

## APPLYING ARTIFICIAL MYCORRHIZAE IN PLANTING URBAN TREES

SZABÓ, K.<sup>1\*</sup> – BÖLL, S.<sup>2</sup> – ERŐS-HONTI, ZS.<sup>3</sup>

<sup>1</sup>*Corvinus University of Budapest, Faculty of Landscape Architecture, Department of Garden and Open Space Design  
1118, Budapest, Villányi út 35-43  
(phone: +3614826308)*

<sup>2</sup>*Bavarian State Institute for Viticulture and Horticulture  
D-97209 Veitshöchheim, Germany*

<sup>3</sup>*Corvinus University of Budapest, Faculty of Horticultural Science, Department of Botany  
1118, Budapest, Villányi út 35-43  
(phone: +3614826379)*

*\*Corresponding author  
e-mail:krisztina.szabo9@uni-corvinus.hu*

(Received 23<sup>rd</sup> July 2014; accepted 8<sup>th</sup> Aug 2014)

**Abstract.** Urban habitats are unique and harsh environments for established plant communities, basically due to the increased stress (disturbance, pollution, drought etc.). However, ever increasing is the need for extending the green areas of the cities. There are several possibilities for urban afforestation, yet most of them require the use of chemicals that burden the soil. A feasible solution is applying artificially modified microbiota to enhance the growth and stress tolerance of the ornamental species. Mycorrhiza is mutualistic interaction between plant roots and fungal hyphae that increases the ecological fitness of both partners, especially among harsh environmental conditions. As a consequence, exploiting the advantages of this symbiosis can be a proper and sustainable strategy for establishing artificial plant communities in urban areas. In the present article we review the most important questions of urban afforestation and describe the most important general features of mycorrhizae that are related to their extended use by the humankind. Afterwards, we sum up the actual knowledge on mycorrhizae of plants living in urban areas. Then we report about the Bavarian research project “Urban Green 2021”. Finally, we introduce an extensive ongoing study aiming to gain new results on this topic among Hungarian conditions.

**Keywords:** *urban trees, afforestation, ectomycorrhiza, endomycorrhiza, inocula*

### Introduction

#### *A brief historical review of Hungarian allées/tree lines*

In the ancient times and the Middle Ages, allées were not mentioned in the European cities. Afterwards, the signs of allées can be discovered on the works of Dutch painters in the 17<sup>th</sup> century. Later, the people disengaged from the castles, so the urban afforestation began (Cséser 1928). The first allées in Hungarian horticulture were planted in the Visegrád Garden, from that time linden was the most frequently favoured tree of the country. In the Primacy Garden of Pozsony, Ágost Keresztély established a French garden (parterre, parkland and a linden tree line) on the site of a previous renaissance garden (in 1735). In the 18<sup>th</sup> century, the allées were appreciated much more than in any other centuries, therefore, further beautiful linden lines were planted (Rapaics 1932). In the 19<sup>th</sup> century several species were introduced into urban afforestation, like white mulberry (*Morus alba*), Japanese pagoda tree (*Sophora*

*japonica* or rather lately *Styphnolobium japonicum*), green or red ash (*Fraxinus pennsylvanica*) and the black locust (*Robinia pseudoacacia*) from North America.

Epoches of favoured genera and species used in Hungary (Schmidt & Honfi 2006):

- Early 1900's – the epoch of sycamore (*Platanus*) and horse chestnut (*Aesculus*);
- From 1950 until the early 1960's – the epoch of white linden (*Tilia tomentosa*) and sorb species (*Sorbus*);
- From the end of the 1900's to nowadays - species of hackberry (*Celtis*) and ash (*Fraxinus*);
- The spread of the American species like Indian bean tree (*Catalpa bignonioides*), honey locust (*Gleditsia triacanthos*);
- In the following years several other species extended in the allée from East Asia, e.g. Empress tree or royal paulownia (*Paulownia tomentosa*), China tree or golden rain tree (*Koelreuteria paniculata*), maidenhair tree (*Ginkgo biloba*) and the pear (*Pyrus* species) in the present epoch (Schmidt 2003, Vajna 2012).

The first urban allée in Budapest was established near the VIGADÓ, consisting of unidentified species was watered by prisoners (1789). According to Rapaics (1934) its trees were lindens and black locusts. The second urban allée was founded near the Chain Bridge from the bridgehead of Pest (1856). This was the first urban tree line by Radó's opinion (1981), but copper engravings seem to contradict this opinion. The planted trees were *Robinia pseudoacacia* and one individual of these locusts remained even till today.

