Results of a long-term project using controlled mycorrhization with specific fungal strains on different urban trees

Abstract

Several research projects have been undertaken in the past years to identify the effects of mycorrhiza, which include increased water and nutrient uptake, and protection against drought, salinity, heavy metals and pathogens (Augè, 2001) on widely planted shade trees. However, most experiments were carried out under conditions different to those commonly found in the urban environment. The aim of this work was to investigate the effect of different strains of mycorrhizae-forming fungi specifically selected for the urban environment in different situations (i.e. urban and historical parks, parking lots, boulevards) usually found in cities all over the world. The project began in 2006 and was carried out on several of the most widely planted shade tree species of different ages ranging from newly planted to mature trees located in our historical parks. Trees were inoculated with specific mycorrhyzal inoculi according to species and environmental conditions. Different growing conditions were tested ranging from trees growing in a parking lot, to trees growing in historical or peri-urban parks. Results obtained to date have been variable according to species and environmental conditions. Some of the test species (i.e. *Celtis australis*) responded quickly to mycorrhizal fungi that were extremely effective in increasing plant growth and leaf gas exchange. Other species (i.e. *Tilia* spp.) showed a different response according to plant age and planting site. Other species (i.e. *Fraxinus excelsior*) had a slow response to mycorrhyzal inoculation. In general there has been a positive (sometimes very strong) response to mycorrhizal inoculation and further data will be harvested in 2011.

Introduction

Keywords:

tree physiology, growth, transplanting, photosynthesis

Mycorrhizae-forming fungi are ecologically significant because they form relationships in and on the roots of a host plant in a mutualistic association. The host plant provides the fungus with soluble carbon sources, while the fungus provides the plant with several benefits including enhanced nutrient, especially phosphorus, uptake (Yao *et al.,* 2001; Habte, 2006); protection against drought through increased water use efficiency and enhanced root exploration of the available soil volume (Espeleta *et al.,* 1999; Augè, 2001; Kaya *et al.*, 2003); and reduction in disease incidence (Thygesen *et al.*, 2004), pathogen development (Cordier *et al.,* 1996) and disease severity (Matsubara *et al.,* 2001). It has been reported that mycorrhiza protect the host plant from heavy metals (Smith and Read, 1997; Joner *et al.,* 2000) and salinity by protecting cell membrane integrity through higher root accumulation of P and Ca^{2+} and by increasing the efficiency of sodium-excluding mechanisms in infected roots (Mancuso and Rinaldelli, 1996; Rinaldelli and Mancuso, 1996). However, the urban environment is markedly different from natural and forest environments where mycorrhizal fungi have evolved and adapted and, consequently, the ecological distribution of fungi is probably altered in an urban environment. Recent work analysed mycorrhizal colonization patterns of *Tilia* grown in the urban, nursery and forest environment (Timonen and Kauppinen, 2008). They showed that healthy street and forest trees had higher number of mycorrhiza morphotypes than unhealthy urban trees. Surprisingly, none of the mycorrhizal fungi found in the nursery were found in the urban environment, suggesting that the nursery genotypes are either not adapted to street conditions or they are outcompeted as transplanted trees establish a more mature mycorrhizosphere (Timonen and Kauppinen, 2008). Since drought, use of de-icing salts, lack of nutrients and attack from pathogens are among the main causes of failure of urban trees (Fini and Ferrini, 2007), the inoculation of urban trees with selected, native, competitive and effective mycorrhiza may enhance tree growth and survival in the urban environment. However, studies

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regarding mycorrhizal inoculation of urban trees in Europe are few. The aim of this project was to evaluate the effect of inoculation with selected native mycorrhizal fungi on trees growing in a street environment, in a parking lot and in an historical and peri-urban park. The results are a part of a long-term research project started in 2006 with an initial inoculation and that will conclude in 2011.

Material and methods

Selection, propagation and distribution of the mycorrhizal fungi

Selection, multiplication and distribution of the specific mycorrhizal inocula were as described in Fini *et al.* (2011). Briefly, five to seven healthy mature trees growing in the urban and peri-urban environment were selected and fine roots were sampled by digging holes around the tree. Holes were deep and wide as required to harvest a sufficient amount of fine, absorbing root. Trees were selected on the basis of the following criteria: 1) health; 2) age; 3) same species as the trees to be inoculated; 4) similar environmental conditions to those of the site where the inoculum had to be distributed. Each sample weighed approximately 500 grams of roots + soil. Root samples were analysed at the MycoMax laboratory (MykoMax Gmbh, Wuppertal, Germany). Mycorrhizal species were isolated and multiplied in a greenhouse in non-sterile conditions following the procedure developed by MycoMax in agreement with German FLL standards for mycorrhiza inoculation. Criteria for the selection of mycorrhizal fungi were: 1) frequency of mycorrhizal root tips (ecto-) or intensity of root colonization (VAM); 2) structure and vitality of the Hartig net (ecto-) or arbuscules (VAM); 3) phosphatase activity (VAM). After at least eight months of culture on living and viable roots, roots containing fungal mycelium were harvested and mixed with montmorillonite clay and a hydro-gel to maximize durability. The fungal inoculum was distributed within one month of its production. Three holes exposing the absorbing roots of the tree to be inoculated were dug for each 10 cm of stem diameter (measured at 1.3 m trunk height) of the tree to inoculate. 125 ml of product were placed in each hole to ensure contact between fungal mycelium and absorbing tree roots. Holes were quickly re-filled with a shovel.

