Growth, nutrition and response to water stress of *Pinus pinaster* inoculated with ten dikaryotic strains of *Pisolithus* sp.

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Summary

Reconstituted dikaryons of *Pisolithus* sp. (Pers.) Coker & Couch from South Africa influenced growth parameters (shoot length, shoot/root ratio and leaf area), nutrition and physiological indicators (transpiration rate, stomatal conductance and xylem water potential) of maritime pine (*Pinus pinaster* Ait.) seedlings during drought and recovery from drought. Seedlings colonized with certain dikaryons were more sensitive to water stress and showed less mycorrhiza formation under water stress than seedlings colonized with other dikaryons. Control (uninoculated) seedlings were significantly smaller than those inoculated with dikaryons. Transpiration rate, stomatal conductance and xylem water potential varied among mycorrhizal treatments during the water stress and recovery periods. After rewatering, the controls and seedlings inoculated with dikaryon 34 × 20 had a weaker recovery in transpiration rate, stomatal conductance and xylem water potential than the other treatments and appeared to have experienced damage due to the water stress. Concentrations of various elements differed in the shoots of *Pinus pinaster* colonized by the various dikaryons. It is suggested that breeding of ectomycorrhizal fungi could constitute a new tool for improving reforestation success in arid and semi-arid zones.

Introduction

The rapid increase in the desertification of arid and semi-arid lands is a problem of international scope (Ben Salem 1985, Nelson 1990). Restoration of affected areas by tree planting is an immediate priority. However, trees grown in these regions exhibit both high mortality and poor growth (Felker 1986).

Seedlings planted on dry sites must possess characteristics that confer tolerance to water stress if they are to survive. Nursery management practices, including inoculation with ectomycorrhizal fungi, can improve seedling vigor after outplanting (Duryea and Landis 1984, Duryea 1985). Ectomycorrhizal fungi influence the water, nutritional and physiological processes of their host (Dixon et al. 1980, Dixon et al. 1983, Reid et al. 1983, Mitchell et al. 1984) and can confer a greater tolerance to water stress on the host plant (Hardie and Leyton 1981, Pigott 1982, Brownlee et al. 1983, Boyd et al. 1986, Read and Boyd 1986). For example, survival and early
growth of pine and oak seedlings on a range of site types are significantly improved following inoculation with ectomycorrhiza-forming *Pisolithus* sp. (Pers.) Coker & Couch (Marx et al. 1977, Marx 1980, Dixon et al. 1983, Marx et al. 1985). The ability of ectomycorrhizal seedlings to exploit available nutrients and water in the soil depends greatly on the soil characteristics and on the extension of the extramatrical phase of the fungal mycelium, which is often in the form of mycelial strands (Skinner and Bowen 1974a, 1974b, Finlay and Read 1986).

Ectomycorrhizal fungi differ in their ability to improve host nutrient uptake and tolerance to water stress. Parke et al. (1983) showed that *Rhizopogon vinicolor* conferred greater water stress resistance on Douglas-fir (*Pseudotsuga menziesii* Franco.) seedlings than *Laccaria laccata*, *Pisolithus* sp., or native fungus. The selection of superior genotypes of ectomycorrhizal fungi may further enhance the performance of inoculated seedlings subjected to unfavorable post-planting conditions.

There are substantial differences in the ability of monokaryotic and dikaryotic strains of *Pisolithus* sp. to form mycorrhizae and mycelial strands, and in their effect on the growth of maritime pine (*Pinus pinaster* Ait.) (Lamhamedi et al. 1990, Lamhamedi and Fortin 1991). *Pisolithus* sp. shows great potential for application to reforestation programs on adverse sites (Marx and Cordell 1989). However, little is known about the genetic variation underlying the ability of ectomycorrhizal fungi to tolerate water stress. The objective of this study was to compare the influence of different dikaryotic strains of *Pisolithus* sp. on the growth, nutrition, and ability of *Pinus pinaster* seedlings to tolerate water stress.

**Materials and methods**

**Cultures**

Monokaryotic cultures were obtained by germinating spores from a single basidiomata of *Pisolithus* sp. from South Africa (Kope and Fortin 1990). The spores were paired in all possible combinations on MNM agar medium. After incubation for 3–4 weeks each plate was examined microscopically at the interface of the two colonies for the presence of clamp connections. Number of nuclei was observed by staining with 0.05% DAPI. Clamp connections and number of nuclei were taken as evidence of the dikaryotic state. Dikaryotic cultures used in this research were the same as those used in previous studies (Lamhamedi et al. 1990, Lamhamedi and Fortin 1991). Characteristics of the cultures are presented in Table 1.

