Ecological correlates of seed survival after ingestion by fallow deer

A.M. Mouissie, E. J. van der Veen, G.F. Veen and R. van Diggelen

Abstract

The ability of plant species to tract rapid climate change, persist in highly fragmented landscape and reach habitat restoration sites depends critically on long-distance seed dispersal. Seed ingestion and defecation by large herbivores is a potentially important long-distance seed dispersal mechanism. The ‘foliage is the fruit hypothesis’ suggests that many herbaceous plants have seeds, adapted to survive mastication and digestion, and foliage adapted to attract large herbivores. Here we show that seeds of 24 of 25 fed plant species can survive ingestion and defecation by fallow deer. Seed survival is negatively related to seed mass and variance of unit seed dimensions and positively related to seed longevity, but not related to habitat fertility. The log_{10} transformed product of seed mass and variance of seed dimensions is the best predictor of seed survival, explaining 46% of variance in the data. Our results do not support the ‘foliage is the fruit hypothesis’, because seed survival does not seem related to foliage edibility. The ecological correlates of seed survival, presented in this paper, can help to estimate the ability of plants to disperse long distances.
Introduction

The ability of plant species to track rapid climate change, persist in highly fragmented landscape and reach habitat restoration sites depends critically on long-distance seed dispersal (Clark 1998, Bakker & Berendse 1999, Cain et al. 2000). Some plant species have light plumed seeds, burrs or fleshy fruits that enable long-distance dispersal by respectively, wind, epizoochory and endozoochory. However, most species lack such diaspores and depend on non-standard means of dispersal (Higgins 2003). For example, the ant-dispersed woodland herb, *Trillium grandiflorum* is occasionally dispersed beyond 3 km by white-tailed deer (Vellend et al. 2003). For many plants large herbivores are likely candidates to serve as long-distance seed dispersal vectors, owing to their large home range sizes and long seed retention inside their guts (Pakeman 2001). The ‘foliage is the fruit hypothesis’ even suggest that for a large number of small-seeded herbaceous species, a normal and adapted for mode of dispersal is through ingestion of seeds while large herbivores consume their foliage (Janzen 1984). Surveys showing that herbivore dung often contains high densities of viable seeds (Welch 1985, Malo & Suarez 1995b, Pakeman et al. 2002), seem to corroborate this hypothesis. However, the foliage is the fruit hypothesis has received little experimental testing and there is little quantitative information available on the mechanism of endozoochory.

A crucial phase in the dispersal of plant seeds by large herbivores is the survival of the molar mills, digestive fluids and bacteria after ingestion. Seeds adapted for endozoochory should be small, round and hard. In addition foliage edibility and composition should attract large herbivores to ingest seeds, like fruits attract frugivores (Janzen 1984).

Due to a lower carbon/nutrient ratio, plant species associated to fertile soils are more palatable than species associated to less fertile soils (Bryant et al. 1983, Hobbie 1992, Iason & Hester 1993). Hence, if palatability and seed survival are co-evolved traits there should also be a relationship between habitat fertility and seed survival.

Surveys of the seed contents of herbivore dung indeed show that small, round seeds (Pakeman et al. 2002) and species associated to fertile soils (Mouissie et al, unpublished) are relatively frequently dispersed via dung. However, these surveys do not provide a causal explanation for this. Does it result from higher seed survival, higher seed ingestion or perhaps simply from higher seed production? Seed feeding trials conducted so far do not provide an answer, because they have been directed at specific species or species from a specific system; eg six tropical pasture species (Simao Neto et al. 1987), grass and legume seeds (Gardener et al. 1993).

