

Effect of seed passage through birds and lizards on emergence rate of mediterranean species: differences between natural and controlled conditions

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Summary

1. The ingestion of fruits by vertebrate frugivores produces great variation on seed germination responses which depend on (1) the frugivorous species; (2) several factors intrinsic to the plant species; and (3) the conditions under which germination tests are conducted. Most studies on this topic have used only one or a few disperser species, and have been performed under controlled conditions.
2. The main goals of this study were to determine (1) the effect that birds (*Turdus merula*) and lizards (*Podarcis pityusensis*) have on seed emergence patterns of a group of common mediterranean plants; and (2) whether such patterns differ between field and common garden conditions. Over 2 years we compared seed emergence times with those of controls (pulp-removed seeds).
3. Emergence patterns were inconsistent for most plant species when comparing field vs. common-garden conditions. In some cases results were even contradictory: for instance lizards accelerated the emergence time of *Rubus* seeds in the field, but delayed it in the experimental garden; likewise *Rubia* seeds ingested by Blackbirds emerged more slowly than controls in the field, but faster than those in the garden. The two frugivorous species had also inconsistent effects on the same plant species.
4. Significant changes in seed weight after passage through the animals' digestive tracts were observed in most species. However, seed weight did not explain differences in seed emergence patterns between ingested and non-ingested seeds.
5. This study demonstrates the great heterogeneity in seed-emergence responses of the different plant species to ingestion by different types of frugivore, and to the different experimental conditions. Thus, caution is needed when making generalizations from studies that aim to evaluate the influence of frugivores on seed dispersal quality.

Key-words: endozoochory, mediterranean vegetation, ornithocory, saurochory, seed emergence rate

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Introduction

It has been assumed for a long time that one of the advantages of endozoochory is germination enhancement – that seeds ingested by frugivores germinate in higher proportions, or more quickly, than non-ingested ones (Traveset & Verdú 2002 and references therein). However, an increasing number of studies have shown that such enhancement is far from universal, and that a variety of factors, both biotic and abiotic, may affect the outcome of seed treatment in the frugivores' digestive tracts (Traveset *et al.* 2001a, 2001b; Santamaría *et al.*

2003; Espinar *et al.* 2004; Verdú & Traveset 2005). This is of crucial importance for determining the quality of a particular disperser for a plant.

The conditions under which germination tests are performed are known to influence germination success (Bustamante *et al.* 1993; Figueiredo & Perin 1995; Traveset *et al.* 2001a, 2001b). It is thus important that seed responses to dispersers' gut treatments are examined in the field, as it is in the natural environment where we can test if a germination enhancement is adaptive or not. So far, most reported information comes from studies carried out under controlled (thus favourable) circumstances, usually in the laboratory (see review in Traveset & Verdú 2002), yet this may often obscure significant differences between treatments (Herrera 2000; Traveset *et al.* 2001a, 2001b).

On the other hand, most experimental studies that test the effect of gut passage on seed germination have been performed with only one or a few dispersers from all those available for the plant. Birds and non-flying mammals are the frugivorous taxa for which most data are available, as they are important dispersers for a great variety of plants (see review in Traveset 1998). In contrast, little is known about the effect of reptiles (but see Liu *et al.* 2004, Nogales *et al.* 2005 and references therein), despite the fact that they are also effective dispersers in some ecosystems, mainly in islands (Olesen & Valido 2003). Studies that compare the effect of different groups of dispersers on germination are scarce and have shown either consistent (Krefting & Roe 1949; Lieberman & Lieberman 1986; Mandujano *et al.* 1994; Traveset & Willson 1997) or inconsistent results (Lieberman & Lieberman 1986; Figueiredo & Perin 1995; Engel 1997; Nogales *et al.* 1998; Nogales *et al.* 2005). The latter are often attributed to the different retention times in the animals' guts (Izhaki & Safriel 1990; Barnea *et al.* 1991; Murphy *et al.* 1993; but see Barnea *et al.* 1990; Traveset *et al.* 2001, 2001a, 2001b), although other factors, such as type of food ingested along with the fruits (with variable water content, pH, proportion of plant material, etc.), are likely to influence the level of mechanical or chemical scarification of the ingested seeds (Traveset 1998; Traveset *et al.* 2001a, 2001b; Figuerola *et al.* 2002).