### **Criteria for choosing species and cultivars in brief**

There are two most important viewpoints that should be taken into consideration being in close correlation with each other: the planted area and the planned plants.

Important properties of the planted area are:

- location, exposure;
- climate: particularly the effects of frost or heatwave;
- soil: pH, groundwater;
- size of planted area in view of the size of root system and the crown;
- degree to which area is built up: buildings, sidewalks, overhead wires and subsurface wires;
- air contamination;
- pavements.

Important features of the planned plants are:

- height and width of the crown;
- stability (storm injury, wind tunnel);
- tolerance against drought and the high level of groundwater;
- resistance against pests and causative agents.

Once upon a time carriage drivers thought that the only purpose of planting urban trees was decoration, giving shade without disturbing the traffic. Then a list of requirements rose, like stress tolerance, ornamental value, central leader, trunk remains straight up through the crown, optimal crown, sturdy branch system (not fragile) and even that the tree has no fruits or if it has only little ones and thermo radiation, high concentration of salt and metal finally the capability of long leafy or late defoliation.

Of course, there are no such species or cultivars that fulfils all these requirements. The principal feature should be decided by the user's group (Biza 2006). Nowadays, in

the mass of asphalt and concrete the most important need is that the plant or the tree should survive the urban environment.

### Overview of mycorrhizal interactions

According to the most detailed definition (Brundrett 2004), mycorrhizae are intimate mutualistic interactions between modified absorptive organs (chiefly roots) of a plant (photobiont) and the hyphae of a fungus (mycobiont) with the main purpose of nutrient transfer between the organisms. Though this broadest accepted terminology refers also for symbiotic interactions of thallic plants (mosses or prothallia of pteridiophytes), sometimes called mycothalli or paramycorrhizae (Strullu-Derrien and Strullu 2007), for the purpose of urban plantations much more important are the eumycorrhizae, hosted by vascular plants.

Based on the anatomical structure, two main types of mycorrhizae have been distinguished since as early as the first description of the interaction in the 19<sup>th</sup> century (Trappe 2005). In the evolutionarily oldest type, called endomycorrhiza (Selosse and Le Tacon 1998, Brundrett 2002), the hyphae penetrates through the plant cell wall and form intracellular structures by invaginating the cell membrane (Smith and Read 2009). In the root cells the following structures may be present: *hyphal coils* and/or multiply branching *arbuscules* for the nutrient transfer between the mutualistic partners and *vesicles* for the storage of lipids (Bonfante and Genre 2008). From the root surface, extraradical hyphae run far into the neighbouring soil areas. The now described so-called vesicular-arbuscular mycorrhiza (VAM) is the most widespread mycorrhiza type (Wang and Qui 2006) occurring on the roots of more than 67 % of angiospermous (Trappe 1987) and over 80% of all terrestrial plants (Smith and Read 2009). Though the spectrum of host plants is extremely wide (Harley and Harley 1987, Wang and Qui 2006) the fungal partners belong to a single phylum, Glomeromycota (Schüßler et al. 2001, Brundrett 2002). Due to its frequent occurrence in natural ecosystems, VAM interactions have been quite intensely studied for the last decades (Koide and Mosse 2004, Triparthi and Khare 2012), including its potential application for forestry and agriculture (Peterson et al. 1984, Azcón-Aguilar and Barea 1997, Gosling et al. 2006, Gianinazzi et al. 2010).

The other large group, ectomycorrhizae (EM) are characterised by an obvious mycelial mantle covering the fine roots, from which hyphae grows into the root forming an almost exclusively extracellular interface (Hartig-net) enwrapping the cortical cells (Bonfante 2001, Smith and Read 2009). From the mantle, different emanating elements (cystidia, hyphae, rhizomorphs) derive (Agerer 1999). Though merely 4-5% of vascular plants form ectomycorrhizae (Brundrett 2002), the most important forest trees of all climatic zones are amongst them (Wang and Qui 2006), which reveals a fundamental ecological significance of the symbiosis. Mycobionts of the interaction are either ascomycetes (Maia et al. 1996) or basidiomycetes, with an estimated number of 5-6000 species (Molina et al. 1992). The list of documented EM-forming fungi was compiled by several authors (De Román et al. 2005, Agerer 2006), but the number is continuously increasing.