Container-grown trees in nursery production

A total of 80 two-year-old hedge maples (*Acer campestre* L.), 80 littleleaf lindens (*Tilia cordata* Mill.) and 80 pedunculate oaks (*Quercus robur* L.) were potted in 3 litre containers using a peat:pumice (3:1) substrate amended with 3 kg m^{-3} dolomite to neutralize pH. A reduced dose (1 kg m^{-3}) of a controlled release fertilizer (Ficote®, 15–3, 5–10, 8–9 months formulation, Scotts, Marysville, OH) was used in this experiment to avoid an excessive soil chemical fertility which may decrease mycorrhizal colonization. Container capacity, wilting point and effective water holding capacity of the substrate was determined using the gravimetric method described by Sammons and Struve (2008). 40 plants per species were inoculated with specific mycorrhizal fungi (ECM in oak, VAM in maple and both ECM and VAM in linden). Inoculation was done on March 2008 using 25 ml of inoculum per plant. Plants were either irrigated daily in order to restore container capacity, well watered (WW) or irrigated daily to 30% of container water capacity, water shortage (WS). The experimental was a randomized block design with 6 blocks and 5 plants per species and treatment in each block.

Trees from the nursery to the landscape: plant material and growing conditions

A total of 48 plants (14–16 cm circumference) were selected in Lappen Nurseries (Nettetal, Germany) in winter 2007. In April 2007, 24 plants were inoculated ($+I_N$) with specific ecto- and endomycorrhizal fungi selected in Milan urban area and the remaining 24 plants were not ($-I_N$). In May 2008, all plants were root pruned. Then, plants were grown in the nursery until early spring 2010, when they were moved to Milan. At transplanting, half of the plants were inoculated with the same fungi as in 2007 ($+I_T$) and the remaining half were not ($-I_T$). Therefore, four treatments were compared: 1)$+I_N+I_T$: plants inoculated both in the nursery and at transplant; 2) $+I_N-I_T$: plants inoculated in the nursery but not at transplant; 3) $-I_N+I_T$: plants inoculated only at transplant; 4)$-I_N-I_T$: control plants (never inoculated). Plants were arranged with a factorial randomized block design with 8 blocks and 4 plants per block.

Young trees in urban parks

In November 2005, 62 pedunculate oaks (*Quercus robur*; 10–12 cm circumference) were planted in two rows in an urban park in San Donato Milanese (Milan, Italy). Distance between plants was 8 m within the row and 8 m between the rows. 24 trees were inoculated with selected specific ecto-mycorrhizal fungi and 24 were not inoculated. Inoculation was performed in November 2006, approximately one year after planting. Trees were arranged in a randomized block design with 3 blocks and 8 plants per treatment within each block. 14 remaining oaks were used to separate, on the row, inoculated and control plants, to reduce the risk of unwanted contamination on non-inoculated plants.

Trees in parking lots: plant material and growing conditions

In November 2005, 24 European hackberry (*Celtis australis*; 14–16 cm circumference) were planted in a parking lot in San Donato Milanese (Milan, Italy). Trees were planted in a planting hole with an unpaved surface of about 0.5 m^2, surrounded by asphalt and concrete. Trees were arranged in a randomized complete block design with 6 blocks and 2 plants per treatment within each block. 12 trees were inoculated with species-specific, native strains of ectomycorrhizal fungi, and 12 trees were not inoculated and acted as controls. Inoculation was undertaken in November 2006, approximately one year after planting.

Street trees: plant material and growing conditions

In spring 2004, 20 European ashes (*Fraxinus excelsior* 'Westhof's Glorie'; 20–25 cm circumference) were planted along a street characterized by high traffic and pollution, located in Florence (Italy). The size of the planting hole was about 1 m^2. Trees were planted in a randomized complete block design with 5 blocks. 10 trees were inoculated with species-specific strains of endomycorrhizal fungi and 10 trees acted as control. Inoculation was in April 2006.

Trees in a historical park: plant material and growing conditions

In autumn 2006, 14 mature European linden (*Tilia x europaea*; 170–220 cm circumference) and 14 mature horse chestnut (*Aesculus hippocastanum*; 120–160 cm circumference) were selected in a historical park located in the city-centre of Milan. 14 additional newly planted *Tilia x europaea* (18–20 cm circumference) and 14 *Aesculus hippocastanum* (20–25 cm circumference) were selected in the same location. Trees were planted in a heavily compacted soil. The experimental set-up was a complete randomized design using a single tree per replicate and 7 replicates. 7 mature and 7 young trees of each species were inoculated with selected native and species-specific strains of both ecto- and endomycorrhiza (linden) or with endo-mycorrhizal fungi (horse chestnut). Inoculation was in November 2006.

Measurements of tree growth and vitality

One year after inoculation, a sample of fine root + soil was harvested from inoculated and control trees. Samples were harvested from one (historical park, street trees) or two (nursery) plants per treatment and replication. Roots were carefully separated from the soil and cut into 1 cm long pieces. Frequency of ectomycorrhizal roots was measured on 200 root tips as the ratio of mycorrhizal root tips to total root tips (Newton and Pigott, 1991). To evaluate VAM colonization, roots were stained using 0.05% Trypan blue in lactoglycerol (Koske and Gemma, 1989). Percentage of root colonization was measured by counting cross-hair intersections using a stereomicroscope (McGonigle *et al.,* 1990).

Biomass of container-grown plants was determined after two years from inoculation (2009). To measure biomass, plants were cut at the root flare, roots were cleaned with a flush of compressed air and leaves were excised from stems. Roots, stems and leaves were then oven-dried at 70°C for 72 hours and weighted separately to determine dry weight. Biomass of field-grown trees was estimated measuring shoot and diameter growth. According to the different experiments, the following parameters were measured during the entire duration: Shoot growth was on 20 shoots per treatment per replicate. Stem diameter was measured at 1.3 m trunk height.

