Plant DW 2008 (g)	55.0 a	54.8 a	60.9 a	48.9 b	ns	*	ns
	Plant DW 2009 (g)	160.4 a	153.3 a	190.3 a	123.4 b	ns	**	ns
	Root/shoot 2008	0.8 a	0.9 a	0.9 a	0.8 a	ns	ns	ns
	Root/shoot 2009	0.9 a	0.9 a	0.9 a	0.9 a	ns	ns	ns
	Leaf area 2008 (cm ²)	631.5 a	597.9 a	930.7 a	298.7 b	ns	*	ns
	Leaf area 2009 (cm ²)	4428.0 a	4036.9 b	4833.4 a	3631.5 b	*	**	ns
Oak	Plant DW 2008 (g)	58.7 a	56.5 a	64.0 a	50.9 a	ns	ns	ns
	Plant DW 2009 (g)	187.5 a	201.8 a	233.6 a	155.8 b	ns	**	ns
	Root/shoot 2008	0.7 a	0.7 a	0.6 b	0.8 a	ns	*	ns
	Root/shoot 2009	0.7 a	0.8 a	0.6 b	1.0 a	ns	**	ns
	Leaf area 2008 (cm ²)	1737.2 a	1153.2 b	1885.6 a	1004.8 b	*	**	ns
	Leaf area 2009 (cm ²)	5092.9 a	3875.9 b	5715.4 a	3253.4 b	**	**	ns

Different letters within the same line and factor indicate significant differences between +I and -I plants and between WW and WS plants +I inoculated plants, -I non-inoculated plants, WW well watered conditions, WS water shortage conditions * $P \leq 0.05$; ** $P \leq 0.01$ (differences between treatments)

In linden, water shortage reduced A and E throughout the experiment (Fig. 1c, d). In WW lindens, inoculation had no effect on gas exchange, except 17 months after inoculation when +I plants had higher A and E in comparison to -I plants. Seventeen and 18 months after inoculation, +I WS lindens had significantly higher A than the -I WS treatment. Mycorrhizae increased E of WS lindens 18 months after inoculation.

Leaf gas exchange was affected by mycorrhiza and water shortage in oak (Fig. 1e, f). Water shortage reduced A and E of oaks, even if 17 months after inoculation +I WS plants had similar A and E as WW oaks. In well-watered conditions, inoculation had little effect on plant gas exchange, while in water shortage conditions, inoculated oaks had higher A than control oaks 5, 17, and 18 months after inoculation. +I WS oaks also had higher E than -I WS plants 17 and 18 months after inoculation.

Stomatal limitations to photosynthesis were not affected by treatments in maple and linden (Table 3). Mesophyll limitations (L_m) were significantly affected by mycorrhiza and water shortage. L_m is null by definition in WW plants (Lawlor 2002). In maple and linden, L_m was significantly higher in -I WS than in +I WS plants (Table 3). In oak, L_s was similar in the two WW treatments. In water shortage conditions -I WS had greater L_s than +I WS. L_m was significantly higher in -I WS than in +I WS.

Inoculation increased the apparent rate of carboxylation (V_{cmax}) and the apparent maximum electron transport rate contributing to ribulose 1,5-BP regeneration (J_{max}) in all

species (Table 4). Water shortage decreased V_{cmax} and J_{max} in all species as well. Oak exhibited a lower loss of functionality of Rubisco and electron transport in comparison to linden and maple.

Water use efficiency (WUE) was affected by inoculation and water shortage in all species (Fig. 2). In maple, no clear effect of factors was observed 4, 5, and 7 months after inoculation. Seventeen and 18 months after inoculation, +I WS maples had higher WUE than -I WW and -I WS (Fig. 2a). In linden, 4, 5, and 7 months after inoculation, WUE was affected by water shortage, but not by inoculation (Fig. 2b). Seventeen and 18 months after inoculation, +I WS lindens had higher WUE than +I WW and -I WW plants. Both +I and -I WW lindens had higher WUE than -I WS lindens. In oak, -I WS had lower WUE when compared to the other treatments 5 and 7 months after inoculation, but differences were not confirmed in the following samplings (Fig. 2c).

Chlorophyll fluorescence

In maple and linden, the maximal quantum yield of PSII (F_v/F_m) was significantly affected by inoculation and water shortage (Table 5). +I plants had higher F_v/F_m than -I plants in 2008 and 2009. Water shortage reduced F_v/F_m in both years. In oak, F_v/F_m measured in 2008 was affected by inoculation, but not by water regime, and +I plants had higher F_v/F_m than -I. In 2009, no effect of inoculation and water shortage on F_v/F_m was found in

