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Field performance of *Pinus pinea* and *P. halepensis* seedlings inoculated with *Rhizopogon* spp. and outplanted in formerly arable land

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Abstract – The effect of mycorrhizal inoculation on field performance of *Pinus pinea* and *P. halepensis* seedlings, established in formerly arable land, was evaluated for up to 43 months after outplanting. Containerized seedlings of both tree species were produced in two different substrates, peat:bark and peat:vermiculite, and inoculated with spores of either *Rhizopogon luteolus* or *R. roseolus*. One month prior to outplanting, more than 50% of *P. pinea* short roots were colonized by either inoculated fungi, whereas a maximum of 32% of *P. halepensis* short roots were colonized by *R. roseolus*. Seedlings inoculated with *R. roseolus* were generally taller than non-inoculated ones at transplantation. However, significant differences disappeared or remained small in absolute values during the monitoring period (less than 7 cm in the best case, 34 months after outplanting). Inoculation with *R. roseolus* increased in 20% the survival of *P. pinea* seedlings over non-inoculated ones after 43 months in the field. Under the experimental conditions tested, these differences in survival were large enough to justify inoculation costs since spore inoculation with *Rhizopogon* species in the nursery is easy and inexpensive. Transplant Stress Indices allowed to detect overall post-transplantation stress and further recovery periods although no clear relationship between inoculation and planting check was detected.

afforestation / field performance / mycorrhizal inoculation / *Pinus* / *Rhizopogon*

Résumé – Performances de *Pinus pinea* et *P. halepensis* inoculés avec *Rhizopogon* spp. et transplantés sur un ancien terrain agricole. L'effet de l'inoculation mycorrhizienne sur la croissance et la survie de *Pinus pinea* et *P. halepensis* établis sur un ancien terrain agricole a été évalué pendant 43 mois après la mise en place de la plantation. Les plants des deux espèces ont été produits en conteneur sur deux substrats différents : tourbe:écorce et tourbe:vermiculite, et inoculés avec des spores de *Rhizopogon luteolus*, ou *R. roseolus*. Un mois avant la plantation, tous les plants de *P. pinea* inoculés avaient plus de 50 % des racines courtes colonisées par le champignon inoculé sans distinction d'espèce ou de substrat. Les plants de *P. halepensis* n'avaient au maximum que 32 % des racines courtes colonisées par *R. roseolus*. Les plants inoculés avec *R. roseolus* étaient généralement les plus hauts au moment de la transplantation. Ces différences significatives sont néanmoins restées petites voire ont disparu au cours du temps (moins de 7 cm, dans le meilleur cas, 34 mois après l'établissement de la plantation). La survie des plants de *P. pinea* inoculés avec *R. roseolus* a augmenté de 20 % par rapport à celle des plants témoins après 43 mois en plantation. Dans les conditions expérimentées, les différences de survie étaient assez grandes pour justifier le coût de l'inoculation, surtout parce que l'inoculation avec des spores de champignons du genre *Rhizopogon* est facile à appliquer en pépinière. L'utilisation d'Index de Stress après transplantation ont permis de détecter des stress post-transplantation et des périodes de récupération, mais il n'a pas été établi de relation entre l'inoculation et le stress de transplantation.

inoculation mycorrhizienne / performance en plantation / *Pinus* / reboisement / *Rhizopogon*

1. INTRODUCTION

Mycorrhizal inoculations with several plant-fungus combinations have been intended to improve seedling quality in forest nurseries [5, 18]. Inoculation techniques adapted to conventional nursery tasks have been reported and, in some cases, developed at a pre-commercial level [11, 15, 16, 19, 20]. Sub-

sequent field-performance testing of inoculated versus non-inoculated plants has covered many plant-fungus combinations and field situations [3, 4, 8, 12, 17, 18, 31]. Comprehensive data, however, are difficult to obtain since experimental conditions are very variable and most research is concentrated in small geographical regions and with few fungus-plant combinations [4]. Among the situations where mycorrhizal inoculation

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seems to be effective to promote plant survival and growth after outplanting are the environmentally stressful sites [4, 8, 21, 32].

