

ENDOZOOCHORICAL PLANT SEED DISPERSAL BY RED DEER (*Cervus elaphus*) IN THE POĽANA BIOSPHERE RESERVE, SLOVAKIA

SAM M.J.G. STEYAERT ^{1*}, JAN BOKDAM ¹, WIM G. BRAAKHEKKE ¹,
SLAVOMIR FINĎO ²

¹ Nature Conservation and Plant Ecology Group, Wageningen University, Droevendaalsesteeg 3a, Building 100 'Lumen', 6708PB, Wageningen, the Netherlands; e-mail: sam.steyaert@wur.nl

² Tulská 29, 960 01, Zvolen, Slovakia

Abstract

Steyaert S.M.J.G., Bokdam J., Braakhekke W.G., Findo S.: Endozoochory plant seed dispersal by red deer (*Cervus elaphus*) in the Poľana Biosphere Reserve, Slovakia. *Ekológia (Bratislava)*, Vol. 28, No. 2, p. 191–205, 2009.

From an evolutionary point of view, endozoochory can be considered as a mutualistic process, in which both the plant seed-dispersing animal species and the dispersed plant species benefit. Disperser benefits may be direct, by using the dispersed plant species as a food source, and indirect, by promoting the distribution of these food plants. Despite biomass loss involved, plant species may benefit from endozoochory, because it promotes long-distance dispersal and linked advantages. We tested this mutualism hypothesis in the Poľana Biosphere Reserve, central Slovakia, with red deer (*Cervus elaphus*) as disperser. We collected data on distances covered by red deer between seed ingestion and excretion sites, and the corresponding vegetation by VHF telemetry and GIS analysis. We assessed the viable seed content of red deer dung by a germination test. Information on forage palatability, habitat and seed longevity of the dispersed species were taken from literature. From a total of 400 g dry red deer feces, 1766 seedlings emerged, belonging to 43 species. Most of the observed dispersal distances exceeded 100 m, which is commonly considered as a minimum for long-distance dispersal. Red deer transported mostly seeds from a variety of vegetation types to forests. Our results support the hypothesis that endozoochory is a mutualistic process. We conclude that, since landscapes are changing due to global warming and land use changes, it is important for the maintenance of plant species diversity to promote migration and free ranging of large grazers like red deer, by means of infrastructural measures.

Key words: nature conservation, long distance seed dispersal, co-evolution, mutualism

*Corresponding author

Introduction

Seed dispersal in patchy landscapes has raised many questions among many scientists. As early as 1859, Charles Darwin stated that either adaptations for long-distance dispersal or accidental long-distance dispersal events are of critical importance for plant population dynamics. Many plant species lack apparent morphological adaptations for ballistic dispersal, epizoochory, endozoochory by birds and dispersal by wind or water (Myers et al., 2004; Cain et al., 2000). Yet, such unassisted seeds disperse faster and over longer distances than expected. It has been hypothesized that large herbivorous and omnivorous mammals are important vectors for endozoochorous long-distance (> 100 m) plant dispersal as a result of co-evolution (Pakeman, 2004; Myers et al., 2004; Vellend et al., 2003; Pakeman, 2001). As long-distance dispersal may be important for maintenance of global biodiversity, invasion dynamics, plant population structure and vegetation responses to changing climate and land use, it has become an increasingly important issue in the scope of nature conservation and restoration. It is now generally acknowledged that many plant species and seeds are endozoochorously dispersed, but many aspects are still insufficiently known (Pakeman, 2004; Couvreur et al., 2004; Heinken et al., 2002; Mouissie et al., 2005; Vellend et al., 2003; Cain et al., 2000).

It has been hypothesized that endozoochory is a mutualistic interaction (Callaway, 2007; Begon et al., 1986). This implies that not only the dispersing animal species, but also the dispersed plant species benefit from it. The aim of our study is to test this hypothesis.

Because the benefit in terms of plant fitness is difficult to quantify, we have used the number of seeds per species dispersed, and seed dispersal distances as a proxy. Dispersal distance was considered important, because the further plant seeds are dispersed from a source population the higher the chance on outbreeding and thus improved genetic diversity. Because seeds – especially seeds that have no dormancy – can only establish if they are deposited in a suitable habitat we also recorded the habitat type at the deposition site. Dispersing herbivores and omnivores may benefit from endozoochory in two ways, i.e. immediate and long term. Ingested seeds and their envelope (e.g. fruits and foliage) may provide an immediate nutritional reward. The long-term benefit implies that the disperser promotes the distribution of its own forage plant species.

The mutualism hypothesis was tested in the Poľana Biosphere Reserve in Slovakia with red deer (*Cervus elaphus*) as the dispersal agent. From July 2005 till mid September 2005 we monitored the terrain use of red deer by means of VHF telemetry. Red deer fecal samples were collected during the same period. The seed content of the fecal samples was analyzed by a germination test in a greenhouse in Wageningen from October 2005 till May 2006.

We focused on the following questions:

What is the viable seed load of red deer feces, in terms of numbers and species composition during the summer?

At what distances from the seed source are the seeds deposited?

To what extent is the deposition site suitable for establishment of the dispersed species?

Materials and methods

Study area

The study was carried out in the Poľana Biosphere Reserve in Central Slovakia. The reserve covers 20 360 ha and contains one of the biggest former volcanoes of Europe. The center is situated at 48 39' N latitude and 19 29' E longitude. The tertiary volcanic bedrock is covered with Cambisols and very fertile Andosols. Altitudes range from 460 to 1458 m above sea level. The relief is varied, with steep slopes to the inside of the caldera and less steep slopes to the outer part.

The climate varies from cold mountainous on the highest elevations to a mildly cold climate in the lower parts. Annual average temperature varies from 2.5 to 8 °C. The air humidity is high to very high and annual precipitation ranges between 650 and 1300 mm. Most precipitation falls during spring and summer.

65% of the reserve is covered by broadleaved deciduous forest (mainly *Quercus-Fagetum* communities), native coniferous woodland (mainly *Picea abies*), mixed woodland (mainly beech-fir forests) or tree line ecotone. 20% of the area is covered with grassland and 15% of the area is man-made landscape (arable land, orchards and urban areas). Grassland presence in the reserve is conditioned and maintained by human activity since historic times. Different management types resulted in several species rich grassland types. According to moisture and nutrient conditions, the grasslands can be divided in four main communities: wetland communities; wet and mesophyllic communities; pasture communities and their successional stages; and nitrophilous communities on intensive managed pastures. The reserve is scarcely inhabited with about 320 inhabitants in three settlements (Sorokova, Pichler, 2002).