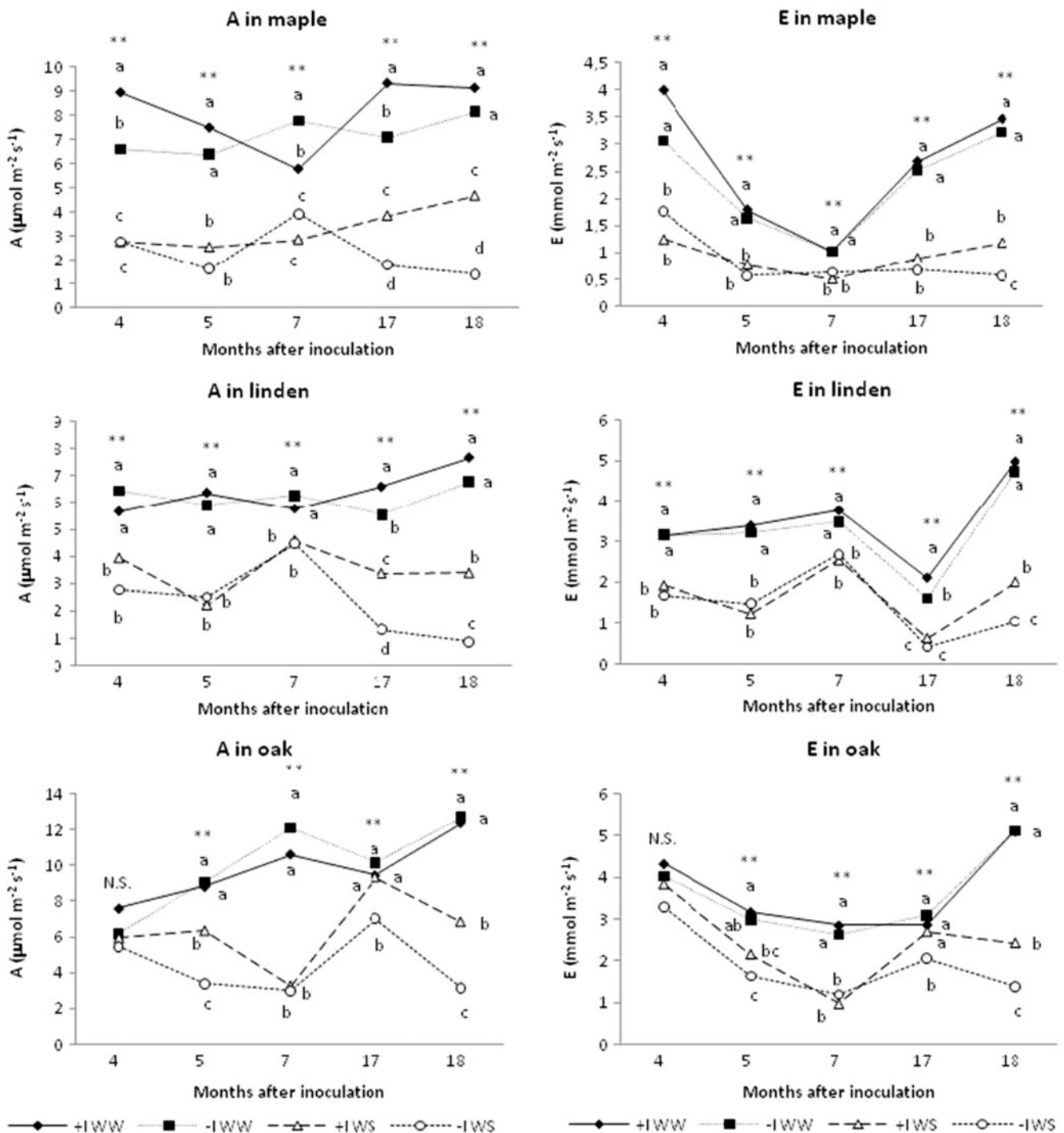


Fig. 1 Carbon assimilation (A , $\mu\text{mol m}^{-2} \text{s}^{-1}$) and transpiration (E , $\text{mmol m}^{-2} \text{s}^{-1}$) of hedge maple (a, b), littleleaf linden (c, d), and pedunculate oak (e, f) inoculated (+) or not inoculated (-) with native mycorrhiza and growing in well-watered (WW) or water

shortage (WS) conditions. **Significant differences among treatments within each sampling date at $P \leq 0.01$. Different letters within the same sampling date indicate significant differences among treatments using Duncan's MRT

oak. No interaction between factors was found in any of the species studied.

