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# EFFECTS OF REAFFORESTATION TECHNIQUES ON THE NUTRIENT CONTENT, PHOTOSYNTHETIC RATE AND STOMATAL CONDUCTANCE OF *PINUS HALEPENSIS* SEEDLINGS UNDER SEMIARID CONDITIONS

E. DÍAZ* AND A. ROLDÁN

*Departamento de Conservación de Suelos y Aguas, CEBAS-CSIC, Murcia, Spain*

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## ABSTRACT

A field experiment was carried out to evaluate the effect of different soil preparation techniques for *Pinus halepensis* afforestation on physiological parameters in Mediterranean summer conditions. The soil preparation treatments consisted of terracing (mechanical and manual) and the addition of an organic amendment (urban solid refuse). The mycorrhizal treatments consisted of nursery inoculation with *Pisolithus arhizus* and the addition of forest soil to the planting holes. Six years after planting the study revealed significant differences in heights and basal diameters, phosphorus and potassium concentrations in the leaves and photosynthetic rate and stomatal conductance. The pines grown in the manually prepared terraces were subjected to strong water stress, as reflected by the high $A{:}g_s$ ratios and highest levels of phosphorus and potassium in leaves. Mechanical terracing and the addition of urban solid refuse produced a higher photosynthetic rate, and a combination of these treatments was particularly effective. Copyright © 2000 John Wiley & Sons, Ltd.

KEY WORDS: semiarid conditions; water stress; photosynthetic rate; stomatal conductance; intrinsic water use efficiency; urban solid refuse

## INTRODUCTION

In forest ecosystems of semiarid areas, water availability is considered to be the major limitation to net primary production (Woodward, 1987; Kozlowski and Pallardy, 1996). In Mediterranean areas, many species which have adapted to poor but reliable habitats become efficient and 'specialized' in the use of resources and are able to survive long periods of physiologically unfavourable conditions (Boer, 1999). One example is *Pinus halepensis*, an autochthonous species of the western and central Mediterranean Basin, which is frequently used in reafforestation schemes in Spanish Mediterranean areas (Pastor-López, 1995; Vallejo, 1997). Its resistance to drought (the principal object being the economical use of the little water available) is based on regulation of the stomatical system, resistance of photosynthetic apparatus to mid and high levels of water deficits, and the accumulation of assimilates within the leaf and root system (even at the cost of wood production). *Pinus halepensis* progressively closes its stomata to limit transpiration in drought conditions (Quezel, 1985). Another xeromorphic feature which helps to reduce water loss during stress periods in *Pinus* sp. is its sunken stomata (Bolhàr-Nordenkampf, 1987). Due to its xerophilous characteristics, *Pinus halepensis* forests occur in the most arid zones, where annual average rainfall is lower than 350 mm.

Different soil preparation techniques prior to reafforestation affect hillslopes in different ways and create different microenvironments for the growing trees by altering the physical and chemical characteristics of the soils. This can affect tree growth and the success of the reafforestation programme (Herrero-Borgoñón and Rubio, 1994). In arid and semiarid zones with degraded soils, terracing has been demonstrated to be

* Correspondence to: E. Díaz, Departamento de Conservación de Suelos y Aguas, CEBAS-CSIC, Apdo. de Correos 4195, 30080 Murcia, Spain. E-mail: ediaz@natura.cebas.csic.es

the most successful way of preparing the soil (Serrada, 1990). Organic amendment, too, can improve the physical properties of soil, particularly the capacity to hold water and soil moisture (Querejeta *et al*., 1998), although it also has an important fertilizing effect (Albaladejo *et al*., 1994).

Studies on leaf gas exchange and the ecophysiology of evergreen Mediterranean species have been conducted in a variety of Mediterranean areas (Tetriach, 1993). In the course of a field experiment (Roldán *et al*., 1996a, b), it was found that the combination of soil terracing, organic addition and mycorrhizal inoculation considerably improved growth and survival of *Pinus halepensis* seedlings even under severe drought conditions. However, the effect of the combination of these techniques on the ecophysiological status of *Pinus halepensis* have not been clearly identified. In this paper, we determine the height and basal diameter, nutrient content (nitrogen, phosphorus and potassium), photosynthetic rate, stomatal conductance and intrinsic water-use efficiency of seedlings in order to better understand the mechanisms that influence their establishment in semiarid conditions.

