

## Mechanisms underlying the interaction between *Pinus halepensis* and the native late-successional shrub *Pistacia lentiscus* in a semi-arid plantation

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*Pinus halepensis* has been extensively planted in semi-arid areas throughout the world. This has often led to slow-growth stands that: a) suffer from insect plagues, b) promote nutrient depletion and c) fail to promote the recovery of native vegetation. The introduction of native late-successional shrubs in these stands could stimulate successional processes, improve soil conditions and enhance their resilience against disturbances. The main objective of this study was to evaluate the mechanisms underlying the interaction between *Pinus* and the native late-successional shrub *Pistacia lentiscus* in a semi-arid plantation. By using manipulative field and laboratory experiments, we evaluated direct (competition for soil resources and allelopathic effects) and indirect (competition with herbaceous understorey) interactions between *Pinus* and *Pistacia*. We found no effect of *Pinus* litter and root exudates on *Pistacia* growth. In the field, *Pistacia* seedlings planted under the canopy of *Pinus* showed higher survival than those planted in open areas with sparse vegetation. Girdling of *Pinus* trees did not affect the performance of planted *Pistacia* seedlings, but suppression of the herbaceous understorey significantly enhanced both survival and physiological status of *Pistacia* seedlings planted under the canopy of *Pinus*. The magnitude of the interference by herbaceous understorey was considerably higher than that by *Pinus*. Our results provide evidence that a negative indirect interaction between *Pinus* and *Pistacia*, mediated by the herbaceous understorey, is taking place in the afforestation studied, and can help to explain the low rates of colonisation of late-successional woody shrubs typically observed in semi-arid *Pinus halepensis* plantations.

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Nowadays, tree plantations are one of the most important land uses world-wide. They cover over 115 million ha of the earth's land surface (Anon. 1999), and their extension is expected to increase substantially in the next decades to satisfy timber and wood demands (Sedjo 1999), and to control CO<sub>2</sub> emissions to the atmosphere (Batjes 1998). Plantations are often based on monocultures, a fact that has been

widely debated (Hartley 2002). Single-species plantations may harbour (Brockerhoff et al. 2003) or reduce (Humphrey and Patterson 2000) native biodiversity, act as vectors that spread fires (Moreno 1999) and alien plants (Richardson 1998), and induce losses of soil carbon (Bashkin and Binkley 1998). Recurrence of wildfires and pest outbreaks are expected to increase under current climatic-change scenarios, increasing the

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vulnerability of plantations (Ayres and Lombardero 2000). In recent years there has been growing concern about the way to increase the resilience of plantations (Larsen 1995, Niemelä 1999). Despite being a highly debated topic, functional attributes of ecosystems such as productivity and resilience may be enhanced in accordance with the number of species that the ecosystem contains (Loreau et al. 2002). Thus, it is not surprising that management practices leading to an increase in woody species richness have been proposed as a way to enhance the resilience of tree plantations (Hartley 2002).

*Pinus halepensis* has been planted extensively in dry and semi-arid areas throughout the Mediterranean Basin, especially in its western part (Barbéro et al. 1998). This species has also been introduced in other regions, e.g. South Africa, where it currently invades large areas in the wild (Richardson 1998). In semi-arid areas, *Pinus* plantations are often slow-growing stands that: 1) are unable to allow vegetation to recover significantly (Maestre and Cortina 2004a), 2) suffer from frequent insect plagues (Mendel 1984) and 3) do not show spontaneous colonisation by late-successional shrubs (Barberá et al. 2003). Planting methods traditionally used in these areas, such as mechanical terracing and subsoiling, have often promoted soil erosion and nutrient depletion (Herrero and Rubio 1994, García et al. 1998). Thus, there is great interest in introducing late-successional native shrubs, such as *Pistacia lentiscus* and *Quercus coccifera*, in semi-arid *Pinus* plantations. These shrubs have the capacity to resprout quickly after disturbances, provide suitable habitats for a great number of wild and game animals, and the communities dominated by them accumulate more carbon and nutrients in the soil than *Pinus* plantations (De la Torre and Aliás 1996, Lloret and Vilà 1997, López and Moro 1997). Furthermore, they are also positively associated with species richness and diversity in semi-arid degraded ecosystems (Maestre 2004). Thus, an increase of the cover of late-successional shrubs in semi-arid *Pinus* plantations could foster succession, increase biodiversity, improve soil conditions, enhance resilience against disturbances, and promote the development of faunal communities.

Positive interactions between plant species, or facilitation, are widespread in natural communities, and have been considered as a main driver of plant community dynamics and ecosystem processes, especially in stressful environments (Callaway 1995, Bruno et al. 2003). It has been predicted that that the relative importance of facilitation and competition may vary inversely across gradients of abiotic stress, with facilitation dominating in areas of high abiotic stress (Bertness and Callaway 1994). This prediction has been supported by different studies conducted in semi-arid areas (Pugnaire and Luque 2001, García-Fayos and Gasque

2002, Maestre et al. 2003a, but see Maestre and Cortina 2004b). Contrasting with these studies, Maestre et al. (2003b) reported a negative effect of *Pinus* on late-successional shrub seedlings in semi-arid plantations of SE Spain. They suggested three hypotheses to explain this interaction: 1) competition between introduced seedlings and *Pinus*, 2) allelopathic effects of *Pinus* litter or root exudates, and 3) competition between introduced seedlings and the herbaceous understorey, which is facilitated by *Pinus* (Bautista and Vallejo 2002). In the present study we performed manipulative field and laboratory experiments to test the roles of the above-mentioned hypotheses on the establishment of *Pistacia* in a semi-arid *Pinus* afforestation. If 1) and 2) are true, they would suggest that direct interference between *Pinus* and *Pistacia*, mediated by allelopathy or competition, respectively, regulates the establishment of the latter beneath the *Pinus* canopy. If 3) is true, it would suggest that an indirect interaction (sensu Miller 1994) between *Pinus* and *Pistacia*, mediated by the herbaceous understorey, is taking place in semi-arid plantations.

