EFFECT OF ECTOMYCORRHIZAL INOCULATION AT PLANTING ON GROWTH AND FOLIAGE QUALITY OF TILIA TOMENTOSA

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Abstract. By analogy with the techniques of controlled mycorrhization of forest plantations, an attempt was made to inoculate silver limes (*Tilia tomentosa*) with ectomycorrhizal fungi at planting on a street in Paris, France. In spite of poor colonization of the roots by the introduced symbionts (*Laccaria laccata, Paxillus involutus* and *Cenococcum geophilum*), tree growth was stimulated and yellowing of leaves in autumn was delayed. These results are discussed in terms of biology of the symbioses and practical benefit of this technique applied to ornamental trees in urban plantations.

The ectomycorrhizal symbiosis between higher fungi and roots is known to be an obligate association for most forest trees in the temperate and boreal regions. The fungal symbiont, through its extensive hyphal network, takes up water and nutrients from the soil and transfers them to the root cortex. It also produces plant growth regulators affecting the plant development, and the mycelial mantle protects the short absorbing roots against soil-borne pathogens. The combination of these various mechanisms results in a wide range of tree responses to the symbiosis, depending on the plant and fungal species and on the environmental conditions.

The use of this diversity of response is developing in forestry practice through a technique known as "controlled mycorrhization." It consists of inoculating seedlings in the nursery with mycelia of ectomycorrhizal fungi selected for their better performance, compared to the resident symbionts at the outplanting site, for stimulating tree growth (1,5,6). Controlled mycorrhization is an energy-saving and environment-friendly alternative to soil tilling, chemical fertilizers and pesticides for improving the early growth of forest plantations.

Many ectomycorrhizal tree species are also planted in cities as ornamentals but their mycorrhizal status is poorly documented (2). Among them, *Tilia tomentosa* (silver lime, native to south-eastern Europe) is massively used as an ornamental in French cities, particularly in Paris. The aim of the present work was to test the possibility of adapting controlled mycorrhization to *T. tomentosa* planted along streets.

Urban conditions differ from forest ones in that trees are much bigger when outplanted and are submitted to more severe stresses such as air and soil pollution by exhaust fumes, de-icing salts, and soil compaction. Their root systems are also generally confined in a smaller volume and the air and water supply is often limited by sealed pavements. The aim of the present study was to use controlled mycorrhization to alleviate these stresses. Moreover, as trees are bigger and older when outplanted (ten years or more), it is difficult to control their mycorrhizal status in the nursery. In this experiment, the fungal inoculum was thus applied at planting.

Material and Methods

The trees used for the experimental plantation were *Tilia tomentosa* 7-9 cm in trunk diameter at 1.30 m above ground. They had been grown for 8 years under Atlantic climate in a specialized nursery in western France. They were delivered to the planting site with a root ball 70 - 80 cm in diameter, wrapped in iron net. When planting, the net was open on top but not removed (it is designed to rust and break apart in the soil within a few years) and fine roots were sampled for mycorrhiza examination; about 50% of the short roots were typical jet-black ectomycorrhizas due to the Ascomycete *Cenococcum geophilum* while others were miscellaneous, unidentified morphotypes, with a dominance of the *Scleroderma* type. The trees were delivered with a full crown and were not pruned at planting.

The fungal inoculum was mycelium aseptically grown in a vermiculite-horticultural sphagnum peat mix moistened with nutrient medium (7). Three ectomycorrhizal fungi were used: *Laccaria laccata* strain S238 (isolated from a sporocarp in a mixed conifer stand in Oregon), *Paxillus involutus* strain NAU (from a sporocarp in an oak forest nursery in France) and *Cenococcum geophilum* strain NCY (from an ectomycorrhiza of a lime tree planted along a street of the city of Nancy in France).

The trees were planted 10 m apart and 8 m from the buildings, in the wide footpaths on both sides of Boulevard Suchet (between Place de Colombie and Rue Boilly) in the 16th Arrondissement of Paris between January 28 and January 31, 1991. The traffic is heavy in this street and many cars park between the trees.

The planting holes, 10 m^3 in volume (3 x 3 m, 1.2 m deep), were filled with natural top-soil from the outskirts of Paris (50% sand, 35% silt, 15% clay, 15% organic matter, C/N 12, pH 6.5). No fertilizer was added. During the summer of 1991, the trees were watered during the dry periods (100 liters water per tree per week).