## MATERIALS AND METHODS

### *Field Site Description*

The experimental area was located in El Aguilucho (UTM: 30SXG5395) in the Carrascoy range in Murcia Province (southeast Spain) at a mean height of 180 m a.s.l. The climate is semiarid Mediterranean, with extremely hot and dry summers. The average annual rainfall, which falls mostly in autumn and spring, is 300 mm. The mean annual temperature is 18 °C and the potential evapotranspiration reaches 900–1000 mm year$^{-1}$. The predominant soils are Haplocalcid and Petrocalcid (Soil Survey Staff, 1998) with a sandy loam texture. The vegetation consists mainly of slow-growing shrubs with some *Pinus halepensis*. The groundcover is sparse, and the predominant species are *Rosmarinus officinalis* L., *Anthyllis cytisoides* L., *Thymus* sp., *Helianthemum* sp. and *Fumana* sp.

### *Experimental Design and Layout*

The experiment was conducted with a $2 \times 2 \times 3$ split split–plot design with five replication blocks (Snedecor and Cochran, 1989). Two mycorrhization treatments plus control were tested (factor M), with and without organic amendment (factor R), in mechanically terraced and manually terraced plots (factor T). The main plots, mechanically ($T_1$) or manually terraced ($T_2$), were replicated in five blocks of two plots.

The experimental area of 2400 m$^2$ was established on a homogeneous hillside (25 per cent slope) facing east. Mechanical terracing was carried out in June 1992, when 4 m wide and 60 m long terraces were excavated by a bulldozer. The thick subsoil lime crust existing in these terraces was broken by deep ploughing along the planting line, using a single-tooth subsoiler mounted at the rear of the bulldozer. Manual terracing produced terraces 0·5 m wide and 60 m long with strips of natural vegetation between adjacent terraces. Both the manual terracing and the subsequent pitting were done by ordinary hand-hoes.

The organic amendment used was urban solid refuse (USR) which was applied to one subplot in each main plot (two main plots per block). This USR was a solid fresh material, neither composted nor ground, but allowed to mature naturally for 15 days. The refuse was supplied by the Murcia Municipal Waste Treatment Plant. The analytical characteristics of the USR, determined by standard methods (Page *et al*., 1982) are shown in Table I. The refuse was applied in a single addition of 100 Mg ha$^{-1}$ to both the mechanical and the manual terraces at the beginning of the experiment in October 1992. The USR was incorporated into the top 30 cm of the soil of the terraces using a rotovator in the case of the mechanical terraces and in the planting holes only, using hand-hoes, in the manually made terraces.

Seeds of *Pinus halepensis* Mill. from El Valle nursery (Murcia) were sown in 300 ml plastic bags in a 3:1 soil/peat mixture. The seedlings were grown in El Valle nursery for one year without any fertilization. One-third of the seedlings were inoculated with basidiospores of the ectomycorrhizal fungus *Pisolithus arhizus* (Pers. I) Rauschert (= *P. tinctorius* [Pers.] Coker and Couch) at the nursery following Castellano and Molina (1989). Spores were applied three times, 1 month apart, 12 weeks after sowing. Each plant received $5 \times 10^5$

 

Table I. Composition of the urban solid refuse (USR) used in the field experiment

| Variable | |
|---|---|
| Initial moisture content (%) | 45·4 |
| Ash (% dry weight) | 40·6 |
| Loss of ignition at 750 °C (%) | 59·3 |
| Organic carbon (% oven dry weight) | 25·3 |
| Total nitrogen (% oven dry weight) | 1·19 |
| Total phosphorus (% oven dry weight) | 0·55 |
| Extractable C (%) | 4·81 |
| C fulvic acids (%) | 3·17 |
| C humic acids (%) | 1·64 |
| Carbohydrates (%) | 4·95 |
| Available phosphorus (mg $kg^{-1}$) | 500·0 |
| pH (1:10 aqueous extract) | 6·8 |
| Polysaccharide (% glucose) | 13·0 |
| Total Cu (mg $kg^{-1}$) | 237·0 |
| Total Zn (mg $kg^{-1}$) | 650·0 |
| Total Cr (mg $kg^{-1}$) | 365·0 |
| Total Cd (mg $kg^{-1}$) | 2·0 |
| Total Ni (mg $kg^{-1}$) | 328·0 |
| Total Pb (mg $kg^{-1}$) | 235·0 |
| *Aqueous extract* (1:10) | |
| Electrical conductivity (s $m^{-1}$) | 0·44 |
| $K^+$ (% oven dry weight) | 0·550 |
| $Na^+$ (% oven dry weight) | 0·680 |
| $Cl^-$ (% oven dry weight) | 0·880 |
| $SO_4^{2-}$ (% oven dry weight) | 1·400 |

spores per application. At the time of planting, 150 ml of pine forest soil was added (S) to the planting holes of another third of the seedlings. This inoculum was taken from an established *P. halepensis* stand located 300 m from the experimental plots. The transferred soil was collected three hours before planting from the feeder-root zone (top 20 cm of mineral soil) of randomly selected mature pine trees. The remaining third of the seedlings did not receive mycorrhizal inoculum and served as a control (C).

