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Ecological Engineering 26 (2006) 224–230
ECOLOGICAL ENGINEERING
www.elsevier.com/locate/ecoleng

Is seed availability enough to ensure colonization success? An experimental study in road embankments

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Received 31 March 2005; received in revised form 18 July 2005; accepted 4 October 2005

Abstract

We tested the hypothesis that seed availability is a limiting factor for plant colonization of road embankments under Mediterranean climate conditions. Experimental sowing on 10 road embankments was carried out to compare the colonization success of plants that successfully colonize the road embankment and species that appear only occasionally in the road embankments. After sowing, we measured plant establishment, biomass production, and reproductive capacity of the species.

The species that appear only occasionally in the road embankments had lower emergence rates (l.l ± 0.3%) than species that were successful colonizers (18.8 ± 2.9%). None of the species of the former group survived or reproduced. The results did not support the hypothesis that seed availability was the main factor limiting plant colonization in the road embankments. We concluded that the arrival of seeds to road embankments under Mediterranean climate conditions was not enough to ensure colonization success of plants. Other factors, like hydric stress, appeared to affect seedling establishment and plant growth. Reclamation measures such as species selection should be taken in account to ensure revegetation success of road embankments.

Keywords: Colonization; Restoration; Road embankments; Seed availability; Aspect; Semi arid; Sowing experiments

1. Introduction

Plant colonization is a key issue in the restoration of road embankments because it is widely accepted that vegetation has a role in controlling soil loss and runoff (Snelder and Bryan, 1995; Andrés and Jorba, 2000). Plant colonization is also essential in the stabilization of motorway slopes (Andrés and Jorba, 2000). The first barrier to successful plant colonization is seed availability (Foster and Tilman, 2003; Donath et al., 2003). However, in the natural systems the presence of seeds is not a guarantee of colonization success, since other factors, such as climate, lack of microsites, or lack of appropriate soil conditions, are also important (Primack and Miao, 1992; Eriksson and Ehrlen, 1992; Rosales et al., 1997). Once available seeds have

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doi:10.1016/j.ecoleng.2005.10.003

germinated, competition for resources such as light, water, and nutrients and/or pollinators (Ayalsew et al., 1992) also influence all further stages of a plant's life history.

Road slopes in semi–arid regions represent good experimental areas to study such phenomena in areas disturbed by humans. The low vegetation cover of road slopes in semi–arid areas of Spain has been attributed to unfavorable soil properties (low fertility and low water retention) and the steepness of the slopes (Bochet and García-Fayos, 2004). In addition, aspect has proved to be an important factor in determining the species composition of road embankments in these Mediterranean areas (Andrés et al., 1996).

In contrast to the reports above about the role of seed availability, Alborch et al. (2003) suggested that environmental factors are more influential than seed availability for the colonization of road embankments. These authors reached their conclusions from observational data by comparing the flora and the soil seed bank of road embankments with the flora of the surrounding areas.

We tried to evaluate if plant colonization in embankments is more limited by seed dispersal than by seed germination or development of species. We defined two groups of species: (1) those that grow abundantly in road embankments and in surrounding habitats (called "successful" species) and (2) those species that appear on surrounding habitats but grow only scarcely in embankments (called "unsuccessful" species).

The hypothesis is that if seed availability is the main limiting factor to plant colonization in road embankments, similar establishment rates should be obtained by sowing seeds of successful species and unsuccessful species. On the contrary, if road embankment conditions are hindering plant establishment, the addition of seeds of unsuccessful species should lead to lower colonization success compared with the addition of seeds of successful species. In order to test it, seedling emergence, biomass production, and reproduction of both groups of species were analyzed. North- and south-facing embankments were included in the design to test the importance of aspect in colonization success, because better conditions for plant establishment and growth were expected on north-facing slopes than on the south-facing ones under Mediterranean conditions (Andrés et al., 1996; Bochet and García-Fayos, 2004).

2. Materials and methods

2.1. Study area

The study area was located in "La Plana de Utiel-Requena" (Comunidad Valenciana, East of Spain) between km 285 and 286 of the A3 dual carriageway that links Valencia with Madrid (39°29′N; 1°06′W). Lithology, slope angle, and date of construction were similar for all the selected embankments. These were built on calcareous marls and clays of tertiary origin (García, 1996) with slopes ranging between 28° and 34°. Their construction was completed during summer 1994. The climate was Mediterranean with 418 mm and 14.2 °C of mean annual precipitation and temperature, respectively (Pérez, 1999). Inter- and intra-annual rainfall distributions were highly variable and show one peak in May and another in October.

