Influence of infection by an endomycorrhizal fungus on root development and architecture in *Platanus acerifolia*

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**Abstract**

Morphological analysis, modelling and topological methods have been used to investigate the influence of a vesicular-arbuscular (VA) endomycorrhizal infection on the root system of *Platanus acerifolia*, a very common tree species in urban environments. Root systems of endomycorrhizal plants did not differ during the earliest growth period, but at five weeks’ growth and onwards the overall effect of mycorrhiza formation was to increase lateral root frequency, giving rise to a more branched root system. During the earliest growth phase, root systems of *P. acerifolia* developed a herringbone pattern, which then tended towards a more dichotomous pattern in mycorrhizal plants after five weeks when infection was maximum and a mycorrhizal growth response occurred. This study shows for the first time that VA mycorrhizal infection can considerably affect root morphogenesis in a tree species.

Reference

Influence of infection by an endomycorrhizal fungus on root development and architecture in *Platanus acerifolia*

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Morphological analysis, modelling and topological methods have been used to investigate the influence of a vesicular-arbuscular (VA) endomycorrhizal infection on the root system of *Platanus acerifolia*, a very common tree species in urban environments. Root systems of endomycorrhizal plants did not differ during the earliest growth period, but at five weeks' growth and onwards the overall effect of mycorrhiza formation was to increase lateral root frequency, giving rise to a more branched root system. During the earliest growth phase, root systems of *P. acerifolia* developed a herringbone pattern, which then tended towards a more dichotomous pattern in mycorrhizal plants after five weeks when infection was maximum and a mycorrhizal growth response occurred. This study shows for the first time that VA mycorrhizal infection can considerably affect root morphogenesis in a tree species.

Key words: endomycorrhizal infection, *Platanus acerifolia*, root development.

Knowledge about the architecture and morphology of a root system can provide useful information for appreciating not only its nutrient absorbing capacity, but also the energy cost to the plant of a given type of root system structure vis-à-vis nutrient uptake. Root system development can be analyzed using different mathematical models (Picard et al., 1985; Jordan 1987a,b; Pages e Aries 1988) or a developmental model based on axes (Rose 1983). These, however, are not adequate for generating hypotheses about root function (Fitter 1985, 1986) nor for visualizing or quantifying rooting strategies and topological analyses are necessary to obtain information about these aspects.

Many soil factors, in particular nutrients and microorganisms, influence root development (see for example Torrey 1986). Several studies have shown that infection by symbiotic VA mycorrhizal fungi can cause morphological changes in the root systems of different herbaceous plants (Fitter 1985, 1986; Berta et al., 1990; Hetrick 1988), and more recently VA mycorrhizal infection was also reported to influence the root architecture of *Vitis vinifera* L., a micropropagated woody species (Schellenbaum et al., 1991).

This paper reports the use of morphological analysis, modelling and topological methods to investigate the influence of VA mycorrhizal infection on the root system of *Platanus acerifolia* Willd., a (non-micropropagated) tree species which is very common in urban environments and quite well known from an ecological point of view (Von Sury e Fluckiger 1991).

**MATERIALS AND METHODS**

**Plant growth conditions**

Seeds of *P. acerifolia* were cold-treated (4 °C, 8 weeks) and then germinated on humid filter paper in Petri dishes in a controlled environment (20 °C, 70% r.h., 16h day, 220 μErg.s⁻¹.m⁻²). After ten days, home-
geneous seedlings were transplanted into individual pots containing 400g of a disinfected clay-loam soil, gravel and Terra-green (Oil Dry SA, RFA) mix (2:1:1). Half of the seedlings were inoculated with *Glomus fasciculatum* (Thaxter sensu Gerdemann) Gerd & Trappe amend. Walker and Koske (LPA 7) by introducing 1g of chopped mycorrhizal onion roots into the planting hole, and half received filtered mycorrhizal root washings as control seedlings. Plants were grown in the controlled environment described above and harvested at 3, 4, 5, 6 and 7 weeks after transplanting. All plants received weekly 16 ml of long Ashton solution (Hewitt 1966) without P and were watered daily. At each harvest, roots of 5 replicate plants were washed and measured (see below) before staining with trypan blue to visualize and estimate the intensity of the VA mycorrhizal infection (Trouvelot et al., 1986). Shoot height and leaf surface area, using a «Delta-T- Area Meter System» (Toshiba), were also recorded.