In maple, the polyphasic shape of the chlorophyll a fluorescence was affected by inoculation and water shortage (Fig. 3). Differences among treatments started at the J-

step, but became more apparent in the I and P steps. In linden, OJIP transient was unaffected by treatments (data not shown). In oak, differences between WW and WS plants became apparent at the J step. At the P step, +I WW had higher fluorescence than the other treatments (Fig. 3)

Table 3 Stomatal limitation (L_s) and relative mesophyll limitation (L_m) in maple, linden, and oak in response to mycorrhizal inoculation and water shortage

Species	Parameter	Treatment				Significance		
		+I WW (%)	-I WW (%)	+I WS (%)	-I WS (%)	I	W	I × W
Maple	L_s	16 a	11 a	25 a	13 a	ns	ns	ns
	L_m	–	–	45 b	76 a	*	–	–
Linden	L_s	11 a	15 a	16 a	24 a	ns	ns	ns
	L_m	–	–	62 b	82 a	**	–	–
Oak	L_s	9 c	10 c	20 b	36 a	**	**	**
	L_m	–	–	28 b	64 a	**	–	–

L_s and L_m were calculated from A/C_i curves as described in Lawlor (2002). L_m is null by definition in WW plants. Different letters within the same line indicate significant differences between treatments

+I inoculated plants, -I non-inoculated plants, WW well-watered conditions, WS water shortage conditions

* $P \leq 0.05$; ** $P \leq 0.01$ (differences among treatments)

Water relations

Pre-dawn leaf water potential was affected by treatments. A significant inoculation × water management interaction was found. In linden, WW plant had less negative Ψ_{pd} than WS plants, and no difference was found between +I WW and -I WW. In water shortage conditions, +I lindens had less negative Ψ_{pd} than -I (Fig. 4). In maple, +I WW, -I WW, and +I WS had less negative Ψ_{pd} than -I WS (Fig. 4).

Soil-to-plant, root-to-xylem and leaf water conductances were not affected by inoculation in maple (Table 6). Water shortage significantly reduced K_{sp} , K_{sx} , and K_l in maple. In linden, K_{sp} and K_l were increased by inoculation, while K_{sx} was unaffected. Water shortage significantly reduced K_{sp} , K_{sx} , and K_l in maple and linden (Table 6).

Discussion

In this experiment, we inoculated container-grown maples (*A. campestre*), littleleaf linden (*T. cordata*), and pedunculate oak (*Q. robur*) with native selected mycorrhizal inocula obtained from healthy, mature trees growing in the urban area of Milan. These three species were selected because they are widely used for urban and landscape planning in Northern Italy. Our data suggest that the mycelium-based inoculum used in this experiment was able to infect the roots of the shade tree species growing in containers in a peat/pumice substrate. We worked in non-sterile conditions, as reflected by a certain degree of colonization occurring in control trees. Mycorrhizal infection in untreated trees was also found by other researchers who worked in non-sterilized environments in both nursery (Wiseman and Wells 2009) and street trees (Appleton et al. 2003).

Table 4 Effect of mycorrhizal inoculation, water regime, and their interaction on the apparent maximum rate of carboxylation by Rubisco (V_{cmax}) and on the apparent maximum electron transport rate contributing to ribulose 1,5-BP regeneration (J_{max})