Restoration of low-productive formerly arable land has been promoted in the European Union (Regulation EEC 2080/92) by supporting afforestation and related management practices. Agricultural soils are generally altered by over-fertilization and their structure, chemical composition and microbial communities might not be suitable for the establishment of forest trees [13, 32]. Also, the abundance of competing weeds is especially detrimental in Mediterranean conditions under short water availability [2, 30]. Together with plant quality, the restoration of the below-ground microbial community has been shown to be important for the establishment of above-ground species in later successional stages [10]. Under this situation, it can be hypothesized that controlled mycorrhizal inoculation may be an advantage for forest plants to be established in formerly arable agricultural soils. To test this, we have established two experimental plantations with inoculated and non-inoculated seedlings in an abandoned cereal field representative of the Mediterranean area. *Pinus halepensis* Mill. and *Pinus pinea* L. were chosen for the study according to ecologic (adaptability) and economic (establishment and maintenance costs, production) criteria to fit the productive objectives considered in the Regulation 2080/92 [37]. *Pinus halepensis* became the most planted conifer in Spain under this framework [9]. Also, *P. pinea* plays an important ecological role in arid and semi-arid zones by preventing erosion and is greatly valued for its edible nuts [26, 27]. Fungal inoculations in the nursery were performed with two *Rhizopogon* species. The genus *Rhizopogon* is considered a good candidate for controlled mass inoculations in nurseries since it occurs both in young and old stands [25], can be easily inoculated in the nursery as spore suspension [5, 22, 29] and there are reported effects on increasing field performance of inoculated plants [4, 31]. In this study, we present the plant performance data monitored in the two established plots along 34 and 43 months after transplantation.

2. MATERIALS AND METHODS

2.1. Seedling production and inoculation

Seeds of *P. pinea* and *P. halepensis* from the Mediterranean region collected in years 1992 and 1993, respectively, were provided by Vilmorin® (Alicante, Spain). Seedlings were produced in 400 cc Forestpot containers (Vivers La Fageda, Santa Pau, Girona, Spain) and filled with a 1:1 (v:v) mixture of either Floratorf peat (Floragard, Oldenburg, Germany) plus horticultural grade 2 vermiculite (Asfaltex, Barcelona, Spain) (p:v) or peat plus locally-composted pine bark 1:1 (v:v) (p:b). The seedlings were produced in a commercial shadehouse (Forestal Catalana S.A., Breda, Girona, Spain) in spring 1996 and 1997. Inoculation was performed one month after seeding by spraying a water suspension of spores of each *Rhizopogon* species prepared by blending dried sporocarps collected the previous autumn in the Montseny Range (Girona, Spain) under *Abies* spp. (*Rhizopogon roseolus* (Fr.:Fr.) Th. Fr.) or under *Pinus sylvestris* L. (*Rhizopogon luteolus* Fr.). Fungal identification was performed according to peridium and spore characteristics described by Smith [38]. For both fungal species, spore suspensions were adjusted to provide 10^6 spores per seedling. Batches of 400 seedlings were produced for each combination of mycorrhizal fungus, tree species and potting substrate including non-inoculated control seedlings.

Table I. Production data of the seedlings established in the two experimental plots.

Seedling data	Experimental plots	
	P-1	P-2
Tree species	<i>P. pinea</i>	<i>P. pinea</i> <i>P. halepensis</i>
Fungal inoculation treatments	<i>R. roseolus</i> <i>R. luteolus</i> non-inoculated	<i>R. roseolus</i> non-inoculated
Year of production	1996	1997
Container substrate (proportions 1:1, v:v)	Peat:bark Peat:vermiculite	Peat:bark Peat:vermiculite
Fertilization method	Soluble fertilizer (Peters 20-7-19)	Osmocote Plus (16-8-12 + 2 MgO)
Transplantation time	April 1997	March 1998