In the present study we chose fleshy fruited plant species common in the Mediterranean Basin, plus one endemic to the Balearic Islands, to examine the effect of seed passage through frugivores' digestive tracts on emergence rate (speed at which seeds emerge), simultaneously examining both in the field and in an experimental garden over 2 years. Here we use the terms 'germination' and 'emergence' time indiscriminately, although what we actually measured was the time at which the seedling emerges. For five of the species known to be dispersed both by birds and lizards (Sáez & Traveset 1995), we compared the effect of these two types of disperser. Given the much longer time seeds are retained within the digestive tract of lizards (2–4 days) compared to birds (usually 20–30 min), we hypothesized that the former were more likely to have a significant effect on germination patterns. We further predicted that differences in such patterns would not be detected in the field similarly to the experimental garden, due to the more favourable conditions – mainly temperature and humidity – in the latter.

Materials and methods

STUDY PLANT SPECIES

From August 2000 to January 2001 we collected fruits from 11 fleshy fruited species common in the western mediterranean flora, plus one endemic species from the Balearic Islands. Fruits of each species were obtained from a minimum of 10 individuals, simultaneously and

from the same site to avoid any effect of seed age or source on germination. The species were: *Crataegus monogyna* (Rosaceae), *Ephedra fragilis* (Ephedraceae), *Juniperus phoenicea* (Cupressaceae), *Osyris alba* (Santalaceae), *Pistacia lentiscus* (Anacardiaceae), *Rhamnus alaternus* and *R. ludovici-salvatoris* (Rhamnaceae, the latter endemic to the Balearic Islands), *Rubus ulmifolius* (Rosaceae), *Rubia peregrina* (Rubiaceae), *Solanum luteum* and *S. nigrum* (Solanaceae), and *Smilax aspera* (Smilacaceae). Hereafter we refer to them by their generic names only, except for the two *Rhamnus* and two *Solanum* species. The main fruit characteristics of all these species are given in Table 1.

FRUGIVOROUS SPECIES

The bird species chosen for the study was the Blackbird (*Turdus merula*; Turdidae), one of the most important dispersers of fleshy fruited plants in the Balearic Islands: it is sedentary and relatively abundant in these islands. We captured three individuals during summer 2000, and two during summer 2001, with mist-nets at several sites within an orchard site (in the latter year this species was especially rare due to a fungus infection that killed many individuals). Captured birds were kept in an indoor aviary, and housed separately in cages (60 × 60 × 60 cm) until the end of the experiment, when they were released at their sites of origin. They were maintained on a mixed diet of beetle larvae (*Tenebrio molitor*), commercial food for insectivorous birds and wild fleshy fruits. Blackbirds swallowed entire fruits of all species tested.

The lizard species used for the experiments was *Podarcis pityusensis* (Lacertidae), endemic to the Pithyusic Islands (Balearic Archipelago, western Mediterranean). This lizard (snout-vent length *c.* 10 cm) is omnivorous, including an important fraction of plant material in its diet (N.R., unpublished data). Twenty individuals were captured during spring 2000 in Formentera Island and transported to our laboratory in Esporles, Mallorca. They were kept in two indoor terraria (30 × 70 × 40 cm) and fed on a diet consisting of beetle larvae, tomatoes and commercial cat food.

During the frugivore ingestion experiments, fresh fruits of each species were collected as they became available in the field. They were given to each individual bird or lizard with an interval period of 24 and 36 h, respectively, to avoid any seed-mixing effects.

SEED-GERMINATION EXPERIMENTS

Seeds from bird droppings were collected from cages while still fresh and cleaned using paper towel. A similar sample of seeds was extracted and cleaned from fruits which were randomly chosen from the same source used for bird and lizard feeding. All seeds were individually weighted to the nearest 0.1 mg to determine if passage through the digestive tract of birds modified

Table 1. Main fruit characteristics for each study species (mean \pm SD)