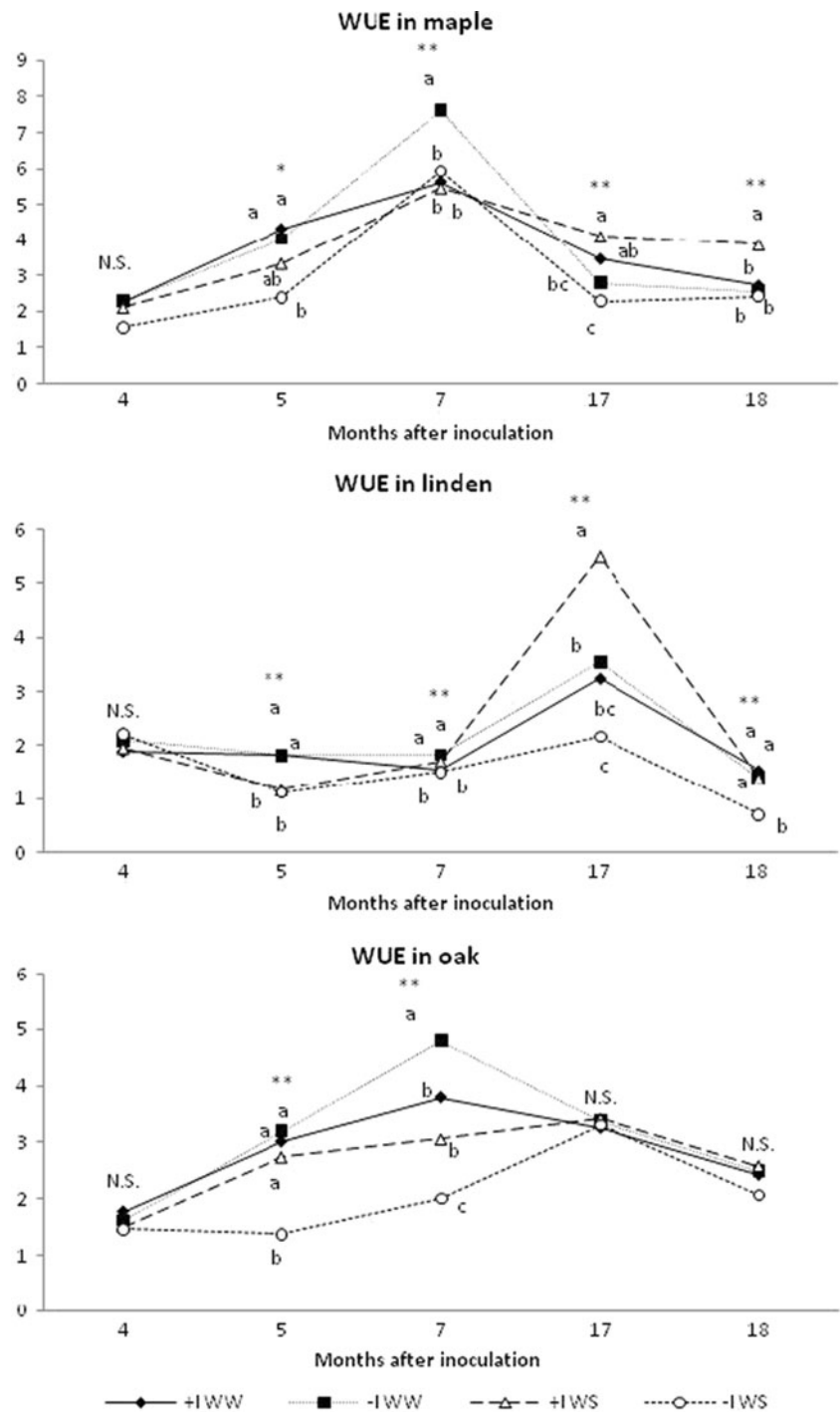
Species	Parameter	Inoculation (I)		Water regime (W)		Significance		
		+I	-I	WW	WS	I	W	I × W
Maple	V_{cmax}	29.1 a	16.0 b	33.0 a	12.1 b	*	**	ns
	J_{max}	72.5 a	36.5 b	74.4 a	34.6 b	*	*	ns
Linden	V_{cmax}	43.0 a	24.4 b	49.9 a	17.3 b	*	*	ns
	J_{max}	68.8 a	40.7 b	80.1 a	29.4 b	*	**	ns
Oak	V_{cmax}	80.1 a	60.3 b	81.9 a	58.5 b	**	**	ns
	J_{max}	98.6 a	75.8 b	96.9 a	77.5 b	**	*	ns

Different letters within the same line and factor indicate significant differences between +I and -I plants and between WW and WS plants

+I inoculated plants, -I non-inoculated plants, WW well-watered conditions, WS water shortage conditions

* $P \leq 0.05$; ** $P \leq 0.01$ (differences between treatments)

Fig. 2 Water use efficiency (WUE , $\mu\text{mol CO}_2/\text{mmol H}_2\text{O}$) of hedge maple (**a**), littleleaf linden (**b**), and pedunculate oak (**c**) inoculated (+I) or not inoculated (-I) with native mycorrhiza and grown in well-watered (*WW*) or water shortage (*WS*) conditions. * $P < 0.05$ and ** $P < 0.01$, significant differences among treatments per each sampling date. Different letters within the same sampling date indicate significant differences among treatments using Duncan's MRT



Although we did not identify fungal species in untreated plants, microscopy revealed that the ectomycorrhizal fungi associated with control seedlings were exclusively contact or short-distance exploration types, characterized by the absence of rhizomorphs (Agerer 2001). This mycorrhizal condition is typical of plants colonized by “nursery mycorrhiza” (Kutscheidt, personal communication). Therefore, it is likely that the symbiosis started during cultivation in the commercial nursery and that control plants were

representative of standard nursery stock infected by native nursery mycorrhiza. Despite a certain degree of colonization, there is evidence that mycorrhizae native to the nursery are generally unable to survive in the urban environment (Timonen and Kauppinen 2008). A controlled inoculation of small seedlings and saplings in the nursery with mycorrhizae of the outplanting site can be useful since it has been shown that some mycorrhizal fungi species may tolerate nursery conditions (Rincon et al. 2007). This may

Table 5 Effect of mycorrhizal inoculation, water regime, and their interaction on maximal quantum yield of PSII (F_v/F_m)

Species	Parameter	Inoculation (I)		Water regime (W)		Significance		
		+I	-I	WW	WS	I	W	I × W
Maple	F_v/F_m 2008	0.767 a	0.737 b	0.764 a	0.740 b	**	**	ns
	F_v/F_m 2009	0.780 a	0.739 b	0.770 a	0.751 b	**	**	ns
Linden	F_v/F_m 2008	0.773 a	0.752 b	0.769 a	0.756 b	**	*	ns
	F_v/F_m 2009	0.789 a	0.762 b	0.798 a	0.753 b	*	**	ns
Oak	F_v/F_m 2008	0.783 a	0.748 b	0.774 a	0.757 a	**	ns	ns
	F_v/F_m 2009	0.784 a	0.789 a	0.789 a	0.784 a	ns	ns	ns