Leaf gas exchange was generally measured on three fully expanded leaves per treatment and block/replicate with an infrared gas analyser (Ciras-2, PP-System, Hertfordshire, UK). Measures were taken at saturating (1300 mol m^{-2} s^{-1}) light intensity, ambient temperature and 360 ppm CO_2. Water use efficiency was calculated as the A to E ratio (Fini *et al.,* 2009). Chlorophyll fluorescence was measured with a portable Plant Efficiency Analyzer (Hansatech Instruments Ltd, King's Lynn, UK) on the same leaves as gas exchange. Fluorescence values were obtained after adapting leaves to darkness for 30 min by attaching light-exclusion clips to the leaf surface of whole trees. Upon the application of a saturating flash of actinic light (3000 mol m^{-2} s^{-1} for 1 sec), 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 (Fv/Fm).

Chlorophyll content was measured two times during the growing season in 2007 (only on *Fraxinus excelsior*) and 2008 with a SPAD-meter (Konica Minolta Holding Inc., Tokyo, Japan). Nine measurements per treatment per replicate were undertaken. Readings were taken in the medial section of the lamina, taking care not to include leaf veins in the measurement chamber.

Statistics

All data were analysed with one- or two-way ANOVA using the SPSS statistical package for Windows (SPSS Inc., Chicaco, IL, USA). Differences between means were determined using Duncan's Multiple Range Test.

Results and discussion

Root colonization in inoculated and control plants

Inoculation with selected mycorrhiza increased root colonization in container-grown maples, lindens and oaks (Table 1). Even if control trees were not inoculated, some mycorrhiza were also found on their roots. Morphotyping of control plant roots classified these mycorrhiza as 'nursery mycorrhiza' (Fini *et al*., 2011). It is common to find 'nursery mycorrhiza' on nursery stock, and in any case these fungal species have been reported to be unable to thrive and provide benefits to the host tree in urban conditions (Timonen and Kauppinen, 2008). Similarly, inoculation of oak trees in an urban park increased the frequency of mycorrhizal root tips. This indicates the ability of selected fungal strains to compete with native microorganisms and efficiently form a symbiotic relationship, even when the native mycorrhizal population is well developed (control had 76% colonized root tips; Table 1). The endomycorrhizal inoculum developed for ash was found to be effective in street environments, even in those characterized by a well-developed native mycorrhiza population. This is important because native mycorrhizal populations are likely to provide lower benefits to plants than selected fungal strains. If fungi in the inoculum are quickly outcompeted by native microorganisms or their infection is slowed down by adverse environmental conditions, colonization of the host plant is reduced and little or no benefit can be expected from inoculation. Possibly this was the case for the newly planted lindens and horse chestnuts in a historical park where poor soil conditions such as heavy soil compaction and lower carbon availability for the mycorrhizal fungus due to lower carbon assimilation (thus lower availability of C to support fungal growth and activity) of newly planted trees resulted in a low inoculum efficacy (Nadian *et al*., 1997). When the same ECM (linden) and VAM (horse chestnut) inocula were used on mature, established trees, root colonization was increased (Table 1).

Table 1 Percentage of colonization by ectomycorrhizal and endomycorrhizal fungi in inoculated and non-inoculated tree species planted in the nursery or in different urban sites.

Site	Species	Treatment	% colonization (ECM)	% colonization (VAM)
Nursery (in container)	*Acer campestre*	Inoculated	-	53%
		Control	-	24%
		P	-	**
	Tilia x europaea	Inoculated	81%	17%
		Control	59%	10%
		P	**	*
	Quercus robur	Inoculated	80%	-
		Control	41%	-
		P	**	-
Urban park	*Quercus robur*	Inoculated	85%	-
		Control	76%	-
		P	**	-
Street trees	*Fraxinus excelsior*	Inoculated	-	81%
		Control	-	71%
		P	-	*
Historical park	*Aesculus hippocastanum* (newly planted)	Inoculated	-	59%
		Control	-	51%
		P	-	n.s.
	Aesculus hippocastanum (mature trees)	Inoculated	-	76%
		Control	-	63%
		P	-	**
	Tilia x europaea (newly planted)	Inoculated	45%	37%
		Control	44%	28%
		P	n.s.	n.s.
	Tilia x europaea (mature trees)	Inoculated	49%	39%
		Control	36%	32%
		P	*	n.s.

Data were collected one year after inoculation. * and ** indicate significant differences between treatments within the same species and planting site at P<0.05 and P<0.01

Container-grown trees in nursery production

Inoculation with specific mycorrhiza did not enhance biomass accumulation of maple, linden and oak saplings growing in containers (Table 2). Plants growing in water-stressed conditions had lower leaf, stem and root (except for oak) dry weights than well-watered plants of the same species, regardless of whether inoculated or not (Table 2). Similar results were found by other authors on several landscape trees (Gilman, 2001; Wiseman and Wells, 2009). Induction of greater stress tolerance and therefore the possibility to grow nursery crops with lower resource input has been reported as the major benefit of mycorrhizal technology in plant production systems (Davies, 2000). In 2009, water-stressed inoculated plants of maple and linden showed higher carbon assimilation and similar transpiration rates and therefore higher water use efficiency than water-stressed control plants (Table 3). Water-stressed inoculated oak had higher transpiration and similar carbon assimilation and water use efficiency than controls. In well-watered conditions, differences between inoculated and control maple and linden were not significant (except for assimilation in maple). Data indicated that in optimal conditions the benefits of mycorrhiza are not always conclusive. The inoculation-induced increase in photosynthetic rate and water use efficiency may become clearer under stress conditions and this may play a major role in determining plant survival when plants are moved from the optimal growing conditions of a nursery to the suboptimal or stressful conditions of an urban environment.