The present vegetation in the region was formed mainly by vineyards, but small patches of shrubland still remained.

2.2. Road embankment selection

Ten separate road embankments were selected for the study: five north-facing and five south-facing slopes. Soil sampling was carried out to test for differences between north- and south-facing slopes. Soil analyses were performed using the procedures published in Page et al. (1982), except electrical conductivity that was measured by Richards (1964) method.

2.3. Species selection

Species selection was based on previous vegetation surveys performed on a larger set of 26 embankments and their corresponding surrounding areas between km 267 and 307 of the A3 highway. Twelve species were selected for the sowing experiment. Six of them were successful colonizers: *Avena barbata, Bromus rubens, Bromus diandrus* (*Poaceae*), *Anacyclus clavatus* (*Asteraceae*), *Medicago minima* (*Fabaceae*), *Plantago albicans* (*Plantaginaceae*), and six of them were unsuccessful colonizers: *Brachypodium retusum* (*Poaceae*), *Santolina chamaecyparissus* (*Asteraceae*), *Medicago orbicularis, Genista scorpius* (*Fabaceae*), *Plantago lanceolata*, and *Plantago sempervirens* (*Plantaginaceae*). Seeds were obtained from local areas and harvested by the authors except for *S. chamaecyparissus*, which was obtained from a local seed supplier (Intersemillas S.A)[1].

2.4. Laboratory experiments

Observations of the presence of endosperm and embryo in 100 seeds per species were made in order to determine the germination potential of the species sowed. Laboratory germination experiments were performed to obtain reference germination rates. Four hundred seeds per species were placed in Petri dishes (50 per dish) with one filter paper moistened with 5 ml of de-ionized water. The seeds were incubated in a light-temperature–controlled chamber with a photo-period of 12 h light–12 h dark and at temperatures between 5 and 15 °C, respectively. The germination was surveyed for 7 weeks. *A barbata* seeds were stratified (at 4 °C for 2 weeks) and legume seeds were scarified (submerged in sulfuric acid, 96%, for 5 min) to improve germination. Seeds used in all laboratory and field experiments were randomly selected from the same set of seeds for each species.

2.5. Sowing experiments

In July 2002, one 60 cm × 65 cm plot per species was established, in each selected embankment, at 20 cm intervals. The area of the plots and about 2 m of the surrounding vegetation was weeded to simulate the barren soil conditions immediately after the built up of road embankments. Ant traps were set up to prevent seed predation.

In September 2002, 50 seeds of each species were sowed in the upper half of the plots. The lower half of the plots was left free for us to control seed movements down the slope through soil erosion (García-Fayos and Cerdà, 1997). However, rainfall was not sufficiently high during the period of the study to cause seed erosion.

Controls were established to survey interference in germination of the studied species from the soil seed bank of the road embankments. This consisted in counting the number of germinations in the lower half of each plot as well as in the plot on the immediate right side.

Seedling emergence was recorded monthly from 23 October 2002 to 7 July 2003. In July 2003, we collected the plants to determine the vegetative and reproductive variables. Seedling emergence, above–ground biomass, and number of "flowers" per plant (spikeletes for *Poaceae,* capitula for *Asteraceae*, and individual flowers for all oiher plant families) were measured and used as dependent variables.

2.6. Statistical analyses

When comparing two means, unpaired Student's *t*-tests or Mann–Whitney *U*-tests were used. Generalized linear models (GLM) were used to analyze the influence of species, colonization success and aspect on the measured variables. GLM with normal error distribution was used to analyze plant biomass and GLM with Poisson error distribution was used to analyze seedling emergence and flower production. GLM with Poisson error distribution analysis was done using R v. 1.8.1. (http://cran.r-project.org/) and Mann–Whitney, Student's *t*-tests and GLM with normal error distribution (ANOVA) were done using SPSS v.l 1.0.1 (SPSS Inc., Chicago, IL, USA) statistical package.

3. Results

3.1. Soil data analyses

There were few differences in soil properties between the north- and south-facing slopes. Only soil available phosphorous was statistically different between the two aspects (Table 1). These results were similar to that obtained with a larger size of soil samples ($n = 20$) taken in a 40 km homogeneous area along the A3 that included the study site (Tormo, Bochet, and García-Fayos unpublished data) and agree with that found by other authors in Mediterranean habitats (Kutiel, 1999).

3.2. Laboratory and field experiments

More than 80% of the seeds sown were considered potentially germinable in all species and no differences were observed in laboratory germination between

[1] The nomenclature of species follows Castroviejo et al. (1986) and Mateo and Crespo (2001) for those species not included in the former reference.

