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Germination success of temperate grassland species after passage through ungulate and rabbit guts

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Summary

1 Dispersal of endozoochorous seed involves uptake by a herbivore and exposure to different kinds of digestive fluids during passage through the gastrointestinal tract. Assessment of the ecological significance of endozoochory therefore requires examination of the survival rate of seeds during this phase.

2 A feeding experiment was conducted with seeds of 19 plant species that are important constituents of temperate semi-natural grasslands and five animal species (two ruminants, two colon fermenters and a caecum fermenter). Mean retention time of germinable seeds was determined and seed characteristics that might affect germination success were examined.

3 Gut-passed seeds had a much lower germination success (0–26%) than non-gut-passed seeds either sown directly on dung (2–79%) or bare soil (7–89%).

4 Relative germination success differed considerably between both plant and animal species. This may result from complex, herbivore-specific interactions between animal behaviour (chewing, digestion) and seed characteristics.

5 Germination success was positively related to seed longevity and, remarkably, also to seed mass and seed shape. Retention time of germinable seeds varied from *c*. 12 hours (rabbit) to 72 hours (ungulates), potentially allowing long-distance seed dispersal. This study highlights both the complex interaction between animal species and seed characteristics and the considerable differences in germination success of gut-passed seeds, which exist between plant species. The loss of seed germinability after gut passage calls into question the ecological significance of endozoochory, although the costs of other dispersal mechanisms remain to be tested.

Key-words: endozoochory, gut survival, herbivore, indicator parameter, ruminant, seed dispersal, seed ecology, seed germination, temperate grassland

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Introduction

Several studies show the potential of dispersal following ingestion by ungulates and rabbits in the temperate and Mediterranean regions of Europe (e.g. Welch 1985; Malo & Suárez 1995; Dai 2000; Pakeman *et al*. 2002) and one of the most critical phases of such endozoochory is the passage of the seeds through the animal's digestive system. The impact of vertebrate frugivores on seed germination is well documented, but similar studies of the effects of grazing herbivores are rare. More information on the effect of gut passage on seeds from dry fruits is therefore needed, particularly for seeds from life-forms other than tropical trees (Traveset 1998; Traveset & Verdú 2002). Seed feeding experiments are important for determining which plant or herbivore traits influence seed survival after gut passage, and hence determine colonization and dispersal capacities. Most such experiments involve only one animal species or are strongly biased towards domestic ruminant species, i.e. sheep and cattle. Including different sized herbivore species, with different digestive physiology

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and morphology, would, however, allow us to study the effects of their specific gastrointestinal system. Studies may also be biased because the plant species used are often limited to graminoids and legumes.

In most ecological studies, assignment of a plant species to a dispersal category is still based on a simple binary scheme, classifying each species as either being of a certain dispersal mode or not. Tackenberg *et al*. (2003) have shown that, in the case of anemochory, the binary classification system will often result in misleading conclusions regarding a species' dispersal potential. Therefore there is a need for parameters that describe the continuous variation in dispersal potential across plant species (e.g. terminal velocity for anemochorous species, buoyancy for hydrochorous and germination success for endozoochory species (Bonn *et al*. 2000; Tackenberg *et al*. 2003)).

Seed feeding experiments have shown that passage through the gastrointestinal tract reduces the germination success of ingested seeds in some (e.g. Lennartz 1957; Özer 1979; Simao Neto *et al*. 1987; Gardener *et al*. 1993a) but not all (Russi *et al*. 1992; Ghassali *et al*. 1998) cases. This may be the result of the particular effect each herbivore species has on the germination response of seeds of particular plant species, but the existence of any such specific effect has not yet been explicitly demonstrated.

Many variables can influence germination success of gut-passed seeds. Two mechanisms that could determine how herbivores affect germination in dry-fruited plants are mechanical and/or chemical scarification of the seed-coat, which may depend upon chewing behaviour and gut retention time (Fredrickson *et al*. 1997) or on the type of food ingested with seeds (Jones & Simao Neto 1987), and the effect of surrounding faecal material on germination and/or future seedling growth (Quinn *et al*. 1994; Ocumpaugh *et al*. 1996).

