

Critical Stages in the Recruitment Process of *Rhamnus alaternus* L.

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• **Background and Aims** *Rhamnus alaternus* is a Mediterranean shrub commonly used in reforestation programs. Although several aspects of its reproductive biology have been studied, little is known about the importance of the different recruitment stages in the overall regeneration process of this species, which limits its proper use in Mediterranean forests and shrubland management. The aim of the present work was to quantify the importance of the different recruitment stages in the regeneration process of *R. alaternus*.

• **Methods** Two populations of *Rhamnus alaternus* on the island of Mallorca that differ in climatic conditions, type of habitat and sex ratio were studied. The importance of seed production, seed dispersal and predation, seedling emergence and seedling survival for the regeneration of this species were quantified.

• **Key Results** In both populations, fruit set and fruit removal by animals were not critical stages, since almost half of the flowers became mature fruits and 90 % of those were dispersed. Most seeds were deposited under female conspecifics (86 and 47 %, at Lloret and Esporles, respectively), and very few were found in open interspaces (1 and 5 %). Post-dispersal seed predation (mostly by ants and rodents) was very high in both populations. Seedling emergence took place during autumn and early winter and it ranged from 31 to 68 % depending upon year and microhabitat. The majority of emerged seedlings died during the first year, mainly due to desiccation; such mortality was influenced by rainfall and differed among microhabitats (varying from 67 to 100 %). The general spatial distribution of seed rain was concordant with the seedling emergence and survival pattern in both populations.

• **Conclusions** The recruitment of *Rhamnus alaternus* appeared to be mainly limited by seed and seedling survival, regardless of the type of habitat in which the species is found. © 2004 Annals of Botany Company

Key words: Mediterranean shrubs, pollination, recruitment, *Rhamnus alaternus*, seed dispersal, seedling survival.

INTRODUCTION

Environmental and physiological factors affecting seed production, seed dispersal and predation, seed germination and seedling establishment influence the regeneration ability of a species (Kiviniemi and Eriksson, 1999). Usually, studies take into account just one of these stages, limiting an overall understanding of the recruitment process (Herrera *et al.*, 1994). However, the number of studies considering several stages has increased in recent years (see for instance, Kaye, 1999; Herrera, 2000; Rey and Alcántara, 2000; Vilà and Lloret, 2000; Traveset *et al.*, 2003), allowing the identification of the bottlenecks for the recruitment of particular species.

In Mediterranean ecosystems, climatic conditions reduce the efficiency of several processes involved in plant regeneration. Summer drought and the infrequency and short duration of periods in which temperature and humidity are suitable for plant growth are the major environmental constraints for plant regeneration under Mediterranean conditions. Seedling survival, specifically, has been identified as one of the main bottleneck processes in several Mediterranean plant species such as *Pistacia lentiscus* (García-Fayos and Verdú, 1998), *Phillyrea latifolia*

(Herrera *et al.*, 1994), *Olea europaea* (Rey and Alcántara, 2000) and *Rhamnus ludovici-salvatoris* (Traveset *et al.*, 2003). Other processes, like post-dispersal seed predation, have also been found to limit plant recruitment, as is the case in *R. ludovici-salvatoris* (Traveset *et al.*, 2003). In addition to the importance of single processes, the role played by the spatial coupling between seed dispersal and subsequent processes has been highlighted by several reports (Herrera *et al.*, 1994; Schupp, 1995; Rey and Alcántara, 2000; Traveset *et al.*, 2003).

One of the species commonly used in reforestation programs in the Mediterranean, due to its fruit characteristics and ability to survive in xeric environments, is *Rhamnus alaternus*, a perennial dioecious shrub distributed along the Mediterranean Basin, that flowers during late winter and early spring, with a peak in mid-February. It produces fleshy fruits that ripen in late spring and early summer, and thus represents an important water and nutrient source for birds and small mammals. The fruits usually bear two or three seeds that are covered by an endocarp that opens when the fruit pulp is removed. *Rhamnus alaternus* has been described as a predominantly entomophilous species. Aronne and Wilcock (1995) have reported that pollen availability limits fruit set in populations in southern Italy, although Guitián (1995) found this not to be the case

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in north-western Spain. Likewise, masting was found in populations in southern Italy but not in those from north-western Spain. These apparently contradictory results suggest that its reproductive behaviour is highly variable depending on environmental characteristics and population life history. In addition, little is known about the importance of the different recruitment stages in the overall regeneration process of this species, which limits its proper use in Mediterranean forest and shrubland management.

The main objective of the present work was to quantify the importance of the different recruitment stages (seed production, seed dispersal and predation, seedling emergence and survival) as key points in the regeneration process of *R. alaternus*. Several specific questions were addressed: (1) is seed production limited by the pollination process or by any other factor affecting seed viability; (2) how important are the effects of post-dispersal predators, either vertebrates (mainly rodents) or ants; (3) how do the patterns of seed-rain overlap with those of seedling emergence and establishment in different microhabitats; and (4) do the critical life stages vary between populations differing in habitat, plant density and climate conditions?