In the present paper, we address this issue by feeding fallow deer known seed quantities of 25 plant species and recovering of seedlings from their dung. We test how seed survival relates to seed size, seed shape and habitat fertility. Such ecological correlates of seed survival give insight in the evolution of endozoochory and can help to estimate the ability of plant species for long-distance dispersal.
Methods

Experimental design

The experiment was conducted at a deer enclosure (450 m²) ‘De Bosrand’ in Lieren, the Netherlands. Five fallow deer (Dama dama) inhabited this enclosure, one adult male, two adult females and two yearling males. The adult male refused to be hand fed and could not be used in the experiment, but its dung served as a control for seed ingestion. The deer grazed on short non-seeding grass covering the enclosure. In addition they were fed daily with commercial deer fodder. The yearlings ate the same as the adults.

We selected 25 plant species native to the European flora, obtaining a large range in seed mass, seed shape, Ellenberg Nitrogen indicator values (Ellenberg 1974, Ellenberg et al. 1992) and seed longevity index (Bekker et al. 1998). Seed shape was quantified as the variance of unit seed dimensions, following Thompson et al. (1993a). Ellenberg Nitrogen indicator values (0-9) are designed to estimate the relationship between vascular plant species and the availability of nutrients in the soil. Plant species with low Ellenberg nitrogen indicator values are associated to nutrient poor soils, whereas species with high values are associated to nutrient rich soils. Although these estimates are derived from the distribution of species in Central Europe, they are shown to be valid in a larger area including the Netherlands and the British Isles (Thompson et al. 1993b, Schaffers & Sykora 2000). The seed longevity index (0-1) indicates persistence of seeds in the soil, from transient through long term persistent.

We obtained most seeds from commercial sources but we collected seven species in the field. Each of the four individual deer ingested 1000 seeds of most plant species. However, some species were fed in lower numbers, either because we could not obtain enough seeds or because the seeds were too large to feed in such quantity (Table 5.1). We encapsulated seeds of each plant species in four bread balls, one for each animal. However, we divided the large seeds of Arctium lappa and Tragopogon pratensis over two balls per animal, keeping the balls small enough to be accepted by the animals.

Feeding was divided over three days: November 4, November 18, and December 2, 2002. The division of seeds to the individual animals and feeding days was a random block design with animals and feeding days as blocks. However, we biased the design such that on a single feeding day a plant species was not fed to both adults or both yearlings. This enabled us to identify the animal to which a species was fed (droppings of the adults were larger than droppings of the yearlings).

Prior to feeding, we removed all droppings from the enclosure. During four days following the feeding of seeds, we collected all droppings of each individual animal separately. After daylight hours the collection of dung could not be continued. Hence, seeds excreted during the night were collected the next morning.
Droppings were stored four weeks at 5°C for stratification of the seeds, concentrated using 2 mm and 212 µm mesh sieves stacked on top of each other and put into containers with layers of potting soil, sterilized potting soil and sterilized white sand according to a standard seed bank analysis technique (Ter Heerdt et al. 1996). Droppings of each animal and collection period (morning, afternoon and night for each of the sampling days) were put in separate containers.

Three containers with droppings of the adult male deer served as a control for contamination of seeds ingested at the enclosure (‘seed ingestion control’). Three containers without droppings, but with the same soil layers, served as controls to test for contamination of outside seeds in the greenhouse (‘outside seed control’). Three samples of 100 seeds of each plant species, sown in similar containers, served as a test of the fraction germinating seeds prior to ingestion (‘pre-ingestion control’). All containers were placed in a glass house and supplied with artificial lighting and automatic daily watering. We identified and removed seedlings from the containers until no more seedlings emerged during a month (recording period was nine months).

Data analysis

Because seeds were stratified and allowed to germinate for a long enough period, we could assume that all viable seeds were recovered. Hence, the difference between the fraction of seeds germinating prior to ingestion and after is the fraction of seeds killed by the fallow deer. Therefore, ‘seed survival’ was analyzed as the ratio between the fraction of seeds germinating after ingestion and the fraction of seeds germinating in the pre-ingestion control.