Table 1
Few differences were found between soil properties according to aspect

	North	South	*p*-value
Organic matter (%)	1.42 ± 0.30	1.51 ± 0.19	0.811
Nitrogen content (mg g^{-1})	0.08 ± 0.02	0.09 ± 0.01	0.724
Phosphorous content (mgP_2O_5 100g $soil^{-1}$)	1.06 ± 0.21	2.95 ± 0.45	0.005
Electrical conductivity (1:5) (μS cm^{-1})	137.60 ± 9.43	150.20 ± 10.40	0.396

Mean, standard deviation and *t*-test *p*-values are shown ($n = 5$).

Table 2
Percent germination potential and mean ± standard error germination rate obtained in laboratory experiments (see Section 2)

	Colonization success	Germination potential	Laboratory germination
Anacyclus clavatus	S	95	94 ± 3
Avena barbata	S	95	73 ± 4
Bromus diandrus	S	100	99 ± 1
Bromus rubens	S	94	81 ± 7
Medicago minima	S	98	71 ± 3
Plantago albicans	S	86	48 ± 3
Brachypodium retusum	U	95	76 ± 7
Genista scorpius	U	86	42 ± 1
Medicago orbicular is	U	93	52 ± 3
Plantago lanceolada	U	100	72 ± 1
Plantago sempervirens	U	82	96 ± 2
Santolina chamaecyparissus	U	97	82 ± 7

Colonization success of species is specified as unsuccessful (U) or successful (S) as defined in text.

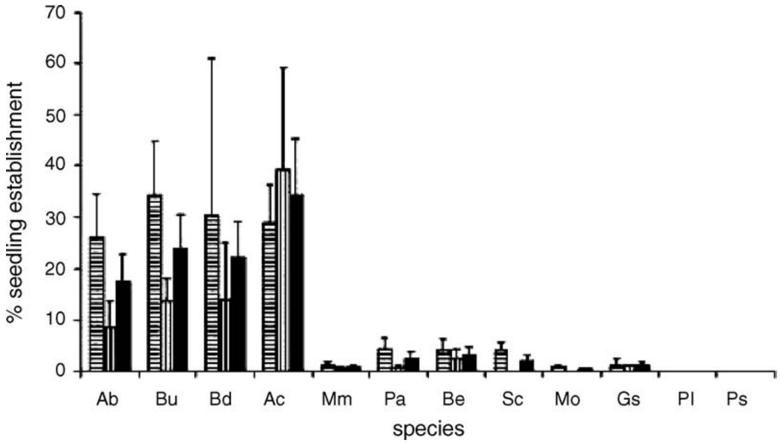

Fig. 1. Field seedling establishment of the studied species. Mean and standard errors of pooled results (solid bars), north aspects (horizontal lines) and south aspects (vertical lines). Germination rates and standard errors of species studied grouped by both aspects (black bars), north-facing slopes (bars with horizontal lines) and south-facing slopes (bars with vertical lines). Species codes are as follows: Ab = *Avena barbata*, Bu = *Bromus rubens*, Bd = *Bromus diandrus*, Ac = *Anacyclus clavatus*, Mm = *Medicago minima*, Pa = *Plantago albicans*, Be = *Brachypodium retusum*, Sc = *Santolina chamaecyparissus*, Mo = *Medicago orbicularis*, Gs = *Genista scorpius*, PI = *Plantago lanceolata*, Ps = *Plantago sempervirens*.

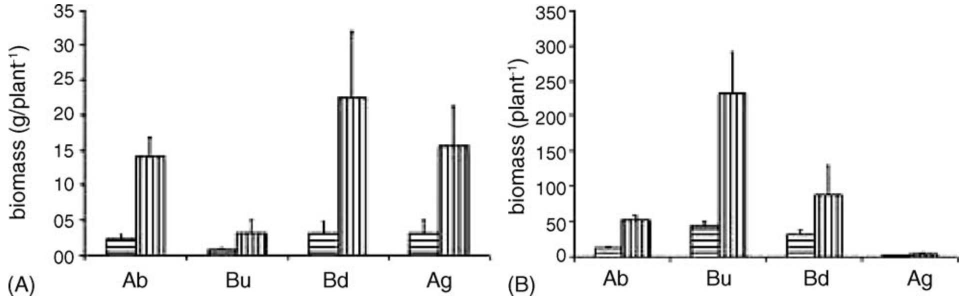

Fig. 2. Mean and standard error of: (A) the above ground biomass of successful species and (B) the flower production by successful species (bars with horizontal lines correspond to north-facing and bars with vertical lines correspond to south-facing slopes).

successful and unsuccessful species (Mann–Whitney, $U = 228.00$, $p = 0.157$) (Table 2).