**Plant inoculation and growth**

The fungal inoculum of each dikaryotic strain included a vegetative mycelium grown in a peat and vermiculite substrate (solid inoculum) and a mycelial suspension grown in a mineral solution (liquid inoculum) prepared according to Gagnon et al. (1987, 1988).

Seeds of *Pinus pinaster*, obtained from an open-pollinated provenance in Morocco.
RESPONSES OF PINE SEEDLINGS TO *Pisolithus* STRAINS

Table 1. Characteristics of different reconstituted dikaryotic cultures of *Pisolithus* sp.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Mycorrhizal colonization</th>
<th>Diameter of mycelial strands</th>
<th>Mycelial spread</th>
<th>GI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(%)</td>
<td>(μm)</td>
<td>(cm² day⁻¹)</td>
<td>(%)</td>
</tr>
<tr>
<td>3 x 28</td>
<td>64.5</td>
<td>40.3</td>
<td>1.27</td>
<td>100</td>
</tr>
<tr>
<td>37 x 34</td>
<td>32.5</td>
<td>23.7</td>
<td>2.28</td>
<td>-</td>
</tr>
<tr>
<td>34 x 25</td>
<td>67.9</td>
<td>49.7</td>
<td>2.14</td>
<td>52</td>
</tr>
<tr>
<td>2 x 36</td>
<td>61.6</td>
<td>63.9</td>
<td>2.30</td>
<td>64</td>
</tr>
<tr>
<td>8 x 28</td>
<td>89.9</td>
<td>123</td>
<td>2.99</td>
<td>100</td>
</tr>
<tr>
<td>11 x 15</td>
<td>32.5</td>
<td>33.2</td>
<td>1.64</td>
<td>0</td>
</tr>
<tr>
<td>34 x 20</td>
<td>47.2</td>
<td>-</td>
<td>1.07</td>
<td>0</td>
</tr>
<tr>
<td>9 x 22</td>
<td>66.0</td>
<td>99.5</td>
<td>1.36</td>
<td>92</td>
</tr>
<tr>
<td>27 x 34</td>
<td>59.7</td>
<td>66.3</td>
<td>1.12</td>
<td>-</td>
</tr>
<tr>
<td>17 x 20</td>
<td>79.9</td>
<td>49.7</td>
<td>2.43</td>
<td>66</td>
</tr>
</tbody>
</table>

1 Ectomycorrhizal colonization was studied using the growth pouch method (Lamhamedi et al. 1990).
2 Diameter of mycelial strands (n = 5) on MMN agar in the absence of the host plant after 5 weeks of growth (Lamhamedi and Fortin 1991).
3 Rates of mycelial spread (n = 5) of dikaryons determined axenically with their host plant on soil using a large petri dish (150 x 15 mm).
4 GI is germination inhibition (%) of conidia of *Truncatella hartigii* after 24 hours of incubation in culture filtrates of dikaryons (Kope 1990).
5 Dikaryon did not produce mycelial strand.

(F. W. Schumacher Co, Inc., USA), were stratified in tap water at 4 °C for five days, surface sterilized in 30% hydrogen peroxide for 15 min and rinsed with sterilized distilled water. Seeds were germinated on a peat and vermiculite substrate (1/10 v/v) saturated with mineral solution (Lamhamedi et al. 1990). Five weeks after germination, 1100 seedlings were transplanted to soil-filled plastic trays (80 x 15 x 15 cm), ten seedlings per tray, and inoculated by placing 10 ml of each inoculum type in direct contact with the roots of each seedling. Ten trays were used for each dikaryotic strain. A single strain was used within each tray. Seedlings in 10 trays were left uninoculated and served as controls of the inoculation treatment.

The soil used in the trays was collected from the C horizon of a podzolic soil, in an area dominated by *Abies balsamea*, north of Québec City. Granulometric analysis showed that it contained 83.8% sand, 15.8% loam and 0.4% clay. The soil was fumigated with methyl bromide one month before planting (Gagnon et al. 1987).