## Materials and methods

### Study area and species employed

Field experiments were conducted in a *Pinus* plantation located in the province of Alicante, SE Spain (Orgegia, 38°23'N, 0°28'W, 80 m a.s.l.; 3° slope; 110° SE aspect), representative of this type of stand in the western Mediterranean. Climate is semi-arid, with a 30-yr average annual precipitation and temperature of 358 mm and 18°C, respectively. Soils are loamy, lithic calciorthid (Anon. 1994). Pines were uniformly planted in the early 1970s with a between-row distance of  $5.6 \pm 0.4$  m (mean  $\pm$  SD,  $n = 12$ ), and a between-tree distance of  $3.8 \pm 1.8$  m (mean  $\pm$  SD,  $n = 12$ ). Most of these trees are poorly developed, and are <4 m in stem height. Understorey vegetation is composed of a dense (>80% cover) herbaceous layer dominated by the perennial sprouting grass *Brachypodium retusum*. The open areas between the pines have a sparse vegetation (<30% cover) constituted by *Brachypodium* and annual grasses and forbs like *Helictotrichon filifolium*, *Asphodelus fistulosus*, *Helianthemum violaceum*, *Carrichtera annua* and *Fagonia cretica*. Early-successional shrubs, such as *Anthyllus cytisoides* and *Thymelaea hirsuta*, are also present in open areas. There is no evidence of spontaneous colonisation by sprouting shrubs like *Pistacia*, *Quercus* and *Rhamnus lycioides*, that dominate late-successional vegetation in semi-arid coastal areas of SE Spain (Rivas Martínez 1987).

*Pistacia lentiscus* is an evergreen, dense-leaved, shrub with a compact cushion-like canopy. Seedlings of this species typically develop a dense root system that can reach depths up to 30 cm during the first year after

planting in semi-arid conditions (Fonseca 1999). They can also maintain leaf biomass and increase leaf water storage and photochemical efficiency under severe drought conditions (Vilagrosa et al. 2003). In addition to these morpho-functional characteristics, this species improves soil physical conditions under its canopy, is resistant to disturbances such as fire and clipping, and bears fleshy fruits that are dispersed by birds (Verdú and García-Fayos 1996).

## Field experiment

We performed a manipulative field experiment to test for interference in *Pistacia* seedlings by *Pinus* or by a herbaceous understorey. *Pistacia* seedlings were planted in a fully randomised factorial experiment with four treatments ("Pine", "Herbicide", "Girdling", and "Open"), and 27 replicates per treatment (Appendix 1). The first three treatments were located under the canopy of *Pinus* individuals. To set up them, we selected 81 *Pinus* individuals of homogeneous size prior to the application of treatments, and then 27 trees were randomly selected for the Pine, Girdling and Herbicide treatments, respectively. We used one individual tree for each *Pistacia* seedling that was planted in these treatments (see below). Similarly, 27 inter-pine areas (outside the crown projection of pines) of homogeneous characteristics were randomly selected to introduce the *Pistacia* seedlings in the Open treatment. To ensure independence between replicates in this treatment, each replicate was separated by at least 1.5 m. In the Pine treatment, we left the vegetation intact. In the Herbicide treatment, we killed the herbaceous understorey by applying herbicide (glyphosate 36% [weight:volume]), avoiding any damage to the pines. In the Girdling treatment, we killed the pine by girdling the trunk with a saw, avoiding any damage to the herbaceous understorey. After carrying out the Herbicide and Girdling treatments, we did not remove the killed plants. One year after the application of treatments, nor the herbaceous understorey nor the pines had recovered (Maestre, unpubl.).

Nine-month-old *Pistacia* seedlings from a local provenance and with homogeneous size (diameter:  $2.9 \pm 0.6$  mm; height:  $7.5 \pm 1.9$ ; mean  $\pm$  SD,  $n = 10$ ) were planted in all the treatments using manually dug holes ( $25 \times 25 \times 25$  cm) on 14 February 2001 (one seedling per planting hole). The seedlings were purchased from a commercial nursery located 60 km from the study sites (Viveros Todolí, Palmera, Spain), and were grown in containers in 150 ml plastic containers with a mixture of peat and coconut fibre (50:50%). To ensure successful establishment, seedlings were irrigated on planting with 2 l of water per hole. Due to the low rainfall registered during the first months of the experiment, we performed

additional irrigations with the same amount of water on 13 April and 16 July 2001.