MATERIAL AND METHODS

Study sites

The study was performed in two populations of *Rhamnus alaternus* L. that differ in climate conditions (rainfall and illumination), type of habitat, predominant vegetation and sex ratio. The first population is located at Esporles, approx. 200 m above sea level on the north slope of a hill in the west of Mallorca island, with a mean annual precipitation of 629 mm, mean maximum temperature of the warmest month (August) of 30.2 °C and mean minimum temperature of the coldest month (January) of 7.4 °C (Guijarro, 1986). The *Rhamnus* population at this location is in the understorey of a mixed pine–oak forest with surrounding vegetation that includes *Pistacia lentiscus*, *Ampelodesmos mauritanica*, *Olea europaea* and *Cistus albidus*. Fifty males and 58 females were recorded (and thus the sex ratio does not vary significantly from 1 : 1; $\chi^2 = 0.593$, $P > 0.05$). The second population is located at Lloret, approx. 150 m above sea level, in the central part of Mallorca, with a mean annual precipitation of 557 mm and monthly mean maximum and minimum temperatures of 31.7 and 7.8 °C, respectively (Guijarro, 1986). This *Rhamnus* population is in the understorey of a pine forest. The vegetation of this area also includes *Pistacia lentiscus*, *Olea europaea*, *Cistus monspelliensis* and *Phyllirea angustifolia*. The sex ratio here was found to be 46 males vs. 16 females, significantly different from 1 : 1 ($\chi^2 = 14.516$, $P < 0.0001$).

Sexual system

The sexual system of *R. alaternus* was examined over two years (1999–2000) in the two populations. In 1999, buds on each of 11 female individuals were bagged at Esporles and on seven females at Lloret in order to assess whether pollen arrival limits fruit set (flowers were hand-pollinated at

anthesis with pollen from different males), and also to determine the potential role of wind as a pollinating vector (bags allowed pollen to pass through but not insects). A minimum of three branches per plant were used for each treatment. Another group of non-manipulated (open-pollinated) flowers, also from a minimum of three branches per plant, served as controls. A similar number of flowers were used for treatments and controls.

In 2000, the pollination experiments were repeated in both populations to assess the importance of pollen limitation. Nine females were selected from each population, some of which were the same individuals used the previous year. Unfortunately, most data from Esporles was lost this year due to extremely high herbivory by goats (*Capra hircus*). In both years, the flower crop was estimated and the distance to the nearest reproductive male for each female individual studied was measured to determine the influence of these parameters on fruit set.

Seed dispersal and seed rain

Both the rate of fruit removal and seed rain were studied during the fruiting period of 1999 in both populations. Removal was assessed every 2 weeks in the same branches in which fruit set was monitored, until the fruits had dried or were dropped. In the system under study, removal is equivalent to dispersal since fruits were only removed by birds that do not act as fruit predators but as legitimate dispersers, i.e. swallowing the berries and defecating the seeds intact. These birds were mostly the Sardinian warbler (*Sylvia melanocephala*) and blackbird (*Turdus merula*), which are non-migratory and remain in the Balearic Islands all year round. No pre-dispersal fruit predation by insects or any other predator was observed in any of the two populations studied.

Seed rain was monitored in five microhabitats: open areas of the forest, under female conspecifics, and under each of the three most usual tree or shrub species in the population. Specifically, *Pistacia lentiscus*, *Quercus ilex* and *Pinus halepensis* were considered in Esporles, and *Pistacia lentiscus*, *Olea europaea* and *Pinus halepensis* in Lloret. Seeds and fruits were collected in aluminium trays of 0.043 m² that were covered with a grid (1 cm wide) to avoid predation by vertebrates. Ten replicates per microhabitat and two trays per replicate were used, so the total surface area assessed per microhabitat was 0.86 m². The sampling interval was every 2 weeks throughout the ripening period, from early June to early August in Esporles, and from early June to mid-July in Lloret.

Fruit predation

Fruit predation was examined over the same period and in the same microhabitats and populations as those considered in the seed rain study. Berries, which dry up very quickly as they are mostly water, were placed in Petri dishes and two treatments were applied: one (control) in which they were available to all predators (mostly rodents and ants in this system), and one in which they were excluded from vertebrate predators (by means of a grid of 1 × 1 cm).

Fruits were employed instead of seeds for the experiment due to the high proportion of intact fruits found under female individuals of *Rhamnus*. Berries that had been dropped by birds (with a beak mark on them) were occasionally found in the trays. Five dishes were placed per microhabitat and treatment, with five fruits on each dish. The number of remaining fruits and seeds was recorded after 2 weeks.

Seedling emergence and seedling survival

Seedling emergence and survival were monitored at the two sites from the autumn of 1999 to the autumn of 2001. Three microhabitats were studied: open areas of the forest, under female conspecifics, and under one of the most usual tree or shrub species in the population, i.e. *Q. ilex* in Esporles and *P. lentiscus* in Lloret. These microhabitats were considered in order to include the whole range of microclimatic conditions in each population. Five replicates per microhabitat were used and 24 seeds per replicate were planted, at a depth of 0.5 cm, in an area of 0.068 m². Seeds were collected the same year from the same population in which they were sown. Apparent non-viable seeds were discarded and only healthy and well-developed seeds were used. Seedling emergence and death were followed every 2–3 weeks until the end of summer and the onset of autumn rain. Seedling emergence stopped at the beginning of spring. Thereafter, seedling mortality started, so separation of both processes was reliable because they did not overlap.