Trees from the nursery to the landscape

Inoculation had no effect on stem diameter growth during the nursery period (2007–2009) and in the first year after planting into the landscape (2009–2010: Table 4). In 2007, inoculation had no effect on shoot growth. In May 2008, roots were pruned in the nursery according to best management practices and this resulted in some degree of stress to linden trees, as shown by a large decrease in shoot growth in 2008 compared to 2007. When above-ground growth was limited by root pruning, inoculation with

Table 2 Effects of inoculation, water stress and their interaction on leaf, stem and root dry weights (DW, g) in inoculated and control *Acer*, *Tilia* and *Quercus* growing in containers in well-watered (WW) or water shortage (WS) conditions.

2009	*Acer*			*Tilia*			*Quercus*		
	Leaf DW	Stem DW	Root DW	Leaf DW	Stem DW	Root DW	Leaf DW	Stem DW	Root DW
Effect of inoculation									
Mycorr.	35.1	92.9	120.3	19.9	64.8	75.6	30.0	84.3	75.2
Control	32.3	90.2	116.4	19.3	63.3	70.7	28.0	83.0	88.7
P	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Effect of water stress									
WW	38.1	111.5	142.4	22.9	77.6	89.8	34.2	111.4	88.1
WS	29.3	71.6	94.3	16.3	60.5	56.6	24.0	55.6	75.9
P	*	**	**	**	**	**	**	**	n.s.
Inoculation x water stress									
P	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

* and ** indicate significant differences between treatments within the same species at P<0.05 and P<0.01.

Table 3 Effects of inoculation, water stress and their interaction on carbon assimilation (A, μmol m^{-2} m^{-1}), transpiration (E, mmol m^{-2} m^{-1}) and water use efficiency (WUE, μmol CO_2/mmol H_2O) in inoculated and control *Acer*, *Tilia* and *Quercus* growing in containers in well-watered (WW) or water shortage (WS) conditions.

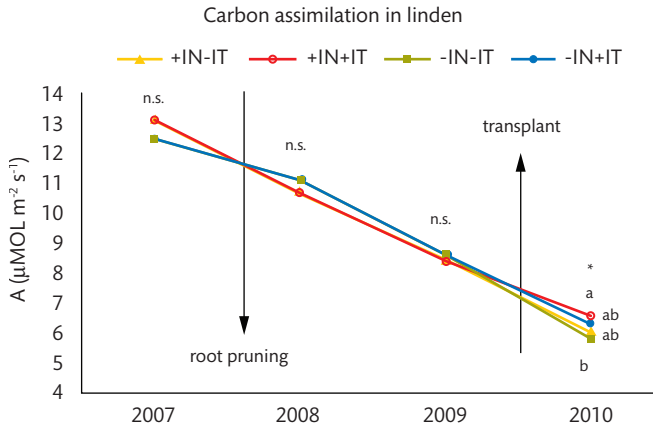
2009	*Acer*			*Tilia*			*Quercus*		
	A	E	WUE	A	E	WUE	A	E	WUE
Myco. WW	9.24 a	3.07 a	3.01 b	7.12 a	3.55 a	2.01 ab	10.90 a	3.99 a	2.74
Contr. WW	7.64 b	2.87 a	2.66 b	6.15 a	3.17 a	1.94 ab	11.43 a	4.11 a	2.78
Mico. WS	4.27 c	1.05 b	4.08 a	3.38 b	1.33 b	2.57 a	8.08 ab	2.57 b	3.15
Contr. WS	1.60 d	0.62 c	2.58 b	1.11 c	0.75 b	1.50 b	5.09 b	1.73 c	2.95
P	**	**	**	**	**	**	**	**	n.s.

Data are the average of two samplings done in 2009. Different letters within the same column indicate significant differences between treatments at P<0.01.

selected mycorrhiza resulted in significantly longer shoots than with untreated plants (Table 4). One year after root pruning (2009), shoot growth recovered to levels similar to 2007 and no significant differences between treatments were recorded. Lindens were transplanted into the urban environment in spring 2010. Transplant stress occurred in the following growing season and greatly reduced shoot growth (Table 4). Again, when stress occurred, an inoculation-induced increase in shoot growth was found. In particular, shoot growth was higher in plants inoculated in the nursery and both in the nursery and at planting when compared to control and plants inoculated only at planting (Table 4).

Carbon assimilation was not affected by inoculation with specific mycorrhiza during the nursery phase, even after a root pruning treatment (Figure 1). After planting in the landscape, plants inoculated both in the nursery and at planting showed higher carbon assimilation than non-inoculated control plants. Inoculating plants both in the nursery and at transplanting possibly contributed to a greater root colonization by mycorrhizal fungi, which resulted in higher photosynthetic rates. Transpiration, stomatal conductance and water use efficiency were little affected by mycorrhizal treatment, during both the nursery period and after transplanting (data not shown). Therefore, we can speculate that trees inoculated both in the nursery and at planting had a higher photosynthesis on a plant-scale basis (higher A) and this may have contributed to greater shoot growth. Previous research in this area has shown that whole-plant photosynthetic rate under resource-unlimited conditions is proportional to shoot growth and leaf area (de Palma *et al.*, 2004).

Figure 1 Carbon assimilation (A, µmol m^{-2} s^{-1}) in linden inoculated in the nursery ($+I_N$-I_T), in the nursery and at transplanting ($+I_N$+I_T), not inoculated ($-I_N$-I_T) and inoculated only at transplant ($-I_N$+I_T). Different letters within the same sampling date indicate significant differences at P<0.05.



