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ORIGINAL PAPER

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Continuous measurement of stem-diameter growth response of *Pinus pinea* seedlings mycorrhizal with *Rhizopogon roseolus* and submitted to two water regimes

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Abstract Linear variable differential transformer (LVDT) sensors were used to detect continuous diameter growth responses of *Pinus pinea* (stone pine) seedlings inoculated with the ectomycorrhizal fungus *Rhizopogon roseolus*. Colonised and non-colonised seedlings provided with sensors were submitted to different water regimes in two consecutive experiments established in a controlled-temperature greenhouse module (cycle 1), and in an adjacent module without temperature control (cycle 2). Under regular irrigation, colonised seedlings showed significantly higher growth than non-colonised seedlings. Water-stressed seedlings showed no benefit from inoculation in terms of growth. Also, seedlings with a high colonisation level recovered more slowly from water stress than control seedlings. A significant positive relationship between maximum daily shrinkage (amplitude of the daily stem contraction) and global radiation was observed only in the first water-stress period in cycle 1 and in regularly irrigated seedlings in both cycles. However, no differential responses due to inoculation were observed. The mycorrhizal colonisation of the seedlings at the end of the experiment was related with the initial colonisation level. Mycorrhizal colonisation by *R. roseolus* in old roots was maintained at significantly higher levels in seedlings which had an initial colonisation level >50% than in seedlings with <50% initial colonisation. Also, more newly formed roots became colonised in seedlings which had an initial colonisation level >50% than in seedlings with an initial colonisation <50%, which had almost no new root colonisation. From the results obtained, it can be concluded that LVDT sensors can be used to detect a differential response of plants according to water supply, mycorrhizal status and, in some cases, to their colonisation level. The results are discussed in relation to the predictive possibilities of the method for the selection of efficient mycorrhizal fungi for the promotion of plant growth.

Keywords Fungal selection strategies · Linear variable differential transformer sensors · Plant growth · *Rhizopogon roseolus* · Water stress

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Introduction

It is estimated that some 5,000–6,000 species of fungi are mycorrhizal, most of them forming ectomycorrhizas or ectendomycorrhizas (Molina et al. 1992). Considerable data on ectomycorrhizal fungi and their effects on a broad range of host plants have been reported (Trappe 1962, 1977; Marx 1980; Smith and Read 1997). Also, there is an increasing recognition of the extent of diversity, both structural and functional, within these fungal species (Smith and Read 1997). This natural variability makes it necessary to develop selection strategies for ectomycorrhizal fungi to be used as inocula in forest nurseries (Trappe 1977; Marx 1980). The final objective of the selection process is to obtain competitive, well-adapted fungi, able to readily colonise the feeder roots of the host plant and extend the absorptive potential of the root system (Trappe 1977; Kropp and Langlois 1990). Fungal isolates can be systematically screened for desired characters: growth rate at different cultural conditions, antagonism towards pathogens, production of critical enzymes, adaptability to high moisture tensions, etc. (Trappe 1977). When inoculation is aimed at increasing survival and growth of plants after outplanting, laboratory tests must be followed by field experiments under diverse conditions (Trappe 1977; Marx 1980). Results obtained to date indicate that mycorrhizal inoculation of containerised seedlings, grown under routine nursery conditions, rarely increases growth in the nursery phase (Molina 1980; Castellano and Molina 1989) although the performance of these plants after outplanting can be sig-

nificantly improved (Castellano and Trappe 1985; Pera et al. 1999). Inoculation in bareroot nurseries with fungi such as *Hebeloma cylindrosporum* Romagn. (Le Tacon et al. 1985), *Laccaria bicolor* (Maire) Orton (Le Tacon and Bouchard 1986) and *Pisolithus tinctorius* (Pers.) Coker and Couch (Marx et al. 1976, 1984) resulted in significantly higher growth of inoculated plants compared with that of non-inoculated ones. Field results from outplanting of inoculated bare root plants also indicate an increase or maintenance of the nursery growth effect (Le Tacon et al. 1992; Pera et al. 1999).

Many of the fungi tested so far have been initially chosen by operational criteria such as their ability to grow in culture and to readily colonise the root system. The effectiveness of the fungi to stimulate plant growth in the field has to be detected after several years, so long-term studies are necessary to effectively determine the benefit of inoculation (Le Tacon et al. 1992; Pera et al. 1999). Unfortunately, only around 2% of the literature available on the outplanting performance of inoculated plants include long-term (data gained over 5 years) experiments (Castellano 1996). Field studies are expensive and long lasting, so it is important to establish alternative criteria for the selection of fungi to be used as inocula.

In this paper, we propose a method to continuously measure early growth responses of seedlings to mycorrhizal colonisation by using linear variable differential transformer (LVDT) sensors. This technique was formerly used to detect the amount of water stored in the plant as an indicator of the need for irrigation (Huguet 1985; Cohen et al. 1997a, 2001). Later, LVDT sensors were used to continuously measure plant responses to stress caused by fungal pathogens (Cohen et al. 1997b; Luque et al. 1999).