We used survival and chlorophyll fluorescence to evaluate the response of seedlings to the different treatments. Seedling survival is the variable that ultimately defines seedling establishment, and is commonly used to evaluate the performance of shrubs under semi-arid conditions (Alguacil et al. 2003, Maestre et al. 2003a, b, Maestre and Cortina 2004b). Chlorophyll fluorescence is a good indicator of photoinhibition and stress (Maxwell and Johnson 2000), and has recently been used to evaluate the outcome of plant-plant interactions involving *Pistacia* seedlings (Maestre et al. 2003a). Survival was recorded every 3–4 weeks between February and September 2001, and monthly between December 2001 and March 2002. We performed chlorophyll fluorescence measurements on 13 April, 1 June, 19 July and 18 August 2001 on six seedlings per treatment randomly selected on each sampling date. We used a portable, pulse-modulated fluorometer, equipped with a leaf clip holder and photosynthetic active radiation (PAR) sensors. Pre-dawn and midday measures of maximal ( $F_m$ ) and minimal ( $F_0$ ) fluorescence were used to calculate maximum efficiency of the photosynthetic energy conversion of PSII ( $F_v/F_m = (F_m - F_0)/F_m$ ). Pre-dawn measurements were performed before sunrise; midday measurements were made after a 30-min dark adaptation between 12:30 and 14:00 local time (GMT +2). We measured PAR between 11:00 and 12:30 (local time) on the same dates. We also measured soil moisture in all treatments by using time-domain reflectometry (TDR; Topp and Davis 1985). We selected this method to measure soil moisture because is a non-destructive method that allows continuous measurements in the vicinity of the seedlings with a minimum disturbance of the soil environment. We installed a 10 cm-long TDR probe vertically in each of ten randomly selected planting holes per treatment at a depth of 5 cm (measuring depth = 5–15 cm) at planting. We selected this depth because most of the roots within the first year after planting are usually included within the first 15 cm of the soil profile (Maestre unpubl.). Measurements were performed with a metallic TDR cable tester every 1–4 weeks from February 2001 to March 2002. A site-specific calibration factor for the soils in the study area was employed.

We quantified the negative and net effects of *Pinus* on *Pistacia* in the field between March and September 2001, when most seedling mortality took place. For this task we used pre-dawn  $F_v/F_m$  data. To assess the net effect of *Pinus* on *Pistacia*, we compared  $F_v/F_m$  at the Pine and Open treatments. Negative effects of competition by *Pinus* and by herbaceous understorey were obtained as the difference in  $F_v/F_m$  between Pine and Girdling, and Pine and Herbicide treatments, respectively. These effects were calculated by

using the “Hedges’ d index” as described in Gurevitch and Hedges (2001).

### Laboratory experiments

In the first experiment (LE1) we tested the effects of *Pinus* litter on *Pistacia* growth. A total of 24 nine-month-old *Pistacia* seedlings purchased from a local nursery (Viveros Todolí, Palmera, Spain) were planted in 650 cm<sup>3</sup> plastic containers filled with pure silica sand (0.5–1.5 mm grain diameter) and slow-release fertiliser at a rate of 2.9 mg l<sup>-1</sup>. Half of the containers contained litter in the top 2 cm of the pot surface, and half were set up as controls. After a random assignment, the seedlings did not show significant differences in diameter, height, and number of leaves between treatments (one-way ANOVA,  $p > 0.4$  in all cases). Fresh litter from underneath the *Pinus* canopy was collected in late spring 2001, dried in the laboratory at 65°C to constant weight, and added to the litter treatment containers in the same amount as found in the field (856 g m<sup>-2</sup>, unpubl.). To prevent mulch effects in the litter containers, a layer of small PVC plastic strips (856 g m<sup>-2</sup>) was added to the control containers. During the course of the experiment (6 June–27 December 2001), all the containers were irrigated daily with 100 ml of deionized water and rotated randomly every two weeks.

In the second experiment (LE2) we tested for the effect of *Pinus* root exudates on *Pistacia* development. *Pistacia* seedlings of the same characteristics described in LE1 were planted in 1600 cm<sup>3</sup> plastic containers filled with the same combination of sand and fertiliser as described above, and containing either *Pistacia* ( $n = 24$ ) or *Pinus* ( $n = 24$ ) neighbours. We used three-year-old *Pinus* seedlings of a local provenance with well-developed root systems as neighbours. For each of the two neighbour species, half of the containers contained finely ground activated carbon mixed with the sand at a rate of 20 ml<sup>-1</sup> of sand, and half contained only the sand. We used activated carbon because it retains organic compounds commonly involved in allelopathic interactions, and because of its lack of negative effects on plant growth. After a random assignment, the seedlings did not show significant differences in diameter, height, and number of leaves between treatments (one-way ANOVA,  $p > 0.09$  in all cases). During the course of the experiment (7 June–27 December 2001), all the containers were irrigated daily with 200 ml of deionized water, and they were rotated randomly every two weeks.

In the third experiment (LE3) we tested for the effect of allelopathic soil compounds on *Pistacia* development. One-week-old *Pistacia* seedlings previously germinated from seed were planted in the same containers used in LE1, which were filled with soil from the Pine ( $n = 24$ ) or Open treatments ( $n = 24$ ). Seeds from local provenance

were collected by the local Forest Services (Banc de Llavors Forestals, Conselleria de Medio Ambiente, Generalitat Valenciana) in 1998. For each of the two treatments, half of the containers (12) contained finely ground activated carbon mixed with the soil at a rate of 20 ml per l of soil, and the other half contained only the soil. Soils were collected on undisturbed areas of the field site from 24 randomly selected Pine and Open treatments. One kg of soil was collected for each treatment. Soil texture (0–20 cm depth) is loamy, with 41% sand, 38% silt and 20% clay, and 40% sand, 38% silt and 20% clay for the Pine and Open treatments, respectively (Maestre et al. 2003b). Soil organic carbon is  $28.1 \pm 1.5$  mg g<sup>-1</sup> and  $22.0 \pm 1.2$  mg g<sup>-1</sup> for the Pine and Open treatments, respectively (mean  $\pm$  SE,  $n = 10$ , Maestre et al. 2003b). During the course of the experiment (15 November 2001–26 March 2002), all the containers were irrigated with 50 ml of deionized water twice a week, and rotated randomly every two weeks.