Young trees in an urban park

Stem diameter growth of newly planted pedunculate oak (*Quercus robur*) was not affected by inoculation with selected specific ectomycorrhiza throughout the experiment (Table 5). Shoot growth was increased by inoculation in the first growing season after inoculation, although shoot growth was very low due to transplant stress (Table 5). In 2008 and 2009 shoot growth was significantly greater in inoculated oaks when compared to control, which indicates a beneficial influence of mycorrhizal inoculation regarding the establishment of oak trees. Even after establishment, differences between treatments were confirmed and inoculated plants showed higher shoot growth in both 2008 and 2009 compared to control plants (Table 5). SPAD values were higher in inoculated plants in both years. Recent papers on some woody species showed that SPAD readings are highly correlated to leaf chlorophyll content (measured using traditional destructive methods) (R^2>0.82), leaf carotenoids (R^2>0.82) and leaf N-content (R^2>0.53) (Luh *et al.*, 2002; Percival *et al.*, 2008). Therefore, higher SPAD readings in treated leaves may indicate a higher nutritional status of inoculated oaks than control ones when planted into an urban park. After September 2008, carbon

Table 4 Effect of inoculation in the nursery phase and/or at planting with specific mycorrhiza on linden trees growing in the nursery (2007–2009) and after transplant in the landscape (2010). In 2008 trees were root pruned to prepare them for transplant.

Inoculation		ΔØ (cm)			Shoot growth (cm)			
Nursery	Transplant	07/08	08/09	09/10	2007	2008	2009	2010
$+I_N$	$-I_T$	0.58	0.74	0.20	51.89	9.78 a	45.75	8.21 a
	$+I_T$			0.33				7.81 a
$-I_N$	$-I_T$	0.47	0.71	0.30	56.08	6.56 b	42.55	6.28 b
	$+I_T$			0.35				5.84 b
P		n.s.	n.s.	n.s.	n.s.	**	n.s.	**

Different letters within the same column indicate significant differences between treatments at P<0.01.

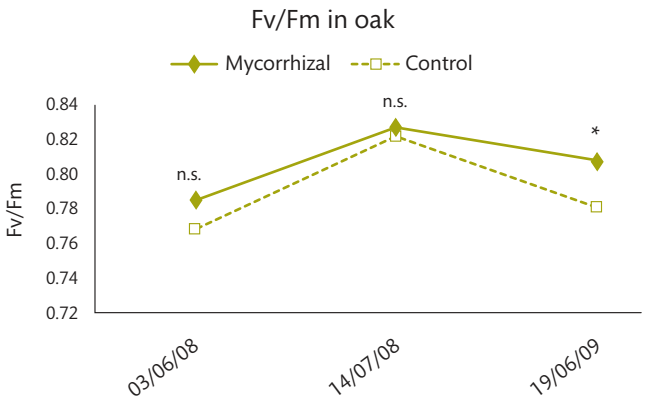
Table 5 Effects of inoculation with selected ectomycorrhiza on diameter and shoot growth and on chlorophyll content of *Quercus robur* planted in an urban park.

Quercus robur	ΔØ (cm)			Shoot growth (cm)			Chlorophyll content (SPAD)	
	06/07	07/08	08/09	2007	2008	2009	June 2008	Sept. 2008
Mycorrhiza	0.70	1.30	1.43	13.52	68.22	71.4	43.2	43.6
Control	0.52	1.27	1.27	4.13	41.38	48.8	39.1	39.8
P	n.s.	n.s.	n.s.	**	**	**	*	**

* and ** indicate significant differences between mycorrhizal and control trees of the same species at P<0.05 and P<0.01.

assimilation was generally higher in inoculated oaks, even if significant differences were found only on 18 May 2009 (Figure 2, left). Also, when significant differences were found, inoculated plants had higher WUE than non-inoculated ones (Figure 2, right). Higher WUE in plants inoculated with selected fungal species were also found in other work and were attributed to stomatal and nutritional effects induced by inoculation (Guehl and Garbaye, 1990; Guehl *et al.*, 1990; Dunabeitia *et al.*, 2004). Taking into consideration that WUE is one of the main growth determining factors in potentially harsh sites such as a urban environments, results obtained in the third growing season after inoculation suggest that ectomycorrhizal colonization may increase long-term tolerance to water stress. Fv/Fm was not affected by inoculation in 2008, and Fv/Fm was higher than 0.80 in both treatments, a value indicative of healthy plants (Percival, 2005; Figure 3). In 2009, inoculated plants had higher Fv/Fm than non-inoculated plants. The higher maximum yield of PSII (Fv/Fm values) measured in 2009 in inoculated plants may explain the higher gas exchange values found in treated oaks in 2009.

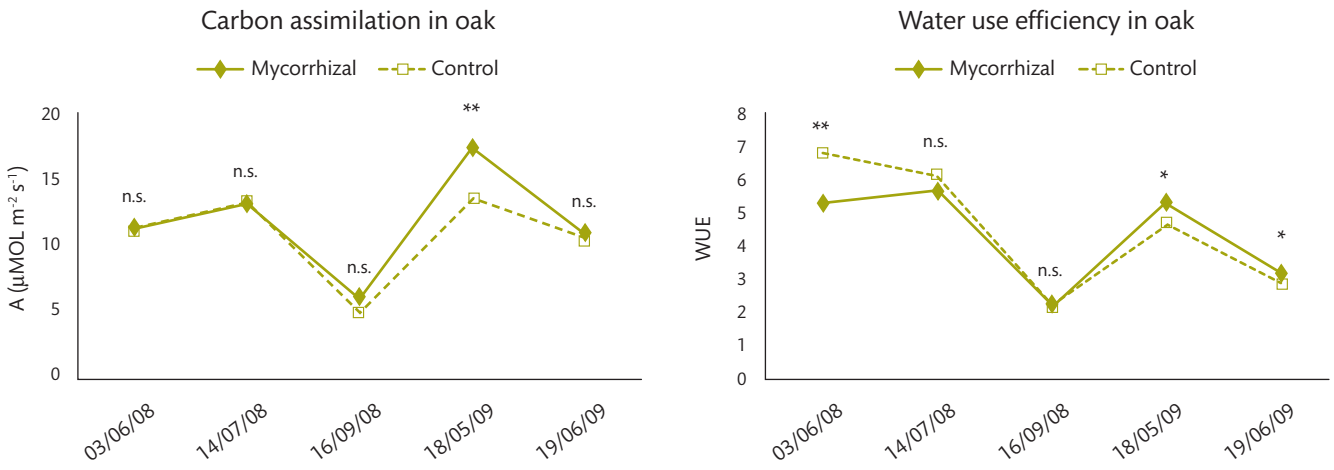
Figure 3 Maximal quantum yield of photosystem II (Fv/Fm) in inoculated and non-inoculated pedunculate oaks planted in an urban park. * indicates significant differences between mycorrhizal and control trees within the same sampling date at P<0.05.