In addition to the above mentioned mycorrhizal types, further categories are distinguished based on special anatomical features. A transitional state between ecto- and endomycorrhizae is the ectendomycorrhiza bearing fungal mantle together with intracellular hyphal structures (Yu et al. 2001). Some mycorrhiza types of unique

anatomy are restricted to certain plant taxa, such as the ericoid (Straker 1996), arbutoid, monotropoid (Zelmer et al. 1996) and orchid (Rasmussen 2002, Dearnaley 2007) mycorrhizae. In addition to the structure-based classification, mycorrhizae can be categorised on the grounds of the quality of the ecological relationship between the fungus and its host (Brundrett 2004, Egger and Hibbett 2004). In this system fungus-plant relationships form a continuum from the parasitic interactions formed by non-green mycorrhizal plants exploiting their fungal partners (Taylor et al. 2002, Selosse et al. 2006) through commensalisms (represented by endophytes – Jumpponen 2001, Newsham et al. 2009) right to the clearly mutualistic mycorrhizae.

The reciprocal benefit of the mutualistic partners is manifested at different levels (Johnson et al. 2006). The basic advantage is the nutrient transfer via the interface: the mycobiont gets organic molecules from the plant (Rosling et al. 2004, Grunze et al. 2004), while owing to their extrametrical mycelia, the fungi represent an enlarged water and mineral absorptive surface for the plant (Bucher 2007, Lee et al. 2013) together with the ability to degrade and so transport the of the stored nutrients of dead organic materials (Smith and Read 2009). Furthermore, the presence of mycorrhizae protects either directly or indirectly against different forms of stresses (Nadeem et al. 2014) including both abiotic factors (drought – e.g. Di Pietro et al. 2007, Wu et al. 2013, heavy metals – e.g. Ott et al. 2002, Hildebrandt et al. 2007 or cold – Tibbett et al. 2002, Wu and Zou 2010) and pathogens (e.g. Pozo and Azcón-Aguilar 2007, Cameron et al. 2013). As a consequence, on the population level, the benefit for the partners is the increased overall fitness (Johnson et al. 2006).

The advantageous relationship between the fungal and plant communities of a certain area is hidden in the diversity of species and ecological functions (Buscot et al. 2000, Feddermann et al. 2010). In natural assemblages poor in plant species may bear their diversity potential belowground in their fungal (often mycorrhizal) microbiota (Dahlberg 2001). Mycorrhizae may be sorted into different functional groups, even within a single anatomical category, based on the nutrients mobilised (Feddermann et al. 2010) or the way how they explore their habitat (Agerer 2001). The high species diversity causing redundancy in ecological functions may stabilise the community of formed by mycorrhizal plants. Besides, high species richness enables the development of diverse ecological relationships at a certain habitat. Since the mycelium of a fungus may form mycorrhizae with roots of several individuals (Lian et al. 2006) and on the root system of a single plant may host mycorrhizae of several fungal species at a certain time, and the mycelia of certain fungi may anastomose with each other (Jakobsen 2004), plants are interconnected into a physiologically continuous system ('common mycelial network', CMN) (Selosse et al. 2006). The experimentally proven ecological consequences of CMN are manifold, from the nursing of suppressed (e.g. shaded) plants right to such indirect parasitic interactions as those of myco-heterotrophic and myxotrophic plants gaining organic molecules or nutrients without an equal compensation (Selosse et al. 2006, Simard et al. 2012).

As members of ecosystems, mycorrhiza and mycorrhizal fungi have profound impacts on the abiotic environment. The role of fungal mycelia (including emanating hyphae of mycorrhizae) on soil characteristics is studied by the field of geomycology (Burford et al. 2003, Gadd 2007). Mycorrhizal fungi contribute significantly to soil development (Johnson et al. 2006) by improving the soil structure via adhering soil particles (Moreno-Espindola et al. 2007, Miransari et al. 2009), mobilising insoluble nutrients from minerals (Landeweert et al. 2001, Winkelmann 2007) or dead organic

matter (Smith and Read 2009) as well as taking part in the biogeochemical cycles of the elements (Millard et al. 2007, Paul et al. 2007, Veresoglou et al. 2012). Beside their effect on the soil, mycorrhizal interactions have profound consequences on the resilience of natural ecosystems against the components of the global climatic change (reviewed e.g. by Mohan et al. 2014).

