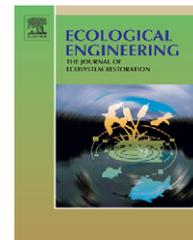




ELSEVIER

available at [www.sciencedirect.com](http://www.sciencedirect.com)journal homepage: [www.elsevier.com/locate/ecoleng](http://www.elsevier.com/locate/ecoleng)

## Improving revegetation of gypsum slopes is not a simple matter of adding native species: Insights from a multispecies experiment

Silvia Matesanz<sup>a,\*</sup>, Fernando Valladares<sup>a,b</sup>

<sup>a</sup> Instituto de Recursos Naturales, C.C.M.A.-C.S.I.C., Calle Serrano, 115 dpdo, 28006 Madrid, Spain

<sup>b</sup> Unidad de Biodiversidad y Conservación, Escuela Superior de Ciencias Experimentales y Tecnológicas, Universidad Rey Juan Carlos, c/ Tulipán s/n, 28933 Móstoles, Spain

### ARTICLE INFO

#### Article history:

Received 29 May 2006

Received in revised form

15 November 2006

Accepted 25 January 2007

#### Keywords:

Multispecies experiments

Gypsum motorway slopes

Hydroseeding mixture

Competition

Survival

Cover

### ABSTRACT

A common practice in the revegetation of motorway slopes is to hydroseed broad-purpose, fast-growing, usually exotic species, without particular attention to soil, climate and general features of each site. The importance of using native species is becoming widely acknowledged and restoration projects are gradually considering native species for the hydroseeding mixture, particularly under adverse climatic and soil conditions. However, the selection of species may not take into account the competitive interactions among commercial and native species, which can dramatically affect the outcome of the hydroseeding. We carried out a multispecies controlled experiment simulating eight different communities with species typically used in the revegetation of gypsum motorway slopes in Mediterranean Spain. The effect of the presence, relative density and emergence time of *Lolium rigidum*, a fast-growing and highly competitive introduced grass, on the growth and cover of each community and on the performance of six individual gypsum species was assessed. Survival and performance of the gypsum species was always hindered by *L. rigidum*. Mean height of the gypsum species was maximal at the combinations without *L. rigidum* and the same was true for aboveground biomass. Same kind of significant effect, although reduced in extent, was obtained when *L. rigidum* was sown 1 month after the emergence of the gypsum species. On the contrary, mean height, aboveground biomass, root biomass, and cover of the whole community (gypsum species + *L. rigidum*) was higher at the combinations with more individuals of *L. rigidum*, due to the fast growth of the latter. Our results showed that fast-growing commercial species outcompeted slow-growing gypsum species even on real gypsum soils and even if the community gets started with less individuals of commercial than of gypsum species, or if the former is sown 1 month after the germination of the latter. These results suggest that the inclusion of native species in the hydroseeding mixture may not improve the revegetation of gypsum slopes in Mediterranean conditions if used in combination with commercial, fast growing species, which can quickly cover the ground during the spring but are not likely to survive over the summer drought. Further studies should focus on the suitability of using herbaceous species tolerant of gypsum soils, as their growth rate is likely to be higher and could be used together or even instead commercial species.

© 2007 Elsevier B.V. All rights reserved.

\* Corresponding author. Tel.: +34 917452500x1204; fax: +34 915640800.

E-mail address: [silvia@ccma.csic.es](mailto:silvia@ccma.csic.es) (S. Matesanz).

0925-8574/\$ – see front matter © 2007 Elsevier B.V. All rights reserved.

doi:10.1016/j.ecoleng.2007.01.005

## 1. Introduction

The construction of linear infrastructures such as motorways leads to the creation of bare and steep surfaces in which vegetation establishment is of crucial importance to provide stabilization or reduce erosion (Andrés and Jorba, 2000; Tormo et al., 2006). Although the assessment of the success of restoration is of central importance in restoration projects (Lane and Lejeune, 2005; Martin et al., 2005; Ruiz-Jaen and Aide, 2005a), the criteria to fix the goals and success of the restoration of motorway slopes are not clearly defined, and are usually limited to the establishment of an unspecific plant cover by means of hydroseeding (Andrés and Jorba, 2000; Matesanz et al., 2006). However, some attempts have been carried out to further our understanding of the type of community that gets established in the slopes, and thus to increase our capacity to improve their ecological restoration (Matesanz et al., 2006). Some studies have emphasized the advantages of using native species in the revegetation of motorway slopes (HarperLore, 1996; Brindle, 2003; Petersen et al., 2004), not only because of the concern regarding the introduction of exotic genotypes (Brown and Rice, 2000; Tinsley et al., 2006; Andel, 2006), but also to avoid possible failures of commercial species due to adverse local conditions, mainly climate and soil properties (Bochet and García-Fayos, 2004). The latter fact is particularly important in the revegetation of motorway slopes on gypsum soils. The existence of a specialized flora in gypsum soils is well known (Escudero et al., 1996; Mota et al., 2003), and these abiotic filter is exacerbated by aridity, limiting the establishment of many plant species (Meyer and García-Moya, 1989).

Therefore, the selection of species for the hydroseeding in areas of gypsum soils under Mediterranean conditions is essential to ensure the development of a durable plant cover of the slopes. However, most restoration projects do not include a formal *a priori* study and selection of the species to be used in each area. Although the use of small percentages of native species in the hydroseeding has recently increased (Paschke et al., 2000; Tormo et al., 2006; Tinsley et al., 2006), the mixtures are still mainly composed by a blend of herbaceous legumes and grasses, most of them highly competitive species (Picon-Cochard et al., 2001; San Emeterio et al., 2004). In addition, the criteria to select the species usually include economic and esthetic, but not ecological factors, and restoration projects may not specify the type of native species to be used in the revegetation. The competitive interactions between native and commercial species remain highly unknown (Navas and Moreau-Richard, 2005), despite the fact that they can significantly influence the outcome of the hydroseeding. This lack of precise guidelines in the selection of the species for the hydroseeding leads to the combined use of fast-growing commercial species with native species, frequently slow-growing camephytes or shrubs, as if the positive features of each group of species (fast growth of the former, high stress tolerance of the latter) were additive properties that are directly transferred to the hydroseeding. While the use of native species can render poor results – lower survival, cover and growth – due to competition with the commercial species, species from gypsum soils are better adapted to cope with gypsum soils

and could outcompete commercial species in this kind of substrate. Thus, the net outcome of the interaction between these two groups of species is particularly uncertain under adverse climate and soil conditions. Knowledge on how plants from different seed mixtures establish and develop under controlled conditions may be very helpful to further our understanding of hydroseeding success and improve the design of effective hydroseeding mixtures for gypsum slopes.

The general objective of the current experiment was to address the influence of species composition, density, relative abundance and timing of emergence of each species separately on the overall performance of the community that gets initially established after hydroseeding with different seed mixtures for gypsum soils. These factors have been recognized as highly important ones affecting the success of revegetation and restoration activities (Ruiz-Jaén and Aide, 2005b). Among the potential set of species, we selected *Lolium rigidum* Gaudin – a widely used, broad purpose, fast-growing commercial grass – as the introduced species, and six species restricted to or tolerant of gypsum as the native species (hereafter gypsum species): *Colutea arborescens* L., *Helianthemum squamatum* (L.) Pers., *Lepidium subulatum* L., *Gypsophila struthium* Loefl., *Thymus zygis* Loefl. ex. L and *Launaea resedifolia* (L.) O. Kuntze, which are beginning to be used in the hydroseeding of certain gypsum slopes in Spain.