To test this technology for detecting seedling growth responses to mycorrhizal colonisation we have chosen the symbiosis between *Pinus pinea* L. (stone pine), and the ectomycorrhizal fungus *Rhizopogon roseolus* (Corda ex. Sturm) Th. Fr. *P. pinea* is a representative conifer of the Mediterranean region which tolerates hot and dry conditions. It is valued for its edible nuts and plays an important ecological role in arid and semi-arid zones by preventing erosion (Montoya 1990; Montero et al. 1997). Among the range of mycorrhizal fungi able to colonise *P. pinea*, *R. roseolus* isolates were the most infective under controlled conditions (Rincón et al. 1999). Also, *Rhizopogon* species produce large quantities of rhizomorphs which have been associated with the enhancement of water uptake (Duddridge et al. 1980; Brownlee et al. 1983; Read and Boyd 1986; Cairney 1992). The purpose of this work was to detect differential seedling growth responses related to fungal colonisation rates and growing conditions. The results are discussed in relation to the predictive possibilities of the method to select efficient fungi in promoting plant growth.

Materials and methods

Plant material

P. pinea seedlings were grown in Forest-Pot (Vivers La Fageda, Santa Pau, Girona, Spain) plates (30 alveoles of 400 cm^3 each) filled with a 1:1 (v:v) mixture of Floratorf peat (Floragard, Oldenburg, Germany) and vermiculite (Termite grade 2; Asfaltex, Barcelona). Fertilisation was applied by adding Osmocote Plus 16-8-12 NPK+2 MgO, slow release (Grace Sierra, Tarragona, Spain) to the substrate at the recommended proportion of 2 g/l. Sowing was done in March 1997 and seedlings were maintained under regular irrigation in a commercial forest nursery greenhouse (Forestal Catalana) in Breda (Girona) during the growing season.

Fungal inoculation and colonisation assessment

Sporocarps of *R. roseolus* were collected in October 1996 under *Abies* sp. in a commercial Christmas tree plantation located in Arbúcies (Girona). The fungi were dried at 35°C for 48 h and kept in a paper bag at room temperature until used. Seedlings were inoculated in May 1997, 2 months after sowing, using a water suspension of spores prepared by blending chopped sporocarps. The spore suspension was adjusted to 10^5 spores/ml and 10 ml per container was applied to provide 10^6 spores per seedling. Non-inoculated control seedlings were irrigated with the same amount of water.

Plant growth parameters (height and root collar diameter) and percentage of feeder root colonisation were assessed from samples of 20 seedlings of each inoculation treatment (*R. roseolus* and non-inoculated) in February 1998. Feeder roots of each seedling were sampled and counted as described by Parladé et al. (1996).

Inoculated seedlings were sorted in two lots according to the proportion of feeder roots colonised with *R. roseolus* (>50% and <50%). A third lot, composed of non-colonised seedlings, was obtained from control, non-inoculated seedlings. In April 1998, a total of 18 seedlings with entire, intact plugs (six from each one of the three lots), were transplanted into 10-l plastic containers filled with a mixture composed of equal volumes of Floratorf peat and sand (32% maximum water holding volume at field capacity). Seedlings were maintained in a greenhouse under controlled temperature (air-conditioner adjusted to 25°C) and irrigated twice a week to field capacity until the installation of the sensors.

Installation of LVDT sensors

LVDT sensors (model DF±2.5 mm from Solartron, Bogno Regis, UK) were installed on each of the 18 transplanted seedlings in June 1998. A special holder (IRTA design, made by Requena, Vilassar de Mar, Barcelona) made of Invar (Fe-Ni alloy with a dilatation coefficient of almost null) and aluminium-based material, and provided with two rubber bands, was used to attach the sensor to the stem of each seedling at 5 cm above the root collar (Fig. 1).

Experimental design and monitoring

Two groups of nine seedlings (three from each lot) provided with their respective sensors were set up randomly on the greenhouse bench. One of the groups was irrigated every other day to field capacity and the other one was initially submitted to water stress (not watered). When the water content of the substrate of non-watered seedlings decreased to 5% in volume, the seedlings were kept without watering for 8 more days (stress period). Then, the seedlings were watered to field capacity and maintained under the same irrigation schedule as non-stressed seedlings. The period of 8 days starting from the day after watering was considered the recovery period. Two consecutive cycles of 38 days each (including the stress and recovery periods in non-irrigated seedlings) were established using the same seedlings under two different environmental conditions. Cycle 1 was established from 15 June to

26 July (calendar days 166–203) in a shaded greenhouse with controlled temperature (under 25°C). Cycle 2 was established from 28 July to 7 September (calendar days 210–247) in an adjacent module of the greenhouse without controlled temperature. Air temperature was measured periodically with a Testostor 171 datalogger (Instrumentos Testo, Cabrils, Spain). Global radiation was measured with a pyranometer SP1110 (Skye Instruments, Powys, UK). Substrate water content was measured with time domain reflectometry equipment (TRIME-FM; IMKO, Ettlingen, Germany).