The experimental design consisted in 54 trees randomly distributed into nine replicates of the six following treatments: 1) Control (no inoculum), 2) Autoclaved inoculum (to check if the substrate used for growing the mycelium has an effect on tree growth), 3) *Laccaria laccata,* 4) *Paxillus involutus,* 5) *Cenococcum geophilum,* 6) Mixed inoculum (co-inoculation with the three fungi).

The inoculation took place at planting, by mixing 1 liter of the vermiculite-peat inoculum described above (3 liters in the case of the Mixed Inoculum treatment, in order to provide the same competitive advantage to each fungus against the resident ones as in the other treatments) into 30 liters of the soil from the plantation holes. This mixture was then distributed under and around the root ball of the tree.

The height increment (length of the top shoot of the year) of each tree was measured annually during the winter resting period from 1991 to 1994 (dates given in the legends of Tables 1 and 2), as well as the diameter at 1.3 m from ground level. The data were subjected to analysis of variance.

The aesthetic value of urban trees depends essentially on the persistence and healthy appearance of their foliage. Therefore, budbreak, yellowing in summer and autumn, and extent of leaf fall were rated on appropriate dates (given in Table 5) according to arbitrary scales (see legend of Table 5). Because this rating was somewhat subjective, it was always performed by the same person and not subjected to statistical analysis.

In August 1992 (i.e. in the middle of the second growing season after outplanting), healthy green leaves from the middle part of the top shoots were sampled for assessing the nutrient status of the trees. The analyses (total N, P, K, Ca and Mg) were performed by the INRA Central Laboratory for Plant Tissue Analyses in Bordeaux (France).

In June 1994 (i.e. three years after outplanting), the mycorrhizal status of all trees was assessed. Two 1 liter soil cores per tree were sampled using a steel cylinder with a cutting rim (8 cm in diameter and 20 cm in depth) 1 m from the base of the tree in opposite directions perpendicular to the street. Roots were seperated from soil, washed, and the thin roots cut into pieces about 2 cm long. The two samples per tree were pooled by thoroughly mixing in water. Pieces of roots were randomly picked and examined using a stereomicroscope. All short roots up to 200 were counted in this subsample, recording separately different morphotypes (*L. laccata, P. involutus, C. geophilum* and Others) according to previous descriptive works (4,8). The results for each morphotypes were expressed as per cent of total short roots and statistically analysed (analysis of variance) after transforming the data by Arcsin square root.

Results

The effects of the fungal treatments on the height and radial increments of the trees during the four years after planting are shown in Tables 1 and 2, respectively. No significant differences were found between the two controls (no inoculum or autoclaved inoculum).

When considering the total height increment from 1991 to 1994 (Table 1), the four fungal treatments had a significant positive effect of about 25%, resulting in trees 45 to 50 cm taller.

Table 1. Effect of the inoculation treatments on the annual shoot length of the trees (cm); mean values of nine replicates. Measurements were made on January 31, 1991 (initial); February 2, 1992; November 9, 1992; March 6, 1994; January 17, 1995.

Treatments	1991	1992	1993	1994	Total 91-94
Control	13.0	17.1	48.8	45.2	124.1
Autoclaved inoculum	13.1	16.8	44.6	36.9	111.4
Laccaria laccata	14.6	25.9	84.4 *	43.0	167.9 *
Paxillus involutus	14.6	27.1	76.4 *	38.0	156.1 *
Cenococcum geophilum	13.7	26.8	75.0 *	52.3 *	167.8 *
Mixed inoculum	10.2	46.2 *	74.0 *	35.0	165.4 *

Values followed by an asterisk differ significantly from that of the Control at the 0.05 probability level (Fisher PLSD test)

This effect is essentially due to the contribution of the third year (1993). No difference was recorded on the first year and the mixed inoculum and *C. geophilum* treatments significantly stimulated tree growth only in 1992 and 1994, respectively.

Table 2 shows that the diameter increment responded positively to *P. involutus, C. geophilum* and the mixed inoculation. *L. laccata* had no significant effect on diameter.

The nutrient status of the trees in 1992, as shown in Table 3 by the mineral content of the leaves, was irregularly affected by the fungal treatments with no obvious relation to the effect on growth. The inoculation with *P. involutus* favored calcium nutrition, that with *C. geophilum* calcium and magnesium content, and the mixed inoculum led to a higher nitrogen concentration in the leaves. There is no difference between the control and the treatment with autoclaved inoculum.