Street trees and trees growing in a parking lot

Inoculation with local strains of species-specific mycorrhizal fungi increased stem diameter growth in young, newly planted European hackberry, growing in a parking lot

Figure 2 Carbon assimilation (A, μmol m^{-2} s^{-1}, left) and water use efficiency (WUE, mol CO_2/mmol H_2O, right) in inoculated and non-inoculated pedunculate oaks planted in an urban park. * and ** indicate significant differences between mycorrhizal and control trees within the same sampling date at P<0,05 and P<0,01.



(Table 6). Significant differences in stem diameter annual growth between inoculated and non-inoculated plants were found both in the first and the second year after inoculation, but not in the third. Inoculation with German, species-specific endomycorrhiza for *Fraxinus excelsior* failed to increase diameter growth in ash trees growing along a road (Table 6). Effect of mycorrhiza on shoot growth was highly significant in 2007 and 2008 in ash and in 2007, 2008 and 2009 in European hackberry (Table 6). Mycorrhizal inoculated ashes had 48% and 42% longer shoots than control trees in 2007 and 2008, respectively. Shoots of mycorrhizal inoculated hackberries were 55%, 98% and 80% longer than those of non-inoculated control trees in 2007, 2008 and 2009, respectively.

Mycorrhizal inoculation increased carbon assimilation and water use efficiency of hackberry in all sampling dates, except in September 2008 (Figure 4). Five months after inoculation (September 2006), no difference in carbon assimilation and water use efficiency was found between mycorrhizal inoculated and non-inoculated control ashes (Figure 5). In 2007, mycorrhizal inoculated ashes had both higher assimilation and water use efficiency than non-inoculated plants, with significant differences confirmed in 2008 (Figure 5). Therefore, possibly, the inoculation-induced increase in WUE allowed mycorrhizal trees to fix more carbon dioxide per unit of transpired water, thus giving greater carbohydrate availability for growth and defence. The maximal quantum yield of photosystem II (Fv/Fm) is a widely used index for measuring plant vitality and early diagnostic measure of stress (Willits and Peet, 2001). Fv/Fm measurement of healthy, unstressed plants is associated with values ranging from 0.75 to 0.85 (Percival, 2005). Both control and inoculated hackberries consistently showed higher Fv/Fm values than 0.75, which indicated a high adaptability of this species to difficult planting sites such as a parking lot. Inoculated plants had significantly higher Fv/Fm values than control plants in July 2008 and June 2009 (Figure 6). This indicated that the phytochemistry of photosystem II was improved by mycorrhizal inoculation, which can result from lower oxidative damage within chloroplasts and/or from a better nutritional status of the leaves. Chlorophyll content was higher in mycorrhizal inoculated hackberries compared to control plants both at the middle and at the end of the growing season (Table 6). The higher SPAD-value measured in mycorrhizal inoculated hackberries reflects a higher nutritional status of plants compared to non-inoculated controls, when grown in a stressful environment such as a parking lot (Luh *et al.*, 2002; Percival *et al.*, 2008). No difference in leaf chlorophyll content and Fv/Fm (data not shown) were found between treatments in *Fraxinus* (Table 6).

Trees in a historical park

In 2006–2007, stem diameter growth was unaffected by mycorrhizal inoculation on both young and mature linden and horse chestnut (Table 7). Mature trees of both species had greater stem diameter growth than newly planted trees. In 2007–2008 mycorrhizal inoculation increased stem diameter growth in mature lindens, but had no significant effect on young trees. In the second year mycorrhizal inoculated mature lindens had 318% higher diameter growth than untreated control. No difference among treatments was found in horse chestnut. In 2008–2009, stem diameter growth of linden trees was similar among treatments, while it was significantly higher in young horse chestnut than in mature ones (Table 7). In 2008, shoot growth was significantly increased by inoculation in mature lindens and horse chestnuts, which had 20% and 55% longer shoots than control trees, respectively (Table 7). No significant difference was found for shoot growth in newly planted linden and

Table 6 Effects of inoculation with selected mycorrhiza on diameter and shoot growth and on chlorophyll content of *Celtis australis* and *Fraxinus excelsior* planted in a parking lot and along a street, respectively.

	ΔØ (cm)			Shoot growth (cm)			Chlorophyll content (SPAD)	
Celtis australis	**06/07**	**07/08**	**08/09**	**2007**	**2008**	**2009**	**June 2008**	**Sept. 2008**
Mycorrhiza	0.57	1.26	0.45	23.86	30.33	36.55	45.37	48.77
Control	0.30	1.07	0.37	15.40	15.25	20.25	39.06	35.68
P	**	*	n.s.	**	**	**	**	**
Fraxinus excelsior	**06/07**	**07/08**	**08/09**	**2007**	**2008**	**2009**	**2007**	**2008**
Mycorrhiza	N.D.	0.71	N.D.	7.05	10.12	N.D.	29.04	30.10
Control	N.D.	0.88	N.D.	4.76	7.11	N.D.	30.03	30.40
P	-	n.s.	-	**	**	-	n.s.	n.s.