The LVDT outputs were recorded by a datalogger (model CR10X with a AM416 multiplexer from Campbell Scientific, Logan, Utah) every 30 s and averaged every 30 min. The system was powered by a standard car battery (12 V, 45 Ah) connected to an automatic charger. The stored data were downloaded to a portable computer periodically.

Data analyses

To obtain increases or decreases of stem diameter in microns, the original data were corrected to the same initial zero value. Data recorded from each seedling were processed to calculate: (1) the average daily growth (DG; difference between the maximum stem diameter measured on 2 consecutive days), and (2) the maximum daily shrinkage (MDS; difference between the maximum and the minimum of each daily curve) (see below). The mean values of accumulated DG in each lot of seedlings throughout the stress and recovery periods were fitted to a straight line by linear regression.

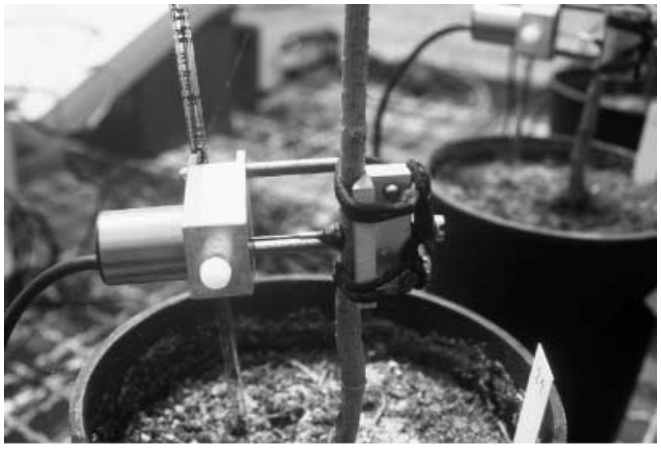

Fig. 1 Detail of the attachment of linear variable differential transformer sensors on the stem of *Pinus pinea* (stone pine) plants

The slopes from each fitted line, corresponding to seedlings with different colonisation levels, were compared by Student's *t*-test (Zar 1984). Growth data of regularly irrigated seedlings measured throughout each cycle were also fitted to a straight line and slopes compared to detect growth differences among seedlings with different colonisation levels. An additional comparison was established in the same way to detect differences in growth between water-stressed and non-stressed seedlings during the recovery periods of each cycle.

Correlation analyses were performed for each cycle to detect linear relationships among the growth variables DG and MDS for each colonisation level and the mean daily global radiation measured in situ.

The mycorrhizal status of all the seedlings was assessed at the end of the experiment. ANOVA was performed to compare the final mycorrhizal colonisation of seedlings. The eventual incidence of mycorrhizal contaminants was also recorded. Colonisation data (percentage of mycorrhizas) were angular transformed (asin$\sqrt{}$%colonisation/100) if necessary to meet the requirements of ANOVA. Differences among means were detected by Tukey's test ($P \leq 0.05$).

Results

Spore inoculation of *P. pinea* provided 95% of seedlings with *R. roseolus* mycorrhizas. Visual estimation allowed us to separate two groups according to the level of root colonisation (high and low). After examining ten seedlings from each group under the stereomicroscope, the first group averaged 83% feeder-root colonisation (SE 2.80) and was named "+50%" (>50% root colonisation), whereas the second group averaged 34% feeder-root colonisation (SE 3.96) and was named "–50%" (<50% root colonisation). The difference between both groups was statistically significant ($P < 0.05$). Of the total inoculated seedlings assessed for mycorrhizal colonisation, 75% of them belonged to the first group (+50%) and 25% to the second (–50%). Average initial growth data of all the seedlings at transplanting, just before sensor installation, were 6.4 mm diameter and 34.6 cm height. No significant initial differences in the above parameters were detected among seedlings belonging to the different colonisation levels including non-mycorrhizal control seedlings.

Mean daily air temperature and global radiation data measured along the two growth cycles are shown in Fig. 2.

