Could recently locally extinct population patches of *Astragalus nitidiflorus* regenerate from the soil seed bank?

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ABSTRACT

Persistence, distribution and dynamics of *Astragalus nitidiflorus* soil seed bank, a critically endangered species of southern Spain, were studied during four consecutive years to determine their importance to regenerate locally extinct patches of the only known *A. nitidiflorus* population worldwide. The spatial distribution of seeds on the ground was highly influenced by the presence or absence of adult plants and by the indehiscent character of the fruit. Results showed that most seeds were present in the surface layer, inside fruit and close to the mother plant. Seed longevity was low in general, but higher for seeds protected by fruit than for single ones after two years of burial. We discuss our results in relation with other patterns of seed dispersal or viability in arid environments, that are generally characterized by high spatial and temporal variability with a short–range dispersal. We concluded that this species is able to form a short–term persistent soil seed bank strongly influenced by environmental factors and population fluctuations. Based on these results, natural regeneration of patches locally extinct some years ago is unlikely from the soil seed bank and recovery should be attempted by sowing seeds or planting new specimens.

Keywords: Barochory; Endangered species; Seed burial; Seed dispersal; Seed longevity; Woody fruit.

1. Introduction

Astragalus nitidiflorus Jiménez Mun. et Pau (Leguminosae) is a perennial herb endemic to the province of Murcia (southern Spain), where it forms the only known metapopulation worldwide. This species is classified as Critically Endangered in accordance with IUCN criteria. The formation of a permanent soil seed bank is favorable in semi–arid ecosystems or in habitats where environmental conditions change dramatically and with unpredictable time patterns [1], ensuring the maintenance of a population in an area even in years with no yield. Seed dormancy and longevity are likely extremely important in the maintenance of soil seed banks, but their formation is probably also highly influenced by the seed input or seed production. Hard–seededness is a widely occurring feature in Leguminosae that imposes physical seed dormancy allowing the long–term burial of seeds and consequently the formation of persistent soil seed banks [2]. Taking into account that plant populations of most semi–arid regions are good candidates for the maintenance of seed banks, and the physical dormancy evidence found in seeds of *A. nitidiflorus*, we hypothesized that this species could form a persistent soil seed bank. In other hand, although seed banks in arid ecosystems are characterized by high spatial and temporal variability [3], presumably in response to the dynamics of processes regulating seed additions and depletions and because most arid plants have short–range dispersal, no data are know about these topics in *A. nitidiflorus*. Therefore, the following questions were arisen. (i) Has *A. nitidiflorus* the ability to form a persistent soil seed bank? (ii) What is the quantitative importance of the seed bank over time? iii) How are seeds distributed in the soil? The answer to these questions will allow us to take the necessary decisions aimed at preserving small patches that disappear over time and to recover them.

2. Materiales y Métodos

Astragalus nitidiflorus is a short–lived legume that colonizes old field in volcanic soils. The dispersion unit is the fruit, a woody indehiscent legume (ca. 2.5–3.0 cm), being barochory the main dispersal mechanism. The species constitutes a classical metapopulation with five small patchily distributed populations located near Cartagena (Murcia province), having a

Mediterranean-type climate with semi–arid conditions. The mean annual rainfall is 246 mm and annual potential evapotranspiration of 1319 mm.

Soil samples were collected from forty 1–m^2 permanent plots in June, from 2009 to 2012, in the largest known population (37°40’06.8”N, 1°08’00.6”E). Plots were 5–m apart from each other along four transects established perpendicularly to the maximum slope in the area of appearance of the species. In each plot two cores (20 cm × 20 cm) were sampled at two different depths: 0–5 and 5–10 cm. Simultaneously, the distance of the nearest adult plant of *A. nitidiflorus* to each plot was measured. Apparently healthy seeds content in soil samples were separated by physical separation method. To test seed viability, seeds extracted from soil samples were scarified with sandpaper and incubated in a growth chamber at 15°C and a 12–h photoperiod. To test if the presence of fruits and seeds in the soil was related to the proximity of adult plants, three plot categories were defined based on the presence/absence of adult plants: (i) presence of the adult near the plot (PA), (ii) absence of the adult near the plot since last year (ALY) and (iii) absence of the adult near the plot for at least two years (ATY). Considering barochory as the dispersal mechanism, a plant was present when it was in a radius ≤ 2 m of the plot.

To determine the viability over time of the seeds incorporated into the soil, two burial experiments were designed using fruits and seeds extracted from fruits (henceforth ‘single seeds’). Two different depths were tested (2 and 7 cm). 64 lots of ten fruits were introduced into small aluminum trays with drainage, which were filled with soil from the habitat until reaching the desired depth. Another 64 lots of 25 apparently healthy single seeds were put into square nylon bags (5 cm × 5 cm) of 0.1–mm mesh filled with soil, and bags were buried into trays at the same conditions than fruits. The experiments were placed in a non–heated mesh irrigated with natural rainfall during two consecutive years. At the end of each season, four replicates of both burial fruits and single seeds were exhumed to count physically undamaged seeds. All apparently healthy seeds were tested for germination at 15 °C and 12–h photoperiod for 30 d*, and those than germinated* were considered the non–dormant viable seed fraction. To know the initial non–dormant viable seed fraction before burial four replicates of 25 apparently healthy seeds were tested for germination at the same conditions. Then, ungerminated seeds were dried for 1 d and then slightly scarified with sandpaper, and incubated for an additional 30 d to determine the initial dormant viable seed fraction. Viability was evaluated by the final cumulative germination percentage. The same process was repeated at the end of the burial experiment to determine the viability of seeds after two years of burial. Also, before burial, 80 fruits were opened and their seeds analyzed to determine the initial percentage of apparently healthy seeds (some seeds are parasitized by *Bruchophagus astragalii)*.