Seedling establishment in field experiments was significantly affected by colonization success ($\chi^2 = 986$, d.f. = 1, p-value < 0.05) but neither by aspect ($\chi^2 = 71$, d.f. = 1, p-value = 0.06) nor by colonization or success versus aspect interaction ($\chi^2 = 3.77$, d.f. = 1, p-value=0.66). Seedling establishment of successful colonizers was over 10 times greater than that of unsuccessful colonizers ($16.84 \pm 2.93\%$ and $1.13 \pm 0.34\%$, respectively) (Fig. 1). No single establishment was recorded in the plots of *P. lanceolata* and *P. sempervirens* although their germination values under laboratory conditions and germination potential exceeded 70 and 80%, respectively (Table 2).

3.3. Biomass production

Biomass production could only be analyzed for the group of successful species because no plants of unsuccessful species survived until cutting time (except five plants of *S. chamaecyparissus).* Plant biomass of the successful species was significantly influenced by aspect ($F_{1,27} = 20.860$, $p < 0.02$) but not by species × aspect interaction ($F_{3,21} = 0.605$, $p = 0.619$). All studied species reached at least four times more biomass per plant in the south than in the north aspect (Fig. 2A).

3.4. Flowering

Species growing on southern aspects produced more flowers per plant than those living on the northern ones (Fig. 2B). No interaction was found between species and aspect ($\chi_1^2 = 396$, p-value = 0.265) which indicated that all successful species studied had produced more flowering on the southern slopes ($\chi_1^2 = 3364$, p-value < 0.05).

4. Discussion and conclusions

There was a clear difference in seedling establishment between unsuccessful and successful species. Since seed viability and germination in laboratory conditions were similar for successful and unsuccessful species, we concluded that road embankment conditions negatively affected the establishment of some plants, and that seed arrival is not a guarantee of successful colonization on road embankments. Unlike other ecosystems (Cantero et al., 1999; Williamson and Harrison, 2002), in our experimental conditions neither competition nor lack of gaps were limiting factors since all competing species were cut before sowing. Therefore, other factors seem to be limiting plant colonization in road embankments at our study site.

Under similar lithologic and topographic conditions, García-Fayos et al. (2000) found that soil salinity and soil moisture limited seed establishment in badland slopes. Since soil conductivity in study site was low (Table 1), we assume that did not affect plant establishment or growth. As regards the soil moisture, Bochet and García-Fayos (2004) in the same study site indicated that soil water remained available for plants for a shorter period in the road embankments than in the surrounding areas.

Moreover, differences in soil moisture might also explain north versus south differences in establishment because water remained available for plants after a rain event up to 41 days in North slopes in front of 6 days in south slopes (Alborch, 2005, same embankments).

The results obtained for biomass production and flowering were opposite to that obtained in establishment, since biomass and flowering were greater on more xeric south-facing slopes. The differences found between seedling establishment and biomass or flower production suggest that the factors limiting seedling establishment (e.g., water availability) may be different than those controlling the plant growth and reproduction. Soil analyses indicated that plant available phosphorous and organic matter tended to be higher in south-facing slopes. These differences in soil nutrients could explain the differences found in plant biomass and flowering.

In conclusion, it appears that plant establishment in road embankments was limited by soil water availability, as suggested by Bochet and García-Fayos (2004) and Alborch (2005), but the next stages in the plant's life cycle depend on other factors such as nutrient availability (Wiegleb and Felinks, 2001) or biotic interactions (Munzbergova, 2004).

4.1. Restoration implications

Our results suggest that plant restoration programs in road embankments need to be based on other considerations, such as: (1) the suitability of local species for colonization (e.g., the selection of species that grow naturally in embankments or similar zones) and (2) abiotic conditions that could hinder plant establishment of the selected species, especially hydric stress or limiting nutrients.

Acknowledgements

We thank the Spanish "Plan Nacional I + D + I del Mnisterio de Cienciay Tecnología" (project REN2001-2313), "Consejo superior de Investigaciones Científicas" and "Fundación Bancaja" for their funding. We also thank José Antonio Bellido for his assistance in sowing, plot weeding and seed collecting; Maite Pardo for her assistance in sowing; the staff of the laboratories of the CIDE for soil analyses; Miguel Verdú and David Conesa for their help with statistical analyses; and two anonymous referees for their considerable help in improving the manuscript.

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