Planting was carried out manually in November 1992. Subplots were divided across the slope into three sub-subplots, and the seedlings subjected to the different mycorrhization techniques were randomly assigned to them. The seedlings were planted in 40 cm wide, 40 cm deep pits at least 1 m apart, in a single row per terrace, with a stocking rate of 1800 pines $ha^{-1}$ (at least 25 seedlings per sub-subplot).

The experiment was developed under strictly natural conditions, with no watering or weeding treatments.

*Sampling and Laboratory Procedures*

Instantaneous gas exchange measurements of the photosynthesis rate (A), and stomatal conductance ($g_s$) were determined using a portable gas exchange system (ADC, LCA4 configured with PLC4C chamber, ADC Inc., Hoddesdon, Hertfordshire, UK) according to the methodology developed by Long and Hällgren (1993), Long *et al*. (1996) and Farage (1998). Measurements were made on whole shoots of one year old from one location of the canopy of each tree with the chamber oriented directly towards the sun. Five plants were considered for each combination of factors. Heights and basal diameters were measured in these plants.

The experiment was conducted on four consecutive days between 15 and 18 June 1998 because of the

 

Table II. Pearson's coefficients of correlation between plant size, nutrients content and physiological parameters measured in *Pinus halepensis* seedlings

| | Height | Basal diameter | P | N | K | A | $g_s$ | A | $g_s$ |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | High irradiance | | Low irradiance | |
| Height | 1 | 0·955*** | −0·231** | −0·004 n.s. | −0·254 n.s. | 0·497*** | 0·532*** | −0·447*** | −0·369*** |
| Basal diameter | | 1 | −0·302*** | 0·088 n.s. | −0·275*** | 0·512*** | 0·566*** | −0·416*** | −0·312*** |
| P | | | 1 | −0·008 n.s. | 0·276*** | −0·196** | −0·286*** | 0·188* | 0·070* |
| N | | | | 1 | 0·027 n.s. | 0·194** | 0·171* | 0·200** | 0·179* |
| K | | | | | 1 | −0·184* | 0·215** | 0·209** | −0·129 n.s. |

*, **, *** significant at $p < 0{\cdot}05$, $p < 0{\cdot}01$, and $p < 0{\cdot}001$, respectively.

sampling intensity in gas exchange measurements. The prevailing climatic conditions on these dates allowed us to collect data at both high and low irradiance.

Measurements were always made in the morning between 8:30 and 10:00 solar time. After the A and $g_s$ measurements had been obtained, the shoots used for gas exchange measurement were taken to the laboratory and their total leaf surface area was calculated according to Johnson (1984). A and $g_s$ were expressed on a total leaf surface area basis.

Shoots were dried at 70 °C for 48 hours. The concentrations of nitrogen, phosphorus and potassium on the needles of the shoots were calculated after digestion in nitric-perchloric acid (5:3), the P content was determined by colorimetry (Murphy and Riley, 1962) and the K uptake was estimated by a flame photometer (Schollemberger and Simon, 1954). The total N content was determined using an automatic N analyser (Carlo Erba Inc.).

### *Statistical Analysis*

The data were tested for normality and subjected to analysis of variance using the SAS Statistical package (Version 6.03, SAS Institute, Cary, NC). The effects of the different treatments and their interactions on the variables measured were tested for significance at the 0·05 level of confidence.

## RESULTS

### *Seedling Growth*

Seedling height and basal diameter were directly correlated and showed the same tendency (Table II). All the factors considered produced significant differences in the growth of the pines (Figure 1), especially factors T and R with mechanical terracing and addition of USR increasing pine growth. Note the strong interaction of T × R (Table III), which resulted in the pines grown in this combination of factors showing the maximum growth. Inoculation with *Pisolithus arhizus* and addition of forest soil yielded a significant increase in seedlings growth when comparing with uninoculated ones. The effect of mycorrhization was favoured by mechanical terracing and addition of USR, as shown by the significant interactions T × M and R × M (Table III).