Specific questions addressed in the experiment were: (i) are there significant differences in the outcome of the different reared communities in terms of cover, height or biomass? and (ii) what is the overall effect of the commercial species on the performance (i.e. survival, growth, biomass) of each individual gypsum species?

## 2. Methods

### 2.1. Conditions of the greenhouse and species

The experiment was conducted from March 2004 to August 2004 in a greenhouse in Madrid (Spain). Air temperature was measured throughout the experiment with a data logger (HOBO model H08-006-04, Onset, Pocasset, MA, USA). Mean temperature from March to July in the greenhouse was  $20.3 \pm 0.15^\circ\text{C}$ , and it ranged from 1 to  $45.7^\circ\text{C}$ . The pots were  $13\text{ cm} \times 13\text{ cm} \times 25\text{ cm}$  (31 capacity) and were well watered throughout the experiment. Soil substrate was collected in different gypsum slopes in the M50 motorway (Madrid, Spain) just after their construction, in order to recreate in the pots the soil conditions that the species from the hydroseeding mixtures would have in the slopes. Due to the recent construction of the road, the substrate in the slopes was mainly regolith, including gypsum gravels. To avoid germination of seeds existing in the substrate, the upper layer of the substrate of each pot (5 cm approximately) was sieved (5 mm pore diameter) and sterilized in an autoclave ( $121^\circ\text{C}$ ) two consecutive times during 30 min. The percentage of soil volumetric water content was regularly measured with a Soil Mixture Sensor (ThetaProbe, Delta-T Devices, Cambridge, United Kingdom) and was  $16.0 \pm 0.3\%$  during the experiment, ranging from 12.1% to 21.3%.

**Table 1 – Species composition and number of individuals of the different experimental mixtures for gypsum slopes**

	Number of individuals							Total
	<i>Colutea arborescens</i>	<i>Gypsophila struthium</i>	<i>Lepidium subulatum</i>	<i>Helianthemum squamatum</i>	<i>Thymus zygis</i>	<i>Launea resedifolia</i>	<i>Lolium rigidum</i>	
Mixtures								
A	6	6	6	6	6	6	–	36
B	18	18	–	–	–	–	–	36
C	6	6	6	6	6	–	6	36
D	4	4	4	4	4	–	18	38
E	1	1	1	1	1	–	31	36
F	4	4	4	4	4	–	18	38
G	4	4	4	4	4	–	18	38
H	18	–	–	–	–	–	18	36

Mixtures D, F and G are identical in composition but differed in the emergence time of *L. rigidum*.

The species used in the experiment representing native species were collected by a road construction company in order to be included in the hydroseeding mixture of the gypsum slopes in the M-50 motorway, in the section linking A3 and A4 motorways, in Southeast Madrid (590 m a.s.l.; 40°20'31.51"N, 3°35'43.63"W). The climate is Mediterranean, with average annual rainfall of 425 mm. The species used were: *Colutea arborescens* L. (Leguminosae), *H. squamatum* (L.) Pers (Cistaceae), *Lepidium subulatum* L (Cruciferae), *Gypsophila struthium* Loefl. (Caryophyllaceae), *T. zygis* Loefl. ex. L (Labiatae) and *Launaea resedifolia* (L.) O. Kuntze (Compositae). These species are small camephytes and also gypsophytes (plants that are restricted to gypsum soils), except *C. arborescens*, and all are typical from the gypsum outcrops of the Iberian Peninsula (Rivas-Martínez and Costa-Tenorio, 1970). Seeds of all the species were collected in 2001 in gypsum patches near Aranjuez (South of Madrid Autonomous Region, 40°01'49.03"N, 3°36'37.11"W). Seeds from the selected commercial species, *L. rigidum* Gaudin (annual grass, Poaceae), were provided by Intersemillas S.A. (Valencia, Spain).

A germination test was performed in order to ensure sufficient amount of seedlings. A pretreatment was carried out for the species with low germination rate (<50%) in the germination test (*C. arborescens*). For this species, an immersion in sulfuric acid (96 vol.%) for 10 min was undertaken to improve the germination rate. Seeds were sown on 15 March. When the pot reached the number of seedling set for each species, new emerging plants were immediately removed from the pot. On 1 April, all the pots had the corresponding number of seedlings to each experiment (Table 1).

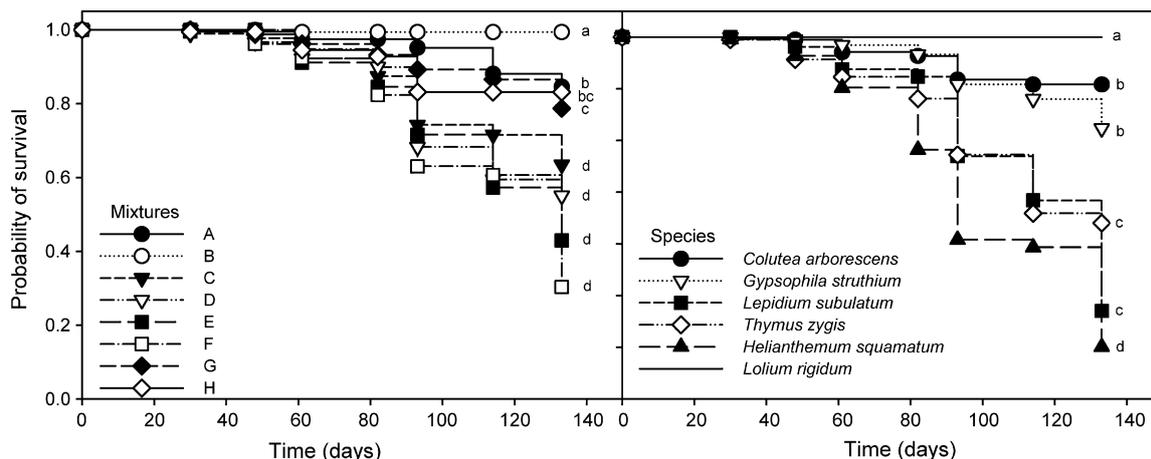
### 2.2. Experimental design

We reared eight different communities differing in the diversity, relative abundance and emergence time of both the gypsum species and *L. rigidum*. All the communities had approximately the same number of individuals (36–38 plants), to allow direct comparisons between mixtures (Table 1). All the mixtures were replicated in 10 randomly assigned pots. Thus, the different mixtures were established to tackle the following four specific questions:

- (1) To assess the effect of the presence of *L. rigidum*, mixtures A (no *L. rigidum*) and C (with *L. rigidum*) were established. Both mixtures had the same number of individuals (six) of each species (six), but differed in the presence of *L. rigidum*, that was substituted by *L. resedifolia* in mixture A (Table 1).
- (2) To assess the effect of the relative density of *L. rigidum*, mixtures C, D and E were established. The three mixtures had the same species composition, but in different proportions: mixture C, six individuals of *L. rigidum* plus six individuals of each gypsum species, mixture D, 18 *L. rigidum* plus four individuals of the gypsum species, and E, 31 *L. rigidum* plus one individual of the gypsum species (Table 1).
- (3) To assess the effect of the emergence time of *L. rigidum*, we established mixtures D, F and G. The three mixtures had the same species composition and proportions of each species, but differed in the emergence time of *L. rigidum*. In mixture D, *L. rigidum* and the gypsum species were simultaneously sowed. In mixture F, the gypsum species were sowed 1 week after sowing *L. rigidum*, as the last species has very quick growth. In mixture G, *L. rigidum* was sowed 1 month after the germination of the target gypsum species, as the growth of these species is slow (Table 1).
- (4) To assess the effect of *L. rigidum* on the performance of the target species *C. arborescens*, mixtures B and H were established. *C. arborescens* was chosen because it is a gypsum-tolerant species able to grow in a relatively wide range of soils and its potential size ( $\geq 1$  m high) makes it very valuable for revegetation projects. Both mixtures had the same diversity (18 individuals of each of the two species in the mixture), but in mixture B, *L. rigidum* was substituted with *G. struthium* (Table 1).

