Benefits of using shrubs as nurse plants for reforestation in Mediterranean mountains: a 4-year study

Jorge Castro\textsuperscript{ab}, Regino Zamora\textsuperscript{a}, José A. Hódar\textsuperscript{a}, José M. Gómez\textsuperscript{a} and Lorena Gómez-Aparicio\textsuperscript{a}

\textsuperscript{a}Grupo de Ecología Terrestre. Departamento de Biología Animal y Ecología, Facultad de Ciencias, Universidad de Granada. E-18071 Granada, SPAIN. FAX: +34 958 243238

\textsuperscript{b}Physiological Ecology Research Group, Botanical Institute, University of Copenhagen. Øster Farimagsgade 2D, DK-1353, Copenhagen K, DENMARK

Author for correspondence: Jorge Castro. E-mail: jorge@ugr.es

Running title: Shrubs as nurse plants for reforestation
Abstract

Shrubs are commonly considered competitors of planted seedlings in reforestation programs. However, shrubs can facilitate the establishment of understory seedlings in environments that, like Mediterranean-type ecosystems, are characterized by harsh environmental conditions. In 1997, an experiment was set up in the Sierra Nevada mountains (SE Spain) to test the use of shrubs as nurse plants for an alternative reforestation technique. Two-year-old seedlings of *Pinus sylvestris* and *P. nigra* were planted in four microhabitats: 1) open interspaces without vegetation (which is the usual method employed in reforestation programs), 2) under individuals of the shrub *Salvia lavandulifolia*, 3) under the north side of spiny shrubs and 4) under the south side of spiny shrubs. Seedlings were also distributed in plots with and without access for ungulates in order to test the effect of herbivore damage. We report here the results of survival and growth after four growing seasons, a time span long enough to draw robust conclusions concerning the suitability of this technique. Pine survival was remarkably higher when planted under individuals of *Salvia lavandulifolia* as compared to open areas (2.6 times for *P. sylvestris*, 1.8 for *P. nigra*). The survival of both pine species was also higher when planted on the north side of spiny shrubs, while mortality on the south side was similar to that found in open areas. The reduction of solar radiation by the canopy of shrubs was likely the main factor determining shrub facilitation. The growth of the pines differed among years. However, growth was not inhibited when planted in association with shrubs as compared to open areas in any of the years. Herbivore damage was low, but was mostly concentrated in the leader shoot, exacerbating the deleterious effect of ungulate herbivores on pine growth. We conclude that the use of shrubs as nurse plants for reforestation is a viable technique to increase establishment success of reforestation in Mediterranean-type ecosystems, and might be similarly useful in other water-stressed environments. In addition, this technique offers the advantage of following natural succession, thus minimizing the impact in the community.

**Key words:** facilitation, Mediterranean ecosystems, nurse plants, *Pinus*, silviculture.
INTRODUCTION

Reforestation is a major environmental goal worldwide (Savill et al. 1997; FAO 2001; Lamb 2001). In areas with a long history of human population such as the Mediterranean basin, intense exploitation has in many cases altered the original composition of species and the physical environment (e.g. erosion) (Bauer 1991; García-Salmerón 1995; Blondel & Aronson 1999). Under such circumstances, planting is particularly necessary in order to recover the vegetation and to ensure the success of regeneration (Bauer 1991; García-Salmerón 1995; Serrada 1995). However, in the Mediterranean basin the prevailing environmental conditions are usually stressful for the survival of planted seedlings. Thus, reforestations usually suffer heavy plant losses that destroy restoration efforts, mostly as a consequence of summer drought and ungulate browsing (Mesón & Montoya 1993; García-Salmerón 1995).