3. Results and Discussion

Most seeds and fruits were distributed in the surface layer and, except for 2011, most seeds were found inside fruits. This is explained by the type of fruit of *A. nitidiflorus*, an indehiscent and hard legume that prevents rapid release of seeds, which remain some years inside the fruit. Moreover, the considerable size of these fruits (ca. 1.9 cm × 0.8 cm) hampers their burial, and nearly 100% of them are on the soil surface.

The highest density of seeds was in PA plots and the lowest density in ATY plots (Fig. 1A). Most plots with seeds or fruits were PA and 83.6% of seeds and 89.2% of fruits were extracted in PA plots (Fig. 1B). So, the spatial distribution of fruits and seeds on the ground was very heterogeneous and highly influenced by the presence or absence of adult plants, showing the typical contagious distribution pattern observed in other semi-arid sites [4]. These data were expected because fruit dispersal is by gravity from prostrate stems. It has been demonstrated that restricted spatial dispersal could be selected under certain conditions to ensure plant survival in situ and establishment, to facilitate plant coexistence and to reinforce spatial aggregation in arid plants.

The soil seed bank density drastically decreased during 2009–2012 and percentage of plots with seeds also showed a continuous decline (Table 1). In the best year a mean of 221.54 viable seeds/m^2 was estimated, similar to that obtained for the shrub legume *Echinospartum algibicum*, another endemic threatened species, although in most years studied the amount of seeds found in soil was very low, and scarcer than other species that tend to form persistent seed banks such as shrubs of the genus *Cistus* (500–9000 seeds/m^2) or some sprouting shrubs of the genus *Erica*

(8500 seeds/m^2), abundant in disturbed forest or scrubland areas [5]. This decrease may be a direct consequence of the exceptionally rainy month of September 2009 (222.4 mm) that resulted in a massive emergence of seedlings from the soil seed bank (although most of them died before reaching the adult stage) as well as the dynamics of the population in the sampled site which showed a great reduction in the number of adult plants, declining from almost 2000 plants in 2008 to 73 and 82 individuals in 2010 and 2012 (personal observation). Taking into account that the soil seed bank was so sensitive to environmental factors and changes in population size, and density values in soil were not maintained relatively constant over time we should consider that this species has a short–term persistent seed bank [6].

The percentage of apparently healthy seeds was significantly affected by the time of burial ($P < 0.001$), but not by depth. The initial percentage of apparently healthy seeds in fruits was only 76.6 ± 3% (Fig. 2A) due to seeds parasitized by *B. astragalii*. The percentage of apparently healthy seeds extracted after each season showed a progressive decrease without significant differences between exhumed single seeds and those contained in fruits (Fig. 2A). In both cases, the major decrease was during the first season of burial remaining around 35–30% apparently healthy seeds after two years of burial (Fig. 2A). The percentage of non–dormant viable seeds was significantly affected by the factors of time of burial ($P < 0.001$) and type of seeds ($P < 0.001$), and their interaction ($P < 0.001$). Depth of burial had no effect. Before burial, the percentage of non–dormant viable seeds was of 29 ± 5.3% (Fig. 2B). After burial, the percentage of non–dormant viable single seeds decreased according to an inverse curve ($F_{1,7} = 632.54$, $P < 0.001$, $R^2 = 98.9\%$) (Fig. 2B). However, in seeds extracted from buried fruits the decline was more gradual following a linear function ($F_{1,7} = 26.3$, $P = 0.001$, $R^2 = 79\%$) (Fig. 2B).

Single seeds showed a significantly higher initial viability than for those contained in fruits (80.7 ± 5.9% versus 61.8 ± 2.5%, $P = 0.005$), mainly because all single seeds used in this experiment were apparently healthy (parasitized seeds were discarded). However, after two years of burial the most significant decline in the viability was for single seeds, only 5.2 ± 0.6% remained viable compared to 29.3 ± 3.1% of seeds contained in fruits ($P < 0.001$).

The woody indehiscent fruits appeared to slow the loss of seed viability, probably due to enhanced protection against fungal and non–fungal diseases, predators, preventing seed scarification by mechanical friction, and also protecting seeds from exposition to climatic factors, e.g. heat from isolation, which is one of the principal drivers of seed senescence. Initially, around 30% of seeds were non–dormant viable, but 100% germinated once they were scarified in the laboratory. This fact, and the seed coat formed by a palisade–cell epidermis (personal observation under scanning electron microscope) like other Leguminosae with hard coats, indicates that seeds of *A. nitidiflorus* have physical dormancy. However, if we consider the high loss of viability experienced in just two years of burial, the hardness of these seeds was not as strong as for other legumes or Cistaceae seeds that have long lifespan once buried in soil.