The seedlings produced in 1996 were fertilized every 15 days with soluble NPK Peters Professional Conifer Grower 20-7-19 (Scott, Tarragona, Spain) plus micronutrients (Fetrilon 13 and Hortrilon, BASF, Barcelona, Spain). Each plant received at each fertilization 3.6 mg N (2.1 mg nitrate, 1.26 mg ammonia, 0.24 mg urea), 1.26 mg P, 3.42 mg K, 0.35 mg Fe, 0.07 mg Mg, 0.06 mg Mn, 0.06 mg Cu, 0.01 mg Zn, 0.01 mg B, and 0.01 mg Mo. The seedlings produced in 1997 were fertilized by mixing Osmocote Plus 16-8-12 + 2 MgO (Scotts, Heerlen, The Netherlands) with the potting substrate at the rate of 2g/L. Total amount of nutrients received per plant along the growing period was equivalent to that provided by the soluble fertilizer. Eleven months after inoculation, 20 seedlings of each treatment were assessed for mycorrhizal root colonization. Morphological identification of ectomycorrhizas was done according to the descriptions reported by Rincón et al. [34]. A homogeneous sample of the root system was taken from each seedling to determine the percentage of mycorrhizal colonization as described in Parladé et al. [29].

2.2. Plantation establishment

One-year-old seedlings were outplanted in spring 1997 and 1998 in two plots, named P-1 and P-2 respectively, established in an abandoned cereal field located in Palau Solità i Plegamans, Barcelona, Spain. *Pinus pinea* seedlings were established in plot P-1 in a factorial design considering two factors: substrate (p:v and p:b) and inoculation (non-inoculated, *R. roseolus* and *R. luteolus*). *Pinus pinea* and *P. halepensis* seedlings were outplanted in plot P-2 considering the same factors as in P-1 but with only two inoculation treatments: non-inoculated and *R. roseolus*. Planting stock data for both plantations are summarized in Table I. The planting area was a flat field located at 90 m altitude and with an average annual precipitation of 467 mm. Soil analyses for both plots are showed in Table II. Soil preparation consisted of a superficial tillage done one month before outplanting. The seedlings were manually planted maintaining the plug intact in a completely randomized design considering a row of 18 seedlings as the experimental unit with 7 replicates per treatment. Final plantation framework was set to 2 × 2 m.

2.3. Field monitoring and data analyses

Seedling height was measured at transplantation and approximately once a year thereafter until February 2000 in plot P-1, and until October 2001 in plot P-2. Differences in growth rates between treatments were determined by comparing annual relative height growth

Table II. Soil parameters for the two experimental plots.

Soil parameter	Determination method	Experimental plots	
		P-1	P-2
pH	Water 1:2.5	8.1	8.1
E.C. 25 °C, 1:5	Extract 1:5, 25 °C	0.12 dS/m	0.19 dS/m
Organic matter	Volumetric, electrochemical	1.6%	2.4%
N	Kjeldahl	0.10%	0.14%
P	Olsen	20 ppm	27 ppm
K	Inductively coupled plasma	152 ppm	543 ppm
Texture	USDA classification	Loam	Sandy clay loam

rates $[(h_f - h_i)/h_i]$, where h_i : height at the beginning of the period considered and h_f : height at the end of the growing period. Seedling survival was also determined every year after outplanting. For each plot and tree species, growth data were analyzed by ANOVA considering the following variables in the model: inoculated fungi, nursery substrate and their corresponding interaction. Differences between means were detected by Tukey's test ($P < 0.05$). Differences in seedling survival between the control and inoculated treatments were detected by chi-square test ($P < 0.05$). Transplant stress index (TSI) as described in South and Zwolinski [39] was estimated yearly for each treatment by determining the relationship between seedling height at the beginning of the period considered and the subsequent height growth. The slope of a linear relationship was interpreted as follows: if the slope was negative, the plants were experiencing planting check or transplant shock. This period lasts until the seedling establishes new roots and the direct effect of transplanting is gradually reduced. When the slope was not different from zero, the stock was recovering from planting check. A positive slope suggested that the stock had recovered from planting check. At this point, the seedlings adjust fully and subsequent growth is the same as younger, non-transplanted trees of equivalent size.