Seed survival data were log10 transformed and plotted against the independent variables: log10 of seed mass \( (M, \text{mg}) \), variance of unit seed dimension \( (V_{sd}) \), longevity index and Ellenberg Nitrogen indicator values \( (N_E) \). A linear regression analysis and Pearson correlation tested how seed survival was related to these independent variables. Similarly, we tested if a series of multiple variable models could explain more variance in seed survival. These models followed the general equation 

\[
\log_{10} (M^x V_{sd}^y N_E^z),
\]

where \( x, y, z \) ranged from -10 to 10, with intervals of 0.1.

We obtained \( M \) of each species by weighing six samples of 100 seeds, while we obtained seed dimensions (length, width and thickness) from the Biolflor database (Klotz et al. 2003). Statistical test were carried out using SPSS software (SPSS Inc. 2001).
Results

Passage rate and recovery of seeds
In total, we recovered 2696 seedlings of 24 species from the droppings. *Arctium lappa* seeds did not survive the molar mills and the digestive tract of fallow deer. Of the other species the recovered fraction of seeds ranged from 0.001 for *Sanguisorba minor* to 0.20 for *Plantago major*. Data on the number of seeds recovered are not shown explicitly, but can be calculated from the number of seeds ingested (Table 5.1), pre-ingestion germinating fraction and seed survival (Figure 5.2).

The deer had excreted about half of the germinating seeds within 24 hours since ingestion and almost all seeds (98%) after 66 hours. They excreted some seeds already within 5 hours and the last seeds after 90 hours. However, the last collected

Table 5.1. Plant species fed to the fallow deer and the ecological correlates tested; variance of seed dimensions, seed mass, Ellenberg Nitrogen indicator value (1 - 9) and seed longevity index (0-1).