### 2.3. Measurements and data analysis

We carried out a total of seven mortality censuses during the experiment. The censuses were performed on 1 May (day 30), 18 May (day 48), 1 June (day 61), 21 June (day 82), 2 July (day 93), 23 July (day 114) and 11 August (day 133), in all the individuals of all the pots. Together with these censuses, seedling height (length of the stem) was measured in three individuals



**Fig. 1** – Cumulative survival probability of all the gypsum species through time for different mixtures (left, mean across gypsum species) and for each individual species (gypsum species + *Lolium rigidum*, mean across treatments, right). Analysis performed with Kaplan–Meier product-limit. Different letters after each line indicate significantly different groups (Cox–Mantel test). See Table 1 and text for composition of each mixture.

randomly selected of each of the gypsum species (except in mixture E, where there was only one individual of each species, and mixtures B and H, where six individuals were measured), and in six individuals of *L. rigidum*. In addition, cover of the pot was visually estimated to fall in one of four classes—1: 1–25%, 2: 26–50%, 3: 51–75% and 4: 76–100%. This estimation was done always by the same observer. Finally, on days 61 and 93, all the individuals of each species were collected from three and four pots of each different mixture, respectively. This was done by clipping all the seedlings and drying the samples at least for 48 h in the oven at 65 °C. On day 93, total root biomass was also measured in four pots of each mixture. In mixtures B and H, each individual of *C. arborescens* root system was collected to test the effect of *L. rigidum* on the gypsum species biomass.

Kaplan–Meier product-limit method was used to estimate the survival function of every species and mixture. Cox–Mantel test was used to test for differences in survival functions among species and among mixtures. To test the effect of the presence, density and emergence time of *L. rigidum* on the mean height of the gypsum species and on total mean height, we performed a nested one-way ANOVA in every date, with mixture as the factor, and pot as the nested effect (within mixture). Tukey’s HSD post hoc test was used to detect differences between groups. To test the effect of the presence, density and emergence time of *L. rigidum* on the aboveground biomass of the gypsum species, the total aboveground and root biomass, we performed one-way ANOVA, with mixture as factor, and Tukey’s HSD as post hoc test. To test the effect of the presence, density and emergence time of *L. rigidum* in the cover of the pots, we transformed each cover class to its midpoint and performed a Kruskal–Wallis one-way analysis of the variance on ranks, and Dunn’s test as post hoc test. Results are expressed as mean ± S.E. throughout the paper, and the level for statistical significance was set at  $p \leq 0.05$ . All the analyses were performed with STATISTICA 6.0 (Statsoft Inc., Tulsa, USA).

### 3. Results

#### 3.1. Gypsum species

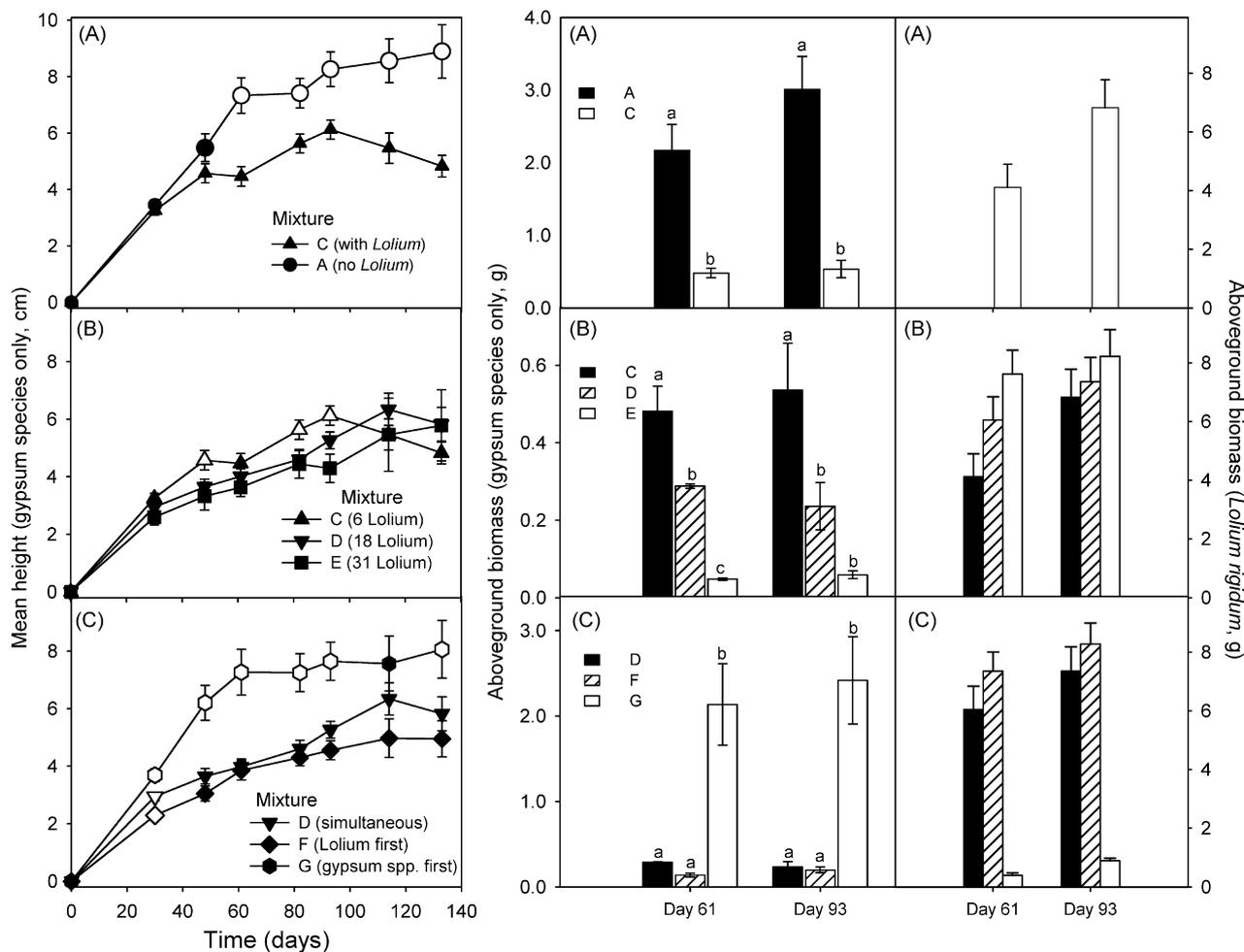
Both species and mixtures differed significantly in survival (Fig. 1). The highest survival rates were found in the individuals of the mixtures where *L. rigidum* was not present. Mixture B (*C. arborescens* + *G. struthium*) yielded the highest survival rate of all the mixtures, followed by mixture A (six individuals of each gypsum species). The mixtures with 6, 18 and 31 individuals of *L. rigidum* (C, D and E) and the mixture where this species emerged first (F) yielded the lowest survival rates. Mixtures H (*L. rigidum* + *C. arborescens*) and G (gypsum species emerged first) had intermediate survival rates. Differences were also found in species surviving rates. While *C. arborescens* and *G. struthium* had the highest surviving rates, *Lepidium subulatum* and *Helianthemum squamatum* had the lowest. *T. zygis* had intermediate surviving rate (Fig. 1).