### *Nutrients Content*

The smallest differences between treatments were seen with nitrogen (Figure 2). No significant differences were observed for the factors T and R, and only the addition of forest soil resulted in a significant increase

 

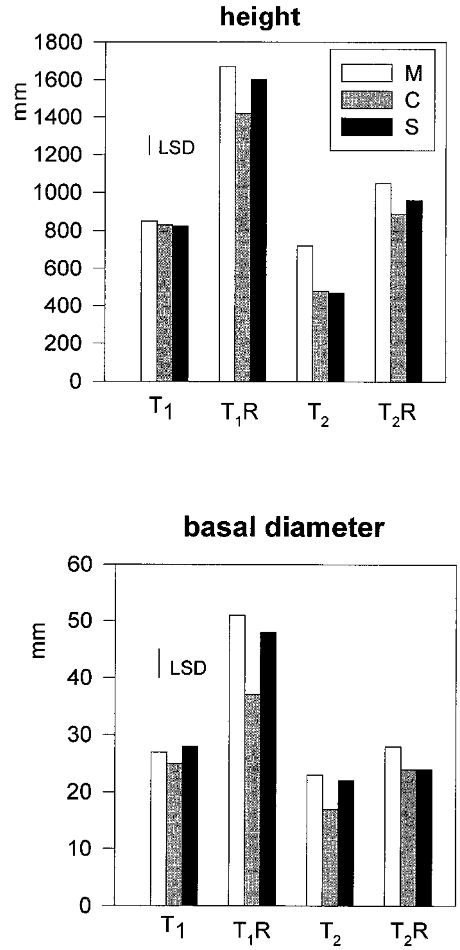


Figure 1. Height and basal diameter of *Pinus halepensis* seedlings subjected to different mycorrhization treatments (M, inoculation with *Pisolithus arhizus*; S, inoculation with forest soil; C, uninoculated) and different soil treatments ($T_1$, mechanical terracing; $T_2$, manual terracing; R, refuse addition).

in the leaf nitrogen content of plants grown in the mechanically dug terraces with and without added USR (as demonstrated by the high degree of significance of the factor M) (Table III).

The lowest degree of phosphorus assimilation by leaves was seen in the plants grown in the mechanically dug terraces, regardless of the mycorrhization treatment, while the highest values were observed in the manually dug terraces with added waste (Figure 2).

This tendency was clearly seen in the factorial analysis, in which the clear effect of factor T and with a high $T \times R$ interaction were evident (Table III). The type of mycorrhization had no significant effect on phosphorus assimilation except in the mechanically prepared terraces with no added waste, where the addition of forest soil led to a significant drop in the assimilation of this nutrient.

As regards potassium assimilation, the highest values were observed in the manually prepared terraces (Figure 2), with a high degree of significance in the factorial analysis. The addition of waste had no significant effect and the type of mycorrhization only had a positive effect on potassium assimilation in an interaction with waste addition (Table III).

 

Table III. *F* value and significance of growth parameter on nutrients content in analysis of variance of the T × R × M (terracing × refuse addition × mycorrhization) split split–plot experiment

| | df | Height | Basal diameter | Foliar concentration | | |
|---|---|---|---|---|---|---|
| | | | | P | K | N |
| Blocks, B | 3 | 0·018 | 2·227 | 3·847** | 2·580 | 6·372*** |
| Terracing, T | 1 | 9701·423*** | 3478·836*** | 42·312*** | 29·148*** | 5·437* |
| Refuse, R | 1 | 16 441·837*** | 2698·423*** | 0·875 | 1·352 | 6·692** |
| T*R | 1 | 1285·641*** | 1054·784*** | 8·760** | 0·352 | 0·504 |
| Mycorrization, M | 2 | 495·820*** | 277·152*** | 1·544 | 1·368 | 7·500*** |
| T*M | 2 | 62·74*** | 18·374*** | 1·967 | 3·231* | 3·979* |
| R*M | 2 | 73·82*** | 1065·093*** | 2·379 | 6·351** | 0·739 |
| T*R*M | 2 | 0·097 | 2·30066 | 0·914 | 2·058 | 1·227 |
| B*T | 2 | 0·015 | 2·154 | 0·085 | 2·964 | 0·215 |
| B*R | 2 | 0·020 | 21·935** | 8·687 | 1·564 | 6·646 |
| B*T*R | 2 | 1·856 | 0·945 | 0·614 | 3·832* | 1·161 |
| B*M | 4 | 0·015 | 8·940* | 0·998 | 0·581 | 0·372 |
| B*T*M | 4 | 8·289 | 2·586 | 3·815** | 1·693 | 2·940* |
| B*R*M | 4 | 2·058 | 5·702 | 0·530 | 2·328 | 0·720 |

*, **, *** significant at $p < 0{\cdot}05$, $p < 0{\cdot}01$, and $p < 0{\cdot}001$, respectively.