Red deer is the largest wild ungulate species that occurs in the study area. It is abundant, with an estimated population density of 0.040–0.045 animals per ha (Findo, personal communication).

Fecal viable seed content

Freshly deposited fecal pellet groups (less than 1 day old) were collected during random walks in the area between July and August 2005. If pellet groups were clustered in a pile, a sample of the internal part was taken to avoid contamination with seeds from the deposition site. If the pellets were not clustered, they were intensively cleaned and brushed to avoid contamination. The samples were stored in paper bags and air-dried. In total, 54 samples were collected, 20 during July (with a total dry mass of 228.84 g) and 34 during August (total dry mass of 680.45 g). Pellets collected in the same month were mixed to form one composite sample for each month.

A germination test was used to determine the viable plant seed content in the fecal samples. 16 sub-samples of each composite sample were spread out on eight plastic trays of 60x40x7 cm, filled with a 4 cm layer of moist, coarse river sand. The trays were subdivided in 6 compartments of 400 cm² each. In each tray two compartments were covered with feces from July, two with feces from August and two were used as a control. The sub-samples weighed 12.5 g and formed a layer of ± 0.5 cm thick. In total, 200 g of both July and August composite samples were used for the germination test. Before the trays were placed in the greenhouse, they were exposed to a dark and moist stratification at 3 °C for 14 days. In the greenhouse the temperature was 15 °C during nighttime and 20 °C during daytime. Relative humidity was 70%. Artificial light was used to assure a 12 hours light period. The trays were covered with plastic lids to prevent seed contamination within the greenhouse. The trays stayed in the greenhouse for 6 months. After 3 months, when no more seedlings emerged, the trays were exposed to another dark and moist stratification for 14 days at –3 °C, to trigger germination of seeds that need frost. A standard broad spectrum fungicide (Rovral® aquaflo, 1.5 ml.l⁻¹ H₂O) was used when fungus appeared in the trays. When seedlings showed nutrient deficiencies, the trays were watered with a standard nutrient solution. Emerged seedlings were identified using the seedling identification key of Muller (1978) and expert knowledge. After identification, the seedlings were recorded and removed as soon as possible. Unidentifiable seedlings were transplanted in individual flowerpots for identification in a later growth stage.

Dispersal distances and deposition sites

Eight red deer individuals, 4 males and 4 females were provided with radio collars. The collars transmitted differential signals, indicating whether the deer were active (foraging) or not. The deer were located by triangulation in an as short as possible time interval (aiming at < 15 minutes). The triangulations were plotted on topographic maps. Of the resulting triangles, the centers were taken as point locations. When one side of the triangle exceeded 100 m, the location was not used for analysis. The locations were plotted on digital orthophotos and analyzed with GIS software (Arcview®) to determine the habitat use of the red deer. In total, 367 locations were obtained, of which 235 (64%) during daytime (8 am–8 pm) and 131 (36%) during nighttime (8 pm–8 am). The number of locations per animal varied from 16, of a male whose collar failed during the study, to 71. More locations of female deer (255) were taken than of males (112), because males were distributed in a more remote and extensive area. All locations were checked on digital orthophotos and assigned to be situated in either forest or open vegetation.

The dispersal distances were calculated as the Euclidian distance covered by deer during a period of 14 h (the assumed gut retention time of red deer), following the start of the triangulation, i.e. when a deer was actively foraging. We used a conservative estimate of the gut retention time to avoid overestimation of the dispersal distances (Vellend et al., 2003; Pakeman, 2001). In total 84 movements were recorded, of which 27 for males and 57 for females. All except one of these records fell in a range of 12 to 16 hours after the moment deer was observed to be feeding (i.e. ingestion of seeds). The deer movement during the interval was assumed to be constant, and the distances were standardized on 14 h in order to decrease variation in dispersal distances caused by variation in time intervals.

Plant species traits

All plant species that emerged from the fecal samples were classified according to habitat preference following Schmidt et al. (2002), who classified 634 vascular plant species in one of three groups: tree layer species (A1), shrub layer species (A2) and herb layer species (B). The herb layer species were further subdivided in habitat preferences as follows:

- B1.1: shade tolerant forest herbs,
- B1.2: herbs of forest edges and gaps,
- B2.1: forest herbs that also occur in open vegetation,
- B2.2: herbs of open vegetation that can occur in forests.

Species that did not occur in the list of Schmidt et al. (2002) were classified as species of short or open vegetation (further one named “species of open vegetation”, group O) (Von Oheimb et al., 2005). Species classified as B2.1 and B2.2 that occur both in forests and in open vegetation were groups as “ecotone species”. Species classified as A1, A2, B1.1, and B1.2 were grouped as “forest species”.

Most of the emerged plant species were classified in three palatability classes for red deer by Dr. Verheyden-Tixier (personal communication, see also Gebert, Verheyden-Tixier, 2001). These classes were: major food items (reported as > 10% of diet in at least one season), minor food items (reported as < 10% of diet in at least one season) and scarcely eaten items (reported as eaten < 1% of the deer diet in at least one season). Plant species not classified by these authors were assigned by us to the same class as the closest ecologically related classified species.

Seed longevity was taken into account in the analysis. Persistent seeds have higher potential for successful dispersal, because, when deposited in unsuitable habitats, they can remain dormant in the soil and wait for better conditions (Couvreur et al., 2004; Pakeman, 2004; Cain et al., 2000). Data on seed longevity was taken from Comparative Plant Ecology by Grime et al. (1988) and a compiled Floristic Database of Central and Western Europe (Vermeulen, unpublished). Seed longevity was classified in four groups: 1. non-persistent seeds; 2. most of the seeds only persistent till the next growing season; 3. a small proportion of the seeds can persist in the soil seed bank; 4. persistent seeds.