**Morphological analysis and modeling**

Root system morphology was analyzed using a developmental model (Rose, 1983). Roots produced directly from the base of the plant were referred to as axes, those arising from the axes as first order laterals, those arising from the first order laterals as second order laterals, etc. This ordering system assumes a distinct identity for each axis, and distinct dimensions and properties for each class of laterals.

At each sampling, the number of axes and of first, second or third order laterals were counted, their individual lengths were measured and total root length was calculated. Lateral root frequency was represented by the number of roots of order n, divided by the length of roots of order n-1.

Data were compared by analysis of variance, treating \( P < 0.05 \) as significant (*) and \( P < 0.01 \) as highly significant (**). Standard errors were calculated for all data.

Logistic analysis of plant growth has often been applied to weight (Causton & Venus 1981); in this paper we have extended it to the number and length of roots. The following linear and non-linear regressions were explored (Causton & Venus 1981; Berta et al. 1990):

- **linear function:** \( y = mt + c \)
- **logistic function:** \( y = \frac{a}{1 + b \cdot e^{-kt}} \)

where \( t \) is time, and \( m, c, a, b \) and \( k \) are constants. The constant \( a \) is the asymptotic value to which \( y \) tends.

**Topological analysis**

Terminology and topological properties are the same as those used by Fitter (1986): magnitude (\( \mu \)) is the number of exterior links in the system (an exterior link is an internode terminating in a meristem), and trees of equal \( \mu \) may be compared by their total exterior path length (\( p, \)), the sum of all path lengths from all exterior links to the base.

Regressions of \( \log p, \) on \( \log \mu \) were made to determine the dependency of these variables on magnitude. When the slopes of these regressions approach maximum values, the systems are described as herringbone, i.e. most branching is confined to the main axes.

\[
p_{\text{max}} = \frac{1}{2} \cdot (\mu^2 + 3\mu - 2) 
\]

(Fitter, 1985)

In order to see whether root systems deviated from a random pattern, i.e. the probability of branching is equal at all links (Fitter, 1985), the parameter \( p, /E(p, ) \), total exterior path length divided by its expected value, was studied assuming random growth:

\[
E(p, |\mu) = N(\mu) 
\]

(Fitter, 1985)

where,

\[
N(\mu) = \frac{1}{2\mu - 1} \cdot \left( \frac{2\mu - 1}{\mu} \right) 
\]

(Fitter, 1986)

If \( p, /E(p, ) \) is greater than 1, the root branching pattern is tending towards a herringbone model, if this value...
Fig. 1 - Leaf area (A) and shoot height (B) in mycorrhizal (●) and non mycorrhizal P. acerifolia plants (○). Bars represent standard errors. (*,**), differences at P<0.05 and P<0.01 respectively. In B the values of mycorrhizal (—) and non mycorrhizal (_-_-) plants are fitted by an exponential function.
TABLE I. Intensity of infection of root cortex (M%) and arbuscule frequency in the total root system (A%).

<table>
<thead>
<tr>
<th>Weeks after transplanting</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>*M%</td>
<td>40a</td>
<td>57b</td>
<td>82c</td>
<td>76c</td>
<td>79c</td>
</tr>
<tr>
<td>A%</td>
<td>32a</td>
<td>47b</td>
<td>58c</td>
<td>45b</td>
<td>51bc</td>
</tr>
</tbody>
</table>

*M% and A% calculated as described by Trouvelot et al. (1986). Different letters following values in a line indicate a significant difference at different times for M and A (P=0.05).

is about 1, random branching has occurred, while if it is much lower than 1, the system approaches a dichotomous pattern.