Seedlings were grown in a greenhouse with a maximum irradiance of 150 W cm⁻² at 400–700 nm and with a natural day length of about 8 h. Day/night temperature and relative humidity were 22/18 °C and 58%, respectively. One month after inoculation, seedlings were fertilized weekly by direct injection of nutrient solution into the irrigation system, applied at a dilution of 1.25/127 (nutrient solution/water, v/v). For each injection, the nutrient solution contained 73 g l⁻¹ of (30/10/10, N,P,K) and 20 g l⁻¹ of K₂SO₄. Seedlings were watered four times each week with tap water until drainage was observed.

After seven months, while all seedlings were still actively growing, a water-stress
treatment was applied to half of the seedlings by withholding water for 19 days (Days 0 to 19), followed by daily irrigation to field capacity (Days 20 to 26). The remainder of the seedlings were watered normally.

Measurements

Seedlings were sampled on 10 different days during both the water stress and the drought recovery periods (Days 1, 4, 8, 15, 19, 21, 22, 23, 24 and 26). Around sunrise, on each of these days, five seedlings were selected randomly from each fungal strain and watering regime treatment. Stomatal conductance and transpiration were measured on the selected seedlings with a Li-1600 (Li-Cor, Lincoln, Nebraska, USA) steady state porometer and a conifer foliage cuvette. The shoots were then severed and shoot xylem water potential was measured with a pressure chamber (P.M.S. Instrument, Corvallis, OR) by the procedures outlined in Ritchie and Hinckley (1975) and Joly (1985).

Growth parameters (shoot length, shoot/root dry weight ratio and leaf area) were measured and mycorrhiza classes were established following plant harvest. Each seedling was rated according to the degree of mycorrhiza root colonization (Class 1 = 0–10%, Class 2 = > 10–25%, Class 3 = > 25–50%, Class 4 = > 50–75% and Class 5 = > 75–100%) as described by Kropp and Fortin (1988).

Leaf area of each seedling was estimated by volume displacement of fresh seedlings (Burdett 1979). A linear regression equation \( y = 15.94x; r^2 = 0.96 \) was used to describe the relationship between displaced water volume and the surface area of needles and twigs. This equation had previously been established on a subsample of 19 seedlings. Projected surface areas were measured with a Leitz Tas Plus images analysis (Ernst Leitz Ltd., Midland, Ontario, Canada).

Soil water content of each tray was determined gravimetrically for each sampling day. These values were converted to soil water potential from soil water curves (Figure 1) obtained by the pressure plate method (Richards 1949).

The needles and twigs of all sampled seedlings were dried at 65 °C for 48 h. Total N of the oven-dried shoots was determined by the standard micro-Kjeldahl procedure, using a Kjeltec auto 1030 analyser (Tecator AB, Hoganas, Sweden). Phosphorus was determined by the molybdenum blue technique (Chapman and Pratt 1961), and K, Ca, and Mg were measured by atomic absorption spectrophotometry.

Experimental design and data analysis

The experiment was analyzed as a split-block design with the two watering regimes (stressed and non-stressed) as the main plots and the 11 strains (10 dikaryotic strains and one uninoculated or control) as the sub-plots. There were also 10 sampling dates and five trays for each watering regime for a total of 1100 seedlings.

Minimum significance differences (MSD) between means were determined with the Waller-Duncan test as shown in Montgomery (1984). Logarithmic \( (x^* = \ln(x + 1)) \) and power transformation \( (Y^* = Y^a) \) were used to achieve homogeneity of variances of potassium concentrations and growth parameters, respectively. The analyses of variance were performed using SAS software (SAS Institute, Cary, NC,
Results

Mycorrhizal colonization
All *Pisolithus* sp. cultures tested formed ectomycorrhizae under both watering regimes. By Day 26, mycorrhiza classes ranged from 3.9 to 4.4 depending on the dikaryotic culture and water regime (Table 2). Seedlings inoculated with dikaryon 11 x 15 showed the highest mycorrhiza formation when non-stressed, but colonization was reduced under water stress. Only dikaryotic culture 8 x 28 showed high colonization regardless of the watering regime. Cultures 37 x 34, 17 x 20 and 34 x 20 were more sensitive to water stress and showed less colonization under water stress than the other dikaryotic cultures.