Data analysis

ANOVA assumptions were not met by the plant species data. Therefore, a non-parametric Wilcoxon Signed Rank test for two related samples was performed to test for differences in the seed density of dispersed species between

July and August. Non-parametric, one sample Kolmogorov-Smirnov tests for uniformity were carried out on the number of species in different classes for palatability, habitat preference and seed longevity.

The dispersal distance records were standardized on 14 hours and merged into 100 m intervals to create a frequency distribution. Differences in dispersal distances due to red deer sex were tested with a non-parametric Mann-Whitney U test. The dispersal distance data set for males was normalized by using a logarithmic transformation. The total data set and the data set for females were normalized using a $\log(x + 150)$ transformation. The seed deposition on a given distance between 0 m and the maximum dispersed distance interval could be predicted after implementing the descriptive statistics for the male, female and general dataset in a Gaussian equation [equation 1]. A conversion index (CI), obtained by dividing 100 by the sum of the expected proportions of seeds deposited for each interval between the minimum and maximum observed dispersal distance was used to express the obtained values in percentages [equation 2]. The predicted percentage of seeds deposited on a given distance ($\%SD_D$) between 0 m and the maximum dispersal distance was then obtained by multiplying equation 1 and 2 [equation 3].

$$E(Y) = \frac{1}{\sigma \cdot \sqrt{2 \cdot \pi}} \cdot e^{-\frac{(D-\mu)^2}{2 \cdot \sigma^2}} + \varepsilon, \quad [\text{eq. 1}]$$

in which

$E(Y)$ = expected proportion of seeds dispersed on a given distance D ,
 σ = standard deviation,
 D = distance,
 μ = mean dispersal distance,
 ε = residual.

$$CI = \frac{100}{\sum E(Y)}, \quad [\text{eq. 2}]$$

in which

CI = conversion index

$$\%SD_D = E(y) \cdot CI, \quad [\text{eq.3}]$$

in which

$\%SD_D$ = the percentage of seeds dispersed on a given distance.

The conditions at the defecation site are of critical importance for germination and survival possibilities for the dispersed seeds. Assuming that light conditions are the main factor for germination, we classified the defecation habitats as either “forest” or “open vegetation”. Seeds from forest species (species group 1: A1, A2, B1.1 and B1.2) were assumed to arrive in a suitable habitat when they were deposited in forests and seeds of open vegetation species (species group 3: O), when they were deposited in open vegetation. Seeds of ecotone species (species group 2: B2.1 and B2.2.) were assumed to arrive in a suitable habitat with every dispersal event. The actual number of seeds dispersed to a suitable habitat per day per deer per species group (P_s) was estimated with the following equation [4]:

$$P_s = DR \cdot DM \cdot P_{s/group} \cdot NS_{group/gdm}, \quad [\text{eq. 4}]$$

in which

P_s = the number of dispersed seeds, arriving in a suitable habitat per day per deer and per species group,

DR = defecation rate of Red deer (# defecations/day),

DM = the average dry mass of a Red deer fecal pellet group,

$P_{s/group}$ = the probability of deposition in a suitable habitat per species group,

$NS_{group/gdm}$ = number of viable seeds of a species group per gram dry deer feces, as found in the germination test.

Table 1. Species that emerged in the germination test, with seedling numbers per 200 g dry deer feces collected in July and August, habitat preference class (A2 = shrub layer species, B1.1 = shade tolerant forest species, B1.2 = species from forest gaps and edges, B2.1 = forest species that can occur in open vegetation, B2.2 = species from open vegetation that can occur in forests and O = open vegetation species), seed longevity class (1 = non persistent seeds, 2 = most seeds only persistent till the next growing season, 3 = a small proportion of the seeds is persistent and 4 = persistent) and palatability class (1 = major food item (> 10% of red deer diet in at least one season), 2 = minor food item (< 10% of red deer diet in at least one season) and 3 = scarcely eaten items (< 1% of red deer diet in at least one season).

Plant species	Plant traits			Seedling numbers		
	habitat preference class	seed longevity class	palatability class	July	August	Total
<i>Agrostis capillaris</i>	B2.1	3	1		2	2
<i>Agrostis stolonifera</i>	B2.2	3	2		11	11
<i>Campanula persicifolia</i>	B1.2	3	3		2	2
<i>Capsella bursa-pastoris</i>	O	4	3	7	9	16
<i>Cardamine hirsuta</i>	O	3	3		1	1
<i>Cardamine impatiens</i>	B1.2	2	3	1		1
<i>Carex sylvatica</i>	B1.1	3	1	1	1	2
<i>Cerastium fontanum</i> spp. <i>vulgare</i>	O	3	2	6	3	9
<i>Chenopodium bonus-henricus</i>	O	4	3	1		1
<i>Crepis vesicaria</i>	O	1	3	1		1
<i>Deschampsia cespitosa</i>	B2.1	3	1	1	1	2
<i>Epilobium tetragonum</i>	O	2	1	5	3	8
<i>Festuca pratensis</i>	O	1	1		1	1
<i>Fragaria vesca</i>	B2.1	4	1	26	5	31
<i>Geranium robertianum</i>	B2.1	?	3	1		1
<i>Hypericum perforatum</i>	B2.2	4	3	1	1	2
<i>Lapsana communis</i>	B2.1	3	2		1	1
<i>Lolium perenne</i>	O	1	2	38	9	47
<i>Luzula multiflora</i>	B2.2	4	1		8	8
<i>Moehringia trinervia</i>	B1.1	3	3	5		5
<i>Myosotis scorpioides</i>	B2.1	3	3	1		1
<i>Phalaris arundinacea</i>	B2.1	3	3	3		3
<i>Plantago lanceolata</i>	B2.2	3	3		5	5
<i>Plantago major</i> spp. <i>major</i>	O	4	3	1	56	57
<i>Poa annua</i>	O	3	1	4	7	11
<i>Poa nemoralis</i>	B1.1	1	1	3	7	10
<i>Poa pratensis</i>	B2.1	3	1	1	3	4
<i>Poa trivialis</i>	B2.1	3	1	2	1	3
<i>Prunella vulgaris</i>	B2.2	3	3		1	1
<i>Ranunculus polyanthemus-nemorosus</i>	O	3	2		1	1
<i>Ranunculus repens</i>	O	4	2		1	1
<i>Ribes rubrum</i>	A2	1	2	5		5
<i>Rumex acetosa</i>	B2.2	1	3		3	3
<i>Scirpus sylvaticus</i>	B2.1	4	3		2	2
<i>Silene vulgaris</i>	O	4	3	2		2
<i>Spergularia rubra</i>	O	4	3	1	20	21
<i>Stellaria media</i>	B2.2	4	3	4	3	7
<i>Trifolium medium</i>	B2.1	3	2		3	3
<i>Urtica dioica</i>	B2.1	4	2	563	863	1426
<i>Vaccinium myrtillus</i>	B2.1	4	1	1		1
<i>Veronica chamaedrys</i>	B2.1	4	3	3	10	13
<i>Veronica montana</i>	B1.1	4	3	10	23	33
<i>Veronica serpyllifolia</i>	O	4	3		1	1
Total				698	1068	1766