The development of suitable strategies for forest regeneration in the Mediterranean basin is an issue of primary concern (García-Salmerón 1995; Scarascia-Mugnozza et al. 2000). Prevailing reforestation techniques used in the region consider the pre-existing shrubby vegetation to be a source of competition for the newly planted young trees (Mesón & Montoya 1993; García-Salmerón 1995; Serrada 1995), and consequently shrubs are cleared by different methods, or seedlings are planted far from shrubs (e.g. García-Salmerón 1995; Serrada 1995; Sternberg et al. 2001). This approach derives primarily from models developed in central and northern parts of Europe (Groome 1989; Bauer 1991), where dense vegetation cover and the lack of severe drought stress makes the pre-existing shrubby vegetation a source of competition for nutrients and light, increasing the mortality of the reforested seedlings (Savill et al. 1997). However, the interactions among plants may be positive in environments that, like Mediterranean-type ecosystems, are characterized by harsh physical conditions such as summer drought accompanied by high temperatures (Bertness & Callaway 1994; Callaway 1995; Brooker & Callaghan 1998). In such settings, the pre-existing vegetation may offer protection to the seedlings by buffering microclimatic extremes (i.e. reduce solar radiation and soil temperature, conserve soil moisture, enrich nutrient content), and in addition may protect seedlings from herbivore damage (Callaway 1995; Rousset and Lépart 1999; García et al. 2000;
Gómez et al. 2001a; 2003a). In fact, a growing body of experimental studies is reporting a facilitative effect of shrubs and grasses for the early establishment of reforested woody species in Mediterranean environments (Maestre et al. 2001; Gómez et al. 2001b; Castro et al. 2002; Gómez et al. 2003b).

In 1997, an experiment was set up in Sierra Nevada mountain (SE Spain) to test an alternative technique of reforestation using two pine species (*Pinus sylvestris* and *P. nigra*) as targets. Previous results showed that shrubs facilitated the early survival of pine seedlings during the first two growing seasons (Castro et al. 2002). The objective of the present work is to analyze the effect of shrubs on survival and growth of pine seedlings four years after planting, considering both the effect of microhabitat and herbivores. This time span may be considered long enough to evaluate establishment success in reforestations, and thus to draw robust conclusions concerning the suitability of this alternative technique of reforestation. Based on the theoretical framework of plant-plant interactions under harsh physical conditions (Callaway 1995), we hypothesize that shrubs provide regeneration niches, thereby increasing reforestation success by ameliorating the microclimate and by protecting seedlings against ungulate herbivores.

**MATERIALS AND METHODS**

**Study area**

The study site was at 1800-1850 m a.s.l. on the northwestern side of Loma de los Panaderos (Trevenque area, Sierra Nevada, SE Spain, 37° 5’ N, 3° 28’ W). Plant cover at the site is patchy, with roughly 36% of bare soil, 28% cover by the shrubby evergreen *Salvia lavandulifolia* (a scrub reaching some 25 cm in height), and 10% deciduous spiny shrubs, mostly *Prunus ramburii*, *Crataegus granatensis* and *Berberis hispanica* (which reach around 150 cm in height) (see Castro et al. 2002 for details on habitat structure). The climate is continental Mediterranean, with hot, dry summers and cold winters. Mean annual rainfall is 830 mm, mean temperature of the coldest month (January) is 3.5 °C, and mean temperature of the hottest month (August) is 21.6 °C (data from 1990 to 2000). Rainfall in 1997, 1998, 1999 and 2000 was 1283,
632, 755 and 1045 mm, respectively. In these areas, forests are formed by the Sierra Nevada Scots pine *Pinus sylvestris* L. var. *nevadensis* Christ and the Salzmann’s black pine *Pinus nigra* Arnold ssp. *salzmannii* (Dunal) Franco.