Values are the average of the measurements obtained in three sampling days (2008) and two sampling days (2009). Different letters within the same line and factor indicate significant differences between +I and -I plants and between WW and WS plants

+I inoculated plants, -I non-inoculated plants, WW well-watered conditions, WS water shortage conditions

* $P \leq 0.05$; ** $P \leq 0.01$ (differences between treatments)

provide benefits to the nursery industry, including: (1) the possibility of nurserymen to grow plants with a lower requirements of fertilizers and water (Carpio et al. 2005) and (2) the possibility of nurserymen to sell plants with higher market value (Davies 2000) because they are already equipped with the best-performing mycorrhizal strains of the outplanting site.

In this experiment, inoculation with specific inocula selected in the urban environment increased the frequency of ECM roots in oak and the percentage of roots colonized by VAM in maple. Inoculation of linden plants with both ECM and VAM fungi increased root colonization by both fungal classes, even if ectomycorrhiza seem to have a greater role than VAM. Inoculation with ECM also altered the pattern of colonization since long-distance exploration structures were found in inoculated plants, but not in non-inoculated ones. The presence of rhizomorphs in inoculated roots was expected since they are typical of the genera *Boletus* (Giovannetti and Fontana 1985) and *Scleroderma* (Waller et al. 1983), and it can be relevant for long-distance exploration and water uptake, especially after outplanting. The overall data of the three species indicated that both the ECM and VAM fungal strains in the inoculum were able to compete with native microorganisms and form the symbiosis with the host plant (Requena et al. 1996). The degree of root colonization is determined by fungus–host compatibility and by environmental conditions. Many previous studies reported that drought has a significant effect on mycorrhiza development, increasing root colonization more often than decreasing it (Simpson and Daft 1990; Augé 2001; Entry et al. 2002). A controlled drought, achieved through the regulation of the amount of water available to plants during the nursery phase resulted in stocky, stress-resistant seedlings able to withstand environmental stresses after transplanting outdoors (Liptay et al. 1998). Shoot-to-root ratio and leaf area are the main parameters which can

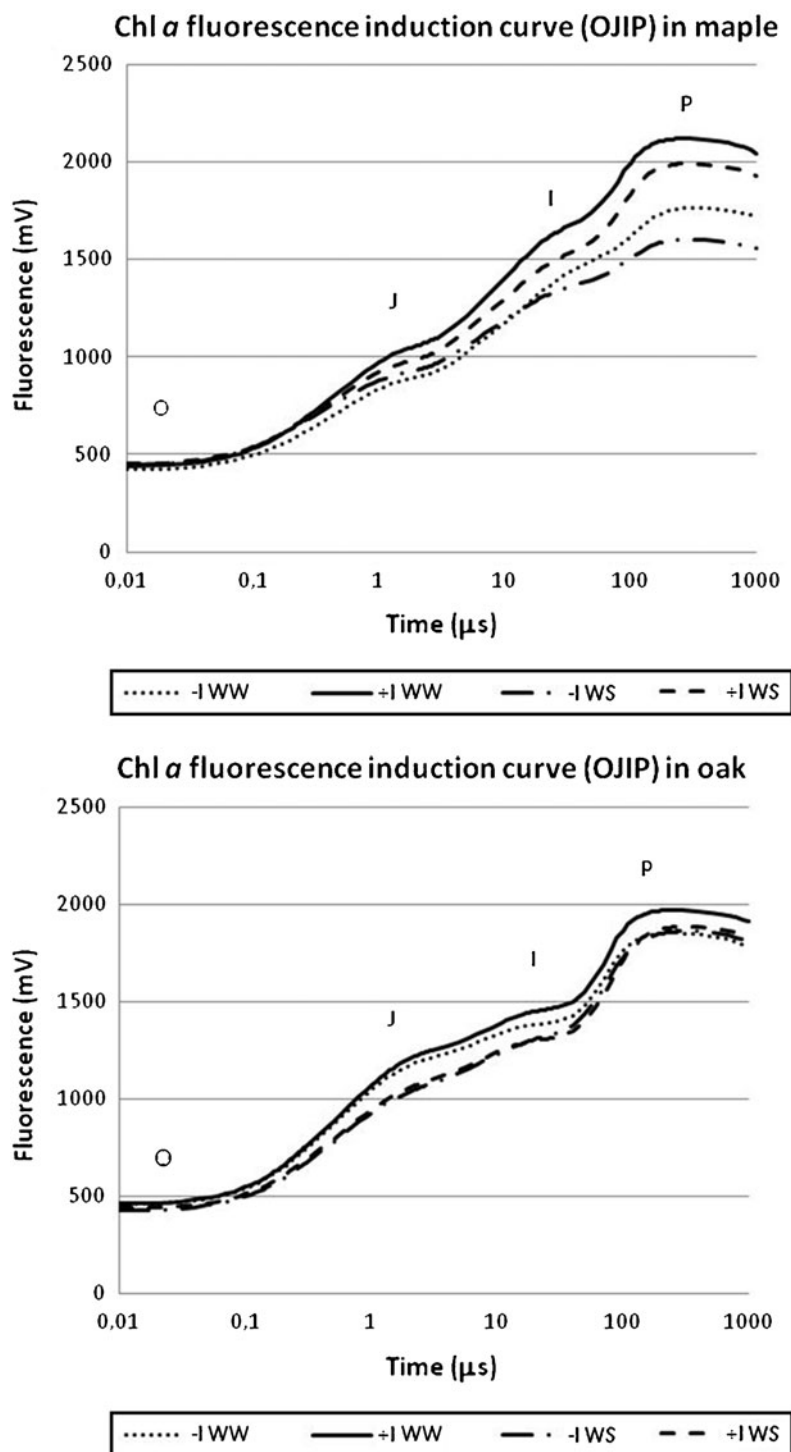
be modified with deficit irrigation practices (Bañón et al. 2006; Franco et al. 2006). We show here that water shortage in the nursery can also be an effective way to improve the efficiency of mycorrhizal inoculation, even if this effect depends on fungal and host species. In fact, water shortage increased root colonization in maple and oak, a vesicular–arbuscular and an ectomycorrhizal species, respectively. On the contrary, when ectomycorrhizal or the vesicular–arbuscular inocula were used in combination (linden), root colonization was not affected by water regime.