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Seeds fed per animal</th>
<th>Variance of seed dimensions</th>
<th>Seed mass (mg)</th>
<th>Ellenberg Nitrogen indicator value</th>
<th>Seed longevity index</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Achillea millefolium</em></td>
<td>1000</td>
<td>0.120</td>
<td>0.154</td>
<td>5</td>
<td>0.11</td>
</tr>
<tr>
<td><em>Arctium lappa</em></td>
<td>300</td>
<td>0.110</td>
<td>9.508</td>
<td>9</td>
<td>0.75</td>
</tr>
<tr>
<td><em>Calluna vulgaris</em></td>
<td>1000</td>
<td>0.072</td>
<td>0.021</td>
<td>1</td>
<td>0.88</td>
</tr>
<tr>
<td><em>Centaurea jacea</em></td>
<td>1000</td>
<td>0.082</td>
<td>2.120</td>
<td>1</td>
<td>0.13</td>
</tr>
<tr>
<td><em>Centaurea scabiosa</em></td>
<td>200</td>
<td>0.107</td>
<td>5.273</td>
<td>4</td>
<td>0.17</td>
</tr>
<tr>
<td><em>Cerastium fontanum</em></td>
<td>300</td>
<td>0.016</td>
<td>0.139</td>
<td>5</td>
<td>0.65</td>
</tr>
<tr>
<td><em>Erica tetralix</em></td>
<td>1000</td>
<td>0.039</td>
<td>0.015</td>
<td>2</td>
<td>0.41</td>
</tr>
<tr>
<td><em>Festuca rubra</em></td>
<td>1000</td>
<td>0.147</td>
<td>0.851</td>
<td>6</td>
<td>0.14</td>
</tr>
<tr>
<td><em>Geum urbanum</em></td>
<td>650</td>
<td>0.166</td>
<td>2.425</td>
<td>1</td>
<td>0.14</td>
</tr>
<tr>
<td><em>Hieracium umbellatum</em></td>
<td>500</td>
<td>0.141</td>
<td>0.376</td>
<td>2</td>
<td>0.56</td>
</tr>
<tr>
<td><em>Holcus lanatus</em></td>
<td>1000</td>
<td>0.103</td>
<td>0.340</td>
<td>4</td>
<td>0.56</td>
</tr>
<tr>
<td><em>Juncus effusus</em></td>
<td>1000</td>
<td>0.057</td>
<td>0.019</td>
<td>4</td>
<td>0.95</td>
</tr>
<tr>
<td><em>Leucanthemum vulgare</em></td>
<td>1000</td>
<td>0.113</td>
<td>0.443</td>
<td>3</td>
<td>0.44</td>
</tr>
<tr>
<td><em>Oenothera erythrosepala</em></td>
<td>1000</td>
<td>0.019</td>
<td>0.602</td>
<td>4</td>
<td>0.88</td>
</tr>
<tr>
<td><em>Plantago lanceolata</em></td>
<td>500</td>
<td>0.095</td>
<td>1.142</td>
<td>1</td>
<td>0.35</td>
</tr>
<tr>
<td><em>Plantago major</em></td>
<td>1000</td>
<td>0.090</td>
<td>0.270</td>
<td>6</td>
<td>0.79</td>
</tr>
<tr>
<td><em>Poa trivialis</em></td>
<td>1000</td>
<td>0.113</td>
<td>0.253</td>
<td>9</td>
<td>0.75</td>
</tr>
<tr>
<td><em>Ranunculus acris</em></td>
<td>1000</td>
<td>0.092</td>
<td>1.966</td>
<td>9</td>
<td>0.24</td>
</tr>
<tr>
<td><em>Rumex obtusifolius</em></td>
<td>500</td>
<td>0.039</td>
<td>1.005</td>
<td>9</td>
<td>0.67</td>
</tr>
<tr>
<td><em>Sanguisorba minor</em></td>
<td>500</td>
<td>0.078</td>
<td>4.108</td>
<td>2</td>
<td>0.33</td>
</tr>
<tr>
<td><em>Silene latifolia</em></td>
<td>1000</td>
<td>0.023</td>
<td>0.800</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td><em>Succisa pratensis</em></td>
<td>200</td>
<td>0.122</td>
<td>1.551</td>
<td>2</td>
<td>0.24</td>
</tr>
<tr>
<td><em>Tragopogon pratensis</em></td>
<td>300</td>
<td>0.193</td>
<td>7.600</td>
<td>5</td>
<td>0.6</td>
</tr>
<tr>
<td><em>Trifolium repens</em></td>
<td>1000</td>
<td>0.024</td>
<td>0.662</td>
<td>6</td>
<td>0.4</td>
</tr>
<tr>
<td><em>Vaccinium vitis-idaea</em></td>
<td>500</td>
<td>0.096</td>
<td>0.260</td>
<td>1</td>
<td>0.03</td>
</tr>
</tbody>
</table>
droppings (90 hours since ingestion) attributed very little to the total seed recovery (<0.1% of all seeds recovered), showing that the sampling period was long enough. There was significant variation in passage rate between plant species (univariate GLM P<0.01). *Cerastium fontanum* passed the digestive tract fastest while *Plantago major* passed slowest (Figure 5.1).

Some seedlings (mostly *Poa annua*) were recorded in the ‘seed ingestion control’. However, none of these seedlings were species used in the experimental feeding. In the controls for contamination of outside seeds no species used in the experiment emerged.

Germinating fraction in the pre-ingestion control was, for most species, higher than 0.3 and ranged between to 0.05 for *Calluna vulgaris* and 0.87 for *Achillea millefolium* (Figure 5.2).

**Seed survival**

Because seeds were stratified and allowed to germinate for a long enough period, we could assume that all viable seeds were recovered. Hence, the difference between the fraction of seeds germinating prior to ingestion and after is the fraction of seeds killed by the fallow deer. Therefore, seed survival was analyzed as the ratio between the fraction of seeds germinating after ingestion and the fraction of seeds germinating in the pre-ingestion control. *Juncus effuses* had the highest seed survival (0.41), followed by *Erica tetralix* (0.27), *Plantago major* (0.24), *Calluna vulgaris* (0.15) and *Oenothera erythrosepala* (0.14). The frugivorous species *Vaccinium vitis-idea* was 9th in rank of best surviving seeds. Half of the species fed to the fallow deer had a low seed survival (<0.05) (Figure 5.2).