**Experimental design**

Two-year-old seedlings of *Pinus sylvestris nevadensis* and *P. nigra salzmannii* were planted during March 1997 in different microhabitats (treatments), and monitored until the fourth growing season. The microhabitats were the following: 1) Open, seedlings planted in areas of bare soil; 2) Salvia, seedlings planted under the canopy of individuals of *Salvia lavandulifolia*; 3) Shrub N, seedlings planted just below the northern edge of the canopy of spiny shrubs (either *Prunus ramburii*, *Crataegus granatensis* or *Berberis hispanica*); and 4) Shrub S, seedlings planted just below the southern edge of the canopy of spiny shrubs. Planting points were distributed in three plots of ca. 6000 m² some 200 m apart. One half of each plot was protected from ungulates (Spanish ibex *Capra pyrenaica* in the study area) with a fence 1.5-m high. In each plot, we planted 50 seedlings of each pine species per microhabitat inside the fence, and the same number outside the fence (1200 experimental seedlings per pine species in total: 50 sampling point x 3 plots x 2 herbivory levels x 4 microhabitats), sampling points being randomly assigned. Planting holes were dug 40 cm deep with a mechanical augur (30 cm in diameter for Open and 12 for the rest of microhabitats). The reforestation method employed in Open treatment was the usual procedure followed in the region. At the end of June 1997, before the onset of summer drought, pines were examined, excluding from the experiment those that had died due to transplant shock (less than 5%). The resulting final number of monitored seedlings was 1115 for Scots pine and 1146 for black pine. See Castro et al. (2002) for additional details on planting procedure and experimental set up. Fenced areas in the experimental design were used in order to analyze the effect of herbivores and their interaction with the microhabitat on reforestation success, given that ungulate herbivory is a major reason of reforestation failures worldwide (García-Salmerón 1995; Savill et al. 1997; Riley & Jones 2003) However, herbivore damage was low during the study period for several reasons and did
not affect survival (see below). Thus, data from the fenced and unfenced area of each plot were pooled for the analysis of survival.

**Pine measurements**

For each experimental pine, we recorded: 1) Survival, which was sampled at the end of the summer period (October) and at the end of the winter period (May) from 1997 to October 2000. 2) Cause of mortality, which was assigned to i) drought, seedlings that dried out during the summer without any visible damage; ii) trampling, seedlings that died from ungulate trampling; iii) herbivory, seedlings that died from ungulate herbivores; iv) vole tunnels, seedlings with roots disturbed by vole tunnels; and v) frost heave, seedlings lifted and loosened from the soil by winter frost. 3) Growth, estimated as the length of the leader shoot after each growing season. 4) Risk of herbivory (only for seedlings outside the fences), percentage of pines that suffered at least one event of ungulate damage over the study period. 5) Damage intensity (only for seedlings suffering herbivory), proportion of the shoots consumed by ungulates, averaged over the years of study while seedlings were alive; this allowed the use of seedlings that died before the end of the experiment. 6) Type of damage, distinguishing herbivory in the leader shoot from that in lateral shoots.

**Data analysis**

Seedling survival was analyzed with a failure-time approach, which measures the time to failure (death) of each individual (Fox 1993). We used the Cox’s Proportional Hazards semiparametric model, which produces estimates of regression models with censored survival data using maximum partial likelihood as the estimation method (Fox 1993; Allison 1995). In addition, cumulative survival after four growing seasons was compared among microhabitats, and for any plot independently, with a contingency analysis in order to explore the final result without the influence of the shape of the survival curve. The growth of the leader shoot was analyzed using a mixed two-way ANOVA, with year as a random factor and microhabitat as a fixed factor (all plots pooled), all herbivore-damaged pines being previously excluded. Risk of herbivory was
analyzed with a contingency analysis, and damage intensity with one-way ANOVA after angular transformation of data (all plots pooled due to low herbivore incidence). Analyses were performed using JMP 5.0 software. Means ± SE are shown throughout the paper.