* and ** indicate significant differences between mycorrhizal and control trees of the same species at P<0.05 and P<0.01. N.D. = not determined.

Figure 4 Carbon assimilation (A, μmol m^{-2} s^{-1}, left) and water use efficiency (WUE, μmol CO_2/mmol H_2O, right) in inoculated and non-inoculated hackberry trees planted in a parking lot. * and ** indicate significant differences between mycorrhizal and control trees within the same sampling date at P<0.05 and P<0.01.

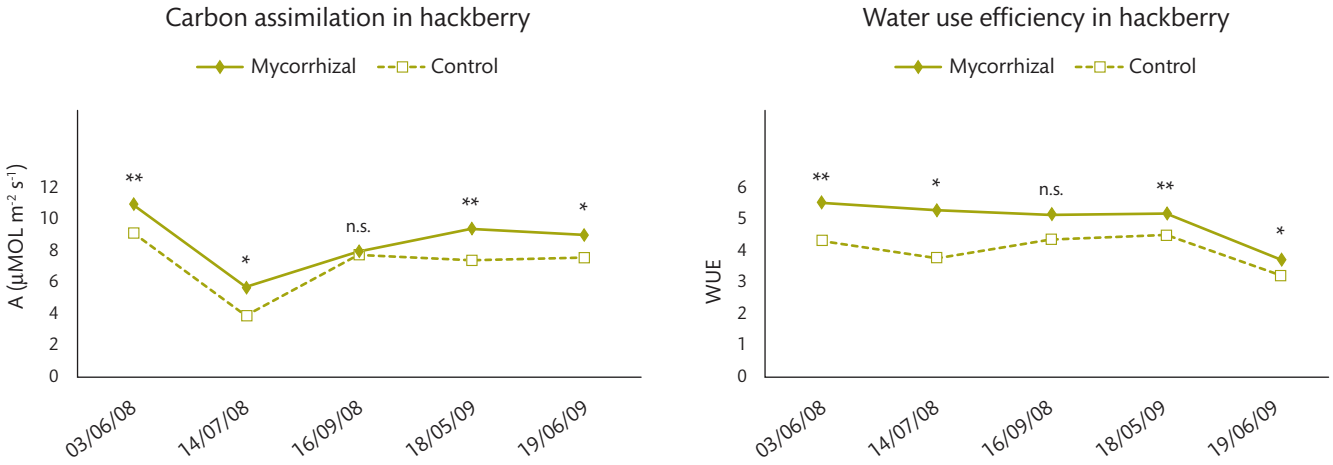


Figure 5 Carbon assimilation (A, μmol m^{-2} s^{-1}, left) and water use efficiency (WUE, μmol CO_2/mmol H_2O, right) in inoculated and non-inoculated ash trees planted as street trees. * and ** indicate significant differences between mycorrhizal and control trees of the same species at P<0.05 and P<0.01.

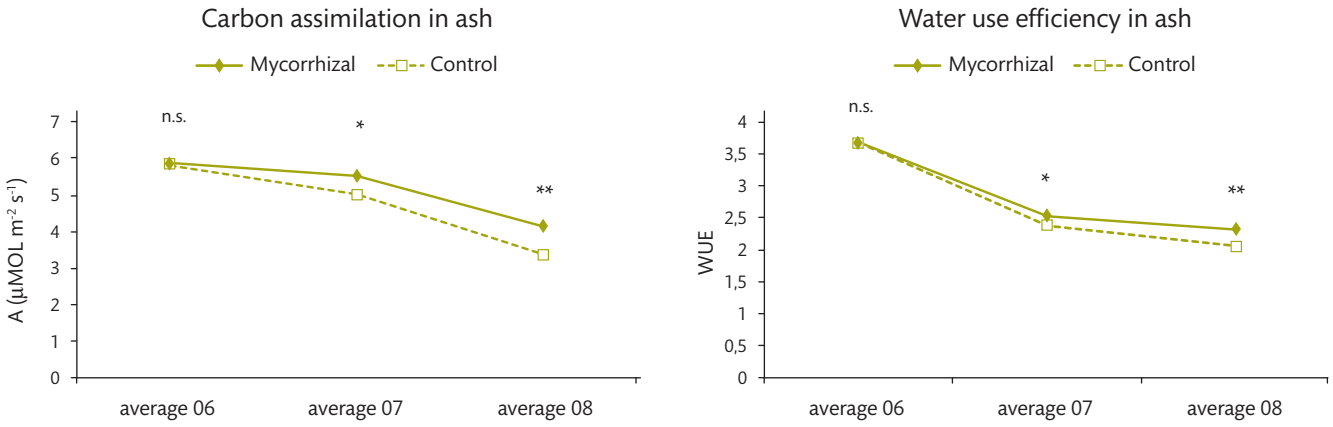
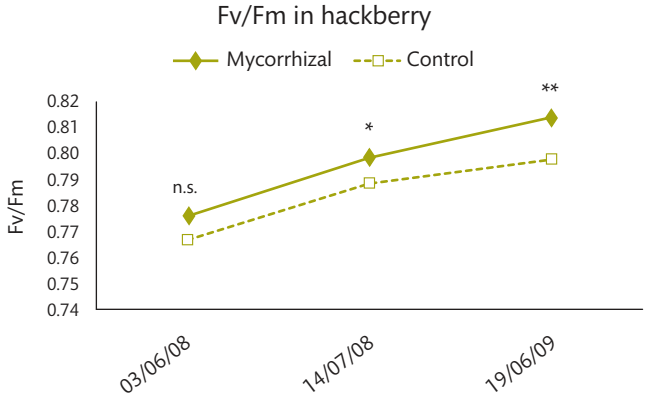


Figure 6 Maximal quantum yield of photosystem II (Fv/Fm) in inoculated and non-inoculated hackberry planted in a parking lot. * and ** indicate significant differences between mycorrhizal and control trees of the same species at P<0.05 and P<0.01.