Species	Fruit length (mm)	Fruit width (mm)	Fruit weight (mg)	N seed per fruit	Seed length (mm)	Seed diameter (mm)	Seed weight (mg)	Pulp water content (%)
<i>Crataegus monogyna</i>	8.9 \pm 0.6 (50)	8.1 \pm 1.1 (50)	416.9 \pm 142.8 (50)	1.1 \pm 0.2 (50)	6.6 \pm 0.8 (53)	4.9 \pm 0.7 (53)	111.2 \pm 46.5 (53)	66.56
<i>Ephedra fragilis</i>	6.6 \pm 0.5 (50)	4.0 \pm 0.6 (50)	55.5 \pm 11.0 (50)	1.0 \pm 0.0 (50)	6.0 \pm 0.4 (50)	2.2 \pm 0.2 (50)	16.7 \pm 4.0 (50)	58.8
<i>Juniperus phoenicea</i>	10.0 \pm 1.2 (50)	9.4 \pm 0.9 (50)	653.7 \pm 182.4 (50)	5.6 \pm 1.4 (50)	5.6 \pm 0.5 (150)	2.8 \pm 0.3 (150)	27.3 \pm 7.1 (150)	18.5
<i>Osyris alba</i>	9.8 \pm 0.8 (50)	8.5 \pm 0.8 (50)	633.0 \pm 139.5 (50)	1.0 \pm 0.0 (50)	6.7 \pm 0.9 (50)	6.0 \pm 0.9 (50)	149.9 \pm 23.5 (50)	64.9
<i>Pistacia lentiscus</i>	4.8 \pm 0.7 (50)	4.4 \pm 0.4 (50)	56.4 \pm 17.9 (50)	1.0 \pm 0.0 (50)	4.4 \pm 0.6 (50)	3.7 \pm 0.5 (50)	16.3 \pm 5.6 (50)	20.7
<i>Rhamnus alaternus</i>	6.3 \pm 0.5 (50)	5.5 \pm 0.6 (50)	129.2 \pm 39.1 (50)	2.1 \pm 0.1 (50)	4.6 \pm 0.3 (68)	2.4 \pm 0.3 (68)	9.5 \pm 1.3 (68)	–
<i>Rhamnus ludovici-sabatoris</i>	5.4 \pm 0.7 (210)	4.2 \pm 0.6 (210)	84.9 \pm 0.1 (210)	2.0 \pm 0.4 (210)	3.8 \pm 0.4 (210)	2.6 \pm 0.2 (210)	14.2 \pm 0.1 (210)	94.6
<i>Rubia perigrina</i>	4.9 \pm 0.7 (50)	4.4 \pm 0.7 (50)	105.8 \pm 49.2 (50)	1.3 \pm 0.5 (50)	3.8 \pm 0.5 (62)	2.8 \pm 0.3 (62)	31.1 \pm 8.2 (62)	68.5
<i>Rubus ulmifolius</i>	11.5 \pm 1.1 (50)	11.0 \pm 1.6 (50)	993.2 \pm 320.4 (50)	27.5 \pm 10.3 (50)	2.7 \pm 0.2 (180)	1.8 \pm 0.2 (180)	4.2 \pm 0.7 (180)	76.8
<i>Smilax aspera</i>	6.7 \pm 0.7 (50)	5.9 \pm 0.6 (50)	193.4 \pm 43.7 (50)	2.4 \pm 0.7 (50)	3.7 \pm 0.3 (117)	3.4 \pm 0.4 (117)	26.1 \pm 8.9 (117)	70.7
<i>Solanum luteum</i>	9.5 \pm 1.3 (50)	7.4 \pm 0.5 (50)	258.8 \pm 47.0 (50)	36.6 \pm 8.2 (50)	1.9 \pm 0.1 (250)	1.4 \pm 0.1 (250)	34.8 \pm 7.9 (48)*	83.2
<i>Solanum nigrum</i>	6.7 \pm 0.6 (50)	6.7 \pm 0.5 (50)	244.8 \pm 41.8 (50)	38.8 \pm 9.4 (50)	2.0 \pm 0.1 (250)	1.5 \pm 0.1 (50)	36.1 \pm 6.2 (50)*	83.6

*Seed weight measurements were obtained by weighing groups of 20 seeds due to the small seed size. Numbers in parentheses represent sample sizes.

seed weight, as found in other studies (Traveset *et al.* 2001a; Paulsen & Högstedt 2002). Seeds from lizard droppings were collected from the terraria and also cleaned, but they were not weighted as they were not considered to be fresh enough at the time of collection. All seeds were kept in paper bags, in dark and dry conditions, until the date of sowing. A total of 200 seeds of each species were used as controls (with the exception of *Osyris* and *Rubus*, with only 195 and 197 seeds, respectively). We also obtained 200 seeds of each species ingested by either birds or lizards, except in the case of *Rubia*, for which we could obtain only 100 seeds of the lizard treatment. Five of the species (*Crataegus*, *Juniperus*, *Osyris*, *Pistacia* and *Smilax*) were fed only to birds, as they were too large for lizards to swallow. For *Ephedra* and *R. alaternus* we could gather only a limited number of fruits, and we thus decided to test these species on a single disperser (birds for *Ephedra* and lizards for *R. alaternus*); the effect of ingestion by *T. merula* on germination patterns of *R. alaternus* had already been tested by Barnea *et al.* (1991). All seeds selected for sowing were apparently viable; aborted seeds (usually of a smaller size and/or different colour) were previously discarded.