RESULTS

The overall mortality rate was 83.2% for *P. sylvestris* and 57.5% for *P. nigra* after four growing seasons (all microhabitats pooled; see Figs. 1 and 2). Drought was the main cause of mortality (85.6% of death seedlings), frost heave was the cause for 14.0% of death seedlings, while only 19 seedlings died due to other causes (vole tunnels, trampling and ungulate herbivory). The highest mortality occurred during the first summer, 46.8% of the *P. sylvestris* seedlings and 29.3% of the *P. nigra* dying (Figs. 1 and 2). The microhabitat had a significant effect for the two pine species (L-R $\chi^2 \geq 51.82$, *P* < 0.0001), and the plot also had a significant effect for *P. nigra* (*P* < 0.0001). There was a significant (*P* < 0.0001) plot x microhabitat interaction in the two pine species as a result of differences in survival patterns among plots (Figs. 1 and 2), but in any case the overall pattern of survival was clearcut: Salvia showed the highest rates after four years of study (28.4% for *P. sylvestris* and 64.4% for *P. nigra*; all plots pooled), Shrub N showed intermediate values (20.3% for *P. sylvestris* and 40.4% for *P. nigra*), whereas Shrub S (*P. sylvestris* = 9.4; *P. nigra* = 30.2) and Open (*P. sylvestris* = 10.7; *P. nigra* = 36.2) registered the lowest values (Figs. 1 and 2). Differences among microhabitats similarly persisted in all the plots when considering the cumulative values after four study years (*P. sylvestris*: $\chi^2$ from 6.79 to 54.96; df = 3; *P* ≤ 0.078; *P. nigra*: $\chi^2$ from 9.68 to 62.70; df = 3; *P* < 0.05).

The growth of the leader shoot differed among years (*P* < 0.0001 for the two pine species), ranging from 4.1±0.1 to 5.4±0.2 cm for *P. sylvestris* and 5.5±0.2 to 7.7±0.1 cm for *P. nigra*. However, there were no differences among treatments for each of the species in any year. The cumulative growth after four growing seasons (sum of 1997-2000 period) was 18.8±0.6 cm for *P. sylvestris* and 26.6±0.4 cm for *P. nigra* (all plots pooled; differences between pine species, *P* < 0.0001).
Herbivore damage was low in the two pine species. Risk of herbivory was 8.1% for *P. sylvestris* and 12.7% for *P. nigra* (all microhabitats pooled), with no differences among microhabitats (*P* > 0.05). Damage intensity was 16.8±4.2 for *P. sylvestris* (i.e. pines suffering herbivory lost 16.8% of their shoots) and 21.4±4.0 for *P. nigra*, with no significant differences among microhabitats for any pine species. When a pine was damaged, the leader shoot was consumed in 89.7% of the cases, and the rest of the damage was in lateral shoots.

**DISCUSSION**

The results show that shrubs did not reduce the survival of planted pines, but rather clearly enhanced seedling establishment. After four years, the survival for *P. sylvestris* and *P. nigra* under the canopy of Salvia was 2.6- and 1.8-fold higher, respectively, than the values reported for the traditional technique in areas of bare soil. This resulted despite that planting conditions were *a priori* more favorable for pines planted in the open areas (larger hole size and additional working around the seedling; see Castro et al. 2002). This facilitative effect of shrubs on woody plants seedling survival has been similarly reported for plantings or sowings in Mediterranean-type ecosystems, as for example Rousset & Lepart (2000) in southern France, or Callaway & D’Antonio (1991), Callaway (1992) and Dunne & Parker (1999) in California (and see also Maestre et al. [2001] for a facilitative effect of a tussock grass in semiarid SE Spain). Furthermore, this positive effect for reforestation has recently been summarized for SE Spain with a meta-analysis considering a wide range of nurse shrubs, target species, and local environmental conditions (Gómez et al. 2003b). In addition, similar facilitative effects of shrubs on seedling survival in woody plants have been reported for other ecosystems having a dry period, such as Chambers et al. (1999) in semiarid piñon and juniper woodlands of western North America, Li & Ma (2003) in mixed oak forests of northern China, or Rodriguez-Trejo et al. (2003) in pine forests of SE United States. Thus, the use of shrubs as nurse plants to boost reforestation success emerges as an alternative technique not only for Mediterranean type-ecosystems, but also for other water-stressed environments.
The first growing season was the most critical period for survival, accounting for more than 50% of the deaths. In addition, the pattern of survival across microhabitats was established mostly during the first growing season, with the rank order of among-microhabitat survival rates remaining similar after four years for the two pine species (Figs. 1 and 2). This supports the contention that the first year is the most critical period for the establishment of planted seedlings (García-Salmerón 1995; Savill et al. 1997; Rey-Benayas 1998), confirming the robustness of conclusions drawn from this four-years study. On the other hand, shrubs did not reduce the growth of the leader shoot in any of the study years. This supports the effectiveness of this reforestation technique, which enhances survival without hampering seedling performance.