Owing to their multi-level beneficial effects, mycorrhizae are utilised in a manifold way by the humankind. By preserving stress caused damages and increasing growth rate and thus the commercial yield and quality, artificial inoculation is applied by agriculture (Stützer et al. 2008, Linderman 2008, Takács et al. 2008, Gianinazzi et al. 2010), for micropropagated plants in orchards (Balla et al. 2008) and in forestry (Peterson et al. 1984, Haselwandter and Bowen 1996) as means of sustainable development (Rooney et al. 2009). Being biological agents, their application can substitute for different chemical treatments, first of all this is the reason why they can be related to sustainable development, but mycorrhizae are even more directly applied by nature conservation for recultivation (Estaún et al. 2008), as well as for *in situ* (Turrini and Giovannetti 2012) and *ex situ* plant protection (Bothe et al. 2010) and as a way of bioremediation (Khan et al. 2000).

### **Mycorrhizae in urban environments**

Fungi of urban environments belong to different ecological functional groups living in manifold interactions with plants and animals. In addition to spores, indoor fungi (living in buildings), parasites and fungi of the phylloplane – similarly to the native habitats – significant is the role of mycorrhizal fungi in these habitats (Newbound et al. 2010). Although the mycorrhizal status of the most important ornamental plants is included in general reviews compiling the known literature on mycorrhizal plants (e.g. Harley and Harley 1987, Wang and Qiu 2006), some publications focus straight on the colonisation of urban samples (e.g. Gáper 1989, Baxter et al. 1999, Timmonen and Kauppinen 2008, Bainard et al. 2011, Karpati et al. 2011, Karlinski et al. 2014).

The comparison of the mycobiota of rural and urban habitats revealed admirable differences between the natural and disturbed areas. In general, the decrease of species diversity and shift in species composition was observed on the level of macrofungi (Newbound et al. 2010, Barrico et al. 2012) as well as when comparing the soil microbiota using high throughput sequencing methods (Jumpponen et al., 2010). For endomycorrhizal glomeromycetes never form sporocarps, studying the EM community seems to be easier due to the presence of their sporocarps. However, it has been accepted already for decades that above-ground view cannot be exactly related to the below-ground composition, either in case of EM fungi (Gardes and Bruns 1996, Dahlberg et al. 1997, Horton and Bruns 2001). Consequently, works based on mycorrhiza samples reveal the real effects of urban disturbances. Nevertheless, trends similar to the fruitbody-based studies were gained from the mycorrhiza based studies. Almost each result indicated decreased species richness yet unchanged EM colonisation level at low disturbance (Baxter et al. 1999, Gebhardt et al. 2007, Bainard et al. 2011, Karpati et al. 2011, Tyburska et al. 2013). Interestingly, EM communities of the disturbed (i.e. urban) sites included a generally shared species group ('Urban EMF suite' – Del Tredici 2010 cit. in Karpati et al. 2011, Timmonen and Kauppinen 2008) in addition to the 'site specific' species (Gebhardt et al. 2007). From the disturbed sites, the EM species of the late successional stages were missing (Karpati et al. 2011).

The level of endomycorrhizal colonisation was found to be decreased (Bainard et al. 2011, Tyburska et al. 2013) or just slightly affected by the urban environment (Karlinski et al. 2014), while the composition of VAM-community became impoverished to different levels depending on the current land-use and land-use history (Stabler et al. 2001, Cousins et al. 2003). A general view is that EM fungi are more seriously affected in urban habitats, and they are suppressed by increasing saprotroph dominance (Newbound et al. 2010) thus VAM might be a more important symbiosis in disturbed urban areas (Bainard et al. 2011). However, it is important to note that occurrences of different fungal functional groups have a seasonal alteration throughout the year (Jumpponen et al. 2010).