3. RESULTS

3.1. Seedling performance in plot P-1

Pinus pinea seedlings produced in 1996 and inoculated with both *Rhizopogon* species showed a relatively high percentage of root colonization (over 50%) before transplantation (Tab. III). However, non-inoculated control seedlings, especially those produced in peat:vermiculite, had a high level of other ectomycorrhizal fungi (mainly *Thelephora terrestris* Ehrh. ex Willd. Fr. and *Rhizopogon*-like mycorrhizas). Analyses of annual height data showed significant interactions ($P < 0.05$) between the inoculation and substrate factors. *Pinus pinea* seedlings produced in peat:bark and inoculated with *R. roseolus* were significantly higher than their controls at transplantation time. Also, the relative growth rate of inoculated seedlings during the first growing season in the field was significantly greater than that of non-inoculated ones (Fig. 1). The differences in height were still significant after 34 months in the field being *R. roseolus*-inoculated seedlings around 15% taller (6.5 cm in average) than non-inoculated ones. On the other hand, *R. roseolus*-inoculated seedlings produced in peat:vermiculite and all the seedlings inoculated with *R. luteolus*, irrespective of the substrate used, did not perform differently

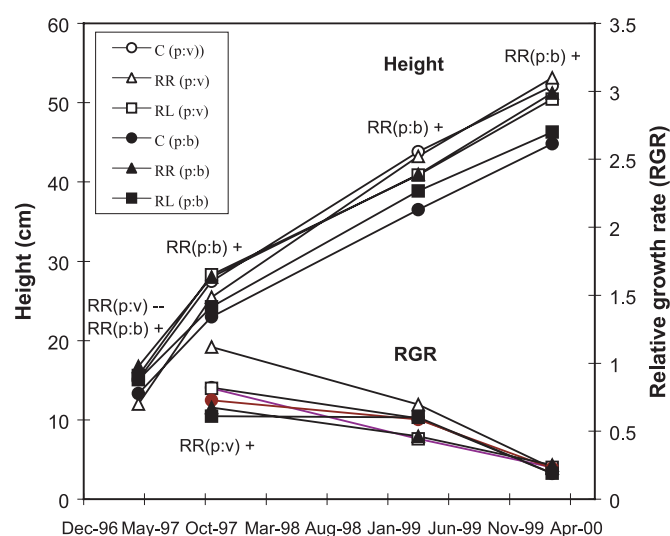


Figure 1. Evolution of height and annual relative growth rate (RGR) of *Pinus pinea* seedlings outplanted in the plot P-1. For each date of assessment, the abbreviations indicate significant differences between inoculated seedlings and their relative control by Tukey's test ($P < 0.05$). C: control, non-inoculated seedlings; RR: *Rhizopogon roseolus*; RL: *R. luteolus*; (p:v): peat:vermiculite substrate; (p:b): peat:bark substrate; + significantly higher than control; - significantly lower than control.

than their respective non-inoculated controls (Fig. 1). Seedling survival was also significantly affected by inoculation. After 34 months in the field, the survival rate of seedlings produced in peat:bark and inoculated with *R. roseolus* was 23% higher than that of non-inoculated controls (Tab. IV). Inoculation with *R. luteolus* also improved the survival of seedlings produced in peat:bark, although this amelioration was only significant in the first measurement, 6 months after transplantation. No significant differences in survival due to inoculation were detected in seedlings produced in peat:vermiculite. In average, seedlings produced in the peat:bark substrate grew significantly less and had lower field survival than those produced in peat:vermiculite. Thus, 34 months after transplantation, non-inoculated seedlings produced in peat:vermiculite were, in average, 7 cm taller and had 25% more survival than seedlings produced in

Table III. Initial colonization data of one-year-old containerized *Pinus pinea* and *P. halepensis* seedlings produced in years 1996 and 1997 to be outplanted in plots P-1 and P-2 respectively. Values are means \pm confidence intervals (95%).

Inoculation treatment	Nursery substrate	Initial colonization (% mycorrhizas)		
		1996	1997	
		<i>P. pinea</i>	<i>P. pinea</i>	<i>P. halepensis</i>
Control ^a	Peat:bark	24 \pm 20.6	24 \pm 15.8	0 \pm 11.7
	Peat:vermiculite	59 \pm 11.4	17 \pm 11.9	2 \pm 2.4
<i>R. roseolus</i>	Peat:bark	69 \pm 12.0	58 \pm 8.4	32 \pm 14.7
	Peat:vermiculite	83 \pm 5.2	64 \pm 8.5	24 \pm 10.2
<i>R. luteolus</i>	Peat:bark	57 \pm 10.0	Not produced	Not produced
	Peat:vermiculite	88 \pm 5.0	Not produced	Not produced

^a Unidentified mycorrhizas.