Topology is influenced by the magnitude of the root system. To compensate for this dependency, an analysis of covariance was carried out using system magnitude as covariate.

RESULTS

VA mycorrhizal infection and plant growth

Intensity of VA mycorrhizal infection was already important 3 weeks after inoculation (table I) and reached a maximum after 5 weeks (intensity of infection of root cortex, M = 82%, arbuscule frequency in the total root system, A = 58%), which corresponded to an important increase in shoot development (Figs 1 and 2). Arbuscules were frequent, being present in 60 to 80% of the infected regions of the root system. No infection was observed in roots of control uninoculated plants. Shoot height and leaf area of inoculated plants were significantly greater than those of uninoculated plants at 5 weeks (Figs 1 and 2), and differences between the two increased with time. Data for mean shoot height fitted a linear function in both mycorrhizal and non mycorrhizal plants (Table II).

Root morphology

Total root length was significantly higher in mycorrhizal plants than in controls from 5 weeks after inoculation onwards (Fig. 3). Mycorrhizal infection did not significantly affect the number of root axes and first order laterals. The number of second order laterals, however, was significantly higher in mycorrhizal plants 5 weeks after transplanting (Fig 4A). Data for the number of second order laterals fitted a logistic function in both mycorrhizal and non mycorrhizal plants (Fig 4A, Table II). The fitted curve of the number of second order laterals had a higher and later asymptotic value (a) in mycorrhizal plants than in controls (Table II). In contrast, the asymptotic values of the curves for the mean length of first and second order laterals were lower for mycorrhizal root systems.

There was no significant difference in the mean length of root axes between mycorrhizal and control plants. First and second order laterals were generally shorter in mycorrhizal than non mycorrhizal *P. acerifolia* root systems (Fig. 4B, 4C) and all data fitted a logistic function (Table II). The mean length of third order root laterals did not vary greatly between plants at 6 and 7 weeks after transplanting (Fig 4D).
Fig. 2 - Mycorrhizal (M) and non mycorrhizal (NM) *P. acerifolia* plants, at five weeks after transplanting. (A) shows the greater leaf area of mycorrhizal plants in comparison with the controls; in (B) the «growth effect» produced by the fungus is clearly visible.
### TABLE II. Values of parameters used in the best fits. $F$ and $R^2$ values refer to each group of data.

<table>
<thead>
<tr>
<th></th>
<th>Non-mycorrhizal plants</th>
<th>Mycorrhizal plants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of parameter</td>
<td>Parameter</td>
</tr>
<tr>
<td>Mean shoot height</td>
<td>(2)</td>
<td>$c = 0.68$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$m = 0.35$</td>
</tr>
<tr>
<td>Number of second order</td>
<td>(1)</td>
<td>$a = 285.50 \pm 29.20$</td>
</tr>
<tr>
<td>laterals</td>
<td></td>
<td>$b = 421.88 \pm 43.15$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$k = 1.06 \pm 0.01$</td>
</tr>
<tr>
<td>Mean length of first order</td>
<td>(1)</td>
<td>$a = 81.00 \pm 10.85$</td>
</tr>
<tr>
<td>laterals</td>
<td></td>
<td>$b = 54.33 \pm 7.28$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$k = 0.89 \pm 0.02$</td>
</tr>
<tr>
<td>Mean length of second order</td>
<td>(1)</td>
<td>$a = 23.70 \pm 3.44$</td>
</tr>
<tr>
<td>laterals</td>
<td></td>
<td>$b = 22.25 \pm 3.23$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$k = 0.92 \pm 0.03$</td>
</tr>
</tbody>
</table>

*Equation (1), logistic function; equation (2), exponential function.
Mycorrhizal plants presented a significantly higher value for any order of lateral root frequency than non-mycorrhizal controls at each harvest. Higher values of lateral root frequency were also observed for each order of laterals (Table III).