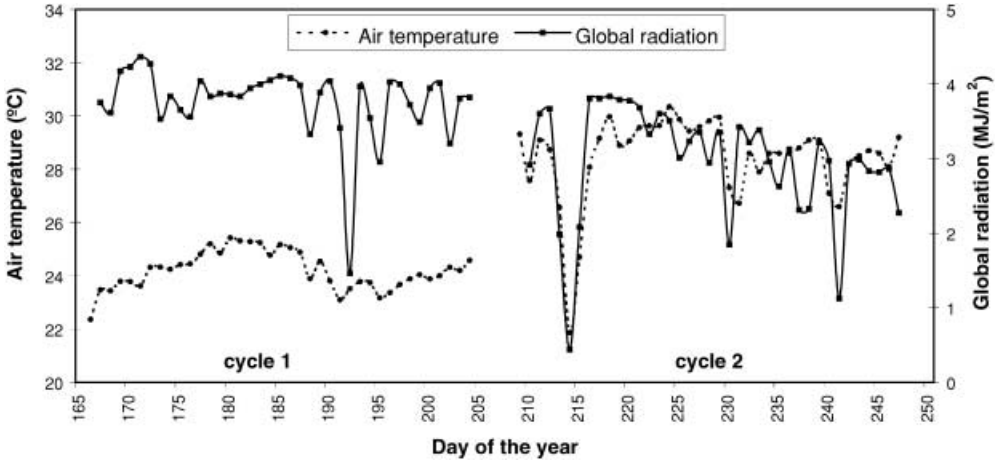

Fig. 2 Mean daily air temperature and global radiation in cycle 1 (*days 166–203*) and cycle 2 (*days 210–247*)

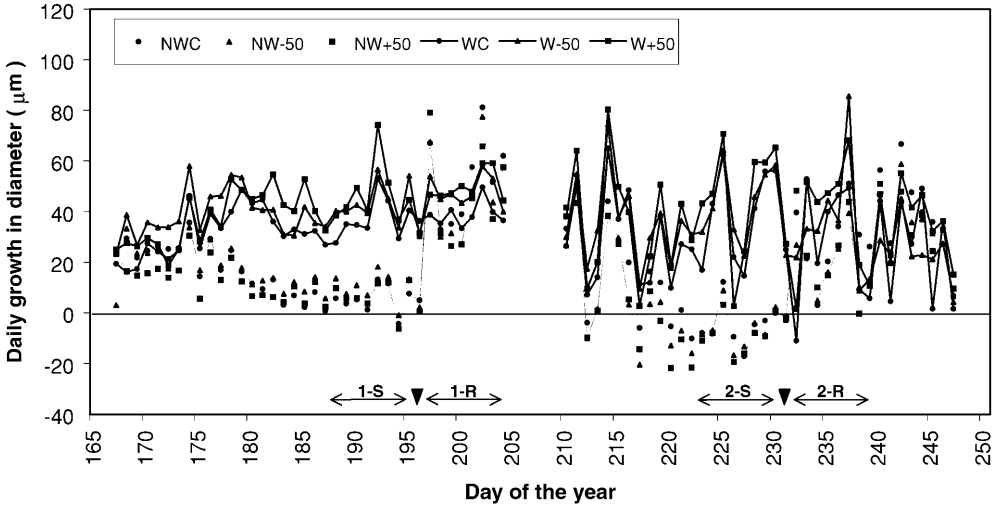

Fig. 3 Mean daily growth in stem diameter of *P. pinea* plants with different colonisation levels of *Rhizopogon roseolus* and submitted to different watering and greenhouse conditions throughout cycle 1 and cycle 2. *Black arrowheads* indicate the days when watering of stressed plants was resumed. Intervals *1-S, 2-S* and *1-R, 2-R* indicate the stress and recovery days throughout cycle 1 and cycle 2, respectively. *C* Control, non-inoculated; *–50* <50% initial mycorrhizal colonisation; *+50* >50% initial mycorrhizal colonisation; *W* watered; *NW* not watered; for other abbreviations, see Fig. 2

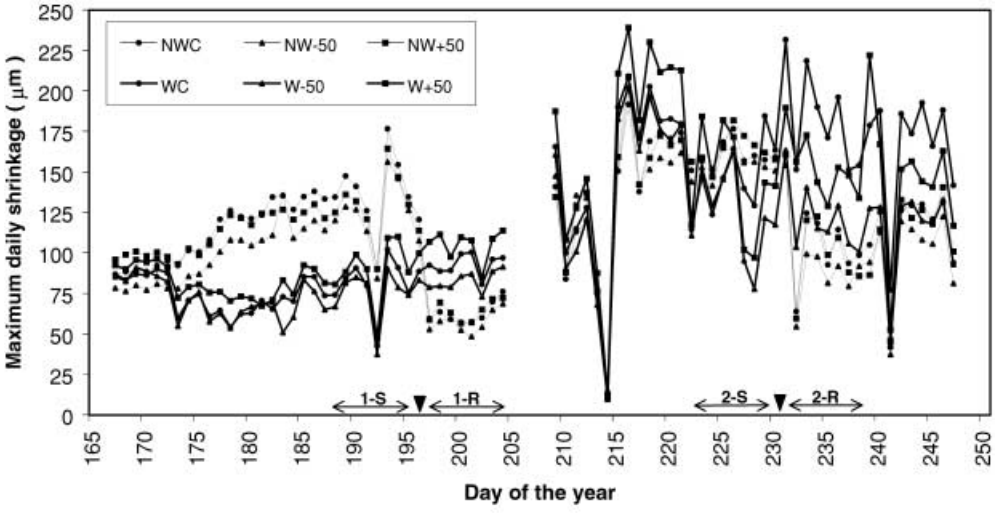

Fig. 4 Mean maximum daily shrinkage of the stem in *P. pinea* plants with different colonisation levels of *R. roseolus* and submitted to different watering and greenhouse conditions throughout cycle 1 and cycle 2. *Black arrowheads* indicate the days when watering of stressed plants was resumed. For abbreviations, see Figs. 2 and 3