This paper focuses on the seed germination success of plant species after passage through herbivore guts. The 19 plant species, which occur regularly in semi-natural grassland communities in Western Europe, have been shown to germinate from dung samples of free ranging herbivores. The five herbivore species included foregut fermenters (i.e. ruminants, sheep and cattle), hindgut or colon fermenters (donkey and horse) and caecum fermenters (rabbits). The main objective was to determine the combined impact of seed intake and gut passage, testing the following hypotheses. (i) Seed passage through a herbivore's gut will reduce the germinability of seeds as compared with uningested seeds either sown on bare soil or on dung + soil substrate (control). (ii) Animal species will have different effects on seed germination because of the differences in theoretical mean retention times of digestive products (Illius & Gordon 1992) and mouth morphology and chewing behaviour (Wallander *et al*. 1995; Mueller *et al*. 1998). (iii) Seed traits may show a relationship with germination success. We expect to find a negative relationship between seed mass and seed shape (expressed as seed length divided by seed width), and a positive relationship between the seed longevity index and seed germination success (Pakeman *et al*. 2002).

Materials and methods

PLANT SPECIES, SEED COLLECTION AND TREATMENT, SEED SIZES AND WEIGHT

The 19 plant species selected either form a substantial part of temperate West European grassland communities (e.g. *Poaceae*, *Juncaceae*, *Cyperaceae*) or are important for nature management, for instance by contributing to the specific floristic diversity (e.g. *Helianthemum nummularium* (L.) Mill., *Thymus pulegioides* L., *Trifolium arvense* L.). The results of previous field studies (Dai 2000; Pakeman *et al*. 2002; Cosyns & Hoffmann 2005) were taken into account to ensure inclusion of a broad range of possible germination responses as well a range of chances of being consumed (e.g. *Poa pratensis* L. has high frequency and abundance whereas *T. pulegioides* is very rare). Seeds with a range of morphological characteristics, possibly related to germination success (differentiation in seed size, shape and mass) were chosen (e.g. *Plantago lanceolata* L., with large seeds adapted to epizoochory, and *Centaurium erythraea* Rafn with very small seed). Seeds were either bought from a commercial supplier (Ecoflora, Halle, Belgium) or were collected from the wild from a large number of individuals during the summer of 2001 (Table 1). Nomenclature follows Lambinon *et al*. (1998).

Mean seed mass was determined by weighing five replicates of 250 air-dried seeds (accuracy ± 0.1 mg). Seed width and length measurements were carried out on 15 seeds without any of their appendices, i.e. plumes, hairs or awns (accuracy ± 0.1 mm). Seed longevity index was calculated according to Thompson *et al*. (1998) and the necessary data were obtained from Thompson *et al*. (1997). Prior to the seed feeding experiment seeds were kept dry and in darkness at room temperature.

ANIMAL SPECIES AND HOUSING

Seeds were fed to domesticated rabbits (*Oryctolagus cuniculus* L.; caecum fermenters) and to four domesticated ungulate species that are regularly used to aid conservation of semi-natural grasslands, two of which are ruminants (cattle (*Bos taurus* L.) and sheep (*Ovis aries* L.)) and two colon fermenters (donkey (*Equus asinus* L.) and horse (*Equus cabbalus* L.)). The animal species were assumed to have a different mean retention time because of variation in body mass and digestive system (Illius & Gordon 1992). Five individuals of each animal species were kept under similar indoor conditions: numbers used in previous studies have ranged from two (Gardener *et al*. 1993b) to four (Simao Neto *et al*. 1987), five (Russi *et al*. 1992), seven (Staniforth & Cavers 1977) and 10 (Ghassali *et al*. 1998).

Rabbits were housed in individual cages (0.8 × 0.8 × 0.5 m), sheep were housed in individual pens (*c*. 3 ×

Table 1 List of 19 plant species that were used in the feeding experiment ordered within families (ordered alphabetically), with indication of the mean mass (mean ± standard deviation), mean seed size (length × width) origin of seed collection and the number of seeds fed to the animal species indicated. The mean seed mass is based on 5 weighings each of 250 air-dried seeds. The mean seed size is based on measurements of 15 seeds each without any appendices, e.g. plumes, hairs, awns. *Indicates which animal species seeds were fed to