In the field

The area used to test seed germination was a mediterranean scrubland located about 1 km from our laboratory, where the experimental garden is located. Seeds of *Crataegus*, *Osyris* and the two *Solanum* species were planted near a temporary stream, as these species are usually found in relatively humid conditions; the rest of species were sown in an open site where shrubs of *P. lentiscus*, *Olea europea*, *R. alaternus*, *R. ulmifolius*, *Ceratonia siliqua* and *S. aspera* are relatively abundant. At each location (open site and near stream), we divided the area into five quadrats (stations) and planted the seeds of all species and treatments in them. Before planting seeds, superficial land and weeds were removed to prevent germinations from the seed bank. In each station we planted groups of 20 seeds of each treatment and species, equally spaced and 5 cm separate from each other. Seeds were planted at a depth of 0.5–1 cm, and each group was protected with a cage (30 \times 20 \times 2.5 cm) made of wire mesh to prevent rodent predation. Stations were separated from each other by at least 5 m. Feeding experiments were performed at different times depending on fruit availability in the field, thus seed sowing was not simultaneous for all species: seeds of *Rubus*, *Rubia*, *Smilax*, *Osyris* and *Crataegus* were planted on 1–5 December 2000; *Pistacia* on 9 February 2001; *Juniperus* and the two *Solanum* species on 7 March 2001; and *Ephedra* and the two *Rhamnus* species on 28 November 2001. Germination and seedling survival were monitored weekly or every 2 weeks, depending on seed-germination rate, until 27 December 2002 when no new seedlings had germinated for 7 months.

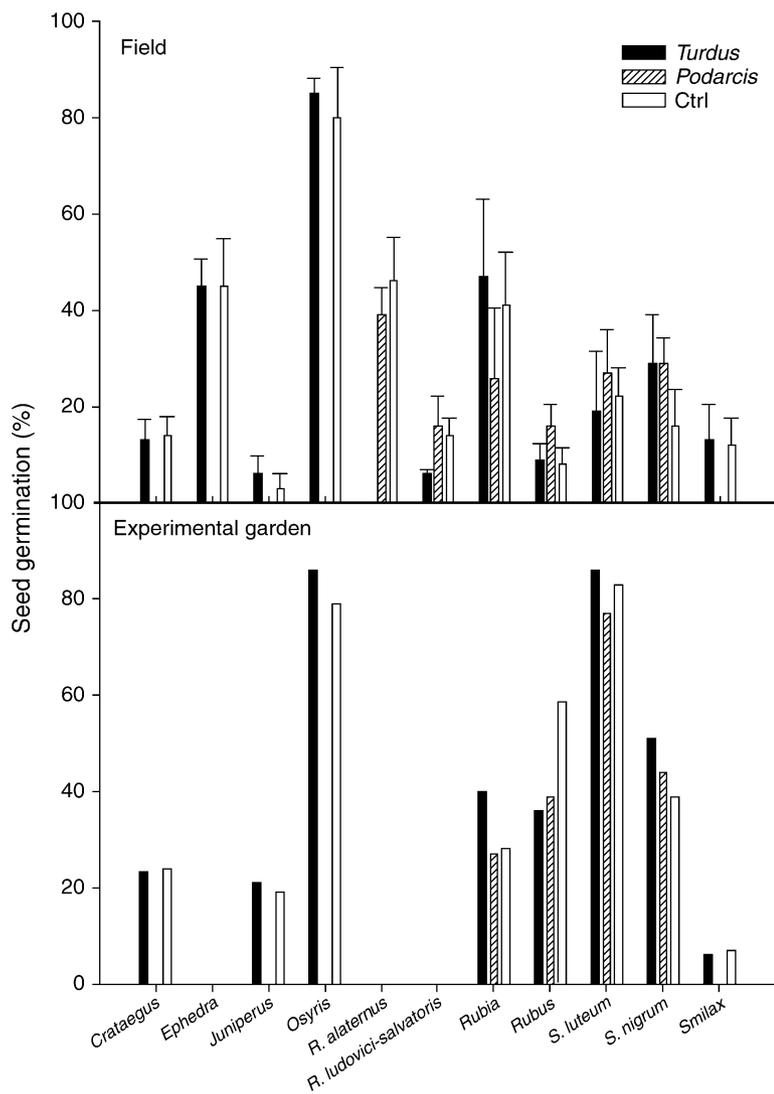


Fig. 1. Final seed germination obtained for each plant species at the end of the study period, under both field and experimental garden conditions. Standard error bars are shown only in the first case, as different stations were used as replicates (see Materials and methods). Note that in some cases (such as the Blackbird treatment in *Rubia*) final seed germination was higher although germination rate (speed) was lower.