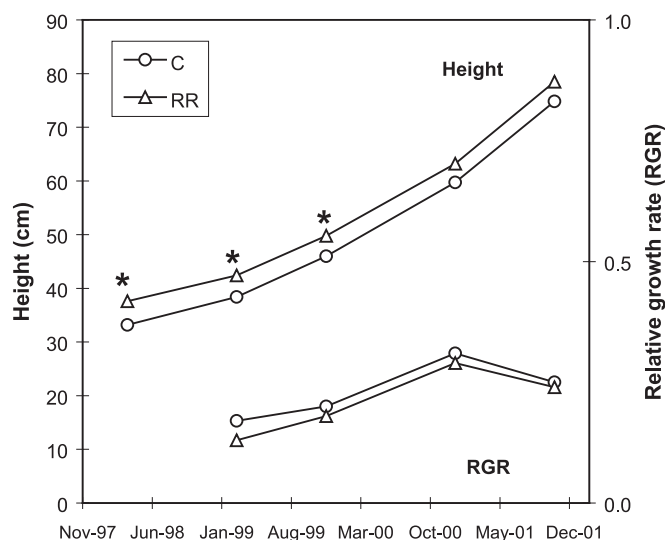
Table IV. Percentage of surviving *Pinus pinea* seedlings at different times after transplantation in plot P-1. For each date, values followed by an asterisk are significantly different from their respective control by the chi-square test with Yates correction ($P < 0.05$).

Nursery substrate	Inoculation treatment	Months after transplantation		
		6	23	34
Peat:bark	Control	81	67	54
	<i>R. roseolus</i>	94*	81*	77*
	<i>R. luteolus</i>	91*	77	68
Peat:vermiculite	Control	93	83	79
	<i>R. roseolus</i>	96	86	83
	<i>R. luteolus</i>	100	92	85

peat:bark. Transplanting stress indices (TSI) for the seedlings established in the plot P-1 are shown in Table V. Seedlings produced in the peat:bark substrate showed values not significantly different from zero in the intervals measured up to 23 months after transplantation. Only control seedlings in the period 23–34 months showed a significant positive value thus suggesting the recovery from planting check. Seedlings inoculated with *R. roseolus* and non-inoculated ones produced in peat:vermiculite showed significant negative values in the period 0–6 months thus indicating that were experiencing planting check. Positive significant values were only detected for seedlings inoculated with *R. roseolus* in the period 6–23 months after transplantation.

3.2. *Pinus pinea* seedling performance in plot P-2

Pinus pinea seedlings inoculated with *R. roseolus* showed a high root colonization level (over 50%) before outplanting (Tab. III). Non-inoculated controls showed less presence of other mycorrhizal fungi than seedlings produced in 1996. In average, seedlings produced in 1997 were around 20 cm taller than those produced in 1996. Analyses of annual height data of outplanted seedlings did not show interactions between the inoculation and substrate factors so data were analyzed together. Inoculated seedlings were significantly higher than controls at transplantation and after 20 months in the field (Fig. 2). Nevertheless, the differences were not significant thereafter. Relative

**Figure 2.** Evolution of height and annual relative growth rate (RGR) of *Pinus pinea* seedlings outplanted in the plot P-2. For each date of assessment, the asterisks next to the symbols indicate significant differences between inoculated seedlings and their control by Tukey's test ($P < 0.05$). C: control, non-inoculated seedlings; RR: *Rhizopogon roseolus*.

experiment and lower than those measured in plot P-1. Overall survival of *P. pinea* seedlings over time was high (Tab. VI). However, seedlings produced in peat:vermiculite and inoculated with *R. roseolus* increased 21% their survival rate compared to non-inoculated controls after 43 months in the field. Seedlings produced in peat:bark were significantly higher than those produced in peat:vermiculite in all the measurements done until 20 months from transplantation and then tended to equal (data not shown). No significant differences in survival due to the type of nursery substrate were detected. Analyses of TSI indices indicated that both inoculated and non-inoculated *Pinus pinea* seedlings experienced planting check up to 20 months after transplantation (Tab. VII). Positive values indicating recovery were detected in the period 33–43 months after transplantation.