The mean height of gypsum species was significantly lower when *L. rigidum* was present in the mixture (Fig. 2A left, Table 2). No significant differences were found in the mean height of the gypsum species in relation to the density of *L. rigidum* present in the pot (6, 18 or 31 individuals). Only in the 2nd, 4th and 5th censuses, the mean height of gypsum species in mixture C (six individuals of *L. rigidum*) was significantly higher than in mixture D and E (Fig. 2B left, Table 2). Also, emergence time had a significant effect on the mean height of the gypsum species. Plants in mixture G (emergence of gypsum species first) had significantly higher height during all the censuses of the experiment but the 6th (Fig. 2C left, Table 2). In all the comparisons, the nested effect (pot within mixture) had no significant effect on the height of the plants (Table 2). Aboveground biomass of the gypsum species during the experiment was significantly lower in mixtures containing *L. rigidum*, and this was more important at increasing *L. rigidum* densities (Fig. 2A and B (centre), Table 2).

**Table 2 – ANOVA results testing for the effect of the presence, density and time of emergence of *L. rigidum* on the mean height of gypsum species, total mean height, aboveground biomass of gypsum species, total aboveground biomass and total root biomass**

Variable	Date	Presence of <i>L. rigidum</i> (mixtures A-C)				Density of <i>L. rigidum</i> (mixtures C-D-E)				Emerging time of <i>L. rigidum</i> (mixtures D-F-G)			
		Predictor	d.f.	F	p	Predictor	d.f.	F	p	Predictor	d.f.	F	p
Mean height of gypsum species	Day 30	Mixture	1	0.36	0.54	Mixture	2	2.80	0.06	Mixture	2	15.44	<0.0001
		Pot (mixture)	18	2.16	0.23	Pot (mixture)	27	1.27	0.17	Pot (mixture)	27	0.74	0.82
	Day 48	Mixture	1	2.20	0.14	Mixture	2	3.54	<b>0.03</b>	Mixture	2	15.57	<0.0001
		Pot (mixture)	18	1.02	0.41	Pot (mixture)	27	0.65	0.72	Pot (mixture)	27	0.38	0.93
	Day 61	Mixture	1	16.35	<b>0.0001</b>	Mixture	2	1.099	0.33	Mixture	2	13.24	<0.0001
		Pot (mixture)	18	0.75	0.60	Pot (mixture)	27	0.95	0.48	Pot (mixture)	27	0.32	0.95
	Day 82	Mixture	1	6.48	<b>0.01</b>	Mixture	2	4.52	<b>0.01</b>	Mixture	2	12.64	<0.0001
		Pot (mixture)	12	1.56	0.14	Pot (mixture)	18	1.78	0.07	Pot (mixture)	18	0.27	0.99
	Day 93	Mixture	1	7.02	<b>0.009</b>	Mixture	2	5.52	<b>0.005</b>	Mixture	2	9.75	<0.0001
		Pot (mixture)	12	0.92	0.49	Pot (mixture)	18	1.79	0.07	Pot (mixture)	18	0.44	0.95
	Day 114	Mixture	1	6.39	<b>0.014</b>	Mixture	2	3.6	<b>0.02</b>	Mixture	2	1.55	0.22
		Pot (mixture)	4	2.59	0.09	Pot (mixture)	6	0.98	0.38	Pot (mixture)	6	1.35	0.27
	Day 133	Mixture	1	16.76	<b>0.0001</b>	Mixture	2	1.17	0.31	Mixture	2	3.20	<b>0.04</b>
		Pot (mixture)	4	2.35	0.10	Pot (mixture)	6	0.82	0.48	Pot (mixture)	6	0.81	0.50
Total mean height	Day 30	Mixture	1	50.40	< <b>0.0001</b>	Mixture	2	2.85	0.06	Mixture	2	16.12	< <b>0.0001</b>
		Pot (mixture)	18	0.54	0.93	Pot (mixture)	27	0.68	0.90	Pot (mixture)	27	0.17	1
	Day 48	Mixture	1	14.87	<b>0.0002</b>	Mixture	2	0.22	0.80	Mixture	2	0.70	0.50
		Pot (mixture)	18	0.37	0.89	Pot (mixture)	27	0.80	0.60	Pot (mixture)	27	1.77	0.06
	Day 61	Mixture	1	1.47	0.22	Mixture	2	3.46	<b>0.03</b>	Mixture	2	0.10	0.90
		Pot (mixture)	18	1.99	0.09	Pot (mixture)	27	1.27	0.24	Pot (mixture)	27	0.64	0.74
	Day 82	Mixture	1	7.06	<b>0.008</b>	Mixture	2	5.08	<b>0.01</b>	Mixture	2	2.36	0.09
		Pot (mixture)	12	0.89	0.52	Pot (mixture)	18	1.04	0.40	Pot (mixture)	18	0.44	0.94
	Day 93	Mixture	1	2.77	0.09	Mixture	2	0.32	0.72	Mixture	2	0.41	0.46
		Pot (mixture)	12	1.09	0.39	Pot (mixture)	18	1.70	0.06	Pot (mixture)	18	1.08	0.38
	Day 114	Mixture	1	0.72	0.40	Mixture	2	1.35	0.26	Mixture	2	0.94	0.40
		Pot (mixture)	4	2.08	0.13	Pot (mixture)	6	1.73	0.16	Pot (mixture)	6	1.33	0.26
	Day 133	Mixture	1	0.008	0.99	Mixture	2	3.93	<b>0.02</b>	Mixture	2	1.37	0.25
		Pot (mixture)	4	1.20	0.31	Pot (mixture)	6	0.64	0.59	Pot (mixture)	6	2.57	0.06
Aboveground biomass of gypsum species	Day 61	Mixture	1	22.16	<b>0.009</b>	Mixture	2	34.19	<b>0.001</b>	Mixture	2	16.28	<b>0.004</b>
	Day 93	Mixture	1	27.87	<b>0.002</b>	Mixture	2	9.38	<b>0.006</b>	Mixture	2	18.19	<b>0.001</b>
Total aboveground biomass	Day 61	Mixture	1	7.25	<b>0.04</b>	Mixture	2	34.19	<b>0.001</b>	Mixture	2	16.28	<b>0.004</b>
	Day 93	Mixture	1	18.01	<b>0.005</b>	Mixture	2	5.851	<b>0.039</b>	Mixture	2	16.77	<b>0.001</b>
Total root biomass	Day 93	Mixture	1	21.38	<b>0.004</b>	Mixture	2	4.096	<b>0.04</b>	Mixture	2	7.07	<b>0.015</b>

Bold values indicate significant results ( $p \leq 0.05$ ).