Poor correlations were established between the nutrients content and the photosynthetic rate and stomatal conductance at high and low irradiance (Table II).

*Physiological Measurements*

*High irradiance*

The range of high irradiance measured for photosynthetic rate and stomatal conductance is shown in Table IV.

*Photosynthetic rate*. The combination of mechanical terracing and the addition of USR produced the highest photosynthetic rate, while mechanical terracing without refuse produced an intermediate level and manual terracing the lowest values (Figure 3). The application of refuse produced no significant differences in manual terracing. The factorial analysis showed that T and R, and the interaction between them, were the factors showing the highest levels of significance (Table V).

The correlation between plant size and the photosynthetic rate was 0·497 for height and 0·512 for basal diameter, respectively, with high levels of significance ($p < 0{\cdot}001$) (Table II).

*Stomatal conductance*. Pronounced significant differences between mechanical and manual terracing existed for the stomatal conductance, the latter showing the lowest values (Figure 3). T and T × R showed the highest degrees of significance (Table V). The correlation between plant size and the stomatal conductance was 0·532 for height and 0·566 for basal diameter, respectively, with high levels of significance ($p < 0{\cdot}001$) (Table II).

*Intrinsic water use efficiency*. Significant differences were apparent between treatments as regards the $A{:}g_s$ (intrinsic water-use efficiency) ratios, the lowest values appearing in mechanical terracing regardless of the other factors (Figure 3).

*Low irradiance*

The range of low irradiance measured for photosynthetic rate and stomatal conductance is shown in Table IV.

*Photosynthetic rate*. The photosynthetic rate was lower in all the treatments for low irradiance conditions, although differences between treatments still existed (Figure 3). The highest values were detected in the

 

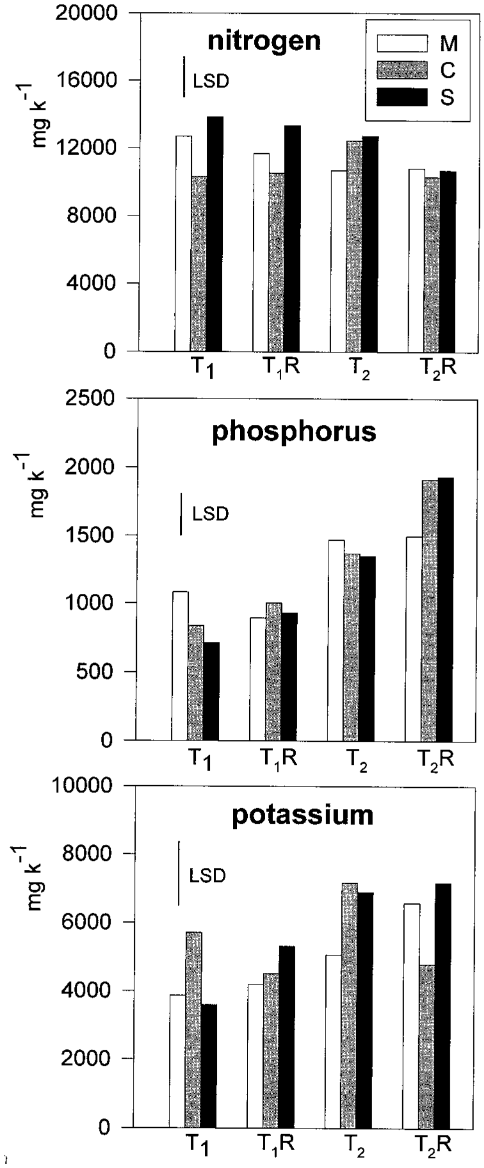


Figure 2. Foliar concentration of nitrogen, phosphorus and potassium of *Pinus halepensis* seedlings subjected to different mycorrhization treatments (M, inoculation with *Pisolithus arhizus*; S, inoculation with forest soil; C, uninoculated) and different soil treatments ($T_1$, mechanical terracing; $T_2$, manual terracing; R, refuse addition).

mechanically dug terraces with no added USR, while the lowest values corresponded to the mechanically prepared terraces to which USR was added. In this last case, the low values observed were possibly due to the significantly higher temperatures recorded during measurements in these treatments compared with the others (Table IV). The factors showing the greatest significance in the factorial analysis were T and $T \times R$ (Table V).