4. Conclusions

All the evidence suggests that *Astragalus nitidiflorus* has the ability to form a short–term persistent soil seed bank, since it was verified that some seeds remained viable in soil for at least two years, although strongly influenced by environmental factors and population fluctuations. Based on this conclusion, natural regeneration from the soil seed bank is not expected for patches that have been absent for > 5 years if there has been no new input of seeds. Recovery of such locally extinct patches should be attempted by sowing seeds or planting new specimens. Knowledge of the morphological and dispersal fruit traits can be as necessary for explaining the demographic patterns of the species as classical soil seed bank studies.

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Tables and Figures

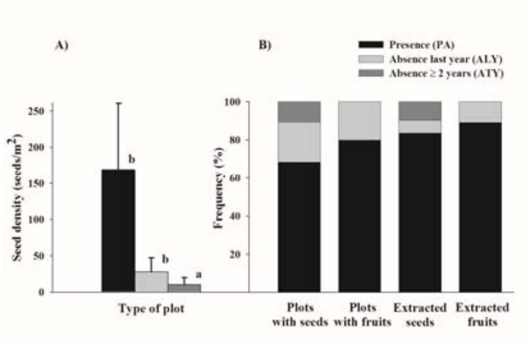

Figure 1. Seed density (A) and frequency of plots with seeds or fruits in relation to the presence of adult plants (B). Bars show standard errors. Different lowercase letters denote significant differences of seed density between types of plots.

Year	Percentage of plots with seeds	Percentage of plots with fruits	Mean seed density in plots ± S.E. (maximum density found in a plot)		
			Layer 0–5	Layer 5–10	*Stat.*
2009	37.5B	10A	208.81 ± 73.75Bb	12.73 ± 7.46Aa	$P_{M\text{-}W}$ = 0.004
	n=15	n=4	n=14 (2240)	n=3 (203.5)	rho = 0.015
2010	22.5AB	7.5A	15 ± 8.48Ab	1.25 ± 0.75Aa	P_W = 0.024
	n=9	n=3	n=7 (312.5)	n=2 (25)	rho = 0.436**
2011	15AB	10A	11.56 ± 8.29Aa	8.44 ± 8.12Aa	P_W = 0.500
	n=6	n=4	n=4 (325)	n=2 (325)	rho = 0.325*
2012	10A	7.5A	55 ± 43.7Aa	0 ± 0Aa	$P_{M\text{-}W}$ = 0.079
	n=4	n=3	n=3 (1725)	n=0 (0)	rho = 0
Stat.	χ^2 = 9.722*	χ^2 = 0.313	P_F = 0.003	P_F = 0.256	

Table 1. Changes recorded in mean seed density and percentage of plots with seeds and fruits of *A. nitidiflorus* in the soil seed bank throughout the study period (2009–2012). (P: non–parametric test). M–W: Mann–Whitney U. W: Wilcoxon signed–rank. F: Friedman. n: number of positive cases of total analyzed. rho: Spearman's rank correlation coefficient. χ^2: chi–squared value. Significance was considered at 0.05(*), 0.01(**) and 0.001(***) P–levels.

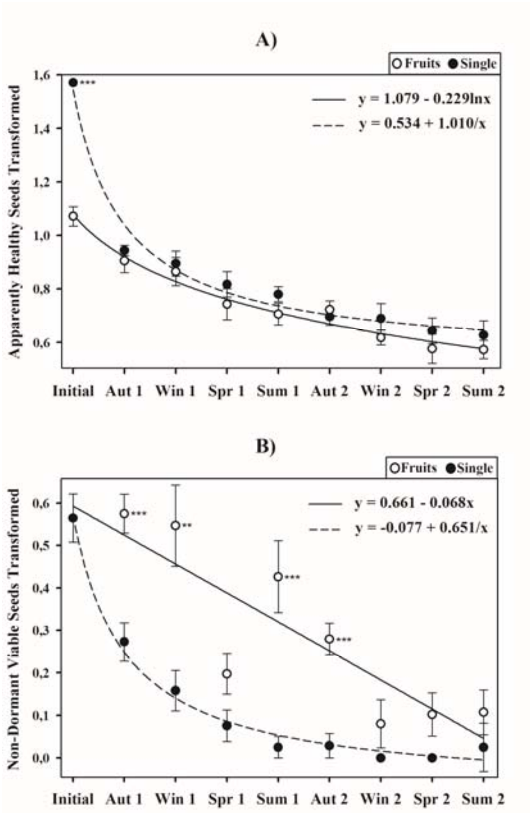

Figure 2. Lost of apparently healthy seeds (A) and non–dormant viable seeds (B) during the seed burial experiment. Mean values are root arcsine transformed. Bars represent the standard error. Significant differences between seeds contained in fruits or singles were considered at 0.05(*), 0.01(**) and 0.001(***) P–levels. Equations of significant regression models are also shown.