All the laboratory experiments were conducted outdoors, under natural light and temperature conditions, at the Univ. of Alicante, located 10 km from the field site. To evaluate seedling response, we measured basal diameter, height and number of leaves at the beginning (LE1 and LE2), and at the end of all experiments (LE1, LE2 and LE3). From these data, the relative growth rate (RGR) was calculated in LE1 and LE2 using the equations provided by Poorter and Garnier (1999). At the end of the experiments, all the plants were harvested and divided into roots, stems and leaves. In LE2, the roots of *Pinus* and *Pistacia* seedlings were separated. In LE1 and LE2, we used the roots outside the root ball as an indicator of root growth. All harvested material was dried at 65°C to constant weight.

We also conducted an experiment to test for differences in evapotranspiration between treatments. In February 2002, total of six, three, and three containers per treatment were prepared as described above for LE1, LE2 and LE3, respectively, but without seedling addition. These containers were irrigated as during the course of the experiment, weighed, placed in the experimental area, and then weighed every 4–12 h until the sand was dry (4 d in LE1 and LE2, and 5 d in LE3). Rates of water loss were calculated as proportional to the percent decrease in total mass of the pot.

### Statistical analyses

Field survival curves were analysed with the Kaplan-Meier procedure, using the log-rank statistic to test for differences between treatments. Fluorometer ( $F_v/F_m$  and PAR) data were analysed by two-way ANOVA, with Treatment as a fixed factor and Sampling Date as a random factor. The effect of *Pinus* and of *Pinus* and herbaceous understorey removal on soil moisture was

evaluated with the ‘‘Hedges’ d index’’ as described in Gurevitch and Hedges (2001). The effect of *Pinus* litter on RGR and roots produced outside the rootball in LE1 was evaluated with one-way ANOVA. In the LE2 experiment, these data were analysed with two-way ANOVA (Neighbour and Activated carbon), with both factors being fixed. In this experiment, we interpret a significant interaction as the presence of an allelopathic effect of *Pinus* roots on *Pistacia* growth. With the aim of taking into account differences in seedling size in the LE1 and LE2 experiments, we included the first axis of a principal component analysis (PCA) performed with the diameter, height and number of leaves of *Pistacia* seedlings at the beginning of the experiment as a covariate in the analyses of root growth. This PCA was based on a correlation matrix. Biomass data from the LE3 experiment were analysed by two-way ANOVA, with both factors (Treatment and Activated carbon) being fixed. In this experiment, a significant interaction would suggest an allelopathic effect of the soil compounds. To assess the effects of the treatments (litter in the LE1 experiment, and activated carbon in the LE2 and LE3 experiments) on water loss we fitted double exponential equations to drying curves. Drying rates for short and long periods of time after irrigation, represented by the first and second exponents of the fitted equations, respectively, were compared separately by using the non-parametric Mann-Whitney U test (LE1 and LE2 experiments), and Kruskal-Wallis tests (LE3 experiment). In all the ANOVA analyses, we used the Student-Newman-Keuls (SNK) test for post-hoc comparisons when appropriate. Prior to these analyses, data were log-transformed when necessary to correct for deviations from normality and to achieve homogeneity of variances. All statistical analyses were conducted with the SPSS 9.0 package (SPSS).

## Results

### Field experiment

The net effect of *Pinus* and the effect of the interference by *Pinus* on soil moisture were negligible throughout the study period (Fig. 1). However, the effect of interference by the herbaceous understorey on this variable was significant during most of the spring and early summer. At the end of the experiment, survival of the planted seedlings ranged from 7 to 63% and showed significant differences between treatments (Fig. 2). Survival in the Herbicide treatment was higher than in the other treatments; it was also higher in the Pine and Girdling treatments than in the Open treatment, but did not show significant differences between the former two. Pre-dawn  $F_v/F_m$  values and PAR were significantly higher in the Herbicide and Open treatments, respectively, than in the other treatments (Fig. 3). Pre-dawn  $F_v/F_m$  values of