Neither the ectomycorrhizal nor the vesicular–arbuscular inocula enhanced plant biomass in nursery conditions. Similar results were found by other authors on several landscape trees (Martin and Stutz 1994; Gilman 2001; Ferrini and Nicese 2002; Wiseman and Wells 2009) and ornamental shrubs (Pinior et al. 2005). However, induction of greater stress tolerance, thus the possibility to grow nursery crops with lower resource input, rather than enhanced plant growth has been reported as the major benefit of mycorrhizal technology in plant production systems (Davies 2000).

Although plant biomass was unchanged, an increase in leaf area was found in the species inoculated with ECM (oak) and both ECM and VAM (linden), while leaf area was unaffected in the species inoculated with VAM (maple). Oak response was very fast and already clear 1 year after inoculation. Linden responded more slowly, and positive effects on leaf area were observed only in the second year. In maple, classified as a facultatively mycorrhizal species (Bundrett 2002) or weak mycotrophes (Lyr et al. 1992), leaf area was not affected by inoculation with VAM.

Lower root/shoot ratios are commonly observed in mycorrhizal plants (Smith 1982; Berta et al. 1995; Cruz et al. 2004). The decline in root/shoot ratio has been attributed to the ability of the fungus to substitute for the relatively greater amounts of root matter required by non-mycorrhizal

Fig. 3 Chlorophyll *a* fluorescence induction curve (OJIP curve) in inoculated (+I) and non-inoculated (–I) plants growing in well-watered (WW) and water shortage (WS) conditions