Seed viability was determined from the seeds of 100 fruits (ten fruits from each of ten different females) from each population. Only those seeds in which the endosperm and the embryo occupied the whole inner space and which were apparently healthy were considered viable.

Transition probabilities (TPs)

Six transition probabilities between different stages, from pollination to seedling survival (TP₁ and TP₂ obtained on a per tree basis, whilst the other four were on a microhabitat basis), were calculated:

TP₁ was equivalent to fruit set, i.e. number of mature fruits relative to flowers produced (it may not be equivalent to 'pollination success' *per se*, as fertilized ovules may have aborted later before reaching maturity).

TP₂, the probability that a fruit was removed (dispersed) by a bird.

TP₃, the probability that a seed was deposited in a particular microhabitat (estimated as the mean density of seeds that arrived in each microhabitat relative to the sum of the mean seed densities deposited in all the microhabitats studied).

TP₄, the probability of escaping predation, by either rodents or ants, in each microhabitat. To calculate this, it was assumed that all seeds removed were actually killed, although this may not always be the case (some might actually be secondarily dispersed). Moreover, fruits or seeds might be easier to detect on Petri dishes than when naturally buried by litter (Hulme 1998), and so this probability is possibly somewhat under-estimated.

TP₅, the number of seedlings emerging relative to the number of seeds planted in each microhabitat.

TP₆, the probability of survival from seedling to sapling in each microhabitat.

TP₁ to TP₄ were estimated from data collected in 1999, while TP₅ and TP₆ were estimated from data collected in 1999–2000 and in 2000–2001 (when germination was slightly greater).

In order to estimate the overall probability of recruitment (OPR) in the population, it was necessary to weigh the cumulative probability of recruitment (CP, which is the product of all TPs) by the relative abundance of each microhabitat in the area. This relative abundance was calculated by performing at each population a total of ten random transects (50 m long and 2 m wide) and recording the number of square meters covered by each type of microhabitat. The CP in each microhabitat was multiplied by the proportion of area covered by that microhabitat, and the OPR was thus the sum of all those products. In order to obtain a value of OPR different from zero (as TP₄ was found to be zero for many microhabitats, although it is likely to be under-estimated), an arbitrary value of 1 % was given to all microhabitats. Hence it was assumed that at least one out of a hundred seeds survive the attentions of predators regardless of the microhabitat where the seed ultimately comes to rest.

Data analysis

Most statistical analyses performed were parametric (ANOVAs and correlations). Angular transformations were used to normalize the proportions in different tests. When data could not be normalized, non-parametric tests (Spearman correlations and Mann–Whitney tests) were employed. For the comparisons of seedling emergence and survival between populations and years and among microhabitats, repeated measurements analyses were performed. The statistical packages employed were Statistica 5.5 (StatSoft Inc.) for the repeated measurements analysis, and SPSS 10.0 (SPSS Inc.) for the other analyses.

RESULTS

The process of pollination

The pollination process occurred in both populations between mid-February and mid- to late-March in 1999 and 2000. However, it was observed that flowering time could vary from year to year depending on climatic variability, especially on winter temperatures. Male and female individuals coincided in their flowering peak in the 2 years and two populations considered, as has been observed in other *Rhamnus* species such as *R. ludovicisalvatoris* (Traveset *et al.*, 2003).

The proportion of flowers that subsequently bore mature fruits varied depending on treatment, population and year (Table 1). Anemogamy produced a significantly lower fruit set than hand-pollination and control flowers in both populations. However, while it was very low at Lloret (4.2 %), anemogamy was rather important at Esporles

