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# Effect of substrate treatments on survival and growth of Mediterranean shrubs in a revegetated quarry: An eight-year study

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#### ABSTRACT

Loss of soil is a major problem that restoration actions must solve in Mediterranean quarries. Soil replacement by a low-quality substrate is often inadequate for revegetation and limits plant establishment and growth in these degraded areas, which are also subject to drought recurrent stress.

In 1998, a field experiment was performed in a limestone quarry at Outão (Serra da Arrábida, SW Portugal) to test different types of substrate improvement: NPK fertilizer, water-holding polymer, mycorrhizal inoculum, and combinations of these. Two-year old plants of three native woody species were planted – carob (*Ceratonia siliqua*), wild-olive tree (*Olea europaea var. sylvestris*) and mastic (*Pistacia lentiscus*). Reports concerning the short-term results showed some differential effects of the assayed treatments on plant growth and physiology. The monitoring program was maintained, and here we report on the survival and growth of the introduced plants over eight years after planting. This prolonged study showed that, with the limited exception of fertilization, none of the assayed treatments added major advantages for plant survival or growth. Regardless of the tested substrate treatment, mortality was low and these native species became established in the revegetated area.

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#### 1. Introduction

Restoration of degraded areas is increasingly demanded and requires adequate procedures. In severely degraded areas such as those resulting from extractive activities, soil loss is often the most difficult problem that restorers face (Heneghan et al., 2008). When the establishment of a plant community is the goal, a substitute substrate is required, but its quality is often quite low and limits the success of revegetation. Treatments with fertilizer, water-holding polymers or mycorrhizal inocula may seem promising procedures to improve plant performance in the field, especially when water and nutrient availabilities are low (e.g. Caravaca et al., 2003a,b; Hüttermann et al., 1999; Sarvaš et al., 2007).

With the increasing demand for restoration, practitioners are looking for the most cost-effective techniques. However, most current theories on plant–soil interactions focus on broad-scale generalizations or "average effects" which often fail to support sitespecific management decisions (Eviner and Hawkes, 2008). Even in similar environments, the responses of plant communities to the

\* Corresponding author. Tel.: +351 21750000x22556; fax: +351 217500048. *E-mail addresses*: g.oliveira@fc.ul.pt (G. Oliveira), alicenunes@yahoo.com same restoration practices may be quite different (García-Palacios et al., 2010). Existing applied ecological studies describe a relatively narrow range of situations (environmental conditions, management constraints, species, etc.), over limited periods. These serious drawbacks limit application of the results to practical restoration.

The scarcity of long-term monitoring of controlled assays also constrains the development and implementation of successful restoration practices, since results obtained from the first months or years after their implementation will not necessarily provide a good indication of longer-term responses (either direct or indirect) to the tested treatments (Cooke and Johnson, 2002; D'Antonio and Meyerson, 2002; Herrick et al., 2006). Moreover, long-term monitoring is particularly significant when slow-growing Mediterranean woody species are used in restoration programs.

Efforts to revegetate a Mediterranean limestone quarry at Outão (Serra da Arrábida, SW Portugal) started in 1983, with the primary goals of reducing the visual impact of bare rock and of landscape integration. To these ends, local shrub species and non-local tree species (*Pinus* spp.) were planted on the excavated platforms.

Low water and nutrient availabilities are the primary factors limiting plant development at this site. Experimental plots were set in 1998 to evaluate the performance of planted shrub species after the addition of a water-holding polymer, a fertilizer, and mycorrhizal inoculum to the substrate used in planting (Clemente et al.,

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2004; Werner et al., 2001). These practices had been proposed by commercial suppliers, and the quarry company was willing to test them and to implement the best one on a large-scale. Short-term results from the first two years following planting showed that the treatments including fertilizer or water-holding polymer had some positive effects on the plants (Clemente et al., 2004). Such effects would be expected to favor plant survival and growth on the long-term, as compared to shrubs which did not benefit from the substrate treatments in the early years of the experiment.

In order to evaluate the long-term consequences of the effects observed during the first two years after planting and/or to detect further responses to the substrate treatments, a monitoring program was maintained at Outão until 2006. Here we present these long-term results and examine the usefulness of the tested treatments for the restoration of this limestone quarry.

#### 2. Materials and methods

#### 2.1. Experimental site and design

The study was performed at the Secil-Outão limestone quarry (Arrábida Natural Park, southwest Portugal; 42°57-59'N, 4°98′–5°02′W). A randomized factorial experimental design was deployed, as described by Clemente et al. (2004). A total of six treatments were established: G (gel – water-holding polymer). M (mycorrhiza), GM (gel and mycorrhiza), F (fertilizer), GF (gel and fertilizer), and C (control - no substrate improvement). Two yearold plants of three native late-successional shrub species, grown in a local nursery, were planted: Ceratonia siliqua L. (carob), Olea europaea L. var. sylvestris (Miller) Lehr (wild-olive tree), and Pistacia lentiscus L. (mastic tree). Three blocks of 18 plots each were sequentially installed along a terrace. Each treatment × species combination was randomly assigned to each plot  $(3 \text{ m} \times 5 \text{ m})$ , and six plants per species were planted in each plot  $(3 \times 6 \text{ plants per})$ treatment). Before planting, a marl layer about 1 m deep was added to the bare rock. In March 1998, plants were installed in holes c. 50 cm deep filled with nursery substrate and marl. Some characteristics of these substrates are shown in Table 1, and comparison with natural, undisturbed surrounding soil indicates that they had lower contents of organic matter and essential elements. Marl material had been disturbed, stockpiled, and leached for several years, thus seriously reducing arbuscular mycorrhiza hyphae and spores, and hindering natural mycorrhizal colonization (Correia et al., 2004). Plants in the nursery were already mycorrhized, but transfer to the field, with drastic changes in substrate characteristics, was