Seedlings growth
Growth parameters were influenced by mycorrhizal treatment and watering regime. At the end of the experiment, on Day 26, statistically significant differences (*P* < 0.05) among mycorrhiza strains were observed in both watering regimes for shoot length, root collar diameter, root dry weight and leaf area of seedlings (Table 2). Non-stressed seedlings generally had greater growth than water-stressed seedlings. The control seedlings were significantly smaller, both in height and in dry matter, than most of the inoculated seedlings in the water-stress treatment. The tallest seedlings in the water-stressed and non-stressed treatments were those inoculated with dikaryotic cultures 2 x 36 and 34 x 20, respectively. Leaf areas were greater in the inoculated seedlings than in the controls. Seedlings inoculated with dikaryotic culture 34 x 20 exhibited the highest leaf areas in both watering regimes; these leaf...
Table 2. Growth parameters and mycorrhizal colonization of water-stressed and non-stressed Pinus pinaster seedlings inoculated with different dikaryotic strains of Pisolithus sp. on Day 26 at the end of the recovery period. Different letters indicate a significant difference ($P = 0.05$) according to the Waller-Duncan $K$ ratio $t$-test.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Shoot length</th>
<th>Total DW (mg)</th>
<th>Root DW (mg)</th>
<th>Shoot/root ratio</th>
<th>Leaf area (cm$^2$)</th>
<th>Mycorrhiza$^1$ class</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-stressed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 x 28</td>
<td>8.9 c</td>
<td>110 a</td>
<td>50.3 a</td>
<td>1.17 bcd</td>
<td>31.9 ab</td>
<td>4.1 bcd</td>
</tr>
<tr>
<td>37 x 34</td>
<td>9.0 abc</td>
<td>101 b</td>
<td>48.8 a</td>
<td>1.05 de</td>
<td>27.73 cd</td>
<td>3.9 d</td>
</tr>
<tr>
<td>34 x 25</td>
<td>8.2 d</td>
<td>97.8 cd</td>
<td>46.8 abc</td>
<td>1.08 cde</td>
<td>28.8 bc</td>
<td>4.2 abc</td>
</tr>
<tr>
<td>2 x 36</td>
<td>9.2 ab</td>
<td>90.8 de</td>
<td>38.5 d</td>
<td>1.35 a</td>
<td>28.7 c</td>
<td>4.3 ab</td>
</tr>
<tr>
<td>8 x 28</td>
<td>8.9 bc</td>
<td>83.0 f</td>
<td>38.5 d</td>
<td>1.14 cde</td>
<td>24.2 e</td>
<td>4.2 abc</td>
</tr>
<tr>
<td>11 x 15</td>
<td>8.7 c</td>
<td>89.1 ef</td>
<td>43.4 c</td>
<td>1.05 de</td>
<td>25.68 de</td>
<td>4.4 a</td>
</tr>
<tr>
<td>34 x 20</td>
<td>9.3 a</td>
<td>101 b</td>
<td>44.4 bc</td>
<td>1.25 ab</td>
<td>33.95 a</td>
<td>4.2 abc</td>
</tr>
<tr>
<td>9 x 22</td>
<td>8.3 d</td>
<td>87.9 ef</td>
<td>42.5 c</td>
<td>1.02 e</td>
<td>24.0 e</td>
<td>4.0 cd</td>
</tr>
<tr>
<td>27 x 34</td>
<td>8.9 bc</td>
<td>103 ab</td>
<td>46.8 abc</td>
<td>1.20 abc</td>
<td>29.6 bc</td>
<td>4.1 bcd</td>
</tr>
<tr>
<td>17 x 20</td>
<td>9.0 abc</td>
<td>98.4 bc</td>
<td>47.8 ab</td>
<td>1.10 cde</td>
<td>25.7 de</td>
<td>4.3 ab</td>
</tr>
<tr>
<td>Control</td>
<td>6.4 e</td>
<td>91.6 cde</td>
<td>44.4 bc</td>
<td>1.05 de</td>
<td>23.6 e</td>
<td>1</td>
</tr>
</tbody>
</table>

$^1$ Mean mycorrhiza classes were determined by visual assessment of short root colonization in percentage classes ($1 = 0-10\%$, $2 = 10-25\%$, $3 = 25-50\%$, $4 = 50-75\%$, and $5 = >75-100\%$) as described by Kropp and Fortin (1988).

areas were 48 and 44\% greater than those of the controls in the non-stressed and water-stress treatments, respectively. Regardless of the watering regime, strain 2 x 36 had the highest shoot/root ratio, primarily because of a low root dry weight.