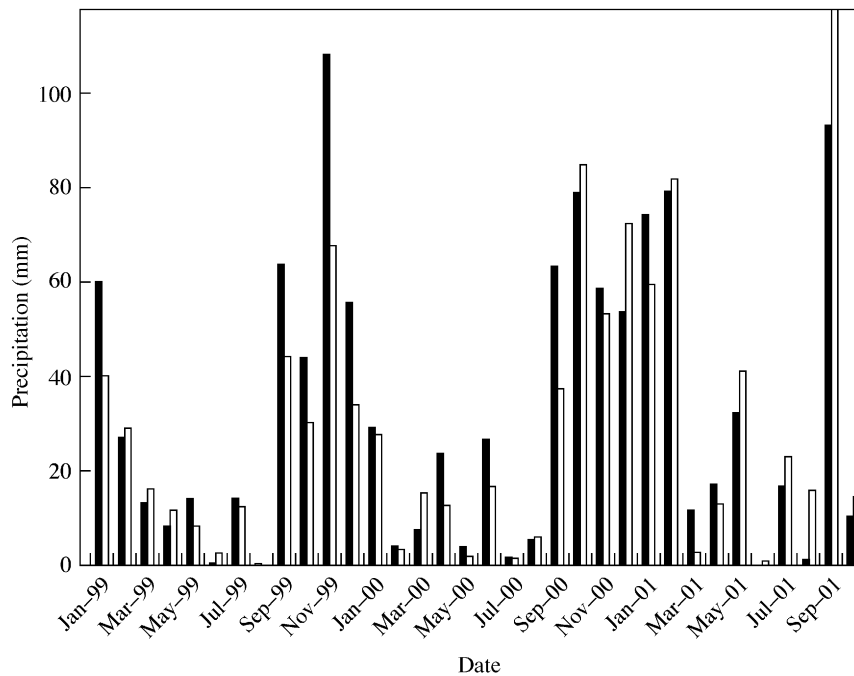


FIG. 1. Monthly precipitation during the study period at the nearest meteorological stations to the sampling sites. Data supplied by Centro Meteorológico Territorial de les Illes Balears. Filled bars correspond to Esporles and open bars to Lloret.

TABLE 1. Percentage of flowers that produced mature fruits of *R. alaternus* in the two study populations (means \pm s.e.)

	Esporles 1999	Lloret 1999	Lloret 2000
Wind-pollinated	16.31 \pm 3.98 ^a	4.19 \pm 0.98 ^a	—
Hand-pollinated	77.39 \pm 3.71 ^b	51.50 \pm 8.84 ^b	40.87 \pm 4.3 ^a
Open-pollinated	65.51 \pm 3.89 ^b	44.77 \pm 4.91 ^b	21.78 \pm 2.87 ^b

In 1999, 11 and seven female individuals were considered at Esporles and Lloret, respectively. In 2000, nine females were taken at Lloret, and data from Esporles were lost because of herbivory. Values within the same year and population with different superscripts differ at $P < 0.05$.

(16.3 %). Hand-pollinated flowers gave a higher fruit set than control flowers in both years and in both populations, but these differences were only significant at Lloret in 2000, and thus pollen limitation was found only on this occasion. Fruit set appears to be associated with rainfall, as it was greatest in the more humid population (Esporles) and in the year with greater rainfall, 1999 (Fig. 1). Fruit set was significantly inversely correlated with distance to the nearest reproductive male ($r = -0.72$; $P < 0.01$) and with flower crop ($r = 0.65$; $P < 0.05$), but only at Esporles.

The viability of seeds in mature fruits varied among years and populations. It was quite high at Esporles in both years (96 % and 73 % in 1999 and 2000, respectively) and also at Lloret, but only in 1999 (98 %), being rather low in this population in 2000 (44 %), presumably due to the extremely dry spring at that site (Fig. 1).

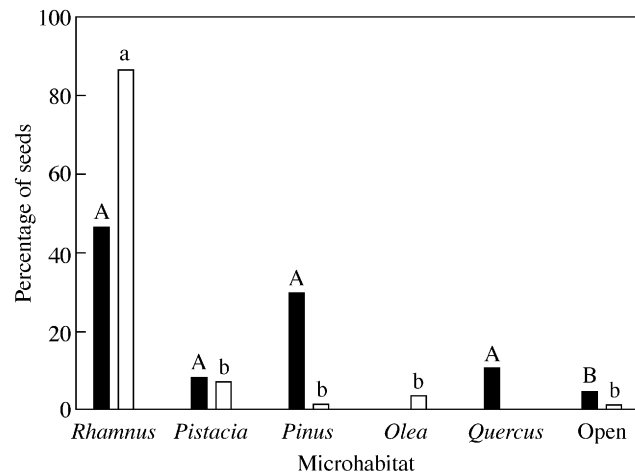


FIG. 2. Distribution of seeds of *R. alaternus* found in five different microhabitats in the two study populations, Esporles (filled bars) and Lloret (open bars). Results are expressed as percentages over the total number of seeds in the five microhabitats. The area considered for each microhabitat was 0.86 m². For each population, different letters indicate significant differences among microhabitats ($P < 0.05$).

The seed dispersal phase

Mature fruits were available from early June to mid-July at Lloret and from early June to late-July at Esporles. The majority of fruits were removed naturally, leaving only 4.2 and 13.8 % that dried on the branches at Lloret and Esporles, respectively. A greater proportion of non-removed fruits was observed at Esporles compared with Lloret, which can be attributed to a major fruit crop in the former population.

TABLE 2. Percentage of fruits and seeds of *R. alaternus* that were removed after 2 weeks in each microhabitat in the two study populations

Microhabitat	Esporles		Lloret	
	Control	Vertebrates excluded	Control	Vertebrates excluded
<i>Rhamnus</i>	100 ± 0	48 ± 15	100 ± 0	100 ± 0
<i>Pistacia</i>	100 ± 0	60 ± 24	100 ± 0	100 ± 0
<i>Quercus</i>	100 ± 0	64 ± 13	92 ± 8	88 ± 12
<i>Pinus</i>	100 ± 0	64 ± 19	100 ± 0	84 ± 16
Open	84 ± 16	72 ± 14	92 ± 8	68 ± 21

Values are means ± s.e. of five replicates

Unfortunately, no information is available on bird densities in either population.