Plant family and species	Seed mass (mg)	Length × width (mm)	Origin of seed collection	Sheep	Cattle	Rabbit	Horse	Donkey
Asteraceae								
Crepis capillaris	0.29 ± 0.004	2.4 × 0.6	Commercial	*	*	*	*	*
Cystaceae								
Helianthemum nummularium	1.1 ± 0.105	1.8 × 1.4	Commercial	*		*	*	*
Cyperaceae								
Carex arenaria	1.0 ± 0.075	1.7 × 1.2	2001 (De Panne, Belgium)	*	*	*	*	*
Fabaceae								
Trifolium arvense	0.36 ± 0.008	0.8 × 0.7	Commercial (NW Germany)	*	*	*	*	*
Trifolium campestre	1.86 ± 0.057	2.6 × 2.2	Commercial	*		*	*	
Trifolium pratense	2.76 ± 0.08	2.1 × 1.8	Commercial	*		*	*	*
Trifolium repens	0.75 ± 0.01	1.0 × 0.9	Commercial	*	*	*	*	*
Gentianaceae								
Centaurium erythraea	0.01 ± 0.001	0.4 × 0.3	Commercial	*	*	*	*	*
Juncaceae								
Luzula campestris	0.7 ± 0.026	1.1 × 1.0	Commercial	*		*	*	*
Lamiaceae								
Prunella vulgaris	0.96 ± 0.029	2.2 × 1.2	Commercial (Belgium)	*	*	*	*	*
Thymus pulegioides	0.14 ± 0.008		Commercial (NW Germany)	*	*	*	*	*
Poaceae								
Agrostis capillaris	0.12 ± 0.003	1.0 × 0.3	Commercial (NW Germany)	*	*	*	*	*
Anthoxanthum odoratum	0.67 ± 0.025	2.2 × 0.9	Commercial (NW Germany)	*	*	*	*	*
Poa pratensis	0.35 ± 0.008	1.5 × 0.7	Commercial (NW Germany)	*	*	*	*	*
Plantaginaceae								
Plantago lanceolata	1.36 ± 0.09	2.9 × 1.3	Commercial (Belgium)	*	*	*	*	*
Rubiaceae								
Galium mollugo	0.53 ± 0.039	1.3 × 1.1	Commercial (Belgium)	*	*		*	*
Galium verum	0.27 ± 0.025	1.0 × 0.9	Commercial	*	*	*	*	*
Scrophulariaceae								
Veronica arvensis	0.11 ± 0.011	1.1 × 0.7	2001 (De Panne, Belgium)	*		*	*	
Veronica chamaedrys	0.27 ± 0.013	1.4 × 1.1	Commercial (NW Germany)	*	*	*	*	*
Number of seeds fed of each plant species/animal				1500	5000	250	2500	2500

3 m), while the other animal species were kept in stables. All enclosures had a flat, concrete floor that allowed accurate dung collection. Animals were fed a highly nutritious and digestible commercial pellet food with additional barley straw for the larger animals, except for cattle, which received additional maize silage. This food regime was applied from 10 days before seeds were fed, until 6 days thereafter. All animal species were fed twice a day (09.00 and 19.00) with equal proportions of the experimental diet: 1 kg of pellets day^{-1} animal^{-1} and 0.7 kg of straw day^{-1} animal^{-1} (sheep, horse and donkey), 0.1 kg of pellets day^{-1} rabbit^{-1} and 5 kg of pellets day^{-1} cow^{-1} plus unlimited silage. All animals had free access to fresh water.

FEEDING EXPERIMENT

In the morning of day 11, a known amount of seeds of up to 19 plant species (see Table 1) was mixed with the commercial food and offered to the animals in a bucket. Each individual animal was observed carefully to ensure that all seeds were consumed and, if necessary, the remaining seeds were mixed in the bucket with a small piece of bread that was then offered to the animal. In all cases animals consumed at least 99.9% of the total amount of seeds.

Immediately before seed feeding, all faeces in cages, pens and stables was removed and a faecal sample was taken from each animal as a control for possible 'external' seed contamination (sample at $t = 0$).