Results

Viable seed load

A total of 1766 seedlings belonging to 43 different species emerged from 400 g dry red deer feces. 698 and 1068 seedlings emerged from 200 g of dry feces collected during July and August respectively. 33 species were found in the August samples and 28 in the July samples. 10 plant species emerged exclusively from the July samples, 15 exclusively from the August samples and 13 species were found in both months. A Wilcoxon Signed Rank test revealed that the difference in seed density found in the red deer dung between the two months was not significant ($N = 43$, $\alpha = 0.05$, $Z = -1.665$ and $p = 0.096$). *Urtica dioica* (stinging nettle) was the most abundant species, with 80.7% of all seedlings. Most of the species (i.e. 38) occurred in very low numbers (< 1% of all emerged seedlings). Only 5 species covered more than 1%, namely *Fragaria vesca*, *Lolium perenne*, *Plantago major* spp. *major*, *Spergularia rubra* and *Veronica montana*. 13 plant species occurred only once (Table 1). No seedlings emerged from the 12 control compartments, revealing that no contamination within the greenhouse occurred.

The ecotone species, which occur both in forests and open vegetation (group B2.1 and B2.2) were most abundant, representing 48.8% of all recorded species and 86.6% of all recorded seedlings. 34.8% of the species belonged to the category of open vegetation, and represented 10.1% of the seedlings. Only 16.3% of the species belonged to the category of forest species, representing 3.3% of the seedlings (Fig. 1). A one-sample Kolmogorov-

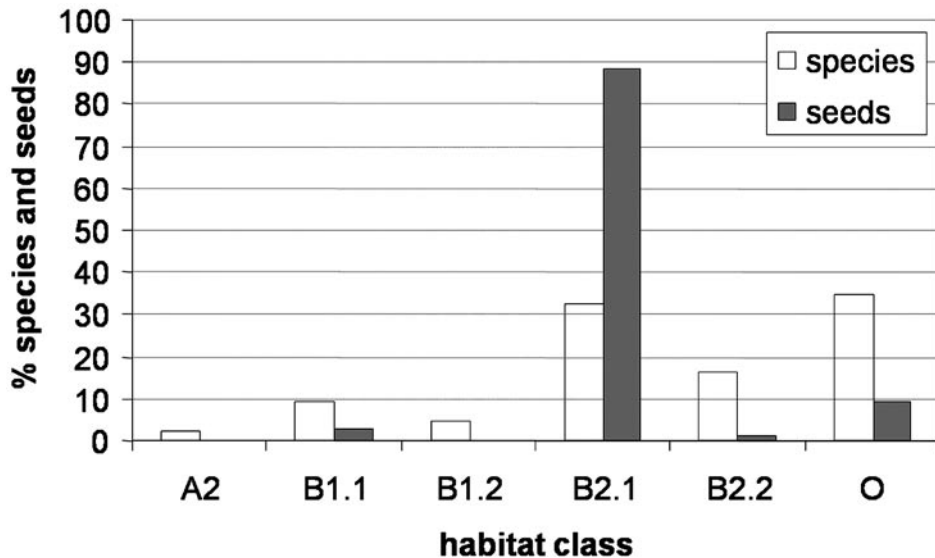


Fig. 1. Distribution of species numbers and seed numbers found in red deer feces during July and August over six habitat classes (A2 – shrub layer species; B1.1 – shade tolerant forest species; B1.2 – species from forest gaps and edges; B2.1 – forest species that occur in open vegetation; B2.2 – species of open vegetation types, occurring in forest; O – species of open vegetation).

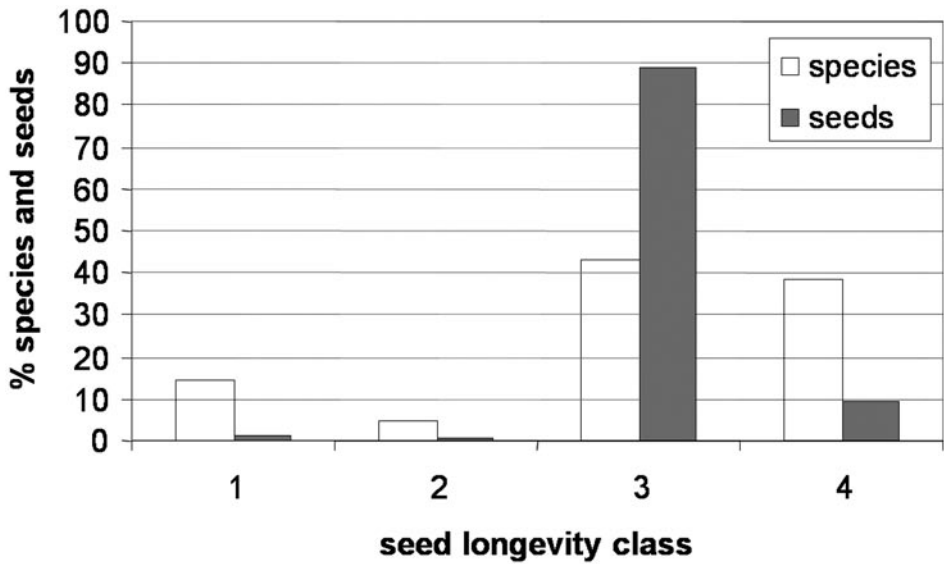


Fig. 2. Distribution of species numbers and seed numbers found in red deer feces during July and August over seed longevity classes (1 – non persistent; 2 – short time persistent; 3 – part of the seeds long time persistent; 4 – persistent).