The correlation between height and basal diameter and photosynthetic rate was respectively $-0{\cdot}447$ and $-0{\cdot}417$ with high levels of significance (Table II).

*Stomatal conductance*. Factor T showed a significant increase in this parameter (Table V) in the mech-

 

Table IV. PAR and temperature and VPD by treatment. $n = 20$

| Treatment* | High irradiance | | | Low irradiance | | |
|---|---|---|---|---|---|---|
| | PAR | $t^a$ | VPD | PAR | $t^a$ | VPD |
| $T_1$M | 1430·8 a | 31·6 bc | 6·2 bc | 334·6 abc† | 24·6 a | 3·5 ab |
| $T_1$C | 1431·6 a | 32·5 c | 5·9 bc | 380·0 bc | 25·2 a | 3·8 b |
| $T_1$S | 1595·0 a | 32·0 c | 6·6 c | 418·2 c | 25·5 a | 3·8 b |
| $T_1$RM | 1587·2 a | 30·0 a | 7·1 c | 320·7 abc | 31·2 c | 3·0 ab |
| $T_1$RC | 1648·8 a | 30·1 a | 7·2 c | 207·7 a | 31·9 c | 2·5 a |
| $T_1$RS | 1610·6 a | 29·7 a | 7·9 c | 247·6 ab | 32·2 c | 2·6 a |
| $T_2$M | 1812·3 a | 30·5 ab | 3·8 ab | 263·8 ab | 28·1 b | 3·4 ab |
| $T_2$C | 1677·3 a | 30·8 ab | 2·9 a | 325·3 abc | 27·8 b | 3·3 ab |
| $T_2$S | 1681·3 a | 30·7 ab | 2·2 a | 306·6 abc | 27·4 b | 3·0 ab |
| $T_2$RM | 1532·6 a | 30·0 a | 2·1 a | 277·1 ab | 27·9 b | 2·8 ab |
| $T_2$RC | 1667·3 a | 30·2 a | 2·7 a | 295·3 abc | 27·7 b | 2·9 ab |
| $T_2$RS | 1778·7 a | 29·7 a | 2·6 a | 309·6 abc | 27·8 b | 3·1 ab |

* $T_1$, mechanical terracing; $T_2$, manual terracing; R, refuse addition; M, inoculation with *P. arhizus*; S, inoculation with forest soil; C, uninoculated.
† Values in columns sharing one or more letters do not differ significantly ($p < 0·05$) as determined by Duncan's test.

anically prepared terraces with no added waste. The addition of USR produced a significant fall in the stomatal conductance recorded.

The correlation between height and basal diameter and photosynthetic rate was respectively −0·369 and −0·312 with high levels of significance (Table II).

*Intrinsic water use efficiency*. Significant differences existed between treatments as regards the A:$g_s$ ratios, the lowest values, regardless of the mycorrhization type, occurring in the mechanically dug terraces to which refuse was added (Figure 3).

## DISCUSSION

Studies of N levels and photosynthetic capacities point to lower N concentrations in plants with a lower photosynthetic capacity (Field and Mooney, 1986; Williams *et al*., 1989; Reich *et al*., 1991). In our case, there was no correlation between leaf N concentrations and the photosynthetic capacities observed.

Observational or experimental evidence offers some support for the assumption that water stress affects the levels of nutrients in conifer tissues (Clancy *et al*., 1995). Kemp and Moody (1984) in Douglas fir found that trees growing in soils with a low available moisture content had higher leaf concentrations of P and K than trees growing in soils moister. The fact that the highest levels of phosphorus and potassium in leaves were recorded in the manually prepared terraces suggests that the pines growing there were exposed to a greater degree of water stress. It is generally accepted that the N content is not so much related with water availability but with the photosynthetic rate.

Semiarid Mediterranean areas are characterized by high levels of irradiance, with an average of 300 sunny days per year. From a performance point of view, therefore, a study of ecophysiological behaviour in high irradiance conditions would make more sense. In such conditions, the highest values for photosynthetic activity were observed in the treatments which most favoured seedling growth (Figure 1), i.e. in the mechanically dug terraces with added USR. However, the same treatment showed the lowest photosynthetic rates when measurements were made with a PAR of below 350, perhaps because of the high temperatures recorded (mean 31·8 °C) which were significantly different from the other treatments. $T_1$RM with similar value of vapour pressure difference and higher PAR than $T_2$S and $T_2$RS had a significant reduction on photosynthetic rate measured at low irradiance (Table IV) because the temperature had a significant increase.