DUNG COLLECTION AND TREATMENT

After seed feeding all dung from each individual animal was collected regularly and put together in trays (40 × 40 × 5 cm) according to an *a priori* fixed time schedule, i.e. at t = +6 h, +12 h, +24 h, +36 h, +48 h, +72 h, +96 h, +120 hours. After air-drying in a

glasshouse (2–3 weeks, < 35 °C), the samples were kept at 2–4 °C for 2 weeks. During air-drying, microbial activity could have caused seed damage (J. E. Malo, personal communication). Air-dried faeces were weighed, slightly crumbled and homogenized. A subsample (150 g except for rabbits and sheep, where this was not always possible) was taken from each collection and spread out over a sand/commercial potting soil substrate (40 × 40 × 2 cm) in a layer of about 0.75 cm. To determine the effect of gut passage, five replicates of 100 seeds of each plant species were sown on a 0.75-cm layer of seed-free (t = 0) crumbled cattle dung (control pots). These control for possible 'crumbled dung effects' and estimate of gut passage effects under given conditions, rather than creating separate controls with seed-free dung of each species. Physical effects, such as crust formation and separation causing mortality of poorly established seedlings due to desiccation, can thus be discounted.

Five further replicates of 100 seeds of each plant species were sown on bare soil substrate (from the same sand/commercial potting soil mix), which enabled us to discriminate between effects of dung and of gut passage. All seeds were kept together with the dung samples at 2–4 °C for 2 weeks and were sown in separate pots in the same glasshouse. Twelve trays without dung or seed addition were used to detect possible germination from the potting soil substrate and contamination in the glasshouse.

All samples were watered twice a day during the whole period. Glasshouse conditions were kept at 20–25 °C with a relative humidity of 50–60% during 16 hours of light (range: 280–410 μmol m^{-2} s^{-1}) and at 10–15 °C and at 80–90% relative humidity during 8 hours of darkness. Seedlings were counted as soon as identification was possible (within 2 months) after which they were removed to avoid competition and to prevent flowering. Seedling emergence was monitored over a period of 6 months. Although more seedlings could have emerged if germination was allowed for a longer period (Malo 2000) there was little emergence during the last 2 months. Underestimation of seed germination success cannot, however, be excluded.

DATA ANALYSIS

Absolute germination success was calculated as

$$\left(\sum_{I=t6}^{t96} n_i \times W_{Di} \times (W_{ssi})^{-1}\right) \times N^{-1}.$$

n_i is the number of seedlings in the subsample (ss) from time period i (+6 hours, +12 hours … 96 hours). W_{Di} is the weight of all dung (D) produced during that period and W_{ssi} is the weight of the dung subsample (usually 150 g). N is the total number of seeds fed of a particular plant species. Absolute germination success was used when comparing gut-passed seeds with non-ingested seeds. When comparing the effect of gut passage between plant species, germination success of the gut-passed seeds was corrected for the germination success of the uningested seeds sown on dung + soil substrate ('relative germination success'). When comparing germination success of the same plant species between animal species, the cumulative germination percentage from all samples (i.e. up to 96 hours after feeding) was used. Prior to statistical analysis, data on germination success were arc-sin square root transformed to approach normality of the proportions.

Mean retention time of germinable seeds (MRT) was calculated by adding the times for passage of 5%, 15%, 25%, … 95% of the germinable seed and dividing by 10 (Gardener *et al*. 1993b; based on Castle 1956). In this procedure only those plant species were included that had an absolute germination success of at least 1%. Therefore only nine plant species allowed a comparison between animal species. More accurate mean retention time estimates could have resulted from a more frequent dung collection, e.g. at 6 or 12 hourly intervals, at least during the first 3 days after seed feeding.

STATISTICAL ANALYSIS

Estimates of absolute germination success were analysed using a two-way ANOVA with seed TREATMENT (ingested vs. uningested seeds) and PLANT species as fixed factors. Possible significant differences in germination success between ingested and uningested seeds on dung + soil (control) and between uningested seeds sown on dung + soil and bare soil were analysed with LSD-test (SPSS 11.0, Chicago, Illinois, USA). Differences in relative germination success were analysed with a repeated structure mixed model ANOVA with PLANT species and ANIMAL species as fixed factors and plant × animal as the interaction factor. Individual animal was the random factor repeated within each plant species. Number of degrees of freedom was estimated by the Satterthwaite method (PROC mixed, SAS system V8). All possible pairwise differences in relative germination success between animal and plant species were analysed with Tukey's honestly significant difference test (SPSS 11.0). The same analysis was used with mean retention time as dependent variable. To test for a possible significant impact of individual animals on germination success the mixed model was run with and without this random factor.