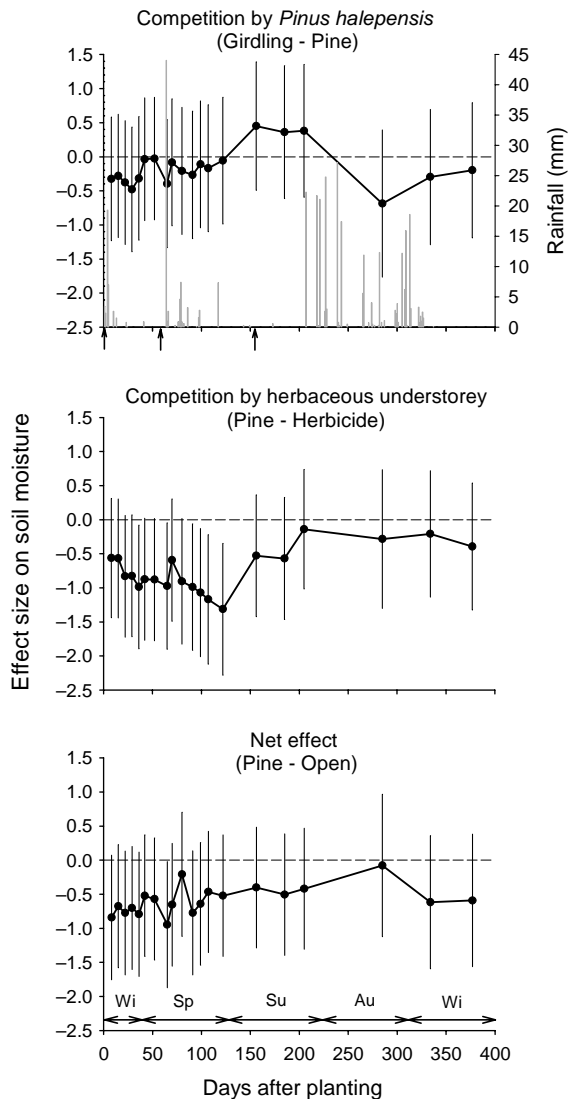


Fig. 1. Dynamics of the negative and net effects of *Pinus halepensis* on soil moisture. Data represent mean effect size (‘‘Hedges’ d index’’)  $\pm$  95% confidence intervals. To calculate each effect we used data from the treatments shown in brackets. The grey bars and arrows in the upper graph correspond to rainfall data and watering dates, respectively. Seedlings were planted on 14 February 2001. Rainfall data come from the Alicante weather station (38°21’N, 0°30’W, 80 m a.s.l.). Wi = Winter, Sp = Spring, Su = Summer, Au = Autumn.

seedlings planted in the Herbicide and Open treatments remained quite constant throughout the sampling period; those of seedlings planted in the Pine and Girdling treatments showed a more fluctuating pattern, with a strong depletion by the end of the summer. Midday  $F_v/F_m$  values showed a similar pattern to that observed at pre-dawn, but they were substantially lower, especially in the Open treatment. When measured using pre-dawn  $F_v/F_m$  values, both the net effect of *Pinus* on *Pistacia* and the negative effect of interference by *Pinus* were not

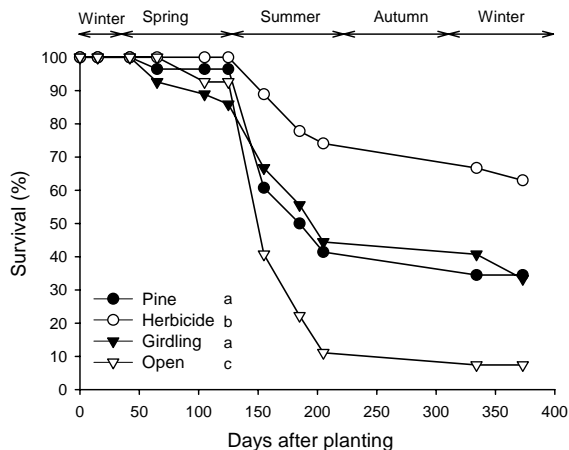


Fig. 2. Survival curves of planted *Pistacia lentiscus* seedlings. Different letters close to the legend indicate significant differences between treatments (log-rank test,  $p < 0.05$ ). Initial  $n = 27$ .

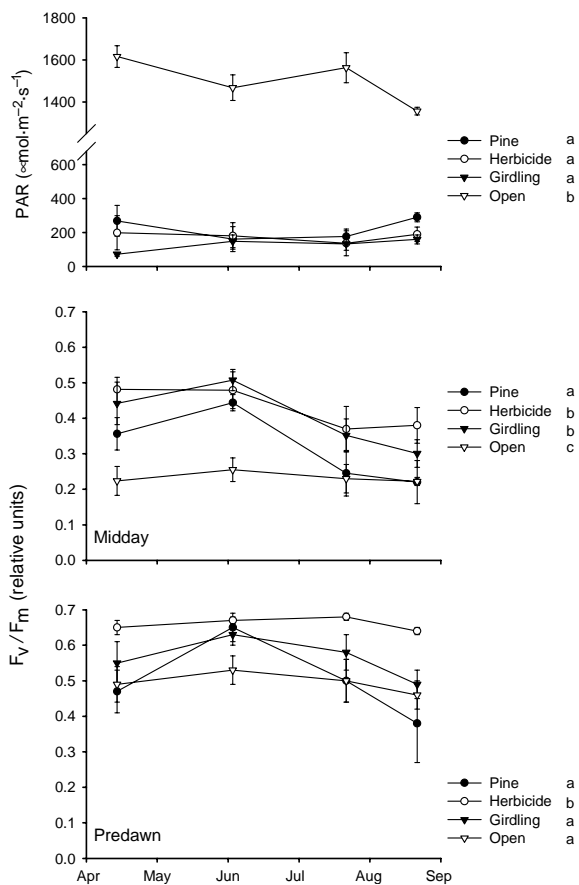


Fig. 3. Dynamics of photosynthetically active radiation (PAR), and of maximum efficiency of PSII ( $F_v/F_m$ ) of *Pistacia lentiscus* seedlings during the spring and summer. Data represent means  $\pm 1$  SE ( $n = 6$ ). Different letters close to the legend indicate significant differences between treatments (SNK test,  $p < 0.05$ ).

significant at any sampling date (Fig. 4). However, the negative effect of interference by a herbaceous understorey was significant on all the sampling dates excepting June.