Table 4 shows the ectomycorrhizal status of the trees on the spring of 1994, i.e. three years after outplanting and inoculating and the year following the maximum growth effect of the fungal treatments. *Laccaria* and *Paxillus* types were present on all roots regardless of the treatments, but they were more abundant in the treatment respectively inoculated with these fungi and to a lesser extent in the mixed treatment. In contrast, the *Cenococcum* type was totally absent in all treatments, in spite of its initial presence at planting and of the positive effect of the *C. geophilum* treatment on tree growth.

Table 2. Effect of the inoculation treatments on the annual diameter increment of the trees measured at 1.3 m above ground (cm); mean values of nine replicates. Measurements were made on January 31, 1991 (initial); November 9, 1992; March 6, 1994; January 17, 1995.

Treatments	1991+1992	1993	1994	Total 91-94
Control	1.18	1.66	1.36	4.20
Autoclaved inoculum	1.25	1.36	1.28	3.89
Laccaria laccata	1.34	1.54	1.50	4.38
Paxillus involutus	1.14	1.86*	1.51	4.51*
Cenococcum geophilum	1.14	1.69	1.70	4.53*
Mixed inoculum	1.82*	1.55	1.28	4.65*

Values followed by an asterisk differ significantly from that of the Control at the 0.05 probability level (Fisher PLSD test).

Table 3. Effect of the inoculation treatments on the mineral content of the top leaves of the trees in August 20, 1992 (per cent of dry matter, mean values of nine replicates).

Treatments	N	P	K	Ca	Mg
Control	2.52	0.16	1.18	3.04	0.43
Autoclaved inoculum	2.41	0.14	1.13	2.66	0.36
Laccaria laccata	2.58	0.16	1.29	2.86	0.39
Paxillus involutus	2.28	0.14	1.17	3.43*	0.44
Cenococcum geophilum	2.55	0.14	1.06	3.68*	0.51*
Mixed inoculum	2.62*	0.15	1.39*	2.57	0.33

Values followed by an asterisk differ significantly from that of the Control at the 0.05 probability level (Fisher PLSD test).

Table 4. Effect of inoculation treatments on the mycorrhizal status of the trees June 7-9, 1994 (identified mycorrhizal morphotypes as per cent of total short roots out of a random sample of 200 short roots); mean values of the nine replicates.

Treatments	Mycorrhizal morphotypes		
	Laccaria.	Paxillus	Cenococcum
Control	7.6	0.4	0
Autoclaved inoculum	7.5	1.0	0
Laccaria laccata	25.9	0.1	0
Paxillus involutus	2.0	11.6	0
Cenococcum geophilum	6.2	3.2	0
Mixed inoculum	17.0	5.4	0

Values followed by an asterisk differ significantly from that of the Control at the 0.05 probability level (Fisher PLSD test).

As shown in Table 5, the overall effect of the treatments on the foliage phenology was essentially marked on the color of the leaves from July on to leaf fall. The yellowing was delayed with all four inoculation treatments, with the magnitude of differences depending on the year. The treatments had no effect on bud break, but *L. laccata* and the mixed inoculum delayed leaf fall in 1992. Local microclimatic data were not recorded. It is thus impossible to attribute this yellowing pattern to any changing environmental factor such as temperature, soil moisture, air relative humidity or pollution.

Discussion

The main aim of this first attempt to adapt the techniques of controlled mycorrhization to urban trees was to improve the early establishment and ornamental value of silver lime. Regardless of the experimental treatments, the planting conditions were particularly favorable: the trees were delivered in excellent health with a large volume of soil around intact roots and they did not suffer from water stress during the first year after outplanting. Even so, the four treatments with fungal inoculation markedly improved tree growth and the persistence of healthy green leaves. It is noteworthy that the treatment with autoclaved inoculum did not induce differences from the non-inoculated control for all the parameters measured, indicating that the substrate used for growing and carrying the mycelium (vermiculite, sphagnum peat and nutrient medium) had no impact on the nutrition of the trees at the dose used.

By analogy with forestry, where controlled mycorrhization is particularly effective under adverse planting conditions (drought, severe mineral deficiency or toxicity of the soil) (3,6), we may speculate that the effect of ectomycorrhizal inoculation of urban trees could be even more interesting under less favorable circumstances. Therefore, this technique may be worth developing in addition to the usual precautions whenever

Table 5. Phenology of the foliage. Rating from 0 to 3 according to the following arbitrary scales.