Table V. Transplant stress indices (TSI) for different growing periods from transplantation (time 0) in the P-1 plot. TSI values significantly different from zero are noted with asterisks: * $P < 0.05$, ** $P < 0.01$.

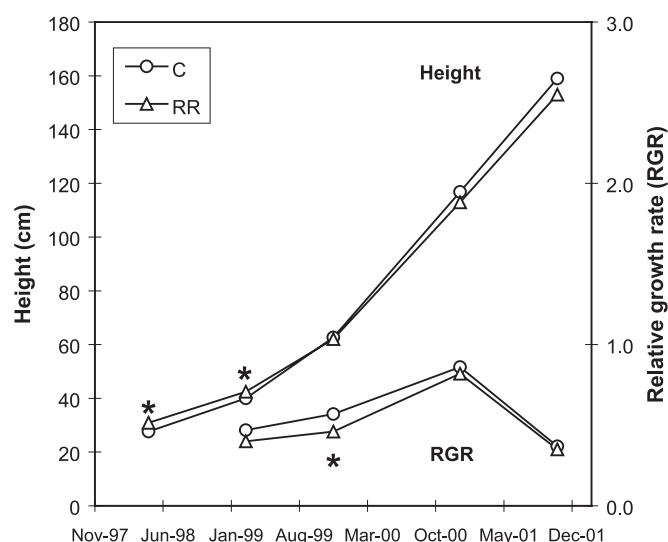
Nursery substrate	Inoculation treatment	Growing period from transplantation (months)		
		0–6	6–23	23–34
Peat:bark	Control	-0.2	-0.2	0.2*
	<i>R. roseolus</i>	-0.2	0.0	0.1
	<i>R. luteolus</i>	-0.0	-0.1	-0.0
Peat:vermiculite	Control	-0.5**	-0.2	0.1
	<i>R. roseolus</i>	-0.5*	0.4*	0.1
	<i>R. luteolus</i>	-0.2	-0.1	-0.1

3.3. *Pinus halepensis* seedling performance in Plot P-2

Pinus halepensis seedlings inoculated with *R. roseolus* showed a lower colonization level than *P. pinea* (Tab. III). Analyses of annual height data of outplanted seedlings did not show interactions between the inoculation and substrate factors so data were analyzed together. Inoculated seedlings were significantly taller than controls at transplantation and after 11 months in the field. However, differences were not significant after 20 months (Fig. 3). Relative growth rate of non-inoculated control seedlings was significantly higher than that of inoculated seedlings during the second year in the field (Fig. 3). At transplantation time, seedlings produced in peat:vermiculite were significantly higher than those produced in peat:bark although they equaled thereafter (data not shown). Survival of *P. halepensis* was not affected by inoculation (Tab. VI) or by the type of substrate. Non-inoculated *P. halepensis* seedlings showed significant negative TSI values in the period 0–11 months after outplanting. Positive significant values indicating recovery were obtained in the period 20–33 months after outplanting. However, in the next measured period (33–43 months) only the seedlings inoculated with *R. roseolus* showed a significant positive value (Tab. VII).

Table VI. Percentage of surviving *Pinus pinea* and *P. halepensis* seedlings at different times after transplantation in plot P-2. For each date, values followed by an asterisk are significantly different from their respective control by the chi-square test with Yates correction ($P < 0.05$).

Tree species	Nursery substrate	Inoculation treatment	Months after transplantation			
			11	20	33	43
<i>P. pinea</i>	Peat:bark	Control	98	96	91	86
		<i>R. roseolus</i>	89	89	84	80
	Peat:vermiculite	Control	89	86	82	70
		<i>R. roseolus</i>	98	95	93	91*
<i>P. halepensis</i>	Peat:bark	Control	97	92	88	86
		<i>R. roseolus</i>	98	93	90	85
	Peat:vermiculite	Control	99	92	89	85
		<i>R. roseolus</i>	95	84	84	74

**Figure 3.** Evolution of height and annual relative growth rate (RGR) of *Pinus halepensis* seedlings outplanted in the plot P-2. For each date of assessment, the asterisks next to the symbols indicate significant differences between inoculated seedlings and their control by Tukey's test ($P < 0.05$). C: control, non-inoculated seedlings; RR: *Rhizopogon roseolus*.