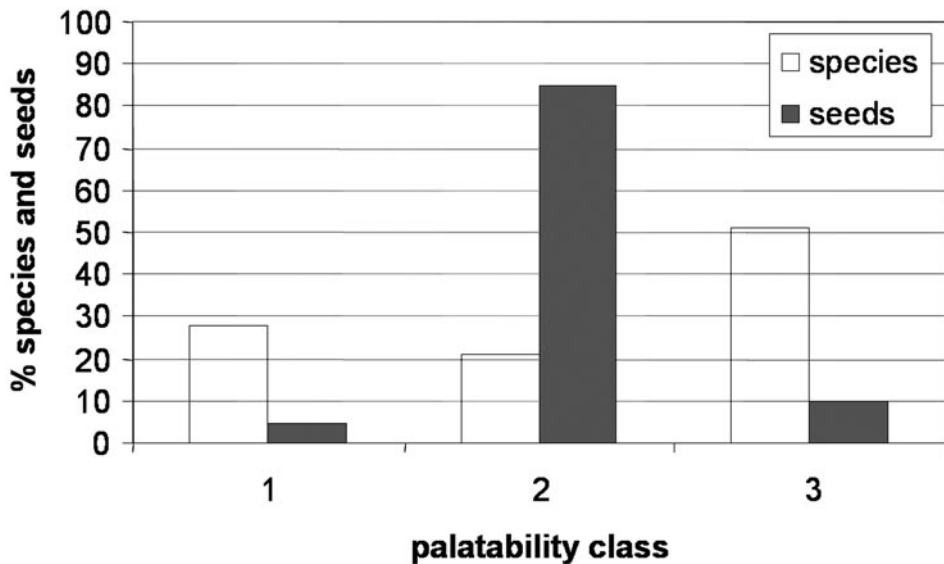


Fig. 3. Distribution of species numbers and seed numbers found in red deer feces during July and August over palatability classes for red deer (1 – preferred (>10%); 2 – minor food item (<10% – >1%); 3 – scarcely eaten (<1%)).

Smirnov test showed that the dispersed species were not uniformly distributed over the habitat preference categories ($N = 43$, $\alpha = 0.05$, $Z = 2.867$ and $p < 0.001$). This is mainly due to *Urtica dioica*, with 80.7% of the seedlings, being assigned to the category of species from forests and open vegetation. When excluding *U. dioica*, species of open vegetation were most frequent in seedling number, but not in species number.

A one-sample Kolmogorov-Smirnov test for uniformity revealed that species with persistent seeds, or with a small fraction of dormant seeds (classes 3 and 4) were more present than plant species with non-persistent or low persistent seeds (classes 1 and 2) ($N = 42$, $\alpha = 0.05$, $Z = 3.086$ and $p < 0.001$). Class 3, of which only a small proportion of the seeds have dormancy capacities, displayed a high species number but a low seedling number. Species with persistent seeds (class 4) were represented by a large number of species as well as seedlings (Fig. 2).

Most plant species dispersed (22) were classified as rarely consumed by red deer (Table 1, Fig. 3). Preferred forage plant species were dispersed in lower species number (12), and in very low number of seedlings (4.7% of all seedlings). Species of intermediate forage preference for deer were dispersed in the lowest species number (9), but in the highest number of seedlings. The large number of seedlings of *U. dioica*, classified in the intermediate forage preference class, caused this peak. A one-sample Kolmogorov-Smirnov test for uniformity showed that the dispersed plant species were not uniformly distributed over the palatability classes ($N = 43$, $\alpha = 0.05$, $Z = 3.355$ and $p < 0.001$) (Fig. 3).

Dispersal distance

The observed Euclidean distances covered by red deer from the locations where they were foraging until 12–16 hours later, ranged from 11 to 4 853 m. Standardizing the data on 14 hours resulted in a dispersal distance ranging from 11 to 3 414 m. The minimum and maximum distance covered by males in the 14-hour interval was respectively 137 and 3 141 m. For females, the minimum and maximum distance covered was respectively 11 and 1 521 m. Males covered significantly longer distances than females during 14 hour intervals, and consequently dispersed plant seeds proportionally further (Mann-Whitney U test, $N = 84$, $Z = -3.070$, $\alpha = 0.05$ and $p < 0.01$).

The models were validated by comparing the expected and observed dispersal distances (in 100 m intervals) with a Mann-Whitney U test. The test showed that the differences between the observed and predicted values for females, males and total were not significant ($N = 16$, $\alpha = 0.05$, $Z_{\text{total}} = -0.796$, $Z_{\text{males}} = -0.079$, $Z_{\text{females}} = -0.604$, $p_{\text{total}} = 0.940$, $p_{\text{males}} = 0.426$ and $p_{\text{females}} = 0.546$). Figure 4 shows the distribution of the dispersal distances for males, females and in total, calculated with equation 3. It appears that 95% of the seeds were dispersed further than 100 m and 25% further than 1 km. Of the seeds dispersed by males and females, respectively 48% and 10% were dispersed further than 1 km. Model fit showed an explained variance for females and in total of $R^2 = 0.647$ and $R^2 = 0.674$ respectively. The explained variance of the “male” model was low ($R^2 = 0.216$).