Characteristic changes of the environmental factors caused by the humankind lie in the background of the observed changes in community traits. Urban sites are intensely polluted, their soil has high nitrogen depositions and it suffer regular, often serious mechanical disturbances (Baxter et al. 1999, Cairney and Meharg 1999, Egerton-Warburton and Allen 2000, Khan 2001, Polanco et al. 2008) each bearing a proven negative effect both on the root parameters (Karlinski et al. 2014) and on the fungal communities (Bernatzky 1974, Stabler et al. 2001, Bainard et al. 2011, Jumpponen and Jones 2010, Jumpponen et al. 2010, Vauramo and Setälä 2010). Although mycorrhizae of natural sites also have to face different disturbances (e.g. Gehring and Whitham 1992, Byrd et al. 2000, Jones et al. 2003), their effect is less deleterious, just like in urban sites in a state close to natural (Lothamer et al. 2014). Therefore, keeping urban stands in a state similar to the native ones may be more resistant to the humankind caused stresses. A certain aspect of this is keeping the soil mycobiota as intact as possible, which can be enhanced by close natural stands that can serve as resources for fungal propagules (Cousins et al. 2003, Schaefer 2011) and from where mutualist fungi can immigrate via wind-dispersal or any other transporting agents (Stabler et al. 2001).

Beside harsh environmental conditions, a further problem of establishing urban ecosystem is the fact that quite frequently non-native species are introduced. The ability of introduced species to utilise native mycorrhizal community is proven for both EM (Tedersoo et al. 2007) and VAM (Cousins et al. 2003) hosts. Nevertheless, introduced non-native stands always had less diverse fungal communities, as it was revealed by both sporocarp (O'Hanlon et al. 2013) and mycorrhizal surveys (Cousins et al. 2003, Dickie et al. 2010, O'Hanlon and Harrington 2012). While native hosts live in mutualism with rare and more exclusive EM taxa (Lothamer et al. 2014), the mycorrhizae of introduced plants are chiefly cosmopolitan and wide-distributed fungi (Trocha et al. 2012). Nevertheless, among certain environmental conditions similar studies led to inconsistent results, for instance, non-native pine trees had higher EM sporocarp and root tip diversity compared to a native *Pinus* (Trocha et al. 2012). The decreased mycorrhizal diversity of introduced species may be traced back to different facts: non-native plants alter the soil microbiota (Bahram et al. 2013, Kohout et al. 2011), these plants (especially the invasive ones) might be less dependent on mycorrhizae (Pringle et al. 2009) and planting aliens means a co-invasion of their endophytic (Knapp et al. 2012) and mycorrhizal fungi at the same time (Cousins et al. 2003, Tedersoo et al. 2007, Vellinga et al. 2009, Dickie et al. 2010) that may overcompete the native species. Unfortunately, the invasion biology of mycorrhizal fungi is just rarely discussed (Vellinga et al. 2009). Besides, since urban sites are mostly dominated by non-mycorrhizal or facultatively mycorrhizal plants (Jumpponen and Egerton-Warburton 2005 cit. in Karpati et al. 2011), the low proportion of mycorrhizal

plant species may also decrease the mycorrhizal diversity of the soil (Bainard et al. 2011). Moreover, according to some authors (e.g. Trocha et al. 2012) native trees had a longer evolutionary time to adapt to the local community. At the same time, introduced ornamental species both use the native fungal community for their survival (Cullings et al. 2000, Bahram et al. 2013), as well as their co-introduced mycorrhizae (see above). A possible solution might be if native hosts are preserved among the introduced ones, because this way a considerably high proportion of the native fungal community can be preserved (Lothamer et al. 2014), and these ‘refugial’ plants aid to keep up a kind of ‘ecological memory’ of the soil microbiota (Schaefer 2011).

In order to enhance the establishment of urban plantations, another rational idea, i.e. the use of artificial mycorrhizae, rose as early as in the 1980’s (Kuhns 1980). Despite these early thoughts, even at the beginning of the third millennium this solution was only a ‘rare practice’ (Pauleit et al. 2002), although a conceptual decision model for predicting its success had been elaborated by then (Findlay and Kendle 2001). The background of this method is the importance of mycorrhizae in plants’ resistance against urban soil disturbances (Polanco et al. 2008) and the low level of available fungal inocula in disturbed soils (Bainard et al. 2011). (However, some results debate this latter observation – Tarvainen et al. 2011.) Knowing that introduced plants may adapt to native microbiota, the mostly recommended technique is using the native fungi in the process (Chan and Griffith 1991, Fini et al. 2011). The positive effect of mycorrhization was found to be even more significant when co-inoculation of rhizobacteria was applied (Rao et al. 2006).