Drought tolerance and recovery from water stress

The effects of the dikaryotic cultures of Pisolithus sp. on physiological parameters during and after recovery from water stress are shown in Figure 2. The decrease in soil water potential between Days 1 and 19 in the water-stress treatments was accompanied by pronounced decreases in transpiration, stomatal conductance and xylem water potential (Figures 2a–c). By comparison, transpiration, stomatal conductance and xylem water potential of non-stressed treatments were very stable between Days 1 and 19 (Figures 3a–c).

Figure 2a shows large variation in transpiration rates among seedlings inoculated...
Figure 2. Transpiration rate (a), stomatal conductance (b) and predawn xylem water potential (c) of *Pinus pinaster* seedlings inoculated with different dikaryons of *Pisolithus* sp. and of control seedlings (T) when subjected to drought. Arrow indicates day of re-watering. Symbols as in Figure 2c.
Figure 3. Transpiration rate (a), stomatal conductance (b) and xylem water potential (c) for non-stressed treatments. Symbols as in Figure 2c.
RESPONSES OF PINE SEEDLINGS TO *PISOLITHUS* STRAINS

with different strains, even on Day 1 of the water stress treatment. Strain 34 × 20 had the highest transpiration throughout the drought period. The transpiration rate of seedlings inoculated with 34 × 25 dropped sharply 4 days after water was withheld. By Day 19, seedlings in all treatments had nearly stopped transpiring. However, in the non-stressed treatments, seedlings inoculated with dikaryon 34 × 20 had a higher transpiration rate than seedlings inoculated with the other strains (Figure 3a). The lowest transpiration rates were measured in non-stressed seedlings inoculated with dikaryon 17 × 20.

By the end of the drought recovery period, three statistically different groups were observed (MSD = 4.21 g cm\(^{-2}\) s\(^{-1}\)). Seedlings inoculated with dikaryons 2 × 36, 27 × 34, 9 × 22, 11 × 15 and those inoculated with 8 × 28, 3 × 28, 34 × 25, 17 × 20 and 37 × 34 exhibited high and medium rates of transpiration, respectively, but seedlings inoculated with 34 × 20, along with the controls, had the lowest transpiration rates and showed signs of severe damage due to water stress.

Stomatal conductance also varied among mycorrhizal treatments during both the water stress and recovery periods (Figure 2b). During the water-stress period, inoculated seedlings showed a lower stomatal conductance than control seedlings, with the exceptions of 17 × 20 and 2 × 36 on Day 15, and 9 × 22 and 17 × 20 on Day 1. Stomata of all water-stressed seedlings were apparently closed by Day 19. By the end of the drought recovery period, controls and seedlings inoculated with dikaryotic culture 34 × 20 had the lowest stomatal conductance. Seedlings inoculated with dikaryons 8 × 28, 34 × 25, 37 × 34, 3 × 28, 9 × 22, 27 × 34 and 17 × 20 had higher stomatal conductances at the end of the recovery period than on Day 1 of the drought cycle. In the non-stressed treatment, significant differences between strains (\(P < 0.05\)) were initially observed in stomatal conductance, but these differences had disappeared by the end of the experiment (Figure 3b).

All fungal treatments had similar xylem pressure potentials at the beginning of the drought cycle (Figure 2c). As the soil dried, xylem water potential declined gradually for both control and inoculated seedlings. At the end of the drying period, on Day 19, a wide range of xylem water potentials was observed, with the control seedlings showing intermediate values. After re-watering, all treated seedlings returned to approximately their original values by Day 25, with the exception of the control and the 34 × 20-inoculated seedlings. The non-stressed seedlings showed xylem water potentials above \(-0.075\) MPa throughout the experiment (Figure 3c).

The after-effects of water stress on transpiration, stomatal conductance and xylem water potential were more pronounced in the controls and in seedlings inoculated with dikaryotic strain 34 × 20.