ANCOVA was used to test for a possible effect of seed characteristics, i.e. seed mass, seed shape (seed length/seed width) and seed longevity index (according to Thompson *et al*. 1998) on germination success and their possible interaction with animal species. ANIMAL species was entered as the fixed factor and seed MASS, seed SHAPE and seed LONGEVITY as covariates in the model. Seed mass and shape were Log_{10} transformed to get a more adequate description of the relationship and hence a better model (Neter *et al*. 1996). Model selection was based on a backward stepwise selection procedure. ANCOVA was run under the univariate SPSS routine (SPSS 11.0).

Results

No seedlings of the experimental plant species were found in any of the trays set up to detect possible contamination from the soil or glasshouse environment or from the t_0 dung samples that were collected just before seeds were fed. In both cases only a few seedlings (< 10) of *Juncus bufonius* L., *Juncus effusus* L., *Epilobium* sp. and *Oxalis corniculata* L. were recorded.

ABSOLUTE GERMINATION SUCCESS OF UNINGESTED AND INGESTED SEEDS

In almost all cases (except *Helianthemum nummularium*), gut-passed seeds showed a significant lower germination success than uningested seeds that were sown on a dung + soil substrate (Fig. 1; LSD-test, $P < 0.05$, Appendix S1 in Supplementary Material). However, absolute germination success varied according to treatment and plant species (Table 2), which was mainly related to the different germination response of uningested seeds that were sown either on the bare soil or dung + soil substrate (Table 3).

Table 2 The effect of seed treatment, plant species and animal species on absolute and relative germination success and on mean retention time of germinable seeds, as revealed by the univariate analyses of variance for these variables

Absolute germination success	d.f.	*F*	*P*
Seed treatment	6	976.26	< 0.001
Plant species	18	89.77	< 0.001
Treatment × plant	100	16.36	< 0.001
Error	500		
Relative germination success	**d.f.**	***F***	***P***
Plant species	18	100.63	< 0.001
Animal species	4	5.45	0.004
	64	5.04	< 0.001
Animal × plant	d.f.	χ^2	*P*
Individual animal (R)	1	53.78	0.001
Error	328		
Mean retention time	**d.f.**	***F***	***P***
Plant species	8	5.382	< 0.001
Animal species	4	133.03	< 0.001
Animal × plant	27	1.781	0.016
Error	139		

RELATIVE GERMINATION SUCCESS OF GUT-PASSED SEEDS

Relative germination success differed strongly between plant species (Fig. 2). Above that, their germination success varied individually between animal species, which is reflected in the significant plant–animal species interaction (Table 2). However, half the number of plant species (generally those with low or very low germination success) did not show significantly different germination success between animal species. Germination success of each of the other nine plant species varied considerably between animal species but no clear pattern across animal species was observed (Appendix S2). Despite this, seeds of some plant species that were ingested by sheep showed a significantly lower germination success compared with at least one of the Equid species or rabbit, e.g. *Agrostis capillaris* L., *Poa pratensis*, *Luzula campestris* and *Prunella vulgaris* L. (Tukey HSD test, $P < 0.05$).

Although relative germination success clearly varied between plant species for each of the herbivore species under study, some general patterns emerged (Appendix S2). Seeds of *Helianthemum nummularium* and *Trifolium*

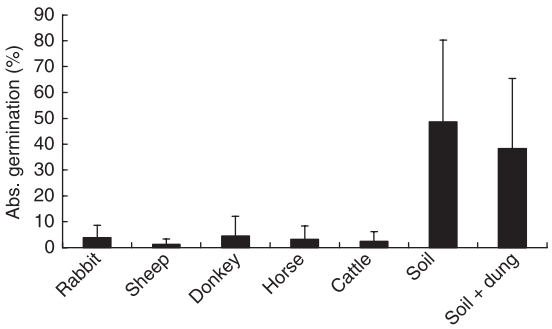

Fig. 1 Overall mean absolute germination success of gut-passed and uningested seeds (± standard deviation). Germination success was calculated across all plant species within each seed treatment (either ingested by rabbit ($n = 18$), sheep ($n = 19$), horse ($n = 19$), donkey ($n = 17$), and cattle ($n = 14$) or not ingested ($n = 19$), and either sown on bare soil or dung + soil substrate).