Although nowadays commercial products with impressive predicted effect are available on the market, and their flexibility allows the inclusion of the mycorrhizal technology to integrated plant production systems and recently has an economically growing market (Feldmann 2008). Some of them are proven to be advantageous for the ornamental hosts (Lanthier 2009), the results of studies using them are inconsistent (Gilman 2001, Ferrini and Nicese 2002, Weissenhorn and Külling 2008). The use of AMF inoculum is recently facing a highly diverse host plant spectrum and diverse substrates for specific uses at the front-end in the market. Recently, more and more positive cost/benefit relationships of mycorrhiza use lead to the development of bioproducts containing AMF (Schneider and Feldmann, 2007; Young, 2005; Gianninazzi et al, 2002) or combination of AMF and bacteria (Ravenskov and Jakobsen, 1999).

Inoculation may be accomplished either in the nurseries or in the target site. When inoculated in nurseries, the lowest mycorrhizal diversity is observed here (Rao et al. 2006), then these fungi are outcompeted by the micorrhizosphere of the urban environment for nursery mycorrhizae are not adapted to street conditions (Timmonen and Kauppinen 2008). In the long run, the most characteristic change of the mycorrhizal community of previously inoculated plants is the increasing proportion of EM of long-distance exploration type (Fini et al. 2011). When inoculating with artificial methods, it should also be born in mind that the target site itself may have the potential for infecting the trees (Appleton et al. 2003), so in such habitats, this procedure would be unnecessary (Findlay and Kendle 2001), depending on the amount of available inocula.

The exact strategy how inoculation is carried out is of admirable importance. Ortaş et al. (2004) tested several techniques to develop suitable inoculum strategies. Ortaş (2008) found in the Eastern Mediterranean Horticultural Region that sometimes if mycorrhizal inoculated seedlings are not well infected with mycorrhizae spores, it may

be necessary to re inoculate them during transplantation to the soil. Most of the time re inoculation seems to give a better response. But it is important to produce well inoculated seedlings. If seedlings are not infected with mycorrhiza, usually there is high mortality.

Fortunately, today we are already aware of the results of long-term monitoring studies on the level of mycorrhization and its result on physiological parameters following artificial inoculation (Ferrini and Fini 2011). These reveal that host species and site characteristics affect the speed of colonisation. When the physiological, growth and nutrition parameters were measured after artificial inoculation, all were found to be improved, in general (Stabler et al. 2001, Zandavalli et al. 2004; Kapoor et al. 2008, Ferrini and Fini 2011, Garcia et al. 2011). Pre-mycorrhization helped some tree species to establish after transplantation, and grew faster during their first season in the field. Mycorrhizal inoculated *Acer platanoides*, *Acer saccharinum*, *Celtis occidentalis*, *Fraxinus pennsylvanica* and *Gleditsia triacanthos* species were observed by Chapdelaine et al (2008) and after two month in the field, non-inoculated control plants generally had smaller trunk diameters than pre-mycorrhized trees and their levels of AM colonization remained lower.

As it was proven, sometimes not the growth itself was enhanced by the artificial inoculation, but physiological traits related to drought stress were ameliorated (Fini et al. 2011). The application of AMF inoculums were examined in semi-arid regions, under greenhouse conditions in Israel for a variety of ornamental crops (herbaceous plants) AMF inoculation can retain plant growth and allow overcoming potential stresses under horticulture practices (Koltai et al 2008). Some authors state that better growth and nutrient response of host plants are not explained by high mycorrhizal colonisation but by the changes in ectomycorrhizal diversity in artificially inoculated hosts (Baxter and Dighton 2001).

As a general conclusion, we can state that mycorrhizal fungi could play an essential role in soil reclamation because they help in recovering biological activity, improving physical properties of soil and mobilizing accumulated minerals (Calvet et al. 2001). Moreover, using artificial inocula is a cost-effective solution for establishing urban plantations of both native and non-native species. Since the use of excess water and fertilisers can be substituted this way, this method means less load on the environment, too (Tschirner et al 2008, Fini et al. 2011) and so applying mycorrhizae can be integrated into sustainable strategies of plant production (Varghese 2000).