**Fig. 2** – Effect of the presence (panels A, mixtures A–C), density (panels B, mixtures C–D–E), and emergence time of *L. rigidum* (panels C, mixtures D–F–G) on mean height and biomass of the five gypsum species. (Left) Mean height (mean  $\pm$  1S.E.) of gypsum species through time in different mixtures. Different colours of the symbols within the same date indicate significant differences between mixtures (nested ANOVA and Tukey's HSD). (Centre) Aboveground biomass (mean  $\pm$  1S.E.) of gypsum species in days 61 and 93 of the experiment. Different letters above bars indicate significant differences among mixtures (one-way ANOVA). (Right) Aboveground biomass of *L. rigidum*.

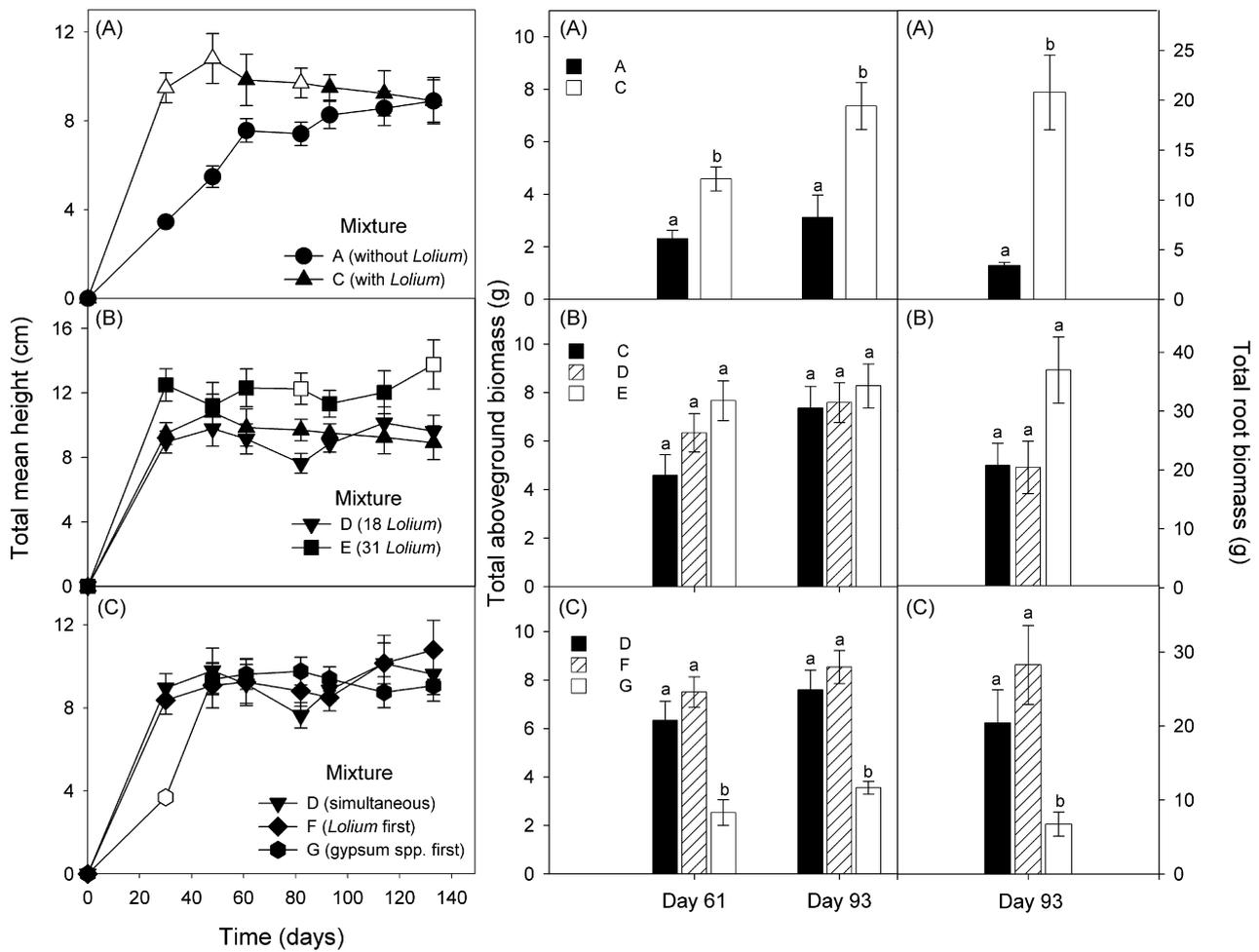
Also, the emergence time had a significant effect: aboveground biomass of gypsum species was higher in both dates when they emerged first (Fig. 2C (centre), Table 2). Aboveground biomass of *L. rigidum* varied across mixtures (Fig. 2 (right)).

### 3.2. Overall community

No significant differences were found in the total mean height of the species in relation to the presence of *L. rigidum*. Only in censuses 2, 3 and 5, total height was significantly higher in mixture C (with *Lolium*) than in mixture A (no *Lolium*, Fig. 3A (left), Table 2). Also, the density of *L. rigidum* did not vary significantly the total height of the species, except in the 4th and last censuses (Fig. 3B (left), Table 2). Emergence time had a significant effect on total height only in the first census (Fig. 3C (left), Table 2). Total aboveground biomass and total root biomass were significantly higher in mixture C (with *L. rigidum*) than in mixture A (no *L. rigidum*, Fig. 3A (centre and right), Table 2).

The density of *L. rigidum* had no significant effect on the total aboveground biomass of the plants in mixtures C, D and E (Fig. 3B (centre), Table 2). However, total root biomass was significantly higher in mixture E (more individuals of *L. rigidum*) than in the mixtures with less individuals of *L. rigidum* (Fig. 3B (right), Table 2). Also, mixture G had significantly less aboveground and root biomass than mixtures D and F (Fig. 3C (centre and right), Table 2).

The presence of *L. rigidum* had a significant and positive effect on the cover of the pot only at the beginning of the experiment (Kruskal–Wallis,  $H=9.42$ ,  $p=0.0021$ ). In the rest of the censuses, there were no significant differences in the cover of mixtures A and C (Fig. 4 (top)). The same was true for the cover of mixtures C, D and E: there were no significant differences among the mixtures throughout the experiment (Fig. 4 (middle)). However, emergence time had a significant effect in the cover of the pots: mixture G had significantly lower cover than mixtures D and F (Fig. 4 (bottom)).