Table 3 Germination success of uningested seeds that were sown either on the bare soil or dung + soil substrate. Two plant species had a substantially higher germination success when sown on dung + soil substrate. Nine plant species were indifferent and eight species showed a significantly higher germination success when sown on the bare soil substrate

Germination success Better on dung + soil substrate	Indifferent	Better on bare soil
Trifolium campestre	*Anthoxanthum odoratum*	*Agrostis capillaris*
Veronica chamaedrys	*Galium mollugo*	*Carex arenaria*
	Galium verum	*Centaurium erythraea*
	Plantago lanceolata	*Crepis capillaris*
	Prunella vulgaris	*Helianthemum nummularium*
	Trifolium arvense	*Luzula campestris*
	Trifolium pratense	*Poa pratensis*
	Trifolium repens	*Thymus pulegioides*
	Veronica arvensis	

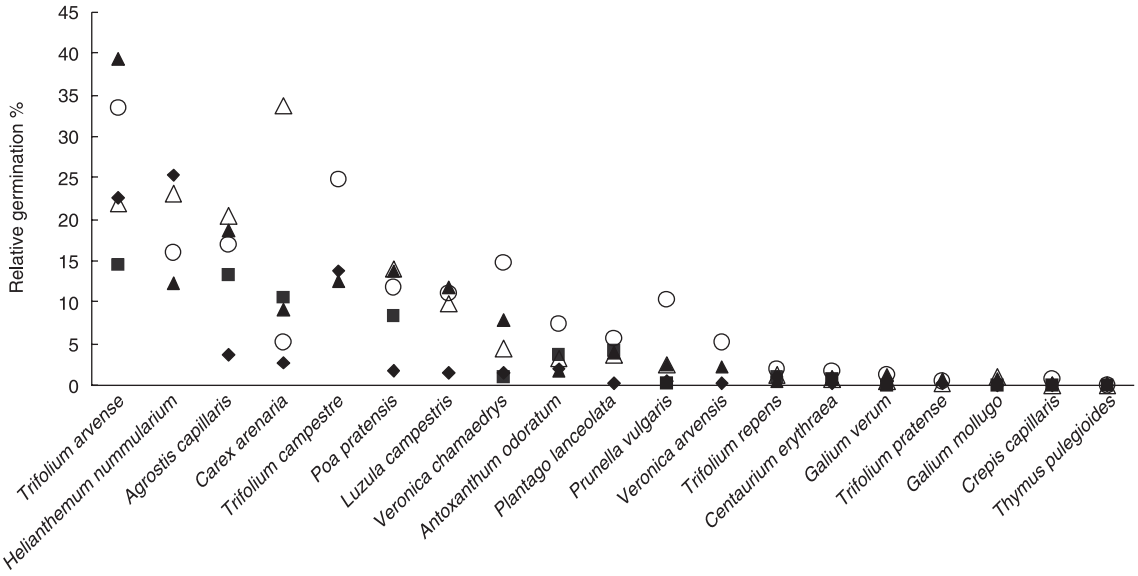

Fig. 2 Overall mean relative germination success of ingested seeds of different plant species. Each point represents the mean relative germination success of a given plant species calculated across the five individuals of each of the indicated animal species (■ = cattle, ○ = rabbit, ◆ = sheep, ▲ = horse, △ = donkey). Error bars were not shown for practical reasons (but see Appendix S1 for detailed information).

arvense had a significantly higher germination success than seeds of most other plant species except *T. campestre* and several graminoids, i.e. *Agrostis capillaris*, *Poa pratensis*, *Luzula campestris* and *Carex arenaria* L.. Gut-passed seeds of those species differ significantly from *Galium verum* L. and *G. mollugo* L., *Crepis capillaris*, *Trifolium pratense* L. and *Thymus pulegioides*, which had among the lowest germination success (Tukey HSD test, $P < 0.05$, Appendix S2).

Within the same animal species germination success varied considerably between individuals and some of the observed variance (*c*. 4%) could be explained by this variation (Table 2).