Seed rain differed significantly among microhabitats and populations (Fig. 2). Both at Esporles and Lloret, most seeds were found under female conspecifics. However, this proportion was much higher at Lloret (87 %) than at Esporles (47 %). At the latter site, seeds were also found in a moderate proportion under pine trees (30 %), and in a lower proportion under oak trees (10 %) and under *Pistacia* shrubs (8 %). In contrast, seeds were more poorly dispersed at Lloret, under *Pistacia* (7.5 %), olive trees (3.5 %) and pine trees (1.5%). Open inter-spaces was the microhabitat with the lowest seed rain in both populations (5 and 1.5 % in Esporles and Lloret, respectively). The distance from any fruiting *Rhamnus* to trays placed in a particular microhabitat was similar in each population, and in all cases the differences were low enough to be considered negligible to a bird (10.54 ± 1.36 m at Esporles; 7.03 ± 0.81 m at Lloret). The greater distance at Esporles than at Lloret might be a consequence of the lower density of plants in the former population, as it corresponds to a pine–oak mixed forest.

Post-dispersal seed predation was very high in both populations. Most seeds had disappeared after 2 weeks at both sites, mainly from the dishes open to all predators (Table 2), although the difference between treatments was significant at Esporles ($F_{1,40} = 17.52$; $P < 0.001$) but not at Lloret ($F_{1,48} = 2.309$; $P = 0.135$). In contrast, there were no significant differences between microhabitats in both populations, and there was no significant interaction between the effects of microhabitat and treatment (all $P > 0.05$).

Seedling establishment

Seedling emergence occurred during autumn and early winter in both populations and years (Figs 3 and 4). No emergence was observed in spring, although the temperature was optimum during this period. In both years, a marginally significantly higher number of seedlings emerged at Esporles than at Lloret ($F_{1,47} = 3.421$; $P = 0.071$). Non-significant differences were observed in seedling emergence between 2001 and 2000 ($F_{1,47} = 0.340$; $P = 0.563$). Similarly, no differences were recorded between microhabitats ($F_{1,47} = 0.093$; $P = 0.911$).

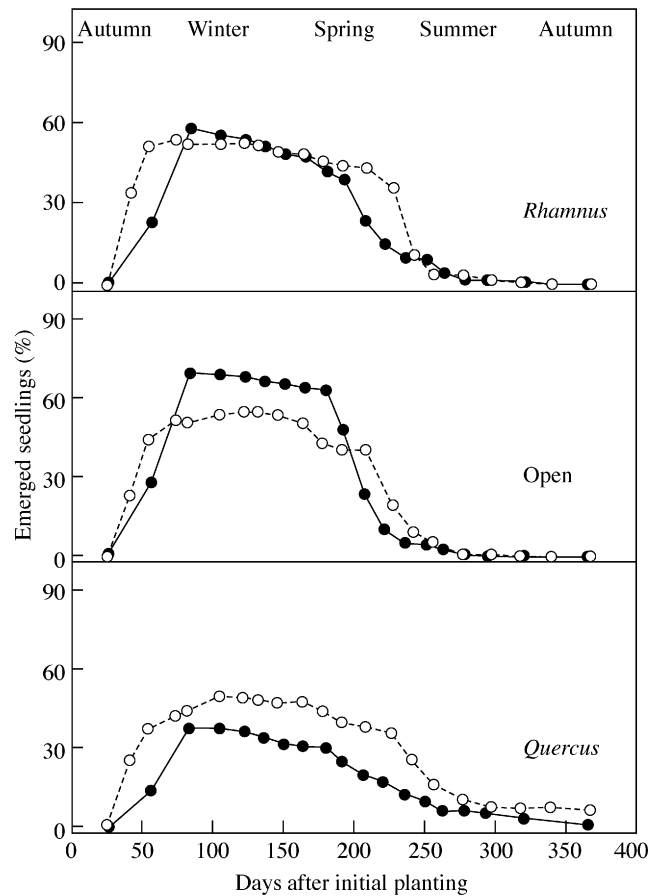


FIG. 3. Course of seedling emergence and survival in the Esporles population of *R. alaternus* from autumn 1999 to autumn 2000 (filled circles) and from autumn 2000 to autumn 2001 (open circles). Each plot represents seedling emergence and survival in the three microhabitats indicated.

Seedling survival (Figures 3 and 4) was significantly higher in 2001 than in 2000 in both populations ($F_{1,45} = 8.382$; $P = 0.006$). Moreover, seedlings disappeared earlier and more quickly during late-winter and spring of 2000, while this disappearance was more gradual during the spring of 2001. This pattern of seedling mortality and survival coincided with the precipitation pattern observed during 2000 and 2001 (Fig. 1). Seedling survival was not significantly different between the two sites ($F_{1,45} = 2.774$; $P = 0.103$). In contrast, marginal significant differences were found between microhabitats in both populations ($F_{1,45} = 2.957$; $P = 0.062$). No seedlings survived to the first year in the open inter-spaces in either population, while seedling survival under *Rhamnus* varied from approx. 2–20 %, and it was also highly variable under oak trees and under *Pistacia* (Table 3).