In the experimental garden

Simultaneously with the planting in the field, seeds of each species and treatment were sown in the experimental garden. They were planted individually in trays of 60 pots (4 × 4 cm) each, filled with horticultural mixture, at a depth of about 0.5 cm. Trays were watered periodically to ensure permanent humidity, and seed germination was monitored every 7–10 days until 9 September 2002, when no seedlings had germinated for about 5 months.

DATA ANALYSIS

Differences in seed weight between treatments were tested by means of a general linear model, after logarithmically transforming the data to reach normality. These analyses were performed using the GENMOD

procedure of SAS VER. 8.02 for Windows (SAS Institute 2001).

The effect of seed ingestion on seed emergence time was tested separately for each plant species using failure-time analysis. A Cox proportional hazard regression model was fitted to the number of days between sowing and emergence for each seed. Using the parametric accelerated failure-time model, results were identical. Non-germinated seeds were modelled as right censored data due to the uncertainty that they could eventually germinate after the study period; for such seeds we thus considered the failure time as the number of days until the end of the experiment. Species such as *Crataegus* and *Juniperus* are known to have physiological dormancy (Baskin & Baskin 1998); *Smilax* is also likely to have such dormancy, given the low germination found in this study and elsewhere (Izhaki & Safriel 1990; Traveset *et al.* 2001a). Other species such as the two *Solanum*, *Rubus*, *Rubia* and *R. alaternus* might not have seed dormancy; however we decided to use a consistent model for all species studied. For the field data, station was added as a random or 'frailty' effect in the model as all treatments were applied to each station. Ties were estimated using the efron method, using the program S-PLUS 2000 (Mathsoft 1999).

Results

GERMINATION IN THE FIELD

Out of the 12 species tested, we found that seed ingestion by dispersers (either birds, lizards, or both) affected seed-germination rate in four species: *Rubus*, *Rubia*, *R. ludovici-salvatoris* and *S. nigrum* (Figs 1 and 2). No seeds of *Pistacia* germinated in the field during the study period, so this species was excluded from the analysis. Differences between treatments were only marginally significant in the case of *Rubus* (between lizards and control seeds), and in the case of *Rubia* and *R. ludovici-salvatoris* (between Blackbirds and control seeds): Blackbirds appeared to delay seed germination marginally in *R. ludovici-salvatoris* ($\chi^2 = 3.47$, $df = 1$, $P = 0.063$) and *Rubia* ($\chi^2 = 3.80$, $df = 1$, $P = 0.051$). However, lizards significantly accelerated germination in *Rubia* ($\chi^2 = 5.87$, $df = 1$, $P = 0.015$) and originally *Rubus* ($\chi^2 = 3.03$, $df = 1$, $P = 0.082$). The two *Solanum* species responded differently to ingestion by dispersers: while seeds of *S. nigrum* ingested by either disperser significantly advanced germination (Blackbirds, $\chi^2 = 4.97$, $df = 1$, $P = 0.026$; lizards, $\chi^2 = 4.20$, $df = 1$, $P = 0.040$), in the case of *S. luteum* no difference was observed between ingested and non-ingested seeds (Fig. 2).

GERMINATION IN THE EXPERIMENTAL GARDEN

Results obtained in the garden were rather different from those in the field (Figs 1 and 2). Under controlled conditions we detected a significant effect of seed