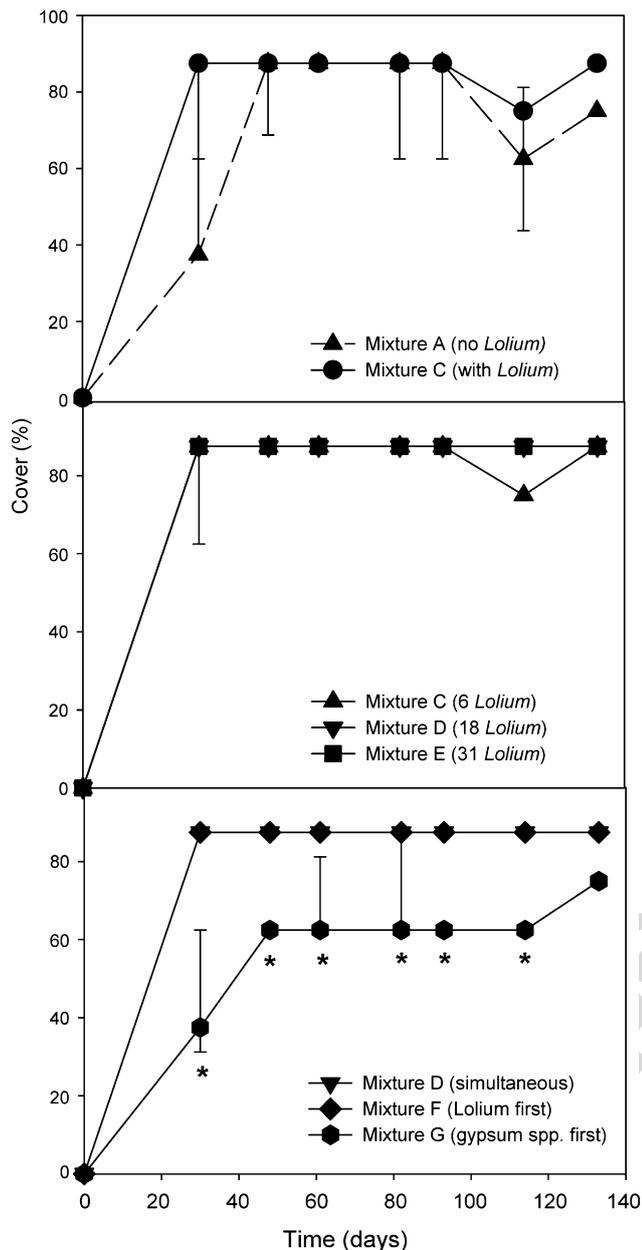


**Fig. 3 – Effect of the presence (panels A, mixtures A–C, top), density (panels B, mixtures C–D–E, middle), and emergence time (panels C, mixtures D–F–G, bottom) of *L. rigidum* on total mean height and total biomass. (Left) Total mean height (gypsum species + *L. rigidum*). Different colours of the symbols within the same date indicate significant differences between mixtures. (Centre) Total aboveground biomass. (Right) Total root biomass. Different letters above bars indicate significant differences between mixtures.**

No consistent differences were found in the mean height of the target species *C. arborescens* between mixtures B (*C. arborescens* + *G. struthium*) and H (*C. arborescens* + *L. rigidum*), although in the last census mean height of this species was significantly higher in mixture B than in mixture H (Fig. 5 (left)). However, plants growing in mixture H had significantly higher mean height than those in mixture B during all the censuses but in the last one (Fig. 5 (right)). In all the comparisons, the nested effect (pot within mixture) had no significant effect on the height of the plants. Aboveground biomass of *C. arborescens* was significantly higher in mixture B than in mixture H in both days 61 and 93 (Fig. 5 (left)). The same was true for the root biomass of *C. arborescens* between the two mixtures on both dates, being significantly higher in mixture B (Fig. 5 (left)). However, total aboveground biomass was significantly higher in mixture H than in mixture B on both dates, and the same was true for total root biomass, which was significantly higher in mixture H (Fig. 5).

#### 4. Discussion

In general terms, our results revealed that the selection of the species to use in gypsum motorway slopes is crucial for the outcome of the revegetation. The highly competitive and fast-growing species *L. rigidum* did not benefit the establishment and development of the gypsum species. In fact, the mixtures that yielded lower survival rates of the gypsum species were those containing *L. rigidum*. Also, the presence of *L. rigidum* reduced both the mean height and the aboveground biomass of the gypsum species. This agrees with other studies suggesting the potential invasiveness and of this species (González Ponce, 1998; San Emeterio et al., 2004). Increasing densities of *L. rigidum* did not significantly influence either the survival or the mean height of the gypsum species, which suggests that even small percentages of this species in the communities can have strong and negative effects on the growth of the other species, particularly in plant communities of highly



**Fig. 4 – Time evolution of the cover (median, 25% and 75% percentile) of the pots with different mixtures (Kruskal-Wallis and Dunn's test). Effect of the presence (mixtures A–C, top), effect of density (mixtures C–D–E, middle), and effect of emergence time of *L. rigidum* (mixtures D–F–G, bottom). Asterisk indicates significant differences.**

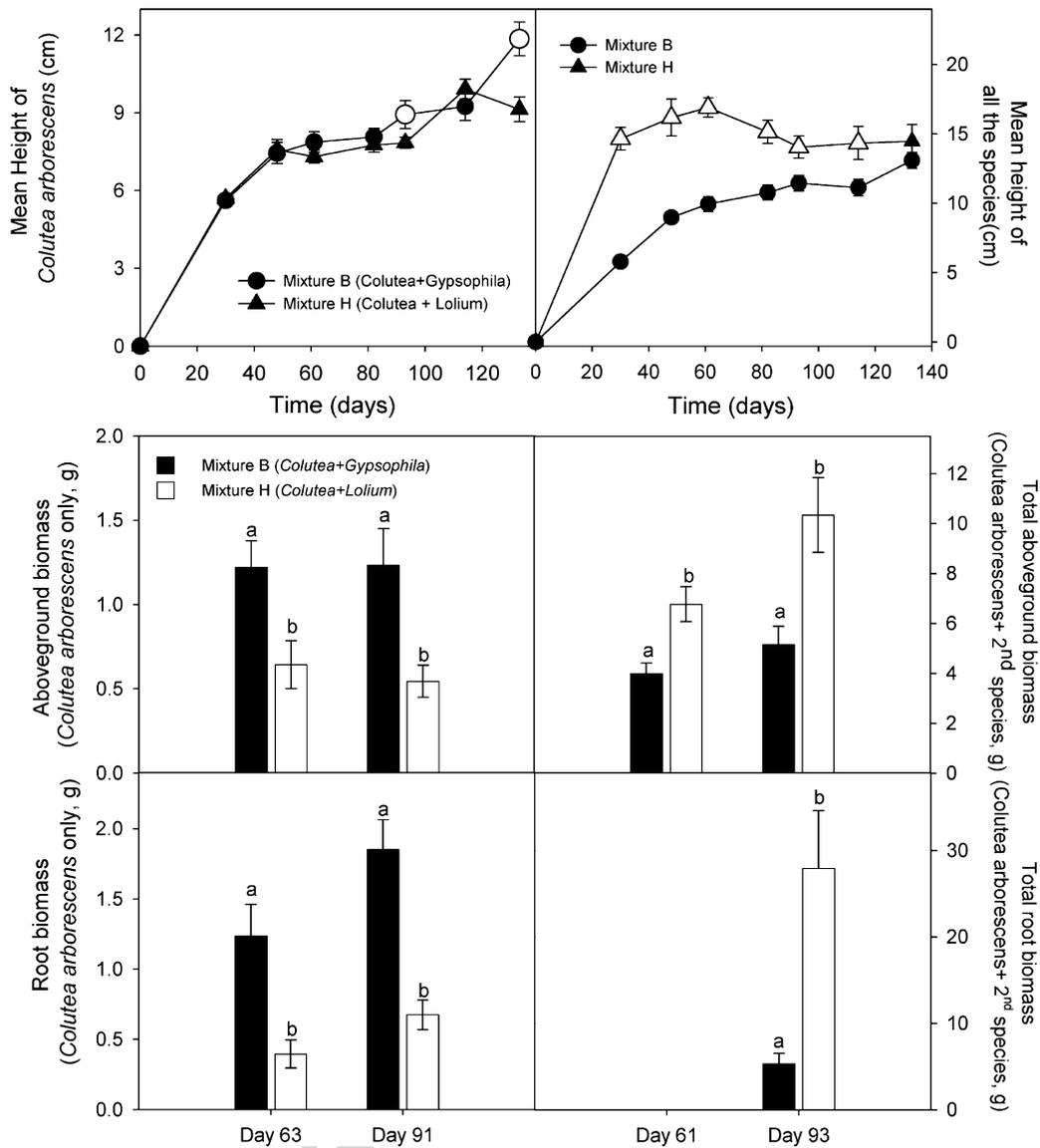
specialized and stress tolerant species. This is also supported by the negative effect of the density of *L. rigidum* on the aboveground biomass of the gypsum species. Other authors have also shown the negative effects of the density of an exotic grass species in the germination, survival and growth of a native shrub under Mediterranean conditions (Eliason and Allen, 1997). Emergence of *L. rigidum* before the gypsum species also had negative effects on the survival, mean height and aboveground biomass of the gypsum species.