### **Testing stress-tolerant tree species in the Bavarian research project „Urban Green 2021“**

Roadside trees have to constantly face a great number of devitalizing stress factors as they are situated in a highly unnatural environment with greatly confined tree pits. During summer months roadside trees often suffer from drought and heat stress, especially at night, owing to the radiation reflection of buildings and paved surfaces. Climate change causing rising temperatures and increased drought stress during summer (see for example 2003, 2006 and 2010) as well as more frequent weather extremes is further aggravating this stress situation. There is some evidence that in our latitudes some of the traditional urban tree species will not always be able to meet future requirements (Roloff et al. 2009), as they won't fit aesthetic standards any more (e.g. *Cameraria ohridella* on *Aesculus hippocastanum*), are becoming increasingly a safety



risk (e.g. *Massaria* on the London plane) or will fail completely (e.g. ash dieback disease on native *Fraxinus* species).

To broaden the presently limited repertoire of urban tree species and to find out, which species or cultivars are able to tolerate the expected future climate conditions in our cities, a long-term project has been started, testing 20 promising tree species in three Bavarian cities with different local microclimates from 2010 to 2021 (*Table 1*). When selecting the various tree species their natural habitat requirements were considered as well as their drought and frost resistance and susceptibility for diseases and pests. Also urbanistic aspects as habit and crown shape of the trees were selection criteria.

**Table 1** Test tree species/ cultivars in research project “Urban Green 2021”, their associated mycorrhiza-types (AM =arbuscular mycorrhiza, EM=ectomycorrhiza) and evaluation of their winter-hardiness (1- very high frost tolerance, 2 – high f.t., 3 – limited f.t., 4 – no f.t., ? – unknown f.t.)

Test tree species/cultivars	Mycorrhiza-type	Frost tolerance "Urban Green 2021"	Winter hardiness Roloff et al. 2009
<i>Acer buergerianum</i>	AM	3	1
<i>Acer monspessulanum</i>	AM	1	2
<i>Alnus x spaethii</i>	EM	2	1
<i>Carpinus betulus</i> 'Frans Fontaine'	EM	2	1
<i>Celtis australis</i>	AM	4	3
<i>Fraxinus ornus</i>	AM/ EM	1	4
<i>Fraxinus pennsylvanica</i> 'Summit'	AM/ EM	1	1
<i>Ginkgo biloba</i> (male selection)	AM	2	2
<i>Gleditsia triacanthos</i> 'Skyline'	AM	1	2
<i>Liquidambar styraciflua</i>	AM	1	3
<i>Magnolia kobus</i>	AM	1	2
<i>Ostrya carpinifolia</i>	EM	2	1
<i>Parrotia persica</i>	AM/EM	2	?
<i>Quercus cerris</i>	EM	1	2
<i>Quercus frainetto</i> 'Trump'	EM	?	2
<i>Quercus x hispanica</i> 'Wageningen'	EM	?	?
<i>Sophora japonica</i> 'Regent'	AM	3	2
<i>Tilia tomentosa</i> 'Brabant'	AM/ EM	3	2
<i>Ulmus</i> 'Lobel'	AM/EM	1	1
<i>Zelkova serrata</i> 'Green Vase'	AM/ EM	3	2

In the different cities, each tree species was planted 8-fold (in some cases only 6-fold for lack of space), altogether summing up to 460 test trees. The different study sites in Bavaria, Germany are located at Würzburg (a city with above average summer temperatures and drought periods (viticultural climate), apt to test the trees for drought and heat resistance), Hof/ Münchberg (the coldest test site influenced by continental climate with the highest number of ice days, suited to test the frost tolerance of the trees), and Kempten (at the foothills of the Alps, with a moderate climate and a high precipitation pattern). At all sites standardized soil substrates with high load bearing properties were used that are in accordance with the recommendations of the Research

Society for Landscape Developing Landscape Construction. These substrates are one-layered to prevent superficial root growth and have a high air capacity as well as a high usable water capacity. The high pore volume guarantees good root penetration into great depth. The tree pits have a standardized size of 8m<sup>3</sup> and a fixed depth of 1,50m. Planting and maintenance procedures were specified according to current practice.