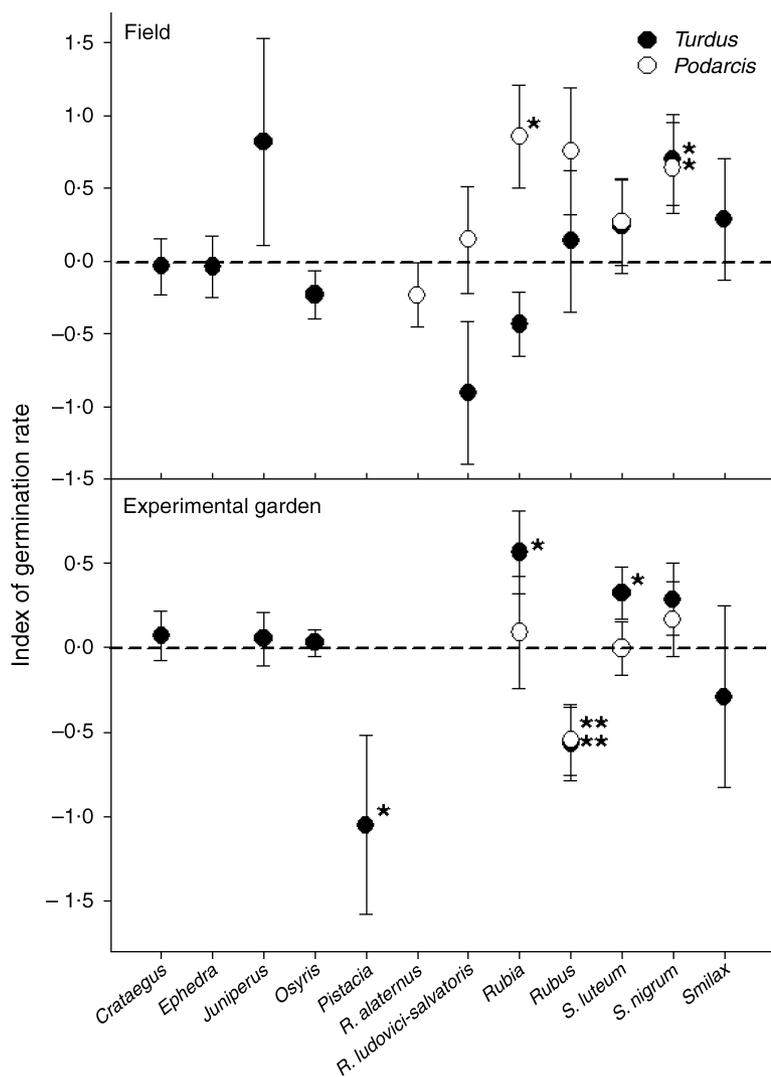


Fig. 2. Index of germination rate of each species in the experimental garden and under field conditions, calculated from the parameter estimates and obtained from separate Cox regressions (mean \pm SE). Data are expressed setting control treatments to zero. Data above and below zero indicate a higher or a lower germination probability, respectively, than the control treatment. Data from different species are not directly comparable as estimates are from different regression curves. Differences between treatments were assessed using the χ^2 test. **, $P < 0.01$; *, $P < 0.05$; †, $P < 0.1$.

passage through frugivores on seed-germination rate in *Rubus*, *Rubia*, *S. luteum* and *Pistacia*. In contrast to the field results, *Rubus* seeds ingested by either birds or lizards delayed germination compared with control seeds (Blackbirds, $\chi^2 = 6.97$, $df = 1$, $P = 0.009$; *Podarcis*, $\chi^2 = 6.86$, $df = 1$, $P = 0.008$; Fig. 2). For *Pistacia*, although seeds ingested by Blackbirds showed a reduction in germination time, only eight control seeds germinated ahead of one from the bird treatment (Fig. 1), thus the statistical difference observed does not make much biological sense. In contrast to field results, seeds of *Rubia* and *S. luteum* ingested by Blackbirds germinated more quickly than control seeds (*Rubia*, $\chi^2 = 4.55$, $df = 1$, $P = 0.023$; *S. luteum*, $\chi^2 = 4.17$, $df = 1$, $P = 0.037$; Fig. 2), but ingestion by lizards had no significant effect (*Rubia*, $\chi^2 = 0.55$, $df = 1$, $P = 0.780$; *S. luteum*, $\chi^2 = 0.06$, $df = 1$, $P = 0.980$).

CHANGES IN SEED WEIGHT AFTER INGESTION

Seed passage through the digestive tract of Blackbirds influenced seed weight in most plant species, although not consistently in the same direction. Most species suffered a slight loss of seed weight after ingestion by Blackbirds, whereas defecated seeds of three species (*Ephedra*, *Rubus* and *Smilax*) were heavier after being ingested (Table 2). Such differences in seed weight were not related to seed size; the correlation between maximum mean diameter and proportional mean weight change between ingested and non-ingested seeds was not significant ($r^2 = 0.042$, $n = 10$ species, $P = 0.561$). All fruits given to birds in the cages were eaten, thus the possibility that birds had selected larger or smaller fruits than controls is also discarded.