4. DISCUSSION

Mycorrhizal inoculation of pines did not cause advantageous growth differences in terms of practical forestry. In the best case, significant differences in mean height were slightly over 6 cm in *R. roseolus*-inoculated *P. pinea* produced in peat:bark compared to non-inoculated controls, 34 months after outplanting. On the other hand, seedling growth was, in average, representative of the normal values registered in Mediterranean areas for both tree species [2, 28, 36]. Nursery inoculation with *R. roseolus* significantly increased seedling height at the end of the nursery phase except in *P. pinea* seedlings produced in peat:vermiculite in 1996. This effect is generally not found

Table VII. Transplant stress indices (TSI) for different growing periods from transplantation (time 0) in the P-2 plot. TSI values significantly different from zero are noted with asterisks: * $P < 0.05$, ** $P < 0.01$.

Tree species	Inoculation treatment	Growing period from transplantation (months)			
		0–11	11–20	20–33	33–43
<i>P. pinea</i>	Control	–0.1**	–0.1**	–0.1	0.3**
	<i>R. roseolus</i>	–0.1**	–0.2**	–0.1	0.3**
<i>P. halepensis</i>	Control	–0.4**	0.1	0.4**	0.0
	<i>R. roseolus</i>	0.0	0.1	0.5**	0.2**

under container nursery conditions with fertilized artificial substrates [5, 23, 24]. Rincón et al. [35] reported that inoculation of *P. pinea* seedlings with *R. roseolus* spores did not increase seedling height under greenhouse conditions. However, Torres and Honrubia [41] found a significant growth effect of *P. halepensis* containerized seedlings 6 months after inoculation with *R. roseolus* spores. In our study, the growth effect promoted by *R. roseolus* in the nursery was maintained for some months after plantation but significant differences were not increasing with time. Differences in relative growth rate, when significant, seemed to be related with the faster growth of small plants tending to equal larger ones.

Reports of experimental plantations with inoculated *P. pinea* and *P. halepensis* seedlings are scarce in the literature. Argillier et al. [1] established *P. pinea* seedlings inoculated with *Suillus collinitus* (Fr.) Kuntze and found initial growth advantages due to inoculation. However, six years after outplanting, the introduced fungus was fully replaced by the native species *Rhizopogon rubescens* [7]. On the other hand, field outplanting of *P. halepensis* seedlings inoculated with *P. arhizus* in semiarid areas in SE Spain showed positive height increases due to inoculation two years after outplanting [33, 36]. The differences, however, were lowered when inoculated plants were compared with those inoculated with native fungi by adding forest soil. In our study, we chose a representative arable land surrounded by natural forests where native fungal propagules are probably to occur. Although the persistence of mycorrhizas was not assessed in this study, De Miguel et al. [6] sampled *P. pinea* seedlings established in the P-2 plot one year after outplanting and found that the number of *R. roseolus* mycorrhizas was significantly higher in inoculated seedlings produced in peat:bark than in non-inoculated ones. This finding is consistent with the growth effects detected in this treatment. However, it is noteworthy that all the seedlings had *R. roseolus* mycorrhizas in some degree. Probably, a plantation framework of 2×2 m with control and inoculated seedlings in adjacent rows caused the colonization of the former by those fungi able to extend rapidly into the soil by means of abundant extramatrical mycelium and rhizomorphs. The ability of *R. rubescens* to dominate ectomycorrhizal communities under adverse conditions was demonstrated by El Karkouri et al. [7].