Increasingly, mycorrhizal fungi products are used for tree maintenance, especially when old trees are restored. There are a number of positive reports (Kutscheidt 2006), however there are hardly any experimental data available on the effectiveness of such products (but see Ferrini & Fini 2011b). Yet, considering the acknowledged significance of mycorrhizal fungi in stressful habitats (Raman & Mahadevan 1996, Feldmann 2008), the application of mycorrhizal fungi products might be particularly effective at urban sites. In this project a controlled application of species-appropriate mycorrhizal fungi preparations to half of the experimental trees was carried out during planting at all sites (*Table 1*). This should supply data to answer the question if the application of mycorrhizal fungi can alleviate the “plant shock” and promote growth and health of roadside trees. At the planting, samples of fine roots of the trees, of the soil of the tree balls, and of the pit substrate were taken to determine the initial mycorrhizal status of the nursery trees as well as the fungal spore content of the pit substrate. At one study site the mycorrhizal status as well as the mycorrhiza species composition is followed up over the years for several experimental tree species. Mycorrhiza composition is determined by morphotyping and by molecular analysis. First results are described by Herrmann et al. (2011, 2013).

Twice a year, during late spring and early fall, a screening of the experimental trees is carried out where frost and drought damages, crown vitality and health status are scored and various growth parameters are measured. In addition, assisted by the municipal garden offices of the partner cities, the phenology of the different tree species are monitored at the different sites, i.e. the calendar week of bud burst, leaf coloring and leaf fall are recorded. This allows not only to assess the risk of late frost damage but also to determine the length of the vegetation period (no. of weeks between bud burst and leaf coloring) of the different experimental tree species at the different sites.

As roadside trees have to be regularly watered during their initial growth phase after planting, data on the drought resistance of the experimental trees are not yet available. However, the pronounced winter of the past years allow to present preliminary results on the frost tolerance of the different tree species which compared to the winter hardiness evaluation of urban tree species by Roloff et al. (2009; *Table 1*). Our results led to a more negative rating of these species/cultivars. However, the evaluation of Roloff et al. (2009) is based on literature of tree species, not specific cultivars, and it does not consider frost splitting risks in its ratings. In contrast, *Acer monspessulanum*, *Fraxinus ornus*, *Liquidambar styraciflua*, *Magnolia kobus* and *Quercus cerris* exhibited a greater frost hardiness in this project than described by Roloff et al. More detailed results are found in Böll et al. (2014).

## **Introduction to the first extensive case-study on urban trees in Hungary**

In Hungary tree species applicable for urban afforestation are continuously searched by improvers, maintainers, gardeners, as well as tutorial institutions. Year by year new cultivars (trademark or registered trademark) were introduced into nursery gardens, but there seems to be so many unfavourable conditions of urban environments that no

species or cultivar have been found so far that fulfil all the requirements and can be applied among all environmental conditions.

In the last few years, several projects were carried out in Europe with the aim of enlarging the spectrum of tree species and cultivars applicable in urban environments. In some of these projects, several tree species were tested under urban conditions, and a set of sample plants were artificially colonised with with mycorrhizal fungi (one of such projects was introduced in the previous part). However, similar experiments on ornamental trees used in urban afforestation were not carried out in Hungary. We started a new Hungarian study in the March of 2014 with the following nine woody species, all of which may have potential roles in Hungarian urban afforestation: *Acer buergerianum*, *Carpinus betulus*, *Fraxinus ornus*, *Ginkgo biloba*, *Magnolia kobus*, *Platanus sp.*, *Sorbus borbasii*, *Tilia tomentosa*, *Ulmus pumila*. The main criterion for the selection was the proper tolerance against polluted urban condition. We used one-year-old seedlings obtained from different nurseries and forestry gardens. In our study, mycorrhizal products available in Hungary (licensed or not yet) are tested in a half-year-long run on 75 specimens of taxa inoculated with ectomycorrhiza and 120 specimens with either only endomycorrhiza or both with ecto- and endomycorrhiza.

The main question of the study is the effect of mycorrhizal treatment on different parameters of the species examined. Three and six months after inoculations, the degree of colonization in different experimental lines and different species will be examined. We will evaluate the degree of rooting, sprouting/shooting and compare the phenological features of different treatments. In our observation we will take root samples of examined taxa from nursery gardens, forestry gardens and old trees in urban conditions and the aim of our experiments is to determine changing quality and diversity of mycorrhizal fungi during plant developing and planting from one site to the other among different climatic and soil conditions. On the other hand, we are also interested in the colonisation pattern of mycorrhizal ornamental plants in the Hungarian nursery gardens.

**Acknowledgements.** The research was supported by the project TÁMOP-4.2.1/B-09/1/KMR-2010-0005. We are grateful to Gábor M. Kovács for his valuable inspiration and advices for the Hungarian project.

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