MEAN RETENTION TIME OF GERMINABLE SEEDS

The variation in MRT of germinable seeds of individual plant species differed between animal species (significant animal–plant species interaction, Table 2). Although rabbits tended to defecate germinable seeds significantly faster than any of the other animal species (Fig. 3), deviations from this general pattern were observed for *Agrostis capillaris*, *Poa pratensis*, *Plantago lanceolata* and *Trifolium arvense*.

MRT only differed significantly between plant species for cattle that defecated germinable seeds of *Plantago lanceolata* faster than seeds of *Carex arenaria* (Appendix S3).

SEED CHARACTERISTICS AND GERMINATION SUCCESS

The selected model explains 18% of the variability in germination success ($r^2 = 0.186$). Overall mean germination success varied significantly between animal species (sheep, 0.07 ± 0.011; cattle, 0.09 ± 0.013; donkey, 0.13 ± 0.012; horse, 0.13 ± 0.011; rabbit, 0.16 ± 0.011) (see also Fig. 1). Animal species ($F_{4,402}$: 7.9, $P < 0.001$), seed mass ($F_{1,402}$: 11.2, $P = 0.001$), seed shape ($F_{1,402}$: 39.3, $P < 0.001$) and seed longevity ($F_{1,402}$: 30.8, $P < 0.001$) had a strong impact on the model. Germination success was positively related to seed longevity (slope: 0.15 ± 0.026, $P < 0.001$), seed mass (slope: 0.04 ± 0.012, $P = 0.001$) and seed shape (slope: 0.19 ± 0.030, $P < 0.001$). No significant interaction terms between seed characteristics and animal species were observed.

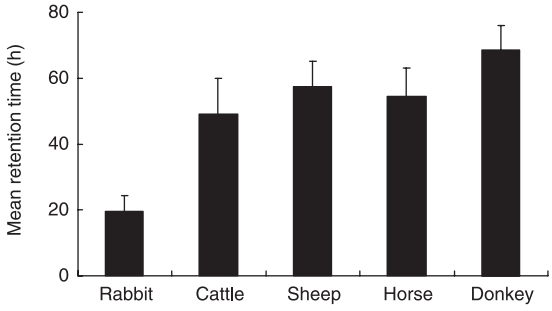

Fig. 3 Overall mean retention time, of germinable seeds that have passed through the gastrointestinal system of the indicated animal species. Mean retention time was calculated across all plant species that had a germination success of at least 1%. Rabbits defecate germinable seeds significantly faster than any of the other animal species, though some deviations of this general pattern were observed (Appendix S3).

Discussion

GERMINATION SUCCESS AFTER GUT PASSAGE

Passage through the gastrointestinal track significantly reduced germination success of all studied plant species, which was in agreement with results from most

other seed-feeding experiments (e.g. Lennartz 1957; Özer 1979; Gardener *et al*. 1993a; but see Ghassali *et al*. 1998 and Russi *et al*. 1992 for opposing conclusions). However, in contrast to previous experiments, five animal species with different gastrointestinal systems were used in this study to test for possible differences in impact on germination success. We expected an inverse relationship between germination success of plant species and estimates of mean retention time (lowest in rabbit, moderate in equid species (30–31 hours) and sheep (41 hours) and highest in cattle (71 hours), based on Illius & Gordon 1992). Such a relationship between germination success and mean retention time of germinable seeds was not evident nor was the predicted pattern in mean retention time. Nor was there any simple relationship between animal species' digestive strategy, body weight and germination success of gut-passed seeds. This may be the result of a complex interplay of several animal and plant species characteristics (e.g. chewing intensity and effectiveness of digestion), which vary independently between animal species (Staniforth & Cavers 1977; Simao Neto *et al*. 1987), and seed characteristics (e.g. seed size, shape, hardness).