**Shoot nutrient analysis**

Elemental concentrations in shoots of *Pinus pinaster* varied according to dikaryons and watering regime (Table 3). In the non-stressed treatments, nutrient concentrations were higher in seedlings inoculated with dikaryon 34 × 20 than in any other seedlings. In the water-stress treatments, only control seedlings had concentrations of N, K and Ca equal to or greater than those in the 34 × 20-inoculated seedlings.
Table 3. Concentrations of elements in water-stressed and non-stressed Pinus pinaster seedlings either uninoculated (control) or inoculated with different dikaryotic cultures of Pisolithus sp. at Day 26 at the end of the recovery period. Different letters for each treatment indicate a significant difference (P = 0.05) according to the Waller-Duncan $K$ ratio t-test.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>P</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-stressed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 x 28</td>
<td>0.78 ab</td>
<td>0.95 ab</td>
<td>5.26 b</td>
<td>1.58 bc</td>
<td>0.86 ab</td>
</tr>
<tr>
<td>37 x 34</td>
<td>0.73 ab</td>
<td>0.91 ab</td>
<td>4.71 bc</td>
<td>1.53 bc</td>
<td>0.84 ab</td>
</tr>
<tr>
<td>34 x 25</td>
<td>0.71 b</td>
<td>0.92 ab</td>
<td>4.94 bc</td>
<td>1.62 b</td>
<td>0.93 a</td>
</tr>
<tr>
<td>2 x 36</td>
<td>0.76 ab</td>
<td>0.87 ab</td>
<td>4.87 bc</td>
<td>1.59 bc</td>
<td>0.84 ab</td>
</tr>
<tr>
<td>8 x 28</td>
<td>0.77 ab</td>
<td>0.89 ab</td>
<td>4.34 bc</td>
<td>1.43 cd</td>
<td>0.76 b</td>
</tr>
<tr>
<td>11 x 15</td>
<td>0.80 ab</td>
<td>0.97 ab</td>
<td>5.08 bc</td>
<td>1.50 cd</td>
<td>0.90 a</td>
</tr>
<tr>
<td>34 x 20</td>
<td>0.92 a</td>
<td>0.99 a</td>
<td>6.87 a</td>
<td>1158 bc</td>
<td>0.95 a</td>
</tr>
<tr>
<td>9 x 22</td>
<td>0.72 b</td>
<td>0.84 ab</td>
<td>4.19 c</td>
<td>1.37 d</td>
<td>0.86 ab</td>
</tr>
<tr>
<td>27 x 34</td>
<td>0.70 b</td>
<td>0.85 ab</td>
<td>4.83 bc</td>
<td>1.48 bcd</td>
<td>0.87 ab</td>
</tr>
<tr>
<td>17 x 20</td>
<td>0.73 ab</td>
<td>0.86 ab</td>
<td>4.60 bc</td>
<td>1.59 bc</td>
<td>0.76 b</td>
</tr>
<tr>
<td>Control</td>
<td>0.71 b</td>
<td>0.74 b</td>
<td>4.24 c</td>
<td>2.15 a</td>
<td>0.74 b</td>
</tr>
<tr>
<td>Water-stressed</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 x 28</td>
<td>0.75 a</td>
<td>0.93 abc</td>
<td>4.2 a</td>
<td>1.51 b</td>
<td>0.82 abc</td>
</tr>
<tr>
<td>37 x 34</td>
<td>0.86 a</td>
<td>0.89 bc</td>
<td>3.90 a</td>
<td>1.38 b</td>
<td>–</td>
</tr>
<tr>
<td>34 x 25</td>
<td>0.86 a</td>
<td>0.94 abc</td>
<td>4.48 a</td>
<td>1.41 b</td>
<td>0.93 a</td>
</tr>
<tr>
<td>2 x 36</td>
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<td>0.93 abc</td>
<td>3.84 a</td>
<td>1.44 b</td>
<td>0.78 bc</td>
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<td>8 x 28</td>
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<td>0.98 abc</td>
<td>4.28 a</td>
<td>1.41 b</td>
<td>0.77 bc</td>
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<td>11 x 15</td>
<td>0.77 a</td>
<td>1.04 ab</td>
<td>4.49 a</td>
<td>1.38 b</td>
<td>0.82 abc</td>
</tr>
<tr>
<td>34 x 20</td>
<td>0.76 a</td>
<td>1.11 a</td>
<td>4.71 a</td>
<td>1.53 b</td>
<td>0.93 a</td>
</tr>
<tr>
<td>9 x 22</td>
<td>0.77 a</td>
<td>0.96 abc</td>
<td>4.24 a</td>
<td>1.49 b</td>
<td>0.86 ab</td>
</tr>
<tr>
<td>27 x 34</td>
<td>0.67 a</td>
<td>0.88 bc</td>
<td>4.10 a</td>
<td>1.44 b</td>
<td>0.84 abc</td>
</tr>
<tr>
<td>17 x 20</td>
<td>0.76 a</td>
<td>0.90 bc</td>
<td>4.09 a</td>
<td>1.44 b</td>
<td>0.76 bc</td>
</tr>
<tr>
<td>Control</td>
<td>0.64 a</td>
<td>0.79 c</td>
<td>4.00 a</td>
<td>2.13 a</td>
<td>0.73 c</td>
</tr>
</tbody>
</table>