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**Figure 5.1.** Fraction of total seed recovery (germinable seeds excreted after x hours/ all germinable seeds recovered) in time (hours) for the slowest passing species (*Plantago major*), the fastest passing species (*Cerastium fontanum*) and the average of all species (25) fed to fallow deer.
Seed mass ($R^2=0.42$) was the best predictor of seed survival in fallow deer, followed by the variance of seed dimensions ($R^2=0.30$) and the seed longevity index ($R^2=0.20$). Seed mass ($P<0.01$) and variance of seed dimensions ($P<0.01$) were negatively related to seed survival, while the longevity index was positively ($P<0.01$) related (Figure 4.3). Seed mass, variance in seed dimensions and longevity index are also interrelated (Table 2.2). The multiple variable model, that explained most variance in seed survival, was the log10 (seed mass $\times$ variance of seed dimensions) (Figure 4.3), but this model explained only 9.5% ($R^2=0.46$) more variance in the data than seed mass alone. None of the tested models combining seed mass and the longevity index correlated better to seed survival than seed mass alone. Seed survival was not related to Ellenberg Nitrogen indicator values (Table 5.2).

![Figure 5.2. Seed survival (fraction of seeds germinating after ingestion/ fraction of seeds germinating prior to ingestion) of 25 species fed to fallow deer. Bars represent averages of feeding trials to four individual deer and three pre-ingestion germination tests. Error bars represent standard error of the mean. Fraction of seeds germinating prior to ingestion is represented between brackets.](image-url)
Figure 5.3. Ecological correlates of seed survival in the digestive tract of fallow deer. Relationship between log10 (seed survival) and log10 (seed mass, mg) (A), variance of seed dimensions (B), seed longevity index (C) and log10 (variance of seed dimensions x seed mass). Each data point represents the average seed survival of a plant species in four animals. Lines represent linear regression models. ** represents \( P < 0.01 \).

Table 5.2. Pearson correlations between log10 seed survival, log10 seed mass (\( M, \text{mg} \)), variance of seed dimensions (\( V_{sd} \)), seed longevity index, Ellenberg Nitrogen indicator value and log10 \( M V_{sd} \).

<table>
<thead>
<tr>
<th></th>
<th>( \text{LOG10 seed survival} )</th>
<th>( \text{LOG10 M} )</th>
<th>( V_{sd} )</th>
<th>Seed Longevity index</th>
<th>Ellenberg nitrogen indicator value</th>
</tr>
</thead>
<tbody>
<tr>
<td>log10 seed survival</td>
<td>-0.66**</td>
<td>-0.56**</td>
<td>0.40*</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>log10 M</td>
<td>-0.66**</td>
<td>-0.44*</td>
<td>-0.44*</td>
<td>0.38</td>
<td></td>
</tr>
<tr>
<td>( V_{sd} )</td>
<td>-0.56**</td>
<td>0.49**</td>
<td>-0.42*</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>Seed longevity index</td>
<td>0.40*</td>
<td>-0.44*</td>
<td>-0.42*</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>Ellenberg nitrogen indicator value</td>
<td>0.05</td>
<td>0.38</td>
<td>0.06</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>( \text{LOG10 (M V}_{sd} )</td>
<td>-0.68**</td>
<td>0.96**</td>
<td>0.67**</td>
<td>0.45*</td>
<td>0.25</td>
</tr>
</tbody>
</table>