### Laboratory experiments

We found no effect of *Pinus* litter on *Pistacia* growth (Table 1). In the LE2 experiment, activated carbon had a positive effect on *Pistacia* above-ground and below-ground growth, but we did not find any indication for allelopathic effects of *Pinus* root exudates (Table 2). In

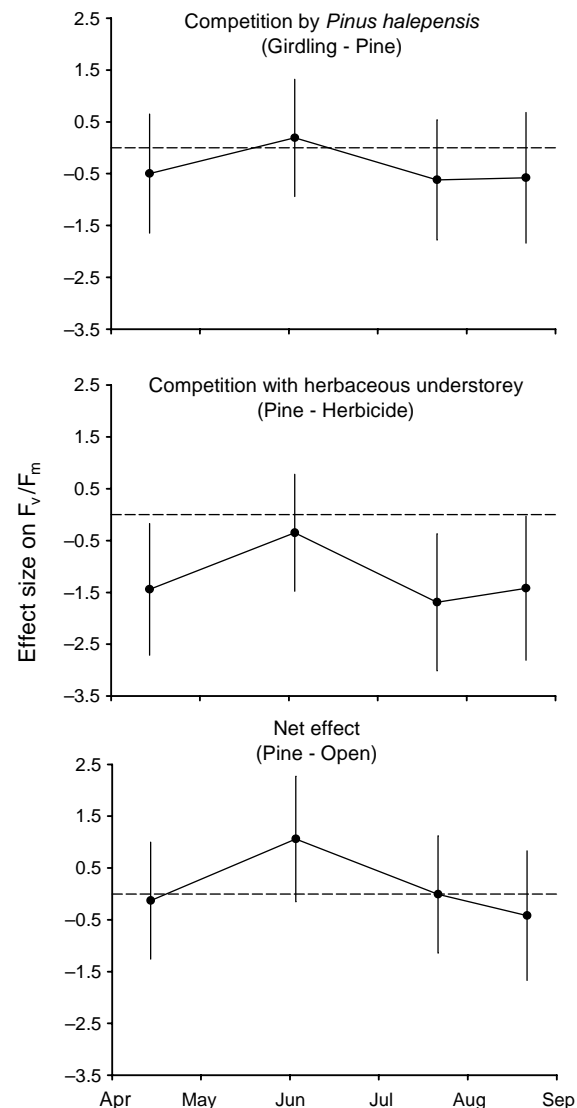


Fig. 4. Dynamics of the negative and net effects of *Pinus halepensis* on *Pistacia lentiscus* pre-dawn maximum efficiency of PSII ( $F_v/F_m$ ). Data represent mean effect size ("Hedges'  $d$  index")  $\pm 95\%$  confidence intervals. To calculate each effect we used data from the treatments shown in brackets.

Table 1. Effects of *Pinus halepensis* litter on the relative growth rate of the diameter, height and number of leaves (RGR<sub>Diam</sub>; RGR<sub>Height</sub>; RGR<sub>Num</sub>, respectively), and on the production of roots outside the rootball of *Pistacia lentiscus* seedlings. Also, the results of a one-way ANOVA. Data represent mean  $\pm$  SE (n = 12).

	Treatment		ANOVA results		
	Litter	Control	F	DF	p
RGR <sub>Diam</sub> ( $10^{-3}$ , d <sup>-1</sup> )	2.07 $\pm$ 0.16	1.74 $\pm$ 0.25	1.17	1,22	0.292
RGR <sub>Height</sub> ( $10^{-3}$ , d <sup>-1</sup> )	1.79 $\pm$ 0.21	1.53 $\pm$ 0.25	0.60	1,22	0.448
RGR <sub>Num</sub> ( $10^{-3}$ , d <sup>-1</sup> )	4.21 $\pm$ 0.55	4.28 $\pm$ 0.51	<0.01	1,22	0.930
Roots (g)	0.56 $\pm$ 0.06	0.40 $\pm$ 0.07	1.64†	1,21	0.214

†Results of an ANCOVA using as a covariate the first axis of a PCA (correlation matrix) performed with the diameter, height and number of leaves of *Pistacia* seedlings at the beginning of the experiment.

the LE3 experiment, we found no allelopathic effect of *Pinus* soil on *Pistacia* growth (Table 3). In this experiment, seedlings showed higher biomass when grown with *Pinus* soil, and activated carbon increased their root biomass and root-to-shoot ratio. Containers with activated carbon lost water more slowly than controls during the first stages after irrigation in LE2 (Mann-Whitney test, p = 0.05), but not in LE3 (Kruskal-Wallis test, p = 0.77). Litter had no effect on water loss during this time in LE1 (Mann-Whitney test, p = 0.248). We found no significant differences between treatments in the second exponent of fitted equations.

## Discussion

Our first hypothesis, i.e. direct interference between *Pinus* and *Pistacia* mediated by competition for water, was not supported by our results. Removal of direct interference by *Pinus* had only small effects on soil

moisture, as well as on introduced seedling performance, suggesting that the introduced seedlings and *Pinus* do not take up water at the same depth. These results agree with previous studies performed in semi-arid afforestations, which have shown a negligible effect of planted *Pinus* individuals on the soil-water balance at depths similar to those evaluated in this study (Maestre et al. 2003b, Bellot et al. 2004). Our second hypothesis, i.e. direct interference between *Pinus* and *Pistacia* mediated by *Pinus* litter or root exudates, was not supported by our results. Container experiments showed that soil compounds, litter exudates, and root exudates had negligible effects on seedling growth. Our results agree with those obtained by Broncano et al. (1998), who found no effect of *Pinus* litter on the emergence and growth of the late-successional tree *Quercus ilex*. They contrast, however, with the situation reported in natural stands of this species after a fire, when allelopathic interactions mediated by chemical components of pine ash may hamper the establishment of species other

Table 2. Effect of neighbor and activated carbon on the relative growth rate of diameter, height and number of leaves (RGR<sub>Diameter</sub>; RGR<sub>Height</sub>; RGR<sub>Num</sub>, respectively), and on roots produced outside the rootball, of *Pistacia lentiscus* seedlings, and results of a two-way ANOVA. Data represent mean  $\pm$  SE (n = 12). PL – = *Pistacia* as neighbor without activated carbon, PL + = *Pistacia* as neighbor with activated carbon, PH – = *Pinus halepensis* as neighbor without activated carbon, PH + = *Pinus* as neighbor with activated carbon. Significant effects (p < 0.05) are in bold-face print.