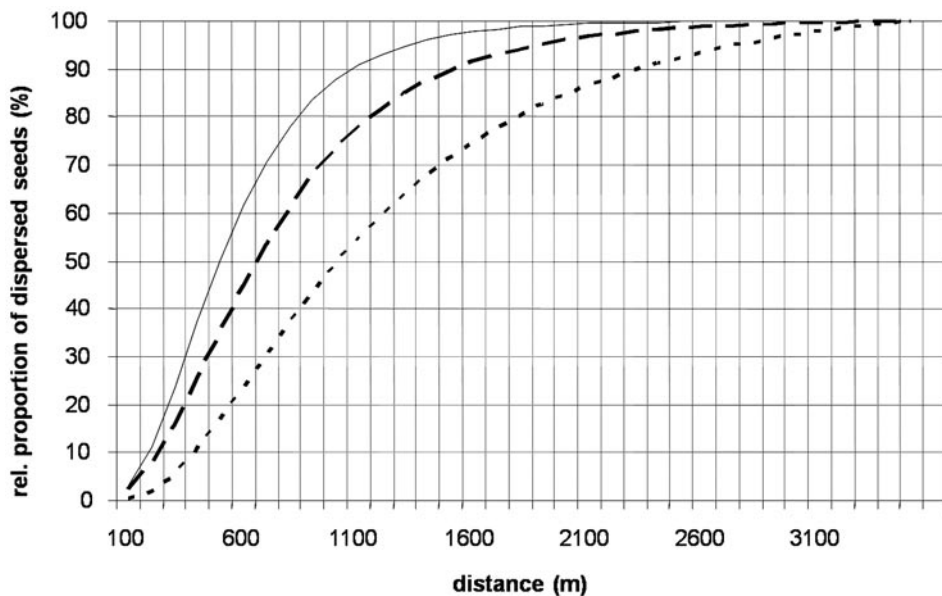


Fig. 4. Predicted proportion of dispersed seeds deposited by red deer through endozoochory as a function of the distance from the seed uptake site (--- = female; — = male; — — = total).

Seed deposition sites

Of the 367 locations of red deer that were observed by radio-tracking 79.6% were situated in forests and 20.4% in open vegetation. Taking into account that 65% of the area is forest and 35% is open vegetation, forest appears to be the favoured habitat of red deer. Only 19.1% of all observation trajectories (14 h periods, starting with a foraging animal) started in open vegetation. The dispersal trajectories, with linked habitat type at the site of ingestion and at the site of defecation revealed the dispersal directions. In 58.3% of the dispersal events, seeds were transported within forests. In 2.4% of the observations, seeds were transported between open vegetation types. Transport from open vegetation to forests occurred in 16.7% of the observations and 22.6% in the opposite direction. Species of open vegetation thus have the lowest chance to be dispersed to a suitable habitat. Forest species have a higher chance of dispersal to a suitable habitat. As species of group B2.1 and B2.2 can be consumed in forests and in open vegetation, and both habitat types are considered to be suitable, these species are considered to be transported to a suitable habitat with every dispersal event.

The number of seeds, deposited in a suitable habitat per day per deer was calculated based on defecation rates of red deer published by Mitchell et al. (1983). They found red deer defecating on average 23.16 times per day, in a range of 13.9 and 34. For the dry weight of the pellet groups we used the values found by Von Oheimb et al. (2005). The median dry weight of the entire pellet groups they collected had a dry weight of 15 g, with a first and a third quartile of 9 and 26 g. Using these figures we calculated that, during July and August, the number of viable seeds that is dispersed to a suitable habitat, expressed per day and per red deer individual, varied from 11 to 75 for forest species, from 1 to 4 for species of open vegetation and from 188 to 1328 for ecotone species. This implies that during the study period the deer population in the study area dispersed between $10 \cdot 10^6$ to $80 \cdot 10^6$ plant seeds to a suitable habitat by means of endozoochory.

Discussion

Fecal seed content

The numbers of species and seeds that we found in the fecal samples is likely to be an underestimation of the real numbers dispersed by red deer. Species with specific germination requirements may have failed to germinate in our germination test, despite the cold treatment. We have used a composite sample of 200 g dry weight red deer feces per month in our germination test, which is only a fraction of the total monthly red deer feces deposition in the study area. A larger sample, collected year round would probably lead to a much higher species number. Despite these methodological flaws, our results were similar to values obtained by Von Oheimb et al. (2005) in Lower Saxony, Germany. They found in three different study sites and a total red deer dry fecal mass of 4 363 g on average 642 seedlings per 100 g. They identified in total 59 vascular plant species in the fecal samples, collected from May to November 2002. The maximum number of species deposited in a single study site was 35. Gill and Beardall (2001) reported in a literature review that 46 vascular plant species have been found in red deer feces on the British Isles. Malo and Suarez (1995) recorded 66 plant species dispersed through endozoochory by red deer in a Mediterranean ecosystem.

Most of the plant species found in red deer feces occurred in very low numbers. *Urtica dioica*, however, occurred in extraordinary large numbers in our samples, with 80.7% of all the emerged seedlings. Only 5 other species contributed more than 1% to the total number of seedlings (*Fragaria vesca*, *Lolium perenne*, *Plantago major* spp. *major*, *Spergularia rubra* and *Veronica montana*). Myers et al. (2004) reported that most of the species that germinate from deer feces were relatively uncommon considering the total number of dispersed seeds. Von Oheimb et al. (2005) found that 74% of all seedlings that emerged from red deer feces were *Urtica dioica*. They found only two other species that contributed more than 1% to the total number of emerged seedlings (*Plantago major* spp. *major* and *Juncus tenuis*).

It is remarkable that *Urtica dioica* was present in such large seed number, because it seems adapted to avoid browsing by its glandular stinging hairs, and it was classified in the intermediate preference forage class for red deer. Field observations revealed that the seed heads of this ecotone herb were commonly consumed after seed ripening in July. Similar defoliation and dispersal patterns are known from cattle. It shows that different plant parts may display significant differences in herbivore traits. Our results suggest that the seeds and top leaves of *U. dioica* are less protected against browsing than the rest of the plant. *U. dioica* seems to be highly adapted to endozoochory. The seeds of *U. dioica* are classified as persistent seeds with dormancy capacities (Grime et al., 1988), which could result in a high survival chance while passing the digestive tract. The dung envelope guarantees a favourable microhabitat for seedlings of this nitrophilous species.

Red deer showed a slight preference for forests, as 79.6% of the radio locations were situated in forests while the forest covered only 65% of the total area. It should be taken into account that the observations during daytime covered 64% of all observations. It cannot be excluded that red deer exploited open sites exclusively during nighttime. Red deer foraged (in hours and proportionally) more in forests than in open vegetation. Therefore, we expected that more seeds of forest species than seeds of species from open areas would be dispersed. Our results show an opposite pattern. The viable seed load of red deer dung is dominated by species and seeds from open and ecotone habitats (the latter dominated by *U. dioica*). When *U. dioica* is excluded from the analysis, the species numbers and seeds of ecotone and open vegetation species are similar. The scarcity of forest species and seeds in the dung may be explained by low seed ingestion and/or by a low survival of the ingested seeds during the passage through the digestive tract. Von Oheimb et al. (2005) stated that forest meadows, forest paths, openings, clearings and agricultural fields were preferred foraging sites for red deer. These are growth sites where especially ecotone species occur, the species type that was most abundant in number of species and individuals in the fecal samples. Von Oheimb et al. (2005) and Myers et al. (2004) attributed the under-representation of forest species in red deer feces to a lower ingestion chance of forest species, because these species have fewer individuals, a lower seed production per individual and they grow in less accessible places than the other species. They also suggested that forest species suffer more seed losses due to mastication, because in general they produce larger seeds.