 

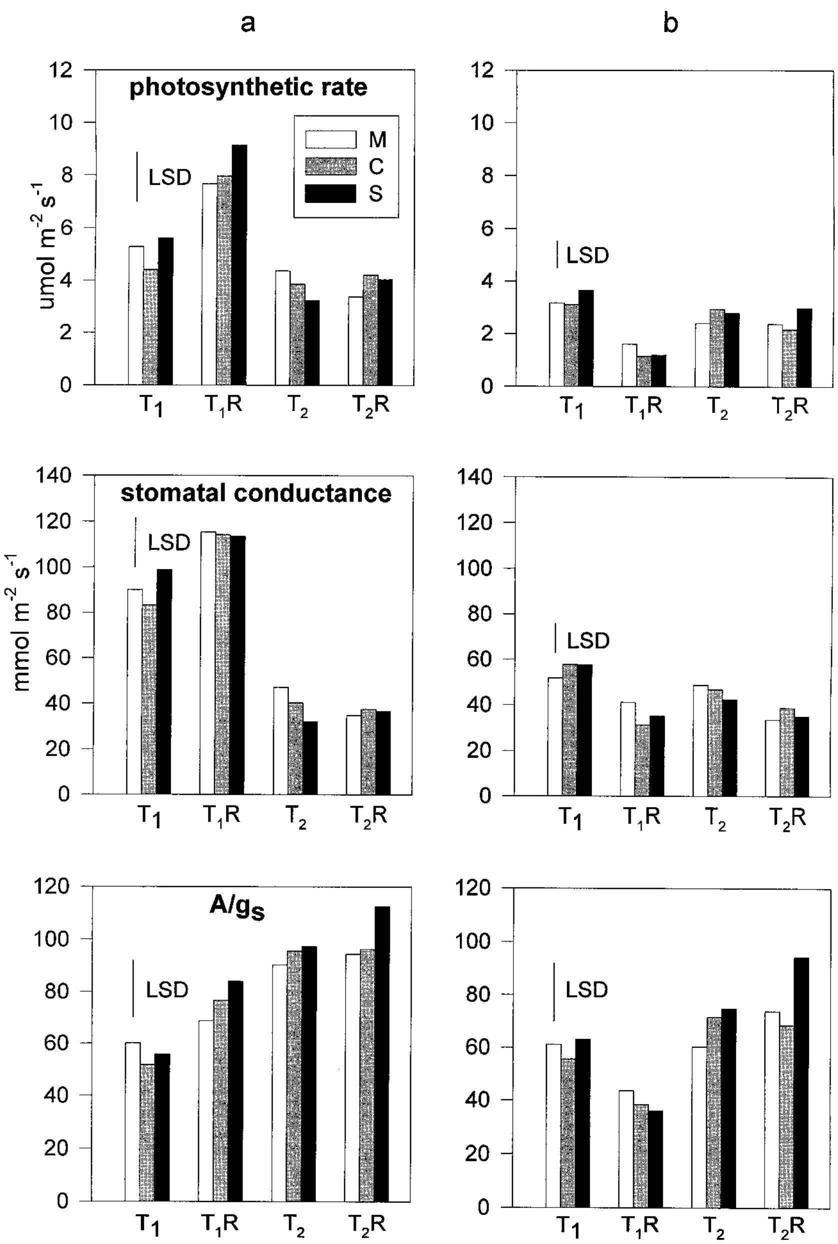


Figure 3. Photosynthetic rate, stomatal conductance and $A/g_s$ ratios measured in *Pinus halepensis* seedlings subjected to different mycorrhization treatments (M, inoculation with *Pisolithus arhizus*; S, inoculation with forest soil; C, uninoculated) and different soil treatments ($T_1$, mechanical terracing; $T_2$, manual terracing; R, refuse addition) (a) measured at high irradiance, and (b) measured at low irradiance.

In natural environments, the regulation of transpiration is strongly dependent upon stomatal responses to both vapour pressure difference and temperature. Stomatal response to temperature *per se* have often been confounded with responses to vapour pressure difference (Schulze and Hall, 1981). When the effects of temperature and humidity are separated, leaf conductance increases with the temperature at temperatures above the optimum for photosynthesis (Hall *et al.*, 1976). Optimum temperatures for photosynthesis in

 