The low ungulate damage during the study may have several explanations. First, these pine species have low palatability, and the patch is furthermore dominated by *Salvia lavandulifolia*, which is not attractive to ungulates (author’s unpublished data). Second, reforestation took place in a sector of the National Park free of domestic livestock (sheep and goats) and that in addition had a low population of the Spanish ibex during the study years. And third, damage intensity on juveniles augments with sapling size (Zamora et al. 2001), meaning that small size of these saplings might not attract the attention of ungulates. In any case, most of the herbivory was on the leader shoot, which considerably slows down pine growth (Hódar et al. 1998; Zamora et al. 2001). These species, particularly slow growing under the dry-summer Mediterranean conditions of the study area, require around 25 years for the leader shoot to grow beyond the reach of ungulates (ca. 150 cm; Zamora et al. 2001). Thus, even the low herbivore damage registered here may imply that around 60% of pines will undergo herbivory before reaching this height threshold (i.e. average of 10% pines browsed in 4 years x 6). In fact, in sectors of these mountains with higher wild and domestic ungulate pressure, most of the juvenile pines suffer herbivory (Zamora et al. 2001). Under such circumstances, tree seedlings surrounded by shrubs are protected from ungulates in the study area (García et al. 2000; Gómez et al. 2001a, 2003a; see also Callaway & D’Antonio [1991], Rousset & Lepart [2000] for other study systems). All this strongly reinforces the contention that nurse shrubs may protect reforested seedlings against ungulates, provided that seedlings are surrounded by the shrubs.
The beneficial effect of shrubs for seedling survival is presumably due to the improved water status of the seedlings through reduction of radiation, lowering soil temperature, and conserving soil moisture (Gómez et al. 2001b; Castro et al. 2002). This is further supported by the fact that survival on the south side of shrubs (where microclimatic conditions were close to Open microhabitat; Castro et al. 2002) was similar to the survival in the Open microhabitat, in contrast to the higher values for the north side of shrubs, supporting a direct effect of shading (Valiente-Banuet & Ezcurra 1991; Kitzberger et al. 2000; Rousset & Lepart 2000). Experimental field studies indicate that the shade provided by the canopy of shrubs or artificial meshes is the main factor favoring the survival of planted seedlings in the same study area (Gómez et al. 2001b; see also Callaway 1992; Rey-Benayas 1998; Dunne & Parker 1999; Weltzin & McPherson 1999; Shumway 2000; Rey-Benayas et al. 2002 for a similar canopy effect). Thus, despite the heliophilous nature of these pine species (Ceballos & Ruiz de la Torre 1971; Nikolov & Helmisaari 1992), the high radiation in Mediterranean environments can impair seedling performance, which may be improved by a moderate light reduction, lowering the risk of photoinhibition and leaf overheating that decreases growth or survival (Valladares 2003). All this suggests that shrubs such as *Salvia*, characterized by a shallow root system and a short, open canopy that moderately reduce radiation, may potentially provide the best functional traits to ensure reforestation success (see also Gómez et al. 2003b). The nurse plant will provide protection during the first years after planting, thereafter being outgrown by the sapling (Fig. 4). *Salvia* species as well as other shrubby species with similar morphological characteristics are common in degraded habitats of Mediterranean mountains, and thus the use of this reforestation technique could be extensively employed. In any case, we should consider that seedling survival after four years was still low in the case of *P. sylvestris* (e.g. roughly 28% in the Salvia microhabitat; Fig. 1). The technique could therefore be used in combination with additional reforestation techniques for species having low survival rates, in particular with procedures directed to reduce drought stress, such as application of water-retention gels, tree shelters, or digging holes of larger size.
The alternative technique of reforestation proposed here emerges from in situ studies of natural regeneration showing that the regeneration niche of woody species in the Mediterranean mountain is positively associated to shrubby species (Rousset & Lepart 1999; Castro 2000; Garcia et al. 2000; Gómez et al. 2001a, 2003a). Under the stressful conditions settled by summer drought and herbivore pressure, competition is compensated by protection against environmental stress, resulting in net facilitation (Bertness & Callaway 1994; Callaway 1995; Brooker & Callaghan 1998). This “facilitative principle” derived from ecological studies is therefore a necessary tool for the development of suitable silvicultural strategies for forest regeneration under Mediterranean scenarios. The technique mirrors therefore the natural, ecological succession, favoring the transition from pioneer shrublands towards mature forests. In addition, a spontaneous, natural shrub cover is already present in most degraded areas where reforestation is required, ensuring the applicability of the technique. All this translates as economic (Castro et al. 2002) and, particularly, ecological benefits, reducing the visual impact of the reforestation, helping to create spatially non-uniform and more diverse stands that are more resistant to pathogens, herbivores and climatic hazards (e.g. Watt 1992; Schönenberger 2001; Hódar et al. 2003). This approach also largely avoids the problems of erosion provoked by traditional, more aggressive techniques that eliminate the shrub cover using heavy machinery (García-Salmerón 1995; García-Latorre 1998; Linares et al. 2002; Pardini et al. 2003). These advantages are particularly relevant in protected areas where conservation and minimization of impact is a major priority, or in mountain areas with complex orography.