Average DG of seedlings belonging to the different treatments in the two cycles are shown in Fig. 3. During the stress periods in cycles 1 and 2 (1-S, 2-S, respectively), DG values of stressed seedlings were consistently lower than those of regularly irrigated seedlings. In cycle 1 the stressed seedlings showed negative DG only one day, whereas in cycle 2 stressed seedlings showed negative DG for several days. SEs of the different treatments varied between 4.98 and 9.00. For a given treatment, variability increased in cycle 2 and in irrigated seedlings. Average MDS data are shown in Fig. 4. In cycle 1, non-watered seedlings had higher MDS than watered seedlings but the values suddenly decreased after watering, even below the values of regularly watered seedlings. In cycle 2 the MDS variations were higher than in cycle 1 and the differences between irrigated and non-irrigated seedlings were not clear. SEs of the different treatments varied between 8.74 and 20.66. As in the case of DG, variability in a given treatment increased in cycle 2 and in irrigated seedlings

The evolution of accumulated seedling growth in terms of stem diameter is shown in Fig. 5 for all the treatments in the two cycles. All the irrigated seedlings grew more than non-irrigated ones. Recovery periods in cycles 1 and 2 (1-R, 2-R, respectively) for water-stressed seedlings were clearly defined by noticeable changes in diameter increase, whereas regularly irrigated seedlings showed continuous and stable growth. Increases in the stem diameter of seedlings adjusted to straight lines during the stress and recovery periods, for both cycles, are shown in Fig. 6. Levels of significance for comparisons of the slopes of the fitted regression lines are shown in Table 1. In 1-S, the slope of the adjusted regression line corresponding to seedlings with <50% of *R. roseolus* mycorrhizas was significantly higher (more positive) than those of seedlings belonging to the other colonisation levels. In 1-R, control seedlings recovered at a higher rate than seedlings with >50% of *R. roseolus* mycorrhizas. During 2-S, all the seedlings showed a decrease in stem diameter. Seedlings with the highest mycorrhizal colonisation showed a significant decrease in stem diameter compared to control seedlings. In 2-R, control seedlings grew at a significantly higher rate than colonised seedlings.

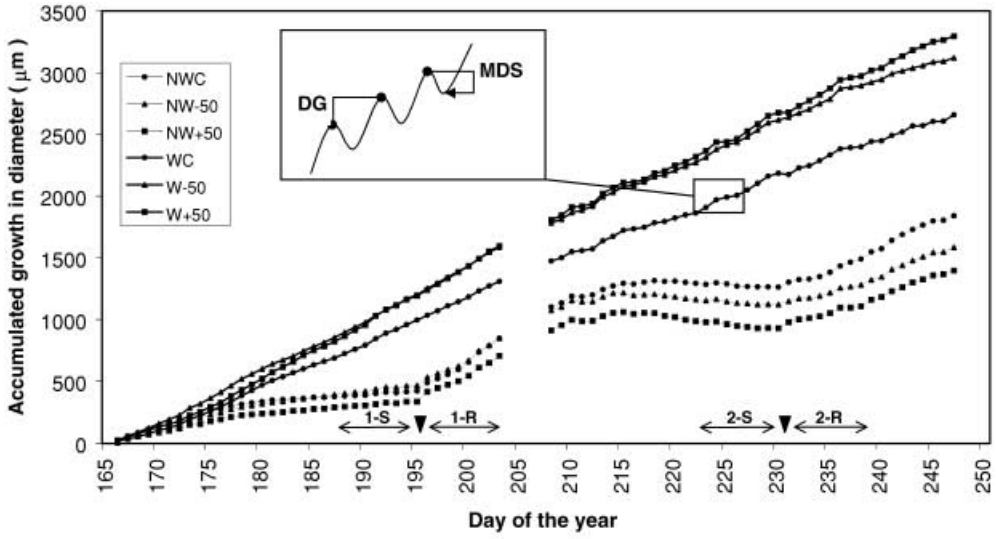

Fig. 5 Accumulated mean daily growth in stem diameter of *P. pinea* plants with different colonisation levels of *R. roseolus* and submitted to different watering and greenhouse conditions. *Black arrowheads* indicate the days when watering of stressed plants was resumed. Detail of the daily evolution of the growth curve and the parameters measured [daily growth (*DG*) and maximum daily shrinkage (*MDS*)] are represented. For other abbreviations, see Figs. 2 and 3