Transition probabilities (TP) between stages and overall probability of recruitment (OPR)

Table 3 shows the transition probabilities between the stages considered in this study and the overall probability of

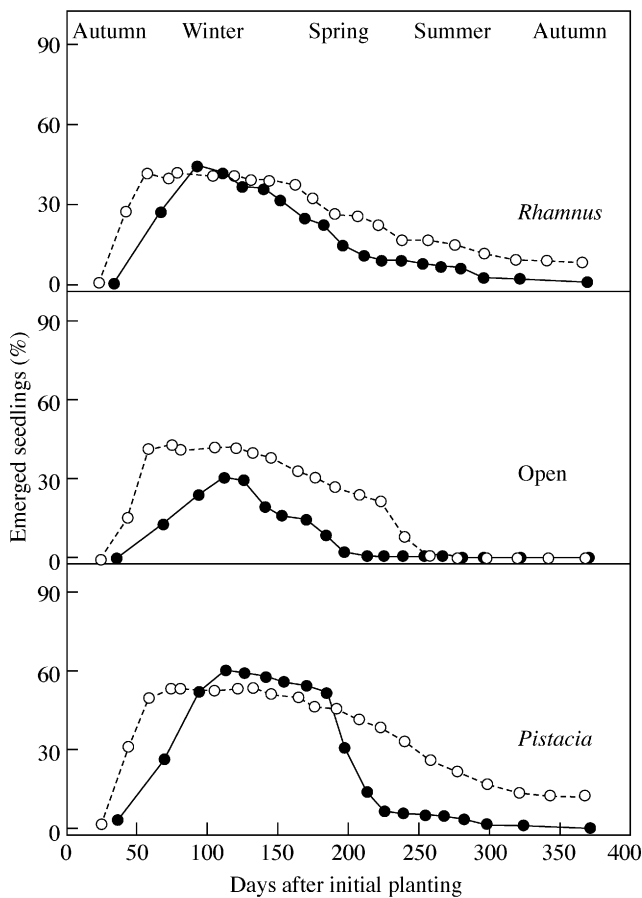


FIG. 4. Course of seedling emergence and survival in the Lloret population of *R. alaternus* from autumn 1999 to autumn 2000 (filled circles) and from autumn 2000 to autumn 2001 (open circles). Each plot represents seedling emergence and survival in the three microhabitats indicated.

recruitment. Fruit set appeared as a limiting stage, mainly at Lloret, where less than half of the flowers became mature fruits. On the other hand, the dispersal of fruits did not seem to represent a limiting process in the regeneration of *R. alaternus*. In contrast, post-dispersal seed predation was very high, and thus it can be considered as one of the main limiting stages in the regeneration of *R. alaternus*. In addition, seedling survival was also a serious limitation in the recruitment process, especially in dry years with a long-lasting summer drought period. In fact, less than 1% of emerged-seedlings survived more than a year in both populations in the 1999–2000 period. These proportions increased the following year, but this stage was still the most limiting, especially in the open inter-spaces microhabitat where all emerged seedlings died during the first year.

The OPR was consistently higher at Lloret than at Esporles for the 2 years of the study. In both populations, the greatest recruitment probability was observed under female conspecifics, where seed rain was also highest. In contrast, there was no recruitment in open inter-spaces in any case due to zero seedling survival. Recruitment under *Pistacia* or *Quercus* was observed only in the second year.

DISCUSSION

Factors influencing the recruitment dynamics of Rhamnus alaternus

Spatial and temporal variation in pollen availability was found in our study populations; this finding is consistent with the variability observed in this species in other areas of its distribution, such as southern Italy (Aronne and Wilcock, 1995) and north-western Spain (Guitián, 1995). Such variation may be attributed to variation in insect densities, which usually depends upon environmental factors such as ambient humidity and temperature, or to variation in the

TABLE 3. Spatial dynamics of recruitment of *R. alaternus* in the two study populations. Transition probabilities (TP) for each monitored process are shown, as well as the cumulative probability for each microhabitat

	Esporles			Lloret		
TP ₁ Fruit set		0.66			0.45	
TP ₂ Fruit removal		0.88			0.96	
		↙	↘		↙	↘
Microhabitat (% vegetation cover)	Open (3.6)	<i>Rhamnus</i> (3.8)	<i>Quercus</i> (6.5)	Open (4.8)	<i>Rhamnus</i> (6.7)	<i>Pistacia</i> (32.9)
TP ₃ Seed arrival	0.05	0.47	0.1	0.01	0.86	0.08
TP ₄ Seed survival	0.01	0.01	0.01	0.01	0.01	0.01
TP ₅ Seedling emergence						
2000	0.68	0.58	0.37	0.31	0.44	0.60
2001	0.54	0.54	0.49	0.43	0.42	0.53
TP ₆ Seedling survival						
2000	0	0.015	0	0	0.019	0
2001	0	0.015	0.120	0	0.200	0.230
Cumulative probability (CP)						
2000	0	2.40 ⁻⁵	0	0	3.10 ⁻⁵	0
2001	0	2.20 ⁻⁵	3.40 ⁻⁵	0	3.11 ⁻⁵	4.20 ⁻⁵
Overall probability of recruitment (OPR)						
2000		9.01 ⁻⁷			2.10 ⁻⁶	
2001		3.06 ⁻⁶			3.47 ⁻⁵	