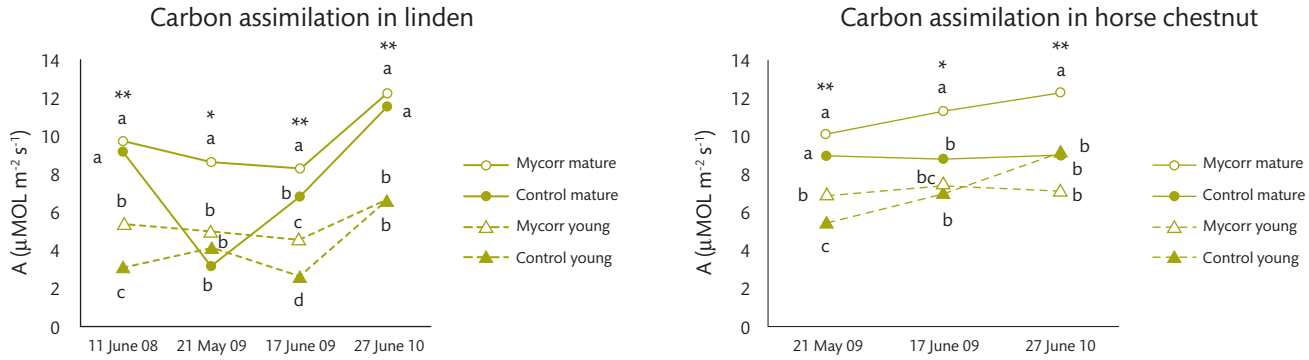
horse chestnut trees. In 2009, shoot growth of linden was higher in inoculated mature trees than in mature untreated trees which, in turn, had higher shoot growth than both inoculated and control young lindens. In horse chestnut, shoot growth was increased by mycorrhizal inoculation in both mature and young trees. As for diameter, shoot growth was higher in young horse chestnut trees than mature ones. Chlorophyll content was affected by mycorrhizal inoculation in mature lindens and young horse chestnuts (Table 7), but was unaffected by mycorrhizal treatments in newly planted linden. Inoculation affected carbon assimilation (A, μmol $m^{-2}s^{-1}$) of linden and horse chestnut (Figure 7). When significant differences were found, inoculated plants always had higher A when compared to control plants of the same age.

Table 7 Effects of inoculation with selected mycorrhiza, tree age and their interaction on diameter and shoot growth and chlorophyll content of Tilia and Aesculus planted in an historical garden in the centre of Milan.

	ΔØ 06/07 (cm)	ΔØ 07/08 (cm)	ΔØ 08/09 (cm)	Shoot growth 2008 (cm)	Shoot growth 2009 (cm)	Chl. content 2008 (SPAD)
Tilia						
Mature mycorrhizal	2.7 a	1.4 a	0.8	14.5 a	21.5 a	52.4 a
Mature control	1.7 a	0.3 b	1.3	12.1 b	14.8 b	47.6 b
Young mycorrhizal	0.6 b	0.2 b	0.6	9.7 c	8.6 c	42.0 c
Young control	0.8 b	0.2 b	1.2	12.6 b	7.7 c	39.8 c
P (inoculation)	n.s.	n.s.	n.s.	n.s.	**	*
P (age)	**	**	n.s.	**	**	**
P (IxA)	n.s.	*	n.s.	*	*	*
Aesculus						
Mature mycorrhizal	1.8 a	0.6	0.4 b	8.8 c	9.5 c	N.D.
Mature control	1.1 ab	0.7	0.4 b	5.7 d	6.1 d	N.D.
Young mycorrhizal	0.6 b	0.3	0.7 ab	13.7 a	15.4 a	43.4 a
Young control	0.9 ab	0.5	1.1 a	12.1 b	10.9 b	40.3 b
P (inoculation)	n.s.	n.s.	n.s.	**	**	*
P (age)	*	n.s.	*	**	**	-
P (IxA)	n.s.	n.s.	n.s.	n.s.	n.s.	-

Different letters within the same column and species indicate significant differences between treatments at P<0.05 (*) and P<0.01 (**).

Figure 7 Effects of selected mycorrhiza on carbon assimilation of young and mature *Tilia* (left) and *Aesculus* (right) planted in an historical garden in the centre of Milan. * and ** indicate significant differences between treatments within the same sampling date at P<0.05 and P<0.01.



Conclusions

Results obtained to date showed that the work of selecting, multiplying and inoculating woody species with site- and species-specific native mycorrhizal fungi can result in greater growth (especially of field-planted trees, as no growth increment was found in container-grown trees) and improved physiology, as can be seen from leaf gas exchange and chlorophyll fluorescence measurements. Time of response was also affected by tree species. For example, *Celtis australis* responded very quickly to mycorrhizal treatment, showing significant differences for shoot growth and chlorophyll content in the first growing season after inoculation, whereas *Fraxinus* required at least two growing seasons before the effect of mycorrhizal inoculation became significant. Tree age also affected success of mycorrhizal inoculum. We tested the same product on newly planted and mature *Tilia* and *Aesculus* growing in a poor, heavily compacted soil and found that symbiosis was more successful on mature trees, compared to newly planted ones. There is evidence that soil compaction limits root growth and activity (Fini and Ferrini, 2007) and reduces mycorrhiza formation (Nadian *et al.*, 1997; Entry *et al.*, 2002). It is possible that roots of young, newly planted trees were more affected by compaction than those of large, established ones. High mortality of fine absorbing roots especially on young linden may explain the

reduced effect of mycorrhizal inoculation. The process of selection of native, specific mycorrhizal strains must be implemented by selecting new strains and fungal species for areas which have already been studied and identifying new fungal species/strains in new geographic areas.

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