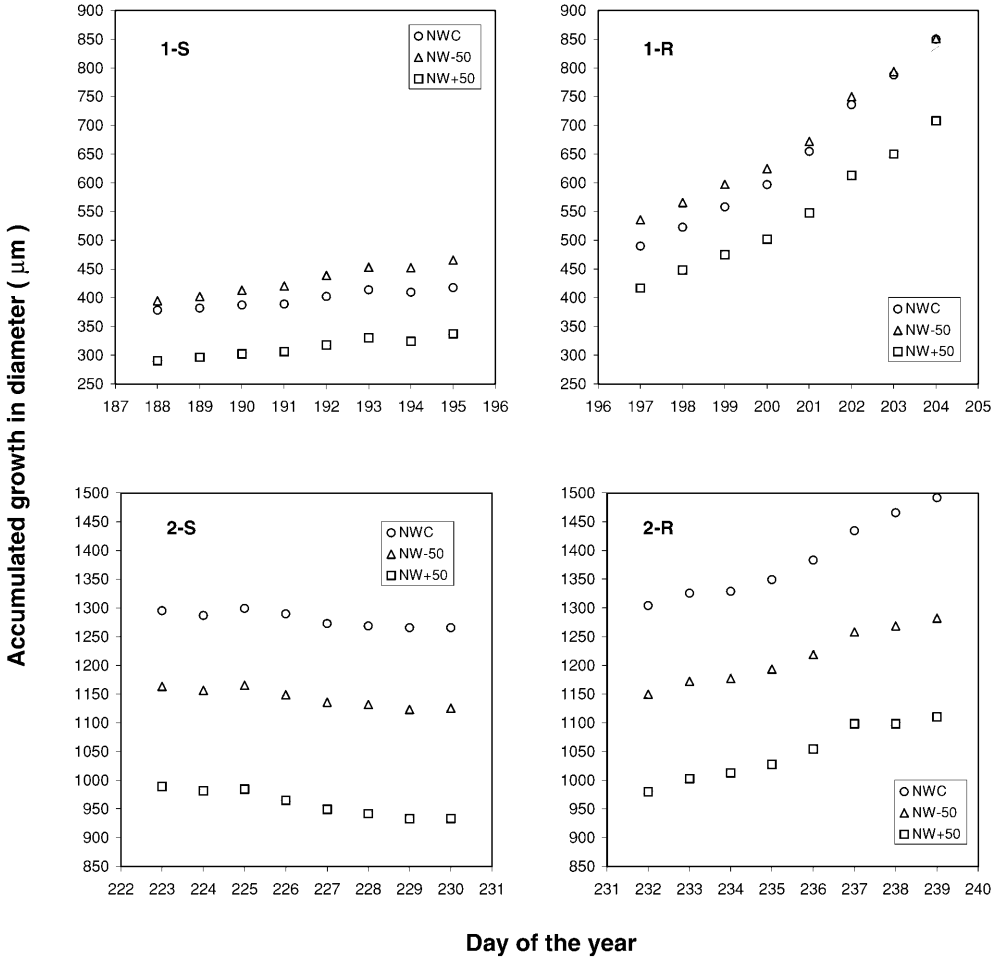

Fig. 6 Fitted regression lines for the mean accumulated growth of *P. pinea* plants submitted to 1-S and 1-R and 2-S and 2-R. For abbreviations, see Figs. 2 and 3

Additional comparisons between the recovery period of stressed seedlings after irrigation and the growth of regularly irrigated seedlings throughout the same days were also performed (data not shown). The recovery rate of control seedlings in the period 1-R was significantly higher ($P<0.05$) than the growth rate of regularly irrigated control seedlings corresponding to the same period. Colonised, stressed seedlings recovered at the same growth rate as regularly irrigated seedlings throughout 1-R ($P=0.47$ and $P=0.12$ for treatments –50% and +50% respectively). Comparisons of the growth rates between stressed and regularly irrigated seedlings in the period 2-