** \( P < 0.01 \) level (2-tailed).
* \( P < 0.05 \) level (2-tailed).
Discussion

Although cited in almost any paper on endozoochory published since Janzen’s publication (1984), the ‘foliage is the fruit hypothesis’ has received little experimental testing (but see Collins & Uno (1985) for a critique). On the one hand this is probably due to its appealing character, putting a large number of species, without apparent adaptation for seed dispersal, into an evolutionary perspective. On the other hand, its formulation is not very specific (“.. all plants [] are not expected to have all these traits”) which makes the hypothesis difficult to test. Many aspects of the ‘foliage is the fruit hypothesis’ can not be refuted. It has been repeatedly shown that seeds of many small-seeded plants are dispersed via dung of large herbivores (Welch 1985, Malo & Suarez 1995b, Pakeman et al. 2002). Furthermore, there is little arguing in Janzens statement that seeds intermingled with attractive foliage are eaten more than seeds not intermingled with foliage, or seeds intermingled by unpalatable or toxic foliage. The controversial part of the hypothesis is in its suggestion that foliage palatability has co-evolved with the ability of seeds to survive the digestive tract of large mammalian herbivores.

Of all 25 species tested, Juncus effuses, known for its unpalatable foliage, survives best in fallow deer. Second best survive seeds of Erica tetralix, a woody dwarf shrub and third are Plantago major seeds which are not intermingled with foliage. Species associated to nutrient rich soils are more palatable than species associated to poor soils (Bryant et al. 1983, Hobbie 1992, Iason & Hester 1993). In addition these species can quickly establish on the open nutrient-rich habitat that dung pats create (Malo & Suarez 1995a, Dai 2000). However, our results show that there is no relationship between seed survival and nutrient availability in the habitat of plant species.

Perhaps there are species in which the dispersal of seeds through ingestion by herbivores has enforced the development of ‘fruit-like foliage’ at the moment of seed maturation. The ‘Stinkolieboom’ (Datura stramonium), which loses its toxicity when its seeds are mature, might be such a species. Buffalograss (Buchloe dactyloides) also seems to fit the foliage is the fruit description reasonably well (Quinn et al. 1994). However, the foliage of most grasses and weeds found in dung of large mammalian herbivores, actually lose palatability as seeds mature (Van Soest 1982). Grazing lawns attract grazers because of their immature state, with high energetic contents (McNaughton 1984). Flowering stalks of most grasses are above the foliage and preferentially avoided by herbivores (WallisDeVries & Schippers 1994). Collins & Uno (1985) concluded that the time was not ripe to consider grasslands or their vegetative components as green fruits. Nineteen year later, we conclude that it still is not.

Nevertheless, Janzen (1984) provides good hypotheses for ecological correlates of seed dispersal through ingestion and defecation by large herbivores. According to Janzen (1984) seeds adapted to endozoochory are small, round and hard. Our results show that seed survival in the digestive tract of fallow deer is indeed negatively related to seed mass and variance of seed dimensions. Previously, it was shown that
the percentage of hard seeds is a good prediction for the survival of legume seeds in the digestive tract of cattle (Gardener et al. 1993).

Seeds that survive the molar mills and digestive tract of herbivores need not be evolutionary molded by endozoochory. Seeds may be adapted to persist in the soil or there may have been selection for high seed numbers (and consequently small seed size). Quite serendipitously such seeds are also well suited to survive seed ingestion.

At least some seeds of almost all species tested survived ingestion by fallow deer. Surveys of seed density in dung usually contain a large proportion of the plant species present in the study site (Welch 1985, Malo & Suarez 1995b). Seed size and shape can not perfectly predict whether a species is present in the dung or not (Pakeman et al. 2002). These observations suggest that a strict classification of species dispersed or not dispersed via the digestive tract of herbivores, is not feasible. Instead, we should consider the ability of plants to disperse via large herbivores as a continuous variable. Movement of fallow deer during the one to three days that seeds retain in their digestive tract can certainly aid long-distance seed dispersal, if seeds survive.

Seed mass is a fair predictor of seed survival, easy to measure, and available in many botanical databases. The linear regression function relating seed mass to seed survival and other ecological correlates of seed survival, presented in this paper, can help to estimate the ability of plant species for long-distance dispersal.

Acknowledgements

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References