Most of the dispersed species have seeds that are long-term persistent. It has been suggested (Cosyns et al., 2005) that persistent seeds cope better with the conditions in the digestive tract than non-persistent seeds. This raises the question whether seed longevity and correlated seed characteristics might be an adaptation to endozoochory.

Dispersal direction and potential dispersal success

We defined potential dispersal success as the fraction of the dispersed seeds that is deposited in a suitable habitat type, i.e. where a species is able to germinate and establish. Within-habitat directions (forest-forest, open-open) were therefore considered as successful, irrespective of the plant habitat type. Ecotone species, occurring in forest and open sites, were considered to be deposited in a suitable habitat with every dispersal event. Evidently,

this is an overestimation, as they cannot germinate and survive everywhere. The dispersal success of species from open vegetation or forests is also overestimated, because they too cannot establish everywhere in the habitat type they are assigned to. Our assumption that light intensity is the most important factor for germination and the distinction of two classes (forest and open) is certainly an oversimplification.

We estimated the potential dispersal success in our study area through endozoochory by red deer to be between $10 \cdot 10^6$ and $80 \cdot 10^6$. Even if this is an overestimation, it is likely that at least some seeds are deposited in a suitable, and – with regard to the parent plant – remote habitat. Dispersal of only a few seeds to a remote habitat can be significant for a species, it is in meta-population dynamics, migration or colonizing new areas and coping global change. When seeds are deposited in unfavourable habitats, they may enrich the soil seed bank and wait for better conditions that allow them to germinate and establish.

Dispersal distance

Cain et al. (2000) consider dispersal as “long-distance” when the dispersal distance exceeds 100 m, a distance in general not reachable by a plant’s own means of dispersal or dispersal through insects or rodents. Herbaceous plant species usually disperse their diaspores less than 10 m far, and their migration rates are usually less than 2.5 m per year (Cain et al., 2000). Long-distance dispersal has often been considered an anecdotal event, caused by extreme weather conditions, accidental dispersal through mammals, humans and machinery, and other accidental ways of dispersal (Myers et al., 2004; Vellend et al., 2003; Cain et al., 2000). In this paper we show that endozoochorous long-distance dispersal by red deer can hardly be called anecdotal, as we observed that 95.9% of the seeds were dispersed further 100 m, and 12.8% even further than 1 km from the seed source. We found a lognormal frequency distribution of the dispersal events in 100 m distance intervals. Our results agree with those from studies of long distance seed dispersal by other ungulates. A lognormal frequency distribution was also found by Vellend et al. (2003) in a study of endozoochory by white-tailed deer. The reported maximum dispersal distance was 3 730 m in a 15 h time interval, 95% of the dispersal events ranged further than 100 m and 25% further than 1 km. Myers et al. (2004) reported that 95% of the seeds dispersed through endozoochory by white-tailed deer were deposited more than 100 m, and 30% more than 1 km from the seed source.

According to our results, both deer sexes dispersed 95% of the seeds further than 100 m and 25% further than 1 km. Males dispersed seeds proportionally further than females. Males dispersed more than 99% of the dispersed seeds further than 100 m and even 48% of the seeds further than 1 km. Females dispersed 93% of the seeds further than 100 m, and 11% further than 1 km. Females with fawn and unsuccessful reproducing females show high site fidelity and have in general smaller home ranges than males. Therefore, they generally disperse seeds less far than male deer. Our field data were collected from July till the end of September. The pre rut and part of the rutting season were thus included. During this period, male red deer show a high intra-specific competition, and they can travel large distances in search of female herds for mating (Georgii, 1980; Clutton-Brock et al., 1985; Carranza et

al., 1991; Conradt, 1998; Szemethy et al., 2003). Therefore, it is likely that seed dispersal distances through endozoochory by red deer peaks in this months.

Conclusion

The results of our study of endozoochory by red deer supported the hypothesis that endozoochory can be a mutualistic relationship. It cannot be excluded that endozoochorous dispersal of unpalatable species (e.g. *Urtica dioica* and other so called pasture weeds) will be disadvantageous for the herbivore. Plant fitness effects have not directly been measured. The proxies we used suggested benefits for a significant number of dispersed species and seeds. All observed dispersal distances were large enough as seed dispersal distance to escape parental competition, increase outbreeding possibilities and increase migration rates. A small – but significant – proportion of the dispersed seed arrives in a remote habitat that is suitable for germination and development. A large proportion is deposited in remote but less suitable habitats, where it adds to the density and diversity of the soil seed bank. Advantages provided by the dung envelope were not included in this study. We found that red deer facilitated itself by ‘sowing’ forage plant species (e.g. *Lolium perenne* *Agrostis* spp. and *Poa* spp.), after the direct nutritional benefits provided by the rewards of ingesting seeds. It cannot be excluded that endozoochorous dispersal of marginal or non-forage plants may induce vegetation shifts from pastures to tall herb or shrub communities with a low forage value and – ultimately – abandonment by the herbivore. Such cases of self-inhibition need further attention.

A rapid shift of climatic zones in combination with free ranging herbivores at landscape scale will put endozoochorous plants at risk. Free ranging wild and domestic herbivores have become rare in most of Europe, due to habitat fragmentation and intensified land use. Therefore, we emphasize the importance to restore free ranging large herbivore assemblages.

Translated by the authors

Acknowledgements

We thank the Forest Research Institute of Zvolen, Slovakia for the opportunity of tracking radio collared red deer, M. Sagat for offering accommodation and the Biosphere Reserve Staff and the forestry offices for granting permission to enter the study area. We thank A. Schaffers for help with the statistical analysis; H. van Oeveren and L. de Nijs for help with seedling identification; the staff of the greenhouse complex ‘De Haaff’ for helping during the germination test, Dr. Verheyden-Tixier and Dr. Storms for classifying the plant species in palatability classes and Dr. Ozinga for his valuable comments.