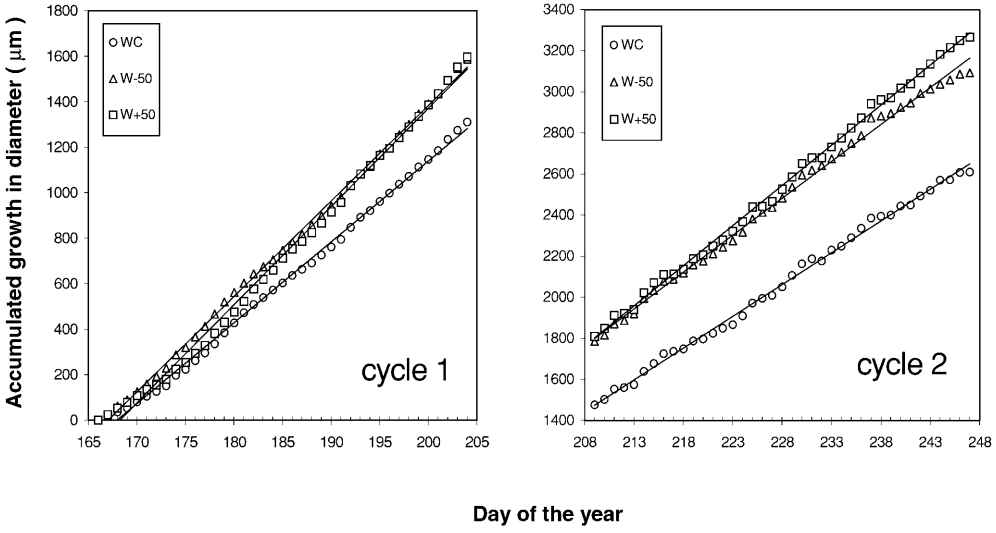

Fig. 7 Fitted regression lines for the mean accumulated growth of regularly watered *P. pinea* plants in cycles 1 and 2. For abbreviations, see Figs. 2 and 3

Table 1 Levels of significance for the comparison of slopes of the fitted lines for each initial colonisation level of *Pinus pinea* by *Rhizopogon roseolus* in the two growth cycles. Intervals *1-S*, *2-S* and *1-R*, *2-R* indicate the stress and recovery periods throughout cycle 1 (days 166–203) and cycle 2 (days 210–247), respectively. *–50* <50% Initial mycorrhizal colonisation, *+50* >50% initial mycorrhizal colonisation

Growth period (days)	Inoculation treatments		
		Control	–50
	Water-stressed plants		
1-S (188–195)	–50	<0.001	–
	+50	0.490	0.001
1-R (197–204)	–50	0.126	–
	+50	0.015	0.316
2-S (223–230)	–50	0.326	–
	+50	0.012	0.067
2-R (232–239)	–50	0.014	–
	+50	0.016	0.973
	Regularly watered plants		
Cycle 1 (166–203)	–50	<0.001	–
	+50	<0.001	0.251
Cycle 2 (210–247)	–50	<0.001	–
	+50	0.001	0.213

Table 2 Coefficients of correlations between different parameters. *Bold numbers* indicate a significant (P<0.05) relationship. *DG* Daily growth, *MDS* maximum daily shrinkage, *RAD* global radiation; for other abbreviations, see Table 1

Growth period (days)	Inoculation treatment	Correlation coefficients		
		MDS/DG	MDS/RAD	DG/RAD
		Water-stressed plants		
1-S (188–195)	Control	–0.280	**0.863**	–0.456
	–50	–0.469	**0.811**	–0.498
	+50	–0.245	**0.833**	–0.330
1-R (197–204)	Control	0.114	0.041	–0.233
	–50	–0.045	–0.158	–0.286
	+50	–0.041	–0.316	–0.018
2-S (223–230)	Control	0.284	–0.167	–0.414
	–50	0.151	–0.166	–0.543
	+50	–0.566	0.102	–0.586
2-R (232–239)	Control	–0.552	0.228	–0.313
	–50	–0.485	0.065	–0.096
	+50	–0.434	0.184	0.155
		Regularly watered plants		
Cycle 1 (166–203)	Control	–0.292	**0.480**	**–0.423**
	–50	–0.253	**0.513**	**–0.432**
	+50	–0.158	**0.441**	**–0.487**
Cycle 2 (210–247)	Control	0.043	**0.579**	–0.148
	–50	–0.158	**0.723**	–0.280
	+50	–0.181	**0.705**	–0.268

R indicated that colonised, stressed seedlings grew at a lower rate than colonised, regularly irrigated seedlings (P<0.05 for both –50% and +50% treatments). No differences were detected between non-inoculated seedlings (P=0.20).

Comparisons among regularly irrigated seedlings showed that, in both complete cycles, the growth rates of colonised seedlings, irrespective of the colonisation level, were significantly higher than those of control, non-inoculated seedlings (Fig. 7, Table 1).

Correlation coefficients among DG, MDS and global radiation parameters are shown in Table 2. No significant relationship between MDS and DG was detected. In water-stressed seedlings, MDS was positively correlated with global radiation in the growth period 1-S. In regularly watered seedlings, MDS was positively correlated with global radiation in both complete cycles. DG was negatively correlated with global radiation only in regularly irrigated seedlings in cycle 1. No differential responses due to the inoculation treatments were detected.