Table V. *F* value and significance of physiological parameters at high and low irradiance in analysis of variance of the T × R × M (terracing × refuse addition × mycorrhization) split split–plot experiment

| | df | High irradiance | | | Low irradiance | | |
|---|---|---|---|---|---|---|---|
| | | A | $g_s$ | $A/g_s$ | A | $g_s$ | $A/g_s$ |
| Blocks, B | 3 | 7·210*** | 1·294 | 20·763*** | 8·000*** | 11·967*** | 16·664*** |
| Terracing, T | 1 | 64·579*** | 222·024*** | 98·934*** | 1·426 | 4·872* | 28·785*** |
| Refuse, R | 1 | 14·497*** | 3·977* | 12·404*** | 52·027*** | 57·242*** | 10·898*** |
| T*R | 1 | 13·884*** | 7·089** | 10·37 | 39·710*** | 6·770** | 33·176*** |
| Mycorrization, M | 2 | 0·535 | 0·017 | 2·657 | 0·150 | 1·072 | 1·154 |
| T*M | 2 | 1·870 | 1·242 | 0·675 | 1·046 | 0·028 | 3·203* |
| R*M | 2 | 1·247 | 0·279 | 0·851 | 0·469 | 0·273 | 0·101 |
| T*R*M | 2 | 0·304 | 0·522 | 0·223 | 0·750 | 2·701 | 1·526 |
| B*T | 2 | 0·267 | 0·468 | 1·256 | 0·152 | 13·026 | 1·953 |
| B*R | 2 | 1·735 | 1·601 | 6·025 | 0·033 | 2·311 | 0·024 |
| B*T*R | 2 | 5·161** | 15·323*** | 0·107 | 4·455* | 1·693 | 4·324* |
| B*M | 4 | 0·412 | 0·346 | 3·573 | 0·317 | 12·133 | 0·062 |
| B*T*M | 4 | 3·220* | 6·144*** | 0·672 | 1·791 | 0·262 | 1·423 |
| B*R*M | 4 | 0·619 | 0·738 | 0·644 | 3·368* | 0·846 | 2·089 |

*, **, *** significant at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

conifers are between 15 and 30 °C (Belous, 1986), although above 25 °C it begins to fall and is up to 50 per cent less at temperatures above 30 °C.

The limiting factor in our case was water stress since previous experiments demonstrated that the water available to plants in the manually produced terraces was less than 40 per cent of that available in their mechanically prepared counterparts (Querejeta *et al*., 1998). The low photosynthetic activity values achieved in manual terraces was due to the water stress. In the morning, plants growing at habitats with water deficit close their stomata, so low values of VPD were obtained for the treatments of manual terraces at high irradiance (Table IV). Flexas *et al*. (1999) showed that hard drought treatment in grapevines produced lowest values of VPD in the morning.

Fernández *et al*. (1998) found that water stress lowered gas exchange values in *Pinus pinaster* while Nelson (1984) argued that conifers do not have inherently low photosynthetic capacity. The lower gas exchange values that we observed in low irradiance conditions in all the treatments have also been reported by other authors, among them Winner *et al*. (1989) in *Pinus halepensis*.

In the conditions prevailing in the study area, for the successful growth of a plantation it is important to measure the efficiency of water use. One of the objectives which one may set in revegetation programmes is to provide soil conditions which favour plant survival. Mechanical terracing with subsoiling provides more water for plants than manual terracing. Such mechanical intervention increases the effective depth of a soil and encourages deeper roots and exploration for water (Querejeta *et al*., 1998). The addition of USR provides nutrients indirectly. In our experiment, the pines grown in manually prepared terraces showed higher A:$g_s$ ratios. High A:$g_s$ ratios indicate that plants are adapted to more arid habitats (Schulze and Hall, 1981).

The development of mycorrhizal associations is also related to acclimation of gas exchange in some conifers (Parke *et al*., 1983; Boyle and Hellenbrand, 1991; Lamhamedi *et al*., 1992). In our case mycorrhization was of little effect on its own except in the case of seedling growth and the levels of nitrogen in leaves in mechanical terracing, but was effective in some interactions with terracing and USR addition. Six years after the experiment was begun all the pines, including the control, showed a high level of mycorrhization (Querejeta *et al*., 1998) irrespective of the inoculation treatment at the outset. Mycorrhizal inoculation favoured seedling performance in the first stages of growth (Roldán *et al*., 1996a, b), but colonization of roots by native mycorrhizal fungi obscured this beneficial effect after six years.

 

In conclusion, it can be said that the ecophysiological variables measured clearly reflect the different treatments. The treatment which best encouraged photosynthetic capacity and stomatal conductance was mechanical terracing because the plants in question were subjected to lower water stress (lower $A/g_s$ values), this effect being strengthened by the addition of refuse. The T × R interaction also showed that a combination of factors is stronger than the addition effect when they are considered separately.

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