ACKNOWLEDGMENTS

We thank the Consejería de Medio Ambiente, Junta de Andalucía, for constant support and facilities. We are also especially grateful to the Direction of the National Park for permission to work in Sierra Nevada, and to Empresa de Transformación Agraria S.A. (TRAGSA) for carrying out the experimental reforestation. Daniel García kindly helped in the field work, and two anonymous reviewers provided helpful comments that improved the manuscript. David
Nesbitt provided linguistic advice. This study was supported by a PFPI-MEC grant to J.C. and projects FEDER 1FD97-0743-CO3-02 and REN2001-4552-E of the Spanish MCYT to R.Z.

**LITERATURE CITED**


Ceballos, L., and J. Ruiz de la Torre. 1971. Árboles y arbustos de la España peninsular. Instituto Forestal de Investigaciones y Experiencias, Madrid.


FIGURE CAPTIONS

Figure 1. Percentage of *Pinus sylvestris* seedling survival at different sampling periods for each plot and microhabitat. X-axis represent days from the start of the experiment (June 1997). S = sampling at the end of the summer period (S1 = summer 1997, S2 = summer 1998, S3 = summer 1999, S4 = summer 2000); W = sampling at the end of the winter period (W1 = winter 1997-98, W2 = winter 1998-99). Sampling of winter 1999-00 (W3) was not done.

Figure 2. Percentage of *Pinus nigra* seedling survival at different sampling periods for each plot and microhabitat. X-axis represent days from the start of the experiment (June 1997). S = sampling at the end of the summer period (S1 = summer 1997, S2 = summer 1998, S3 = summer 1999, S4 = summer 2000); W = sampling at the end of the winter period (W1 = winter 1997-98, W2 = winter 1998-99). Sampling of winter 1999-00 (W3) was not done.

Figure 3. A sapling of *Pinus nigra* outgrowing *Salvia lavandulifolia* four years after planting.
Figure 2

![Graph showing survival percentage over time for different treatments and plots. The graph includes data for Salvia, Shrub N, Shrub S, and Open conditions in Plots 1, 2, and 3. The x-axis represents time in days (0, 90, 365, 545, 730, 910, 1275) and the y-axis represents survival percentage (0 to 100%).]