ANOVA of the mycorrhizal colonisation at the end of the experiments showed a significant effect of the "initial colonisation" factor (P<0.05) and a non-significant effect of the "irrigation" factor (P=0.52). Interaction between both factors was not significant (P=0.78) and data

Table 3 Mycorrhizal colonisation of the plants at the beginning of the experiment (*Initial*) and 6 months later (*Final*). Values followed by *the same letter in the same column* are not significantly different according to Tukey's test ($P<0.05$)

Initial mycorrhizal colonisation	Final mycorrhizal colonisation (%)			
	Old roots		Newly formed roots	
	R. roseolus	Other[a]	*R. roseolus*	Other[a]
Non mycorrhizal	0 a	28 b	0 a	20 b
–50% *R. roseolus*	15 b	29 b	2 a	18 b
+50% *R. roseolus*	67 c	8 a	22 b	3 a

[a] Mycorrhizal contaminants, mainly *Thelephora terrestris*

from both irrigated and non-irrigated seedlings were analysed together. Final mycorrhizal colonisation in old roots by *R. roseolus* was significantly higher in seedlings which had an initial colonisation level >50% than in seedlings with <50% initial colonisation (Table 3). More newly formed roots were also colonised in seedlings which had an initial colonisation level >50% than in seedlings with <50% initial colonisation, which showed almost no secondary colonisation. The incidence of contaminant mycorrhizal fungi other than *R. roseolus* (mainly *Thelephora terrestris* Pers. ex Fr.) at the end of the experiment was significantly lower in seedlings which had an initial colonisation level >50% than in seedlings with <50% initial colonisation and control seedlings. This trend was observed in both old and newly formed roots.

Discussion

The use of LVDT sensors allowed the early detection of a differential growth response of seedlings to variation in water supply. Irrigated seedlings showed a regular growth rate throughout the experiment, whereas stressed seedlings showed a lower growth rate, which was even negative in cycle 2, and a rapid recovery response after watering. This sensitive evaluation of micrometric changes in plant stem diameter has been reported to be very efficient for the measurement of plant water status and irrigation requirements, especially in fruit trees (Huguet 1985; Simmoneau et al. 1993; Cohen 1994; Cohen et al. 2001). Also, LVDT sensors proved to be suitable tools for detecting the effect of pathogenesis on the water relations of forest plants (Cohen et al. 1997b; Luque et al. 1999).

In the present work, short-term differential growth responses due to water regimes and inoculation with ectomycorrhizal fungi have been detected using LVDT sensors. The presence of ectomycorrhizas of *R. roseolus*, irrespective of the colonisation level, resulted in a significant growth increase in irrigated seedlings. However, water-stressed seedlings showed no benefit from inoculation in terms of growth. Moreover, stressed seedlings with a high colonisation level recovered more slowly than control, non-colonised seedlings. It appears that inoculation with *R. roseolus* does not increase the growth performance of *P. pinea* under water stress. Previous research demonstrated that rhizomorphs increase the water uptake of plants (Duddridge et al. 1980). Also, Douglas-fir seedlings inoculated with *Rhizopogon vinicolor* Smith increased their tolerance to water stress (Dosskey et al. 1990). In our experiment, the lower growth and recovery rate of inoculated, stressed seedlings compared to control, non-inoculated seedlings, seem to contradict other results. However, the limited development of the mycorrhizal system in containers, and the fact that Mediterranean plants could benefit from inoculation in terms of survival rather than growth, could explain some of the differences. As Read and Boyd (1986) stated, mycorrhizas may influence the water balance of the host largely through their capability to provide the minimum requirements for survival during stress rather than through their ability to sustain high water flow rates.

Correlation data showed different relationships between global radiation and the parameters MDS or DG depending on the growth period/cycle considered. These different seedling responses could be the result of regulation mechanisms in response to stress. Several authors have described a relationship between MDS and plant water status (Molz and Klepper 1972; Cohen et al. 1997a, 2001). Although further physiological research would be needed to establish this relationship in our experimental set up, the results obtained showed no differential seedling responses due to inoculation.

Mycorrhizal colonisation by *R. roseolus* of old roots at the end of the experiment was directly related to their initial colonisation level. Also, significantly more mycorrhizal contaminants were detected in seedlings with none or low initial colonisation than in seedlings which had a high colonisation rate. Newly formed roots showed secondary colonisation only when initial colonisation was >50%. Seedlings which were not colonised or had <50% colonisation showed significantly more mycorrhizal contaminants in new roots than seedlings with >50% initial colonisation. Results obtained in field outplanting experiments indicate that, in some cases, benefits of inoculation with *Laccaria bicolor* may be maintained after several years in the field, even if the initial fungal colonisation decreases or the fungal diversity in the root system increases (Villeneuve et al. 1991; Selosse et al. 2000). Other authors state that *Pisolithus tinctorius* needs to colonise at least 50% of the feeder roots to persist and maintain their benefit to the host plant (Marx et al. 1991). Therefore, the significance of the colonisation level in the plant response to inoculation is a critical step to consider in the selection of efficient fungi for practical applications.

From the results obtained, it can be concluded that LVDT sensors are sensitive enough to study the relationships between changes in mycorrhizal status and seedling growth responses under different environmental conditions. The fact that these responses may be detected within a few days makes LVDT sensors very useful for

pre-selecting those fungal isolates able to affect the overall plant growth response. Thus, this technique may be a significant improvement in the preliminary screening of a number of fungal candidates for ectomycorrhizal inoculations. However, further research is needed to determine the effectiveness of short-term plant responses, measured in controlled experiments, for the prediction of long-term field performance after outplanting.

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