Despite the conditions in the greenhouse were more favorable in terms of water availability compared to Mediterranean summer drought, the experiment was set to agree with the real schedule and conditions of the restoration projects carried out in similar locations, (i.e. hydroseeding in early spring). In these situations, the slopes are usually watered after hydroseeding to facilitate vegetation establishment. Even though these set of conditions could result in an advantageous situation for *L. rigidum*, this is commonly done in restoration practices and may hinder the establishment of the gypsum species in the very first stages of the community.

Despite the negative effects of *L. rigidum* on the gypsum species, total mean height, total aboveground biomass and total root biomass was higher in the communities containing more *L. rigidum* and, in general, cover was also higher in these communities, due to the fast growth rate of the introduced species. This leads to a conflict between the quick maximization of plant cover and biomass in the slopes and the facilitation of native species, which stress the need of defined goals in the restoration of motorway slopes (Pfadenhauer, 2001; Hobbs and Harris, 2001). While commercial and native species can have different and complementary roles in the establishment of a durable plant cover, with the former quickly stabilizing and enriching the soil and the latter providing resilience and tolerance to summer drought, their simultaneous use in hydroseeding is not likely to render good results.

In general terms, there are no precise guidelines in relation to the selection of the species to use in the revegetation projects, although some studies are recently addressing the importance of species selection (Kobayashi, 2004; Hoy et al., 1994). Traditionally, the main goal of restoration projects is to provide high plant cover rapidly in the slope to minimize erosion (Muller et al., 1998; Bochet and García-Fayos, 2004). A common practice in restoration enterprises is to use fast-growing commercial, frequently exotic species. Due to this, roads have become a contribution to the spread of exotic species (Gelbard and Belnap, 2003; Rentch et al., 2005). Rentch et al. (2005) found that more than half of the species from 13 highways in West Virginia (USA) were non-native, and Gelbard and Belnap (2003) found that road maintenance and improvement (i.e. clearing of vegetation, roadfill, pavement, etc.) promoted the establishment of exotic seeds. Although some studies point to the benefits of the use of native species (HarperLore, 1996; Tyser et al., 1998; Petersen et al., 2004), quantitative results are scant and they are rarely included in the actual restoration projects. Even when the inclusion of native species is explicitly indicated, the selection of native species is not carried out in detail. In our study, the use of a fast-growing species such as *L. rigidum* combined with slow-growing, late-successional gypsum species led to the failure of the latter. In fact, the community that we simulated according to the standard ratio between commercial and native species fixed in real restoration projects in Spain (mixture E) rendered very low survival rates, mean height and biomass of the gypsum species.

Species-specific responses were found in terms of survival in the different mixtures, which suggests that species differed in their competitive ability. However, the effect of *L. rigidum* on *C. arborescens*, which was the species with the highest survival rate, was also negative, in terms of both aboveground



**Fig. 5 – Effect of *L. rigidum* on the performance of *Colutea arborescens*. (Left) Mean height, aboveground biomass and root biomass of *C. arborescens* alone. (Right) Total mean height, total aboveground biomass and total root biomass (*C. arborescens* + second species). Different colours of the symbols within the same date indicate significant differences between mixtures B and H (nested ANOVA and Tukey’s HSD), and different letters above bars indicate significant differences among mixtures B and H (one-way ANOVA).**

and root biomass. Our results, thus, disagree with the idea that commercial species act as *starters* of the plant community (Bautista et al., 1997; Merlin et al., 1999). The presence of pioneer, fast-growing vegetation may affect the establishment of late-successional species in degraded areas by altering microhabitat conditions (light, water, and nutrient availability) that may facilitate or inhibit the establishment of seedlings of late-successional species (Callaway and Walker, 1997; Bellingham et al., 2001; Zanini and Ganade, 2005). In support of the notion that commercial or exotic fast growing species can have negative impacts on the ecological restoration of degraded areas, Forbes and Jefferies (1999) found that the introduction of non-native graminoids species in the restoration of arctic sites decreased the probability of the re-establishment of native species.

Therefore, our results show that it is important to include native species in the hydroseeding mixture but that it is even more important to select *a priori* the appropriate native species and to avoid their simultaneous use with exotic, commercially available species with a high growth capacity. Inadequate species selection can render poor results in the long term. Further studies should focus on the suitability of using herbaceous plants tolerant to gypsum soils, as their growth rate is likely to be higher and, thus, they are less likely to be out-competed by commercial species. The usage of these species instead of generalist species such as *L. rigidum*, would allow a more gradual and stable colonization of the slopes and would reduce the use of highly competitive and exotic species, a commonplace practice in the ecological restoration of motorway slopes in most countries.

## Acknowledgements

We thank P. Lombrail for his inestimable help in the greenhouse and his ideas during data collection. Also, we thank B. Alonso, D.P. Caravaca, D. Bote, D. Sánchez-Gómez, E. Beaumont, and D. Tena for their help and support. J. Oliet and J. Rubio provided fruitful comments on this manuscript. The seeds and field site were provided by Ferrovial-Agromán S.A. One of the authors (SM) was supported by a C.S.I.C. doctoral fellowship (I3P-2003). Financial support was provided by Spanish Ministry for Education and Science (grant RASINV, CGL2004-04884-C02-02/BOS) and Comunidad de Madrid (grant S-0505/AMB/0335 REMEDINAL).