Variable units	RGR <sub>Diameter</sub> ( $10^{-3}$ ) d <sup>-1</sup>	RGR <sub>Height</sub> ( $10^{-3}$ ) d <sup>-1</sup>	RGR <sub>Num</sub> ( $10^{-3}$ ) d <sup>-1</sup>	Roots (g)
PL –	1.26 $\pm$ 0.22	0.92 $\pm$ 0.24	2.47 $\pm$ 1.06	0.42 $\pm$ 0.10
PL +	1.26 $\pm$ 0.22	0.92 $\pm$ 0.24	2.47 $\pm$ 1.06	0.42 $\pm$ 0.10
PH –	0.56 $\pm$ 0.22	0.48 $\pm$ 0.17	1.78 $\pm$ 0.91	0.29 $\pm$ 0.06
PH +	1.69 $\pm$ 0.26	1.34 $\pm$ 0.27	2.97 $\pm$ 0.55	0.51 $\pm$ 0.09

Variable	ANOVA results								
	Neighbor (N)			Activated carbon (C)			N $\times$ C		
	F	DF	p	F	DF	p	F	DF	p
Diameter	2.08	1,44	0.157	7.90	1,44	<b>0.007</b>	0.70	1,44	0.407
Height	1.47	1,44	0.232	7.76	1,44	<b>0.008</b>	0.13	1,44	0.720
Number of leaves	0.46	1,44	0.502	1.46	1,44	0.232	<0.01	1,44	0.960
Roots†	1.23	1,43	0.273	4.36	1,43	<b>0.043</b>	0.65	1,43	0.424

†Results of an ANCOVA using as a covariate the first axis of a PCA (correlation matrix) performed with the diameter, height and number of leaves of *Pistacia* seedlings at the beginning of the experiment.

Table 3. Effect of soil type and activated carbon on the growth of *Pistacia lentiscus* seedlings, and results of a two-way ANOVA. Data represent mean  $\pm$  SE (n=12). PI – =soils from the Pine treatment without activated carbon, PI+ =soils from the Pine treatment with activated carbon, OP – =soils from the Open treatment without activated carbon, OP+ =soils from the Open treatment with activated carbon, R:S =root-to-shoot ratio (below-ground/above-ground biomass). Significant effects (p <0.05) are in bold-face print.

Variable	PI –	PI+	OP –	OP+
Diameter (mm)	0.77 $\pm$ 0.04	0.70 $\pm$ 0.04	0.73 $\pm$ 0.05	0.69 $\pm$ 0.03
Height (cm)	2.63 $\pm$ 0.11	2.48 $\pm$ 0.13	2.56 $\pm$ 0.17	2.20 $\pm$ 0.10
Number of leaves	13.58 $\pm$ 0.58	12.75 $\pm$ 0.84	12.67 $\pm$ 0.51	13.42 $\pm$ 0.72
Leaf biomass (g)	4.69 $\pm$ 0.30	4.33 $\pm$ 0.43	4.02 $\pm$ 0.46	3.64 $\pm$ 0.34
Stem biomass (g)	7.92 $\pm$ 1.02	5.92 $\pm$ 0.74	6.17 $\pm$ 1.15	4.50 $\pm$ 0.48
Root biomass (g)	1.04 $\pm$ 0.12	1.35 $\pm$ 0.23	0.64 $\pm$ 0.09	1.07 $\pm$ 0.16
R:S	1.95 $\pm$ 0.22	2.85 $\pm$ 0.48	1.46 $\pm$ 0.16	2.93 $\pm$ 0.53

ANOVA results									
Variable	Treatment (M)			Activated carbon (C)			M $\times$ C		
	F	DF	p	F	DF	p	F	DF	p
Diameter	0.47	1,44	0.496	2.00	1,44	0.164	0.17	1,44	0.679
Height	1.96	1,44	0.168	3.95	1,44	0.053	0.66	1,44	0.420
Number of leaves	0.03	1,44	0.854	<0.01	1,44	0.951	1.38	1,44	0.247
Leaf biomass	3.22	1,44	0.079	0.93	1,44	0.341	<0.01	1,44	0.983
Stem biomass	4.19	1,44	<b>0.047</b>	1.92	1,44	0.173	0.08	1,44	0.779
Root biomass	5.85	1,44	<b>0.020</b>	5.18	1,44	<b>0.028</b>	0.11	1,44	0.742
R:S	0.93	1,44	0.338	8.74	1,44	<b>0.005</b>	0.94	1,44	0.339

than *Pinus* (Ne'eman 2000). Our third hypothesis, i.e. direct interference between *Pistacia* seedlings and herbaceous understorey mediated by competition for water, was supported by our results; both soil moisture (Appendix 2) and seedling performance increased substantially in the Herbicide treatment as compared to the other treatments. Competition between shrubs and grasses in semi-arid environments has been thoroughly described in the ecological literature (Bush and Van Auken 1989, Aguiar et al. 1992), and may be especially important during the seedling stage of shrubs (Montaña et al. 1995, Köchy and Wilson 2000). The negative effect of herbaceous understorey on soil moisture was maximised during the spring, coinciding with the main growth season for *Brachypodium* – the major component of this understorey – (Caturla et al. 2000). However, it is interesting to note that the negative effects of herbaceous understorey removal on seedling performance were mainly observed when their effects on soil moisture were lower and non-significant. This suggests that *Pistacia* seedlings in the Herbicide treatment may have been able to use the excess water available during the spring to withstand summer drought.