**Topological analysis**

The topological parameter external pathlength ($p_e$) was analyzed in relation to the magnitude ($\mu$) of the root system. The ratio $p_e/\mu$ was submitted to a covariance analysis using $\log_{10}\mu$ as

**TABLE III.** Number (N) of (n) order laterals/total mm (n-1) order laterals (Nn/Ln-1) and of any order of lateral roots/mm of total root length in non-mycorrhizal (NM) and mycorrhizal (M) plants.

<table>
<thead>
<tr>
<th>Weeks after transplanting</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>$N_{y/L_1}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NM</td>
<td>0.214±0.039</td>
<td>0.183±0.044</td>
<td>0.149±0.042</td>
<td>0.143±0.036</td>
<td>0.100±0.012</td>
</tr>
<tr>
<td>M</td>
<td>0.346±0.048*</td>
<td>0.285±0.039</td>
<td>0.170±0.014</td>
<td>0.162±0.032</td>
<td>0.135±0.015</td>
</tr>
<tr>
<td>$N_{y/L_2}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NM</td>
<td>0.123±0.080</td>
<td>0.106±0.090</td>
<td>0.129±0.013</td>
<td>0.122±0.006</td>
<td>0.179±0.016</td>
</tr>
<tr>
<td>M</td>
<td>0.176±0.020*</td>
<td>0.143±0.012*</td>
<td>0.146±0.080**</td>
<td>0.196±0.080**</td>
<td>0.202±0.017</td>
</tr>
<tr>
<td>$N_{y/L_3}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NM</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.092±0.011</td>
<td>0.111±0.005</td>
</tr>
<tr>
<td>M</td>
<td>0.0±0.0</td>
<td>0.122±0.043</td>
<td>0.113±0.070</td>
<td>0.174±0.100**</td>
<td>0.196±0.090**</td>
</tr>
<tr>
<td>N of any order laterals/</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>total root length</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NM</td>
<td>0.61±0.05</td>
<td>0.042±0.05</td>
<td>0.41±0.08</td>
<td>0.44±0.05</td>
<td>0.52±0.06</td>
</tr>
<tr>
<td>M</td>
<td>0.91±0.10**</td>
<td>0.66±0.08**</td>
<td>0.55±0.09**</td>
<td>0.62±0.05**</td>
<td>0.70±0.06**</td>
</tr>
</tbody>
</table>

*, ** values for NM and M are significantly different at $P<0.05$ and $P<0.01$ respectively.

Fig. 3 - Total root length in mycorrhizal (●) and non-mycorrhizal (○) *P. acerifolia* plants. Bars represent standard errors. (*, **), differences at $P<0.05$ and $P<0.01$ respectively.
TABLE IV. Ratio of total exterior pathlength to its expected value (pe/Epe) in non-mycorrhizal (NM) and mycorrhizal (M) plants. Values are means adjusted for covariate (log magnitude).

<table>
<thead>
<tr>
<th>Weeks after transplanting</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>pe/Epe</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NM</td>
<td>1.31</td>
<td>1.03</td>
<td>0.85</td>
<td>0.53</td>
<td>0.46</td>
</tr>
<tr>
<td>M</td>
<td>1.52</td>
<td>1.29</td>
<td>0.73</td>
<td>0.46</td>
<td>0.42</td>
</tr>
</tbody>
</table>

The value of the ratio decreased in the root systems of all plants with time after transplanting. It was greater than 1 at the first two harvests (3 and 4 weeks after transplanting) and was higher in mycorrhizal plants (Table IV). At all subsequent harvests the value of the ratio was less than 1 and was lowest for mycorrhizal root systems (Table IV).