The overall probabilities of recruitment of the populations are calculated by weighting TPs by the relative abundance of each microhabitat

availability of pollen, which is probably lower in drier years. Low rainfall was also apparently responsible for the low seed viability observed at Lloret in 2000. Thus, seed abortion may be a limiting factor in the regeneration process of *R. alaternus* in very dry years. Fruit set was significantly correlated with the distance to the nearest reproductive male only at Esporles, despite this variable being very similar in both populations (4.08 ± 1.07 m and 3.91 ± 1.24 m at Esporles and Lloret, respectively). This might be due to the lower proportion of male individuals (and thus lower pollen availability) at the former site. No pre-dispersal fruit predation in *R. alaternus* was observed in our study, and hence this is not considered to be an important process in the recruitment of this species. In contrast, Bas (2001) reported pre-dispersal seed predation in *R. alaternus* by field mice (*Apodemus sylvaticus*), in the north-eastern Iberian Peninsula.

Fruit removal was quite high in both populations, and hence this is not considered a limiting factor in the recruitment of *R. alaternus*. However, seed shadows generated by dispersers were highly heterogeneous. The majority of seeds were found under female conspecifics in both populations, but especially at Lloret. This factor does not limit *per se* the recruitment process, but it reduces the colonization potential of this species. The absence of other species fruiting at the same time as *R. alaternus* in the populations considered could promote the preference of birds to use this species as a perch, causing the high concentration of seeds in this microhabitat. Many reports have shown greater seedfall under female conspecifics for other bird-dispersed species such as *Cercocarpus ledifolius* (Russell and Schupp, 1998), *Olea europaea* (Rey and Alcántara, 2000), *Rhamnus ludovici-salvatoris* (Traveset et al., 2003), *Pistacia lentiscus* and *Rhamnus palestinus* (Izhaki et al., 1991).

Post-dispersal seed predation was especially high in all microhabitats, and was due to both rodents and ants. Some differences, although not significant, were found between microhabitats. In this sense, post-dispersal seed predation was lower in open inter-spaces than in other microhabitats, which agrees with the results obtained by Hulme (1997) for other Mediterranean woody species. High levels of post-dispersal seed mortality have been reported for many species (Schupp, 1990; Verdú and García-Fayos, 1996a; Castro et al., 1999; Hampe and Arroyo, 2002). At Esporles, a greater removal was found from open than from vertebrate-excluded dishes, which indicates that rodents are the most important predators at this site. In Lloret, in contrast, seeds were removed mainly by ants; one of the commonest species observed on the dishes was *Pheidole pallidula*, a relatively efficient seed-disperser species (Bas, 2001; Gómez et al., 2003). Important levels of seed predation after dispersal have been reported for a variety of species such as *Pistacia terebinthus* (Traveset, 1994), *Cercocarpus ledifolius* (Russell and Schupp, 1998), *Grevillea sp.* (Auld and Denham, 1999), *Crataegus monogyna*, *Prunus mahaleb* and *Taxus baccata* (Hulme, 1997), and also in the more closely related *Rhamnus ludovici-salvatoris* (Traveset et al., 2003). As already pointed out, post-dispersal seed predation in this study

could be over-estimated due to two possible factors. First, secondary dispersal by ants has been reported for some *Rhamnus* species, including *R. alaternus* (Aronne and Wilcock, 1994; Bas, 2001; Gómez et al., 2003). Second, seeds are much more exposed and available to predators in a Petri dish than on the ground where they can be covered by soil or litter (Hulme, 1998). Hulme and Borelli (1999) reported greater post-dispersal predation of surface seeds than of buried ones in small-seeded species. Despite these two factors, the high post-dispersal seed predation observed in this study leads us to consider this stage as highly limiting in *R. alaternus* recruitment.

The seedling emergence recorded in the current study was around 50 % in most cases, which is similar to data reported by Gómez et al. (2003) for intact *R. alaternus* seeds (with elaiosome). However, seedling emergence rates as high as 90 % were recorded from non-manipulated seeds (data not shown), so we do not consider that the presence of the elaiosome could affect seedling emergence to a great extent. Seedlings emerged in autumn and winter in both populations, which suggests that the annual minimum temperatures reached at those sites are not low enough to limit seed germination and seedling emergence. In fact, germination of this species remains unaffected when seeds are exposed to temperatures as low as 5 °C (data not shown). This temperature is similar to the mean minimum temperature of the coldest month recorded in both populations (Guijarro, 1986). In contrast, the zero seedling emergence observed in spring, when the temperature is optimum but water availability is declining, suggests that drought is the most limiting factor in seedling emergence in both populations. Lower water availability might also explain the differences observed in seedling emergence between the 2 study years at Lloret. Seedling emergence differences between populations may be partially attributed to differences in seed viability (higher at Esporles than at Lloret). Seed viability, however, is probably not an important limiting factor, as large differences in this variable were observed at Lloret between the 2 years studied that did not translate into differences in seedling emergence.