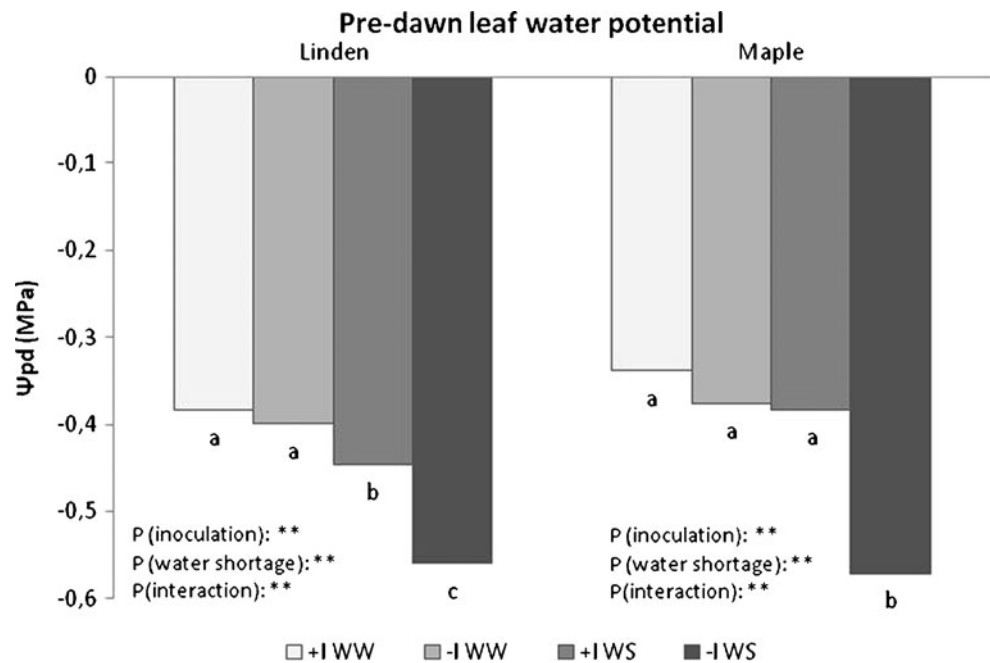


plants for P uptake, and this may allow mycorrhizal plants to allocate a greater proportion of assimilates to shoot production (Bethlenfalvey et al. 1985). In this experiment, root/shoot ratio was not affected by inoculation, as also found on *Fraxinus* grown in a high-P nursery soil (Lamar and Davey 1988).

Reductions of leaf gas exchange in water-limiting conditions have been widely documented in genera such as *Acer* and *Tilia* (Lemoine et al. 2001; Turnbull et al. 2002;

Aasamaa et al. 2004; Fini et al. 2009) and in oaks (Abrams 1990; Drunasky and Struve 2005). If inoculation is effective in reducing the impact of water stress, acclimated plants through mycorrhizal inoculation should be able to sustain higher leaf gas exchange under drought. In fact, in the second year after inoculation, mycorrhiza increased carbon assimilation in all species, and the effect was clearer in water shortage conditions.

Fig. 4 Effect of mycorrhizal inoculation, water regime, and their interaction on pre-dawn leaf water potential (Ψ_{pd} , MPa). Different letters within the same species indicate significant differences at $P \leq 0.01$. +I inoculated plants, -I non-inoculated plants, WW well-watered conditions, WS water shortage conditions



When differences in carbon assimilation are found, they can be caused by stomatal and/or mesophyll limitations. Stomatal limitations indicate the relative importance of stomatal conductance in restricting the supply of CO_2 to metabolism (Lawlor 2002), while mesophyll limitations are caused by the down-regulation of photosynthetic apparatus in response to water stress to match the available carbon substrate (Chaves et al. 2002). Inoculation with ECM of oak saplings resulted in lower stomatal limitations during drought, while L_s was unaffected by inoculation with VAM in maple and both VAM and ECM in linden. In these species, carbon assimilation was mainly limited by mesophyll factors during drought. The down-regulation of photosynthetic rates is an effective control mechanism for protecting the photosynthetic apparatus from photodamage under water stress (Valladares and Pearcy 1997). However, inoculation with ECM and/or VAM resulted in lower

mesophyll limitations to photosynthesis in all species, and this indicates a lower impact on inoculated plants than on the non-inoculated ones.

Mesophyll limitation is determined by changes in mesophyll conductance to CO_2 and by metabolic causes. The latter include Rubisco activity and Ribulose regeneration in the Calvin cycle (Lawlor 2002). We did not measure mesophyll conductance; thus, we could not evaluate the resistance to CO_2 diffusion from the leaf surface to the sites of carboxylation. Therefore, we will refer to the apparent rate of carboxylation (V_{cmax}) and apparent maximum electron transport rate contributing to ribulose 1,5-BP regeneration. Reduced V_{cmax} in water-stressed linden and maple indicates a reversible deactivation of Rubisco in response to altered stomatal conductance (Flexas et al. 2006). Decreased capacity of RuBP regeneration is an early response to drought which, according to

Table 6 Effect of mycorrhizal inoculation, water regime, and their interaction on hydraulic conductance of the soil-to-plant system (K_{sp}), of the soil-to-xylem system (K_{sx}), and of leaves (K_l)

Species	Parameter	Inoculation (I)		Water regime (W)		Significance		
		+I	-I	WW	WS	I	W	I × W
Maple	$K_{\text{sp}} \times 10^{-7}$ ($\text{m s}^{-1} \text{MPa}^{-1}$)	78.9 a	84.1 a	138.2 a	24.8 b	ns	**	ns
	$K_{\text{sx}} \times 10^{-7}$ ($\text{m s}^{-1} \text{MPa}^{-1}$)	169.0 a	164.1 a	281.2 a	52.0 b	ns	**	ns
	$K_l \times 10^{-7}$ ($\text{m s}^{-1} \text{MPa}^{-1}$)	178.2 a	140.5 a	274.8 a	43.9 b	ns	**	ns
Linden	$K_{\text{sp}} \times 10^{-7}$ ($\text{m s}^{-1} \text{MPa}^{-1}$)	155.0 a	115.6 b	234.9 a	35.6 b	*	**	ns
	$K_{\text{sx}} \times 10^{-7}$ ($\text{m s}^{-1} \text{MPa}^{-1}$)	402.9 a	351.0 a	621.5 a	132.4 b	ns	*	ns
	$K_l \times 10^{-7}$ ($\text{m s}^{-1} \text{MPa}^{-1}$)	327.1 a	195.9 b	456.3 a	66.7 b	*	**	ns