Treatments	Bud break				Yellowing						Leaf fall	
	92 Mar.	93 Apr.	94 Mar.	95 Apr.	91 Jul.	91 Sep.	92 Jul.	92 Sep.	93 Nov.	94 Oct.	92 Oct.	93 Nov.
Control	1.4	2.2	2.1	2.2	0.7	0.9	0.2	1.3	1.3	0.9	1.4	1.2
Autoclaved inoculum	2.0	2.2	1.9	2.2	0.6	0.7	0.3	1.4	1.0	0.9	1.8	0.9
Laccaria laccata	1.9	2.9	2.4	2.2	0.4	0.3	0	1.0	1.2	0.7	0.3	1.3
Paxillus involutus	1.9	2.3	2.2	1.8	0.7	0.4	0	1.1	0.7	0.7	1.1	0.8
Cenococcum geophilum	1.8	2.1	2.4	2.4	0.7	0.2	0	1.1	0.7	1.1	1.2	1.2
Mixed inoculum	2.1	2.1	2.2	2.4	0.2	0.3	0	1.1	0.9	1.1	0.8	1.7

Budbreak.: 0: no open buds; 1: some buds open but no emerging leaves; 2: leaves emerged but no elongating axes; 3: most axes elongating. *Yellowing.* 0: whole foliage dark green; 1: whole or part of foliage pale green; 2: whole or part of foliage yellowing; 3: Whole or part of foliage with dry leaves. *Leaf fall*: 0: no fallen leaves; 1: defoliation of less than one third of the crown; 2: defoliation of one third to two thirds of the crown; 3: defoliation of more than two thirds of the crown. Mean values of the nine replicates (no statistics done) ornamental trees are to be planted under difficult conditions or as an alternative to these expensive precautions under more favorable conditions. For instance, optimizing the ectomycorrhizal symbioses could be a way of improving the success of plantations with tree species known for their poor survival after outplanting, such as *Corylus colurna* (Turkish hazel) permitting a wider variety of ornamentals in the cities.

The cost of the inoculum used in this experiment was about FF 30 (US $ 6) per tree, which was marginal compared to the cost of planting such big trees. It would therefore be economically possible to use higher doses of inoculum in order to improve mycorrhizal establishment.

It is remarkable that these promising results were obtained with three fungal strains from various origin, which were initially choosen because of their proven efficiency under completely different conditions (*L. laccata* S238 for controlled mycorrhization of Douglas fir forest plantations in France (5) and P. *involutus* NAU for oaks (3) or because of their urban origin (C. geophilum from a lime tree in a street of Nancy). It is thus reasonable to assume that selection work specifically aimed at urban trees would yield even better performing strains. This is why a survey of the ectomycorrhizal symbionts naturally associated with trees in the streets of Paris is presently being carried on. The collection of new isolates will serve as a basis for a series of experimental plantations in the city.

It is also noteworthy that a positive effect on tree growth and leaf persistence was obtained in spite of the poor competitiveness of the introduced fungi: three years after outplanting, *L. laccata* and *P. involutus* represented only 26% and 12% of the mycorrhizal short roots, respectively. Aggressiveness (root-colonizing ability) and competitiveness against resident symbionts should be important criteria for the selection of better performing strains. The case of *C. geophilum* is particularly clear. *Cenococcum*-type ectomycorrhizas from the nursery were abundant on the roots at planting but completely absent three years later, even in the non-inoculated controls. Because the *C. geophilum* treatment had a significant effect on tree growth, we may conclude that the introduced strain of this species formed mycorrhizas before 1994, then disapeared together with the strain from the nursery. The reason for the poor persistence of *C. geophilum* under the conditions of the experiment might be the irrigation of the trees during the first year after outplanting: this fungus species is known to prefer dry soils.

The mixed inoculum treatment, where the three fungi were introduced together, did not provide any greater benefit than single inoculation treatments, either in terms of mycorrhiza formation or tree growth.

An alternative to inoculation at planting is to inoculate the trees in the nursery one or two years before outplanting, as practiced in forest nurseries. Experiments of this type are under way for future plantations in Paris.

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Zusammenfassung. Es wurde in einer Straße in Paris ein Versuch angelegt, um Linden (*Tilia tomentosa*) mit Ectomycorrhiza-Pilzen während des Pflanzen zu inokulieren. Um die schwache Kolonisation der eingeführten Symbionten (*Laccaria laccata, Paxillus involucratur* und *Cerococcum geophilum*) auf den Wurzeln zu unterstützen, wurde das Baumwachstum stimuliert und die Persistenz der grünen Blätter verbessert. Die Ergebnisse wurden diskutiert in Bezug auf die Biologie der Symbiose und die praktischen Vorzüge dieser Technik in der Anwendung bei Straßenbaumpflanzungen.