Discussion

Seed passage through the digestive tract of frugivores has long been found to affect the germinability and/or

Table 2. Weight of seeds (mg, mean \pm SE) in the two treatments (defecated by *Turdus merula* and control seeds, collected directly from the plant) for each species tested, and percentage of seed mass increase of ingested treatment relative to control seeds

Species†	Control	Defecated	Mass increase (%)	F
<i>Crataegus monogyna</i>	111.21 \pm 6.38 (53)	92.83 \pm 3.0 (183)	-16.5	$F_{1,234} = 4.47^*$
<i>Ephedra fragilis</i>	15.02 \pm 0.34 (140)	15.65 \pm 0.24 (172)	4.2	$F_{1,310} = 4.47^*$
<i>Juniperus phoenicea</i>	27.25 \pm 0.68 (150)	22.71 \pm 0.54 (150)	-16.7	$F_{1,298} = 22.31^{***}$
<i>Osyris alba</i>	149.89 \pm 3.32 (50)	144.22 \pm 1.70 (169)	-3.8	$F_{1,217} = 2.10$ NS
<i>Pistacia lentiscus</i>	14.65 \pm 0.43 (145)	12.91 \pm 0.33 (148)	-11.9	$F_{1,291} = 10.24^{***}$
<i>Rubia peregrina</i>	30.86 \pm 0.99 (65)	23.88 \pm 0.61 (150)	-22.6	$F_{1,213} = 33.20^{***}$
<i>Rubus ulmifolius</i>	4.18 \pm 0.05 (251)	4.48 \pm 0.07 (149)	7.2	$F_{1,398} = 11.28^{***}$
<i>Smilax aspera</i>	26.08 \pm 0.82 (117)	30.28 \pm 0.83 (150)	16.1	$F_{1,256} = 15.97^{***}$
<i>Solanum luteum</i>	34.33 \pm 1.12 (50)	27.52 \pm 0.45 (22)	-19.9	$F_{1,70} = 14.70^{***}$
<i>Solanum nigrum</i>	36.06 \pm 0.88 (50)	24.75 \pm 0.53 (22)	-31.4	$F_{1,70} = 67.24^{***}$

the germination rate of many plant species, which, together with the seed shadow and the quantity of 'intact' dispersed seeds, are essential factors determining plant reproductive and regeneration success (Schupp 1993). However, only a low fraction of studies have performed germination tests in the field, and they have used either one or a few dispersers (Traveset & Verdú 2002). There are also a few studies showing that the experimental conditions under which tests are carried out may lead to different responses in germination, and that such differences are not consistently in the same direction. One group of studies shows that differences in seed germination between treatments are usually magnified under harsh (field) conditions; for example Herrera (2000) detected germination differences between seeds from two pollination regimes only when planted in the field, but not in the glasshouse. Similarly, Traveset *et al.* (2001a) detected a positive effect of bird ingestion on seed germination in outdoor conditions, but not in a growth chamber or in a glasshouse. Another group of studies shows the opposite direction, with a greater effect in laboratory compared with field experiments (Bustamante *et al.* 1992, 1993; Figueiredo & Perin 1995; Yagihashi *et al.* 1998). Finally, a third group of studies finds similar results between the two conditions (Figueiredo & Perin 1995; Figueiredo & Longatti 1997). The present study further confirms, with a larger number of species, that a species may show germination enhancement/inhibition in the field but not under controlled conditions (in the laboratory or glasshouse), and *vice versa*: a significant effect of ingestion detected in controlled conditions may be screened off in the field. For *Rubia*, *Rubus* and *Solanum*, results were inconsistent and showed an inverse trend depending on seed germination conditions (Fig. 2). This demonstrates the caution needed when interpreting germination experiments that aim to evaluate the influence of frugivores on the quality of dispersal they provide to plants. Our suggestion is that future studies examining the quality of dispersal only under laboratory or garden conditions should also be carried out in the field, in conditions as natural as possible to the particular plant. However, under field conditions results may also depend on environmental and temporal stochasticity.