DISCUSSION

The present morphological and topological analyses of root systems of *P. acerifolia* either inoculated with *G. fasciculatum* or uninoculated controls, show that a VA mycorrhizal infection can considerably influence root morphogenesis in seedlings of a tree species. Root systems of mycorrhizal and non mycorrhizal plants of *P. acerifolia* did not differ during the earliest period of infection development, and there was no significant difference in the number and mean length of axes or first order laterals in both mycorrhizal and non mycorrhizal plants throughout the growth period studied. VA mycorrhizal infection did, however, affect the development of second, third and fourth order laterals in *P. acerifolia* at five weeks growth and onwards, so that these roots were produced in a greater number and earlier in mycorrhizal plants. Furthermore, infection decreased the mean length of second order laterals, although these were more numerous. The overall effect of mycorrhiza formation in *P. acerifolia* was, consequently, to increase lateral root frequency giving rise to a more branched root system. Data for mean length of first order laterals, number and mean length of second order laterals fitted a logistic function. Whilst the asymptotic value \((a)\) for the curve of the number of second order laterals was much higher and later in mycorrhizal plants as compared to controls, those for the mean length of first and second order laterals were lower. These results do not differ greatly from those reported for the root system architecture of *V. vinifera* (Schellenbaum et al., 1991), a micropropagated woody plant species, except that a mycorrhizal effect was observed on root axes and first order laterals in this plant, and the data for second order laterals fitted a linear function and not a logistic function as in *P. acerifolia*.

They do, however, contrast with the lower degree of root branching reported in some mycorrhizal herbaceous plants (Hetrick et al., 1988, 1991; Price 1989).

The values obtained for the ratio \(p/E(p)\) suggest a more herringbone pattern for the root system of *P. acerifolia* infected with *Glomus fasciculatum* during the earliest growth phase, which then tended towards a more dichotomous root system in mycorrhizal plants five weeks after inoculation, when infection reaches a maximum and a mycorrhizal growth response is observed. Root systems of the herbaceous plant *Trifolium pratense* L. (Fitter 1987) and of the micropropagated

Fig. 4 - Number of second order laterals (A), mean length of first (B), second and third order (C) laterals in mycorrhizal (●) and non mycorrhizal (○) *P. acerifolia* plants. Bars represent standard errors. (*,***) indicates significant differences at \(P<0.05\) and \(P<0.01\) respectively. In (A), (B), (C) the values of mycorrhizal (●) and non mycorrhizal (○) plants are fitted by logistic functions.
woody plant species *V. vinifera* (Schellenbaum et al., 1991) have also been reported to switch from a herringbone to a more random architecture once endomycorrhizas began to affect shoot growth whilst, in contrast to *P. acerifolia* non mycorrhizal plants retained the former branching pattern as nutrient stress developed. In energy-cost terms, a herringbone structure is more expensive for the plant (Fitter 1987) but more efficient for exploration of soil over long distances. During the early (up to four weeks) growth of *G. fasciculatum*-inoculated *P. acerifolia* or *V. vinifera*, the extent of mycorrhizal infection of the seedlings was relatively high (M = 40-56%). However, the lack of growth responses during this period suggests that the symbiosis was not completely efficient and, in fact, preliminary observations of fungal alkaline phosphatase activity in *P. acerifolia* mycorrhiza indicate that probably only part of the intraradical mycelium is functionally active during this period (Tisserant et al. unpublished data). This could explain the persistence of a herringbone pattern in the root system of these two woody species during the earliest phase of the mycorrhizal infection. The significant growth response after 5 weeks suggests that the mycorrhizal system had reached an efficient level in supplying the plant with an extensive supplementary network for absorbing soil P, so leading to the development of a more random or dichotomous and less costly rooting pattern. Although changes in P nutrition through fertilization have been reported to modify root branching and development in some herbaceous plant species (Hetrick et al., 1988; Fitter et al., 1988, 1991; Amijee et al., 1989; Trotta et al., 1991), the possibility of a hormonal influence on root system development cannot be ruled out. Some authors (Allen et al., 1980, 1982; Edriss et al., 1984; Dixon et al., 1988) have shown hormonal adjustments in herbaceous plants following VA mycorrhizal infection and Barea & Azcon-Aguilar (1982) have reported the production of cytokinin-like substances by *G. mosseae*.

In conclusion, this work shows for the first time the influence of infection by a VA endomycorrhizal fungus on root development, morphology and topology of a tree species. Wider investigations in other plant species and information concerning the physiological basis of the functioning of the symbiosis will give a better insight into the influence of VA mycorrhizal infections on root system strategy.

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