Discussion

Inoculation of seedlings with Pisolithus sp. reduced the detrimental effects of drought on leaf area, shoot length and shoot/root dry weight ratio. Similar effects of Pisolithus sp. on Douglas-fir and white oak seedlings have been reported by Parke et al. (1983) and Dixon et al. (1983). In addition, in this experiment, we have shown that differences in growth, nutrition, and drought tolerance of Pinus pinaster seedlings result when different dikaryotic cultures of Pisolithus sp. are used (Tables 2, 3; Figures 2a–c, 3a–c). This agrees with recent results showing the high intraspecific variability of monokaryotic and dikaryotic cultures of Laccaria bicolor in their ability to form mycorrhizae and to promote the growth of the host plant (Kropp et al. 1987, Kropp and Fortin 1988, Kropp and Langlois 1990). They also extend earlier in vitro observations on the intraspecific variability of Pisolithus sp. (Lamhamedi et al. 1990).

It has been suggested that mycorrhizal associations can increase transpiration flux and the ability to tolerate water stress in their host trees by increasing the absorbing surface area of the roots and by decreasing resistance to water flow from soil to roots.
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(Reid 1979, Nelsen 1987). Seedlings inoculated with certain dikaryons exhibited higher transpiration rates than the control seedlings with no large change in xylem water potential before peak drying on Day 19 (Figures 2a and 2c). When water stress became severe, only uninoculated seedlings (control) and seedlings inoculated with 34 × 20 wilted. Seedlings inoculated with strain 34 × 20 had the largest leaf area at the onset of the drying treatment (Table 2). Strain 34 × 20 had a relatively low capacity for the formation of mycelial strands and for mycelial spread (Table 1). All other dikaryotic strains are capable of extensive mycelial spread and formation of mycelial strands under controlled conditions (Lamhamedi and Fortin 1991).

The poor performance of seedlings inoculated with 34 × 20 suggests that genotypes of *Pisolithus* sp. differ in their ability to confer drought tolerance to *Pinus pinaster* seedlings. It appears that mycelial strands associated with the host root system can transport physiologically significant quantities of water through the soil–plant continuum (Duddridge et al. 1980, Brownlee et al. 1983, Dixon et al. 1983, Read 1984, Boyd et al. 1986, Read and Boyd 1986). Differences in conductance and transpiration among the different dikaryons could therefore have resulted from differences in mycelial spread or strand formation (Table 1), or from differences in the number of mycorrhizal entry points per unit of mycorrhizal root length (Read and Boyd 1986, Wong et al. 1989).

In addition to a possible physical effect on water supply to the plants, dikaryons of *Pisolithus* sp. could have affected stomatal functions through phytohormone concentrations. Xylem concentrations of abscisic acid and proline have been linked to stomatal regulation in mycorrhizal and nonmycorrhizal plants (Levy and Krikun 1980, Johnson 1987, Coleman et al. 1990, Zhang and Davies 1990). Full stomatal function lagged several days behind the recovery of xylem water potential depending on the dikaryotic culture (Figures 2b and 2c), possibly because of the presence of residual abscisic acid in the vicinity of the guard cells (Johnson 1987). Other factors such as stomatal anatomy, temperature and the epidermal cells can also affect stomatal aperture (Spence 1987).

Seedlings inoculated with certain dikaryons (3 × 28, 34 × 20, 17 × 20, 9 × 22, 11 × 15, 37 × 34, 34 × 25) exhibited lower xylem water potentials than the controls, especially when water availability was extremely low (cf. Sands and Theodorou 1978, Dixon et al. 1980 and Parke et al. 1983). Xylem water potentials lower than those of the control seedlings could reflect a greater osmotic adjustment (Nguyen and Lamant 1989). Recently, it has been shown that, in Douglas-fir seedlings, *Rhizopogon* sp. and *Hebeloma* sp. caused decreased leaf osmotic potential compared to controls, whereas *Laccaria* did not (Dosskey et al. 1990). Vesicular-arbuscular mycorrhizae also enable plants to maintain leaf turgor and conductance to lower leaf and soil water potentials than can nonmycorrhizal plants (Augé et al. 1986).