Although improving outplanting performance of seedlings inoculated with *Rhizopogon* species has been reported [25], growth results are generally of low value for the forester in a practical sense. However, we have shown that the significant survival increase of *R. roseolus*-inoculated *Pinus pinea* after transplantation (20% over control seedlings at the end of the

experiment) is high enough to be taken into account, especially when analyzing the cost-benefit balance of nursery inoculations to reduce further field replanting. On the other hand, the slightly higher mortality rates detected in control seedlings in plot P-1 might be due to the high competitive pressure of weeds. Competence of weeds is considered a major problem in restoration of abandoned agricultural lands [2, 10]. It has been reported that weed competition significantly affects the number of ectomycorrhizal root tips and the distribution of ectomycorrhizal morphotypes [40]. Under this situation, mycorrhizal colonization of outplanted seedlings could improve survival by stimulating height growth in the first months after outplanting, even if growth is equalled later by non-inoculated seedlings.

The nursery conditions influenced significantly the growth of seedlings and their subsequent field performance. Transplanted one-year-old *P. pinea* seedlings fertilized with Osmocote were, in average, 20 cm taller than transplanted seedlings fertilized with Peters soluble fertilizer. In average, taller seedlings had higher survival rate although absolute plant height tended to equal with time due to the higher relative growth rate of small seedlings compared with that of greater ones.

The type and quality of the nursery substrate also conditioned seedling performance. Although peat:vermiculite and peat:bark are standard substrates in container-grown seedling production, the variability of peat and, especially, of composted bark is a major drawback to obtain homogeneous quality [14]. In our experiment, the pH of the peat:bark substrate varied from 7.7 in the batch of 1996 to 6.6 in 1997 whereas the peat:vermiculite substrate maintained pH 5 in both cases. Relative nutrient availability in organic substrates depends greatly on pH, being 5.5 the optimal value for conifer production [14]. According to our results, the seedlings produced in 1996 in a sub-optimal substrate (peat:bark with a high pH) benefited more from inoculation with *R. roseolus* than seedlings produced in peat:vermiculite. This effect resulted in a more uniform height of *R. roseolus*-inoculated seedlings produced in either substrates compared to non-inoculated ones. The even production of seedling stock is one of the benefits reported from inoculation in nurseries by other authors [13, 32]. The differences in substrate pH values were not so high in 1997 and the substrate factor did not interact with inoculation. In average, *P. pinea* seedlings produced in peat:bark in 1997 performed better than seedlings produced in peat:vermiculite and only survival of the latter was significantly improved by inoculation.

Transplant stress indices are promising indicators to estimate the intensity and duration of the planting check in both inoculated and non-inoculated seedlings. This method has been

applied to detect differences between progenies, seedling grade, soil treatments, sites, etc. [39]. In this study, we have found that *P. pinea* established in plot P-1 and produced in peat:vermiculite suffered planting check along the first six months after outplanting. Fully recovery was not consistent at the end of the measurements, 34 months after outplanting. Again, the presence of abundant weeds might have impeded to resume normal growth. In plot P-2, we found a similar pattern for *P. pinea* up to 34 months after outplanting but it was detected a full recovery from planting check after that. Planting check period for *P. halepensis* was restricted to the first year after outplanting and full recovery was detected in the second year. No clear relationship between inoculation and planting check period was detected. Although not assessed in this study, full recovery of seedlings could be related with the height of competing weeds. Nevertheless, more studies are needed to determine the usefulness and possibilities of this simple way of estimating plant performance after outplanting. Inoculation of seedlings for the restoration of formerly arable lands under the established experimental conditions has not resulted effective to increase plant growth in a practical sense. However, the increase in survival obtained in *P. pinea* seedlings inoculated with *R. roseolus* makes the inoculation economically feasible since the application of *Rhizopogon* spores in the nursery is an easy and inexpensive practice. It has been calculated that if survival was to be improved by only 5%, ectomycorrhizal technology would be self-financing and even generate savings [13]. In our study, we have obtained by far this improvement due to inoculation. Also, further benefits derived from small height increases to improve competence against weeds in agricultural environments, rather than to increase plant production, need to be investigated. On the other hand, it has to be taken into account that spores are an inoculum more genetically variable than a mycelial one. Also, the collection of sporocarps may be difficult depending on the annual climatic conditions. Further research on the conservation of the ability of meiotic spores to germinate is essential for this inoculum form to be regularly applied in nursery management.

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