## REFERENCES

- Andel, J.V., 2006. Communities: interspecific interactions. In: Restoration Ecology. Blackwell Publishing, Oxford, p. 319.
- Andrés, P., Jorba, M., 2000. Mitigation strategies in some motorway embankments (Catalonia, Spain). *Restor. Ecol.* 8, 268–275.
- Bautista, S., Abad, N., Lloret, J., Bladé, C., Ferran, A., Ponce, J.M., Alloza, J.A., Bellot, J., Vallejo, V.R., 1997. Siembra de herbáceas y aplicación de mulch para la conservación de suelos afectados por incendios forestales. In: Vallejo, V.R. (Ed.), La restauración de la cubierta vegetal de la comunidad valenciana. Fundación CEAM, CEAM-Conselleria de Agricultura y Medio-Ambiente, Valencia, Spain.
- Bellingham, P.J., Walker, L.R., Wardle, D.A., 2001. Differential facilitation by a nitrogen-fixing shrub during primary succession influences relative performance of canopy tree species. *J. Ecol.* 89, 861–875.
- Bochet, E., García-Fayos, P., 2004. Factors controlling vegetation establishment and water erosion on motorway slopes in Valencia, Spain. *Restor. Ecol.* 12, 166–174.
- Brindle, F.A., 2003. Use of native vegetation and biostimulants for controlling soil erosion on steep terrain. In: Proceedings of the Eighth International Conference on Low-Volume Roads 2003, vols. 1 and 2, pp. 203–209.
- Brown, C.S., Rice, K.J., 2000. The mark of Zorro: effects of the exotic annual grass *Vulpia Myuros* on California native perennial grasses. *Restor. Ecol.* 8, 10–17.
- Callaway, R.M., Walker, L.R., 1997. Competition and facilitation: a synthetic approach to interactions in plant communities. *Ecology* 78, 1958–1965.
- Eliason, S.A., Allen, E.B., 1997. Exotic grass competition in suppressing native shrubland re-establishment. *Restor. Ecol.* 5, 245–255.
- Escudero, A., Carnes, L.F., Pérez-García, F., 1996. Seed germination of gypsophytes and gypsosvags in semi-arid central Spain. *J. Arid Environ.* 36, 487–497.
- Forbes, B.C., Jefferies, R.L., 1999. Revegetation of disturbed arctic sites: constraints and applications. *Biol. Conserv.* 88, 15–24.
- Gelbard, J.L., Belnap, J., 2003. Roads as conduits for exotic plant invasions in a semiarid landscape. *Conserv. Biol.* 17, 420–432.
- González Ponce, R., 1998. Competition between barley and *Lolium rigidum* for nitrate. *Weed Res.* 38, 453–460.
- HarperLore, B.L., 1996. Using native plants as problem-solvers. *Environ. Manage.* 20, 827–830.
- Hobbs, R.J., Harris, J.A., 2001. Restoration ecology: repairing the earth's ecosystems in the new millennium. *Restor. Ecol.* 9, 239–246.
- Hoy, N.T., Gale, M.J., Walsh, K.B., 1994. Revegetation of a scalded saline discharge zone in central Queensland. 1. Selection of tree species and evaluation of an establishment technique. *Aust. J. Exp. Agric.* 34 (6), 765–776.
- Kobayashi, S., 2004. Landscape rehabilitation of degraded tropical forest ecosystems—case study of the Cifor/Japan project in Indonesia and Peru. *For. Ecol. Manage.* 201, 13–22.
- Lane, D.R., LeJeune, K.D., 2005. Introduction to the special section on resource heterogeneity and restoration success. *Restor. Ecol.* 13, 390–1390.
- Martin, L.M., Moloney, K.A., Wilsey, B.J., 2005. An assessment of grassland restoration success using species diversity components. *J. Appl. Ecol.* 42, 327–336.
- Matesanz, S., Valladares, F., Tena, D., Costa-Tenorio, M., Bote, D., 2006. Early dynamics of plant communities on revegetated motorway slopes from southern Spain: is hydroseeding always needed? *Restor. Ecol.* 14, 297–307.
- Merlin, G., Di-Gioia, L., Goddon, C., 1999. Comparative study of the capacity of germination and of adhesion of various hydrocolloids used for revegetalization by hydroseeding. *Land Degrad. Dev.* 10, 21–34.
- Meyer, S.A., García-Moya, E., 1989. Plant community patterns and soil moisture regime in gypsum grasslands of north central Mexico. *J. Arid Environ.* 16, 147–155.
- Mota, J.F., Sola, A.J., Dana, E.D., Jiménez-Sánchez, M.L., 2003. Plant succession in abandoned gypsum quarries in SE Spain. *Phytocoenology* 33, 13–28.
- Muller, S., Dutoit, T., Alard, D., Grevilliot, F., 1998. Restoration and rehabilitation of species-rich grassland ecosystems in France: a review. *Restor. Ecol.* 6, 94–101.
- Navas, M.-L., Moreau-Richard, J., 2005. Can traits predict the competitive response of herbaceous Mediterranean species? *Acta Oecol.* 27, 107–114.
- Paschke, M.W., DeLeo, C., Redente, E.F., 2000. Revegetation of roadcut slopes in Mesa Verde National Park, USA. *Restor. Ecol.* 8, 276–282.
- Petersen, S.L., Roundy, B.A., Bryant, R.M., 2004. Revegetation methods for high-elevation roadsides at Bryce Canyon National Park, Utah. *Restor. Ecol.* 12, 248–257.
- Pfadenhauer, J., 2001. Some remarks on the socio-cultural background of restoration ecology. *Restor. Ecol.* 9, 220–229.
- Picon-Cochard, C., Nsourou-Obame, A., Collet, C., Ghuel, J.-M., Ferhi, A., 2001. Competition for water between walnut seedlings (*Juglans regia*) and rye grass (*Lolium perenne*) assessed by carbon isotope discrimination and  $\delta^{18}\text{O}$  enrichment. *Tree Physiol.* 21, 183–191.
- Rivas-Martínez, S., Costa-Tenorio, M., 1970. Comunidades gipsícolas del centro de España. *Anales Instituto Botanico. A. J. Cavanilles*, 27, 193–224.
- Rentch, J.S., Fortney, R.H., Stephenson, S.L., Adams, H.S., Grafton, W.N., Anderson, J.T., 2005. Vegetation-site relationships of roadside plant communities in West Virginia, USA. *J. Appl. Ecol.* 42, 129–138.
- Ruiz-Jaen, M.C., Aide, T.M., 2005a. Restoration success: how is it being measured? *Restor. Ecol.* 13, 569–577.
- Ruiz-Jaén, M.C., Aide, T.M., 2005b. Vegetation structure, species diversity, and ecosystem processes as measures of restoration success. *For. Ecol. Manage.* 218, 159–173.
- San Emeterio, L., Arroyo, A., Canals, R.M., 2004. Allelopathic potential of *Lolium rigidum* gaud. On the early growth of three associated pasture species. *Grass Forage Sci.* 59, 107–112.
- Tinsley, M.J., Simmons, M.T., Windhager, S., 2006. The establishment success of native versus non-native

- herbaceous seed mixes on a revegetated roadside in central Texas. *Ecol. Eng.* 26, 231–240.
- Tormo, J., Bochet, E., García-Fayos, P., 2006. Is seed availability enough to ensure colonization success? An experimental study in road embankments. *Ecol. Eng.* 26, 224–230.
- Tyser, R.W., Asebrook, J.M., Potter, R.W., Kurth, L.L., 1998. Roadside revegetation in glacier national park, USA: effects of herbicide and seeding treatments. *Restor. Ecol.* 6, 197–206.
- Zanini, L., Ganade, G., 2005. Restoration of araucaria forest: the role of perches, pioneer vegetation, and soil fertility. *Restor. Ecol.* 13, 507–514.

Author's personal copy