The present study also provides evidence for inconsistencies in germination responses depending on frugivore species. The effect of Blackbirds and lizards on germination rate was consistently significant only for *S. nigrum* in the field (germination was accelerated in the two treatments compared with controls), and for *Rubus* in the garden (seeds ingested by either frugivore germinated more slowly than controls). A previous study on *S. nigrum* that tested the effect of Blackbirds and Sardinian Warblers (*Sylvia melanocephala*) on germination in a common garden (Mas & Traveset 1999) showed a similar non-significant effect of the two bird species. Similar results to these (and also including a third bird species, *Pycnonotus xanthopygos*) were also

found by Barnea *et al.* (1990), although in that case the test was done under laboratory conditions. In both these previous studies, a significantly positive effect was found for *S. luteum*, which is also consistent with the effect of Blackbirds found in the experimental garden in the present study. In another previous study, seeds of *Rubia* ingested by Blackbirds had been shown to germinate more slowly than if ingested by Sardinian Warblers (Traveset *et al.* 2001a); in such study, *Rubus* seeds passed through Warblers germinated more slowly than those passed through Blackbirds, although neither frugivore affected germinability. One possible explanation for the contrasting results between studies may be that seeds come from different populations, and it is thus possible that they differ in traits related to germination, such as seed coat structure, coat thickness or seed size. The different source of seeds might also be responsible for the contrasting results found for *Osyris* (the previous study had shown an important germination enhancement in Blackbird-ingested seeds compared with controls). These inconsistencies have been reported in a variety of species (Lombardi & Motta 1995; Nogales *et al.* 1999; Nogales *et al.* 2001, 2005). Even seeds of the same population, but of different age, have shown different germination responses to the same treatment (see References in Traveset 1998).

Differences among frugivores have often been attributed to different retention time in the gut (Barnea *et al.* 1991; Murphy *et al.* 1993; but see Barnea *et al.* 1990; Traveset *et al.* 2001a, 2001b). Gut passage time reported for passerine birds usually ranges between 20 and 60 min (see review in Traveset 1998), whereas for lizards it usually varies from 32 to 96 h (L. Santamaría, unpublished data). However, our results do not show any consistent effect of retention time on germination responses, as all possibilities were found: enhancement, inhibition or neutral effect of ingestion by lizards relative to the effect of Blackbirds. This suggests that other factors, probably interacting with seed retention time, are also important in determining germination speed and success. Significant effects of ingestion on germination have often been attributed to the degree of seed coat scarification, associated with morphological and physiological characteristics of the frugivores' digestive tracts (Jordano 1992; Traveset 1998). In addition, differences in the chemical composition of food ingested along with seeds can produce great differences both in seed retention time (Murray *et al.* 1994; Witmer 1996; I. Charalambidou and co-workers, unpublished data), and in mechanical or chemical abrasion of the ingested seeds (Clench & Mathias 1992) – with direct consequences for germination behaviour.

Seeds of most plant species tended to lose weight after passing through Blackbirds' digestive tract, a result consistent with that of Traveset *et al.* (2001a). The outcome probably depends on seed coat structure, not examined in this study. Interestingly, results for the common species (*Rubus*, *Rubia* and *Osyris*) in that previous study and the present one were consistent only

for *Rubia*; in contrast, seed weight of *Rubus* and *Osyris* in Traveset *et al.* (2001a) did not change, or significantly decreased, respectively, after bird ingestion. These inconsistencies may again be attributed to the particular seed traits of each species and population. Moreover, even the gut structure and food composition of each individual frugivore probably affect changes in seed weight. Ongoing research that examines the effects of individual plant and frugivore differences is expected to shed more light on this issue.

In the recent review by Traveset & Verdú (2002), seed ingestion by birds was found to have a significantly greater positive effect on germination than seed ingestion by either non-flying mammals or reptiles, which was mainly attributed to the shorter gut-passage time of the former. The results found in the present study do not follow this trend for most species tested. It is thus clear that a great heterogeneity of results exists, and we are still far from being able to predict the consequences for a given species with particular seed traits and in a particular environment. It is important to note that, even if seed treatment in the frugivore gut enhances germination, this does not necessarily suggest a positive effect on performance of the future plant – specifically on seedling fitness, growth and plant fecundity (Verdú & Traveset 2005).

The microhabitat where the defecated seed is deposited may determine the ultimate success of the seedling and future plant (Rey & Alcántara 2000; Traveset *et al.* 2003), and is perhaps even more important than the effect of seed treatment in the gut. For this reason, to know the quality of a disperser for a given plant we should combine the effect of ingestion on seed germination change with that of depositing the seeds in different microhabitats. A particular microhabitat may be more suitable for germination and seedling establishment than others but, in turn, may enhance germination of an ingested seed more than others. This possible effect of abiotic conditions has been tested recently with two helophytes (*Scirpus littoralis* and *Scirpus maritimus*); seeds ingested by ducks show a higher germination rate than control seeds under low salinity but not under high salinity (Espinár *et al.* 2004). Further research on how the abiotic environment influences seed germination responses to ingestion by frugivorous species is necessary in order to clarify the qualitative importance of seed dispersers for plant reproduction.

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