#### Table 1

Some characteristics of the substrates used for planting in 1998, and of natural soil (*terra rossa*) surrounding the quarry. Values are means  $\pm$  std of values obtained from composite samples (n = 5, plantation substrate and natural soil; n = 3, nursery substrate).

	Substrate		Natural soil <sup>a</sup>
	Plantation	Nursery	
Texture	Clay	Sand	Clay-loam
рН	$8.7 \pm 0.1$	$6.3\pm0.1$	$7.9 \pm 0.1$
Organic matter (%)	$0.54 \pm 0.08$	$\textbf{0.83} \pm \textbf{0.16}$	$7.97 \pm 0.72$
Total N (%)	$0.02\pm0.01$	$0.05\pm0.01$	$0.43\pm0.20$
Extractable P <sub>2</sub> O <sub>5</sub> (mg/kg)	Traces	Traces	$6.2\pm13.9$
Extractable Mg (mg/kg)	$218\pm97$	$681 \pm 159$	$630\pm712$
Extractable K <sub>2</sub> O (mg/kg)	$123\pm18$	$27\pm2$	$351\pm80$
AMF spores (number/g dw substrate)	0.34 <sup>b</sup>	0.67 <sup>b</sup>	c. 0.95 <sup>c</sup>

AMF, arbuscular mycorrizal fungi.

<sup>a</sup> From Correia et al. (2001).

<sup>b</sup> P. Correia (personal communication).

<sup>c</sup> From Correia (2006).

#### Table 2

Cumulative precipitation and mean air temperatures (maximum and minimum) during the monitoring period (1998–2006). Values are means from data recorded by two local weather stations (Secil-Outão). Air temperature data before 2000 are not available (na).

Year	Precipitation (mm)	$T_{\max}$ (°C)	$T_{\min}$ (°C)
1998	413	na	na
1999	847	na	na
2000	915	21.6	11.4
2001	1017	21.1	11.8
2002	836	20.9	12.1
2003	1132	20.9	12.5
2004	638	21.5	12.5
2005	620	21.9	12.2
2006	1079	21.8	12.3

expected to reduce the infectiveness and survival of these mycorrhizal fungi and thus compromise their symbiotic efficiency.

Treatments were applied to the substrate in the holes, according to the experimental design: (i) 100 g dry gel/plant (long-term water-holding polymer: hydrogel, STOCKOSORB 400K, Stockhausen, Krefeld, Germany), (ii) 120 g of a slow-release NPK fertilizer (10:10:10), or (iii) 85 g of Terra-Sorb/plant (mixture of endo- and ectomycorrhizal inoculum and gel: Mycor-Tree, Plant Health Care, Pittsburgh, PA, USA; selection of strains took climate and soil pH and type into account). Watering was applied on the day of planting and one week later (roughly 1 L/m<sup>2</sup>). In April 1998 plants which had died as a result of transplantation shock were replaced.

#### 2.2. Climate and plant monitoring

Meteorological data covering the study period (Table 2) were obtained from two meteorological stations located within the quarry area.

In March 2003, July 2004 and February and December 2006 the plants were assessed to evaluate survival and growth. During the first years of monitoring, plant growth was measured as the increment of the main shoot and diameter at the stem base (marked in 1998, Clemente et al., 2004). However, due to loss or deterioration of materials used in 1998 to mark stems, together with increased branching of trunk bases, these measurements were ceased in 2004. Plant height monitoring began in 2003, to estimate crown growth and replace the previous measurement of leading shoots which was no longer indicative.

#### 2.3. Statistical analyses

Data were log or squared transformed to meet the assumptions of normality and homoscedasticity. Tables and figures present untransformed data. Effects of treatments on survival rates, proportion of plants with positive height increments, and total increment of basal area were tested within species by analysis of variance (one-way ANOVA). Variation of plant height over the studied period was analyzed with repeated measures analysis of variance (ANOVAr). The Dunnett test was used to compare each treatment (*i.e.* G, gel; M, mycorrhiza; GM, gel and mycorrhiza; F, fertilizer; GF, gel and fertilizer) with the control. All statistical analyses were conducted using a critical *p*-value  $\leq$ 0.05 and were performed using the statistical software package (STATISTICA 7.0, StatSoft, Inc., 2004).

#### 3. Results

Reflecting its Mediterranean characteristics, the climate showed marked inter-annual variability in the distribution and amount of rainfall over the study period. The year of planting (1998) was the driest, but the following five years were relatively wet (Table 2).

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