Nevertheless, seeds passing through sheep guts tend to have a lower germination success compared with the other animal species. Similar results were found by Shayo & Udén (1998) and Simao Neto *et al*. (1987), who both recovered lower amounts of seeds of tropical plant species from sheep and goats compared with cattle in their feeding experiments. Like us, Simao Neto *et al*. (1987) found similar seed passage rates for sheep, goats and cattle, and attributed the observed large differences in seed recovery to differences in initial mastication and rumination. Mueller *et al*. (1998) found lower chewing rates and a faster consumption rate of fibre for donkeys than has been reported for equal or greater sized ruminants. Moreover mean retention time of germinable seeds in donkey was 66 hours, which is similar to the observed mean retention time of the larger sized cattle and horse. This was also higher than expected from calculations of mean retention time based on body mass (Illius & Gordon 1992). Germination success on average is relatively high in rabbits, certainly if compared with sheep, which is in clear contrast with the results reported by Lehrer & Tisdale (1956), who found a consistently 10-fold lower germination percentage in rabbit (0–0.64%) than in sheep (1.07–2.47%) for seeds of four forb and three grass species. Although intense initial chewing could be expected in rabbits, we assume that a combination of rumination and the longer retention time of sheep could have caused more seed damage than initial thorough chewing of the fed material.

Within each animal species there was a rather similar basic pattern of differences in relative germination success between plant species (Fig. 2, Appendix S2), suggesting an underlying overall effect of certain seed characteristics. It appeared that ovate-lanceolate seeds, rather heavy seeds and seeds with a high longevity index were likely to have among the highest relative germination success within all animal species. The unexpected positive relationship (e.g. Janzen 1984; Pakeman *et al*. 2002) of seed shape and seed mass with germination success must be interpreted within the context of this experiment, i.e. a limited set of plant species with a limited range of seed sizes (Table 1). The results reflect the high relative germination success of most graminoids, all of which had small, slightly elongated seeds (seed shape: 0.2–0.4). Pakeman *et al*. (2002) concluded from their regression analysis, based on relative seed density data of 21 and 35 temperate plant species, respectively, in dung of free ranging sheep and wild rabbit that only the longevity index was positively correlated with this variable. This is in concordance with the results of this experiment.

CONSEQUENCES FOR SEED DISPERSAL AND NATURE MANAGEMENT

Comparing the results of this feeding experiment with those of several field studies conducted in temperate grassland ecosystems gives rise to further considerations about the significance of endozoochory in the dispersal and regeneration of plant species. The potential for long-distance endozoochory depends on the number of viable seeds consumed by the possible dispersers and the effects of digestive systems on germination success and mean retention time of germinable seeds. Most field studies report the germination of many seedlings of a wide variety of grassland species from ungulate and rabbit dung (Malo & Suárez 1995; Dai 2000; Pakeman *et al*. 2002). This implies that a large number of seeds must have been consumed to compensate for the generally low germination success after gut passage. One may, however, question the efficiency of endozoochory for most temperate grassland species, as the process of mastication and gut passage appears to impose a high cost. Little is known about the relative costs and benefits of other seed dispersal mechanisms. A first insight is offered by Pakeman *et al*. (1998), who calculated that rabbit endozoochory contributed 15% to the developing vascular plant cover in a cool temperate, acidic grassland compared with 40% for other means of dispersal and 45% for regeneration from the seed bank.

On the other hand the observed mean retention times of germinable seeds do favour long-distance seed dispersal (Bonn & Poschlod 1998; Pakeman 2001; Bruun & Fritzbøger 2002). Rabbits have a smaller home range but may still be important seed dispersers at the medium scale (< 150 m), certainly if the germination success observed here is taken into account, together with their sometimes high population density.

To understand, from a plant's-eye-view, the role and relative importance of endozoochory compared with other dispersal modes occurring in West European semi-natural landscapes, we need to quantify the relative contribution of different dispersal modes to later

generations of the plants. This will require an integrated approach, combining information from field observations on the frequency of occurrence of the different dispersal modes with data on the places where seeds are deposited, and considering factors such as the assumed directed nature of endozoochory (between favoured grazing sites) and consequences of seed arriving as part of a dung pat (see Gökbulak 1998).

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Supplementary material

The following material is available from http://www.blackwellpublishing.com/products/journals/suppmat/JEC/JEC982/JEC982sm.htm

Appendix S1 Mean absolute germination success, under glasshouse conditions, of uningested seeds from pots and of gut-passed seeds.

Appendix S2 Relative germination success of gut-passed seeds of different plant species within and between animal species.

Appendix S3 Mean retention times of germinated seeds.

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