In contrast to observations made during previous experiments in the same plantation (Maestre et al. 2003b), we found evidence of a positive net effect of *Pinus* on *Pistacia*. This discrepancy suggests that the direction and size of the net effect of *Pinus* on shrub seedlings may be coupled with abiotic conditions (Greenle and Callaway 1996, Tielbörger and Kadmon 2000, Maestre and Cortina 2004b). During the first eight months after planting, when most mortality took place,

rainfall was 221 mm (plus irrigations), contrasting with the values recorded in 1999 and 2000 (107 and 113 mm, respectively), the years of the two experimental plantings performed by Maestre et al. (2003b). In the light of these results, we hypothesise that, in years with abundant rainfall, the magnitude of the interference between shrubs and herbaceous understorey would be reduced, resulting in a net facilitation because of improved microclimatic and soil conditions underneath the canopy of *Pinus*. In dry years, competition between shrubs and grasses would be more intense, and could not offset the positive effects associated to the microsite provided by *Pinus*. If true, our hypothesis suggests that there is a rainfall threshold defining the transition between negative and positive interactions, as recently suggested for the interaction between *Pistacia* and the perennial tussock grass *Stipa tenacissima* in semi-arid steppes close to our study area (Maestre and Cortina 2004b).

It has been accepted that facilitation and interference act simultaneously, and that the net effect of a plant-plant interaction is the result of unequal negative and positive effects (Chapin et al. 1994, Callaway and Walker 1997). Despite its importance, the temporal dynamics of the positive and negative effects in plant interactions has barely been studied (Holzapfel and Mahall 1999, Maestre et al. 2003a). To our knowledge, no previous study has attempted to evaluate these issues in the context of plantations. Our manipulative experimental approach allowed us to quantify the magnitude of the different negative effects of *Pinus* on shrub seedlings. However, as we did not manipulate the positive effects involved in this interaction (mainly local improvements



in microclimate and soil fertility under the canopy of *Pinus*), their relative importance against the negative effects remain unknown.

In semi-arid *Pinus* stands, observational and experimental data have shown that this species facilitates the development of the perennial herbaceous understorey through improvement in soil conditions and microclimate (Bautista and Vallejo 2002). Despite we did not specifically test for such interaction, the differences in cover of *Brachypodium* found between the open areas and the canopy of pines indicates that it is also occurring in our study area. Our results suggest that an indirect negative interaction between *Pinus* and shrub seedlings, mediated by herbaceous understorey, is taking place in *Pinus* plantations under semi-arid conditions. Indirect interactions are of great importance in determining ecosystem patterns and processes, but their role is just now beginning to be understood (Morin 1999). The findings presented here are among the first to report such an interaction in managed ecosystems like plantations (Mitchell et al. 1999). According to the above hypothesis, the indirect interaction reported would mainly prevail during dry years. High seedling mortality during summer drought commonly acts as a bottleneck for shrub colonisation in semi-arid Mediterranean environments (Escudero et al. 1999). Since dry years are common throughout the Mediterranean Basin, the indirect interaction described could limit shrub establishment in semi-arid *Pinus* plantations, reinforcing the negative effect of drought on this process. The mechanisms driving succession in Mediterranean *Pinus* forests in the absence of disturbances are still poorly understood. It has been suggested that they will be replaced by *Quercus ilex* or *Q. pubescens* forests under dry, sub-humid conditions (Zavala et al. 2000). However, under semi-arid conditions, little is known about community dynamics either in plantations or in natural stands (Barbéro et al. 1998). The indirect interaction described may limit colonisation by late-successional shrubs, and thus greatly influence the evolution of semi-arid *Pinus* stands towards late-successional stages.

Our results also have direct implications for the management of *Pinus* plantations, and contribute to the debate on the appropriateness of the massive use of this species in semi-arid Mediterranean areas (Maestre and Cortina 2004a, Bellot et al. 2004). One of the arguments used for the widespread utilisation of *Pinus* in restoration projects is its role as a pioneer species. It has been largely assumed that *Pinus* will allow for the introduction of late-successional species as its development improves soil conditions and ameliorates harsh microclimatic conditions under its canopy (Ruiz 1973, Montero and Alcanda 1993). Current theory and observational field data support this hypothesis over dry and sub-humid Mediterranean conditions (Lookingbill and Zavala 2000, Zavala et al. 2000), but not in semi-arid

areas (Maestre et al. 2003b). Our results provide the first experimental evidence of the possible mechanisms underlying this interaction. Large-scale herbaceous understorey control to facilitate the introduction of late-successional shrubs may be unfeasible due to the high financial cost and to the undesirable secondary effects of herbaceous species removal. Nevertheless, we believe that this kind of control, combined with the planting of shrubs, would be useful for increasing resilience in plantations established in very degraded areas or located in areas with a high risk of fire or pest outbreak. This control could be especially helpful in slow-growing plantations with poor reproductive capacity, and thus with limited possibilities to regenerate themselves after disturbances.

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