+I inoculated plants, -I non-inoculated plants, WW well-watered conditions, WS water shortage conditions

* $P \leq 0.05$; ** $P \leq 0.01$ (differences between treatments)

the Farquhar model, is due to a decreased electron transport rate (Medrano et al. 2002). Regardless of water regime, plants inoculated with either VAM or ECM, or both of them, had higher apparent rate of carboxylation and apparent rate of RuBP regeneration when compared to non-inoculated plants. This finding was also confirmed by higher maximal efficiency of PSII photochemistry (F_v/F_m) shown by inoculated plants. Similar to that observed by other authors, differences in the polyphasic chlorophyll *a* transient between inoculated and control plants started in the JI phase and ended in the P step (Piniór et al. 2005). OJIP test provided evidence that water regime and inoculation induced changes in the redox state of plastoquinone (JI phase) and of the acceptor side of PSI (IP phase; Schansker et al. 2006; Tóth et al. 2007). The differences observed between inoculated and control plants in parameters related to PSII phytochemistry, even in well-watered conditions, may be attributed to the sink strength of the fungi (Lehto and Zwiazek 2011).

There may be several causes that explain why inoculated plants did not accumulate greater biomass despite better maintenance of photosystem II integrity and functionality and higher assimilation rate. First, one of the main aims of this study was to evaluate the effect of mycorrhiza and water stress on the functionality of the photosynthetic apparatus. For this reason, gas exchange was measured at saturating light intensity, which is probably higher than the natural light intercepted by leaves. Second, mycorrhiza can extract an estimated 5–20% of labile photosynthates from colonized plant roots (Eissenstat et al. 1993), and this may have reduced carbon availability for growth.

Plants must make a compromise between the photosynthetic CO₂ uptake and transpirational water loss, especially when water availability is low. Therefore, in addition to the effects of inoculation on carbon assimilation, changes in water flow through plants as induced by different mycorrhizal fungi deserve attention. Increases in transpiration following inoculation with vesicular–arbuscular and ectomycorrhizal fungi have been frequently reported and reviewed by others (Dixon et al. 1980; Augé 2001; Navarro et al. 2009). When mycorrhizal plants show higher transpiration, it may be related to the increased water uptake by the mycorrhizal root system, delayed stomatal closure in response to decreasing soil water potential, and to increased soil-to-plant hydraulic conductance (Allen 1982; Augé et al. 1986; Ebel et al. 1994, 1996).

Increased plant hydraulic conductance can be achieved by increasing either soil-to-xylem or xylem-to-leaf conductances, or both of them. There is growing evidence that both ecto- and endomycorrhizal association can drastically affect root hydraulic conductance, but no effect of mycorrhiza on leaf conductance has been reported so far (Lehto and Zwiazek 2011). Consistent with the higher transpiration

observed in linden 17 and 18 months after inoculation, we found an increase in soil-to-plant hydraulic conductance in inoculated linden. This increase was caused by higher leaf conductance in inoculated than in non-inoculated lindens during water shortage rather than by changes in root-to-xylem conductance. Although we made no direct measurement of abscisic acid (ABA), increased xylem-to-leaf conductance may be due to lower amount of ABA in the xylem sap in inoculated plants, as previously observed by others (Duan et al. 1996; Goicoechea et al. 1997). On the contrary, when only VAM were inoculated, as in maple, no effect on plant conductance was found. However, this may be due to the weak mycotrophic behavior of maple rather than to a characteristic of VAM symbiosis. The lack of effectiveness of inoculation with VAM or both ECM and VAM in increasing root to xylem conductance has been previously found in experiments where mycorrhiza did not induce changes in plant growth or phosphorus nutrition (Koide 1993).

Since plant conductances are mainly determined by plant water status, any change in plant water relations induced by inoculation may be crucial in determining the response of leaf gas exchange during water stress. Leaf water potential was not affected by inoculation in well-watered conditions (Davies et al. 1993; Ebel et al. 1994; Bryla and Duniway 1997). In any case, at lower soil moistures, leaf water potential declined less in plants inoculated with VAM or both VAM and ECM. Previous literature explained the less negative leaf water potential of mycorrhizal plant as a consequence of fungus-mediated tolerance and avoidance mechanisms which result in increased plant tolerance to drought (Porcel and Ruiz-Lozano 2004).

In conclusion, controlled mycorrhization in the nursery did not enhance growth of container-grown maples, lindens, and oak, but provided several physiological benefits such as the maintenance of less negative leaf water potential, higher apparent carboxylation rate, higher RuBP regeneration, and higher quantum yield of PSII under water shortage. The overall data suggest that inoculated plants were better able to maintain physiological activity of shade tree species during water stress when compared to non-inoculated plants and thus can be considered more drought-tolerant. However, the effects of nursery inoculation and acclimation to water shortage conditions on post-transplant performance need to be evaluated in future research.

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