Seedling survival was the most limiting factor in the recruitment process of *R. alaternus*. Although seedlings mainly died due to desiccation, other factors such as herbivores and/or pathogens cannot be ruled out. Such a high mortality occurs in other Mediterranean species, such as *Olea europaea* (Rey and Alcántara, 2000), *Helianthemum squamatum* (Escudero et al., 1999) and *Rhamnus ludovici-salvatoris* (Traveset et al., 2003). The seedling mortality took place as soon as water became scarce, which happened earlier at Lloret than at Esporles, and earlier in 2000 than in 2001. Moreover, seedling mortality was also higher in 2000 than in 2001, which is attributed to the greater rainfall recorded in the spring of 2001. Water stress reduces seedling growth (Khurana and Singh, 2000), and it has been reported to be the main cause of seedling mortality in other species such as *Frangula alnus* (Hampe and Arroyo, 2002), *Olea europaea* (Rey and Alcántara, 2000), *Pistacia lentiscus* (García-Fayos and Verdú, 1998) and *Phillyrea latifolia* (Herrera et al., 1994). Recruitment of *R. alaternus* is thus highly dependent on the

rainfall pattern, which is highly variable in the Mediterranean climate. In a climate-change scenario, in which a decrease in precipitation is predicted in the Mediterranean region (McCarthy *et al.*, 2001), the recruitment of this species could thus be highly reduced, even to levels that might threaten the species' survival in many areas where it is currently present.

Spatial discordance among stages and differences among populations

The general spatial distribution of seed rain is concordant with the seedling emergence and survival pattern in both populations. The microhabitat under *Rhamnus* was the site where most seeds were deposited and also where a great proportion of seedlings survived. There was also a high seedling survival under *Pistacia* and under *Quercus*, but seed rain was rather poor in these microhabitats. Seedling emergence was similar in all microhabitats, and it cannot be considered to exert a great influence on microhabitat differentiation in the recruitment process. Nevertheless, the proportion of seedlings emerged in open inter-spaces was higher than expected, since water availability and soil characteristics in this microhabitat are less favourable for seed germination and seedling emergence than those commonly found under shrub canopies (Verdú and García-Fayos, 1996b). The larger differences between day and night temperatures that are typically registered in open inter-spaces might well promote seed germination in this microhabitat, as has been shown in other species (Thompson *et al.*, 1977; Fenner and Kitajima, 1999). Moreover, a thinner layer of plant litter in the open might also facilitate seedling emergence compared with other microhabitats (Dzwonko and Gawroński, 2002). The relatively high seedling emergence in open inter-spaces, however, was neither concordant with the low seed deposition nor with the zero seedling survival observed in this microhabitat. Low seed deposition in open inter-spaces is common in avian frugivore deposition patterns (Izhaki *et al.*, 1991; Jordano, 2001 and references therein), and low seedling survival in this microhabitat is expected as water stress is longer and more intense where there is no canopy protection (Ibáñez and Schupp, 2001; Verdú and García-Fayos, 1996b).

The OPR values were rather low for both populations and in both years. In all cases, less than 1 % of *R. alaternus* flowers produced 1-year seedlings, with seedling survival being consistently the most critical stage. In both populations, the highest probability of recruitment was under conspecifics, and the lowest was in open inter-spaces. Therefore, the processes that constrain *R. alaternus* recruitment do not appear to depend upon the type of habitat where the species is found nor upon the population structure. Nevertheless, the OPR values were higher at Lloret than at Esporles in both years. This is surprising since Esporles has lower annual sunshine and higher annual precipitation, which presumably reduces the extent of summer drought, the main cause of seedling mortality. However, a deeper analysis of climatic conditions during the study period showed a higher precipitation at Lloret than at Esporles from May 2001 to August 2001 (Fig. 1), which corresponds

with the most critical period for seedling survival. In fact, seedling survival was considerably greater at Lloret than at Esporles that year. Moreover, the high seed rain accumulation under *Rhamnus* at Lloret (86 %), and its association with a relatively high seedling survival in this microhabitat (2 and 20 % in 2000 and 2001, respectively) could also be responsible for the greater OPR reported for this population.

In conclusion, the recruitment of *R. alaternus* was highly limited by seedling survival and post-dispersal seed predation in the two studied populations. Water limitation was the main cause of seedling mortality, preventing regeneration in some microhabitats, mainly in open-inter-spaces. The identification of the critical stages of the recruitment process of *R. alaternus* has important implications for management policies. In this sense, direct sowing cannot be considered an effective method to regenerate this species, because seed predation and summer drought would to a great extent reduce the emergence and survival of seedlings, respectively. Sapling planting, although much more expensive, could be more effective than direct sowing. In addition, at the two sites studied in particular, an effective management policy must take into account the control of the goat population in order to avoid damage caused by their heavy browsing pressure.

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