This study has shown that preselected dikaryons of *Pisolithus* sp. modified ecophysiological responses during and after an episode of low water availability and improved the host plant’s response to these conditions. Others have reported that differences in leaf water potential, transpiration rate, leaf resistance and the ability to recover rapidly from water stress were due to differences in hydraulic conductivities
and plant nutrition (Nelsen and Safir 1982, Nelsen 1987). Soil water potential at the soil-root interface and hydraulic conductance, in addition to physiological parameters, would have been helpful in evaluating and understanding the ecophysiological responses of the host plant. From the data presented here, it is apparent that certain dikaryons of *Pisolithus* sp. such as $3 \times 28$, $9 \times 22$, $27 \times 34$, $17 \times 20$, $37 \times 34$ and $8 \times 28$ can improve the tolerance of plants to drought conditions and aid their recovery after watering. The results recorded here and in previous studies (Kope and Fortin 1989, 1990, Kope 1990, Lamhamedi et al. 1990, Lamhamedi and Fortin 1991) demonstrate that the variability within *Pisolithus* sp. could be used for breeding improved mycorrhizal fungi with characteristics such as mycorrhiza formation, production of mycelial strands, production of antibiotic substances and tolerance to water stress. The encouraging results obtained on dry and harsh sites by using *Pisolithus* sp. as an inoculum (Marx et al. 1977, Dixon et al. 1983, Marx et al. 1985, Marx and Cordell 1989) could be further improved by breeding the symbiont with a view toward reforestation programs of arid and semi-arid zones.

Mycorrhizal infection also influences total plant dry mass. Lodgepole pine seedlings inoculated with *Pisolithus* sp. have shown significantly greater photosynthesis and growth rates than nonmycorrhizal plants (Ekwebelam and Reid 1983). In contrast, other research has shown interspecific variability in the photosynthesis of Douglas-fir seedlings inoculated with different ectomycorrhizal fungi (Dosskey et al. 1990). Although different dikaryons influence stomatal conductance (Figure 2b), it is not clear whether this response is of a magnitude that will affect photosynthesis and growth. No clear relation could be found between dry mass accumulation and stomatal conductance of seedlings inoculated with the different dikaryons. Read et al. (1983) suggested that ectomycorrhizal fungi influenced photosynthesis and growth through source-sink effects. Differences in growth rates could be due to the effect of different dikaryons on the partitioning of carbohydrates among organs. The low total dry mass of seedlings inoculated with the vigorous dikaryon $8 \times 28$ (Tables 1 and 2) and other dikaryons could have been due to the high energy cost of this strain (Fogel 1980). Bowen (1984) postulated that ectomycorrhizal plants compensate for high energy costs by having greater photosynthesis per day.

The higher nutrient contents of seedlings inoculated with dikaryon $34 \times 20$ may be attributed to the efficiency with which this strain exploits the soil surrounding the roots. Dikaryon $34 \times 20$ produces fine and densely packed hyphae, whereas the other dikaryotic cultures exploit the soil by forming relatively long but widely spaced mycelial strands (Lamhamedi and Fortin 1991). Similar differences among different isolates for nutrient uptake were reported by Mitchell et al. (1984). The increase in explored soil volume by dikaryons having long mycelial strands could have significant effects on the uptake of poorly mobile nutrients. Under such conditions, mycelial strand extension could compensate for decreased root growth during periods of drought. The lower calcium content of inoculated seedlings compared to controls for both watering regimes (Table 3) may be the result of accumulation of this nutrient in the hyphae in the form of polyphosphate granules (Stark 1972, Strullu et al. 1983). Harley and Smith (1983) reported that ectomycorrhizal development
increased the internal phosphate concentration of seedlings but reduced the excessive intake of calcium.

Seedlings inoculated with certain dikaryons maintained physiological functions at reduced tissue water potentials. Thus, the high mortality of non-inoculated *Pinus pinaster* seedlings planted on most sites in Morocco (Ruehle et al. 1981) could be improved by planting seedlings inoculated with preselected dikaryons.

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**References**


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