References

- Begon, M., Firbank, L., Wall, R., 1986: Is there a self-thinning rule for animal populations? *Oikos*, 46, 1: 122–124. [doi:10.2307/3565389](https://doi.org/10.2307/3565389)
- Cain, L.M., Milligan B.G., Strand, A.E., 2000: Long-distance seed dispersal in plant population. *Am. J. Bot.*, 87: 1217–1227. [doi:10.2307/2656714](https://doi.org/10.2307/2656714)

- Callaway, R.M., 2007: Positive interactions and interdependence in plant communities. Springer, Berlin, 415 pp.
- Carranza, J., Hidalgo de Trucios, S., Medina, R., Valencia, J., Delgado, J., 1991: Space use by red deer in a Mediterranean ecosystem as determined by radio-tracking. *J. Zool.*, 211: 681–693.
- Clutton-Brock, T.H., Majot, M., Guinness, F.E., 1985: Population regulation in male and female red deer. *J. Anim. Ecol.*, 54: 831–846. [doi:10.2307/4381](https://doi.org/10.2307/4381)
- Conradt, L., 1998: Measuring the degree of sexual segregation in group living animals. *J. Anim. Ecol.*, 67: 217–226. [doi:10.1046/j.1365-2656.1998.00183.x](https://doi.org/10.1046/j.1365-2656.1998.00183.x)
- Cosyns, E., Delporte, A., Lens, L., Hoffmann, M., 2005: Germination success of temperate grassland species after passage through ungulate and rabbit guts. *J. Ecol.*, 93: 353–361. [doi:10.1111/j.0022-0477.2005.00982.x](https://doi.org/10.1111/j.0022-0477.2005.00982.x)
- Couvreur, M., Christiaan, B., Verheyen, K., Hermy, M., 2004: Large herbivores as mobile links between isolated nature reserves through adhesive seed dispersal. *Appl. Veg. Sci.*, 7: 229–236.
- Gebert, C., Verheyden-Tixier, H., 2001: Variations of diet composition of red deer (*Cervus elaphus* L.). *Mammal Rev.*, 31: 189–201. [doi:10.1046/j.1365-2907.2001.00090.x](https://doi.org/10.1046/j.1365-2907.2001.00090.x)
- Georgii, B., 1980: Home range and activity patterns of female red deer (*Cervus elaphus*) in the Alps. *Oecologia*, 58: 238–248. [doi:10.1007/BF00399224](https://doi.org/10.1007/BF00399224)
- Gill, R.M.A., Beardall, V., 2001: The impact of deer on woodlands: the effect of browsing and seed dispersal on vegetation structure and composition. *Forestry*, 74: 209–217. [doi:10.1093/forestry/74.3.209](https://doi.org/10.1093/forestry/74.3.209)
- Grime, J.P., Hodgson, J.G., Hunt, R., 1988: Comparative plant ecology: a functional approach to common British species. Academic Division of Unwin Hyman Ltd, London, 679 pp.
- Heinken, T., Hanspach, H., Raudnitschka, D., Schaumann, F., 2002: Dispersal of vascular plants by four species of wild mammals in a deciduous forest in NE Germany. *Phytocoenologia*, 32: 627–643. [doi:10.1127/0340-269X/2002/0032-0627](https://doi.org/10.1127/0340-269X/2002/0032-0627)
- Malo, J.E., Suarez, F., 1995: Herbivorous mammals as seed dispersers in a Mediterranean dehesa. *Oecologia*, 104: 246–255. [doi:10.1007/BF00328589](https://doi.org/10.1007/BF00328589)
- Mitchell, B., McCowan, D., Campbell, D., 1983: Faecal deposition as indicators of site use by red deer. *Annual Report Inst. Terr. Ecol.*, 1982: 85–87.
- Mouissie, A.M., Vos, P., Verhagen, H.M.C., Bakker, J.P., 2005: Endozoochory by free ranging, large herbivores: Ecological correlates and perspectives for restoration. *Basic and Applied Ecology*, 6: 547–558. [doi:10.1016/j.baaec.2005.03.004](https://doi.org/10.1016/j.baaec.2005.03.004)
- Muller, F.M., 1978: Seedlings of the north-western European lowland. A flora of seedlings. Wageningen, 656 pp.
- Myers, J.A., Vellend, M., Gardescu, S., Marks, P.L., 2004: Seed dispersal by white-tailed deer: implications for long-distance dispersal, invasion, and migration of plants in eastern North America. *Oecologia*, 139: 35–44. [doi:10.1007/s00442-003-1474-2](https://doi.org/10.1007/s00442-003-1474-2)
- Pakeman, R.J., 2001: Plant migration rates and seed dispersal mechanisms. *J. Biogeogr.*, 28: 795–800. [doi:10.1046/j.1365-2699.2001.00581.x](https://doi.org/10.1046/j.1365-2699.2001.00581.x)
- Pakeman, R.J., 2004: Consistency of plant species and trait responses to grazing along a productivity gradient: a multi-site analysis. *J. Ecol.*, 92: 893–905. [doi:10.1111/j.0022-0477.2004.00928.x](https://doi.org/10.1111/j.0022-0477.2004.00928.x)
- Schmidt, M., Von Oheimb, G., Kriebitzsch, W.-U., Ellenberg, H., 2002: List of northern German typical lowland forest plants (in Germany). *Mitteilungen der Bundesforschungsanstalt für Forst- und Holzwirtschaft*, 206: 1–37.
- Sorokova, M., Pichler, V., 2002: The tourism potentials and impacts in Protected Mountain Areas Polana Protected Landscape Area – Biosphere Reserve, Slovakia. Institute of Forest Ecology, Zvolen.
- Szemethy, L., Mátrai, K., Katona, K., Orosz, S., 2003: Seasonal home range shifts of red deer hinds, *Cervus elaphus*: are there feeding reasons? *Folia Zool.*, 53: 249–258.
- Vellend, M., Myers J.A., Gardescu, S., Marks, P.L., 2003: Dispersal of *Trillium* seeds by deer: implications for long-distance migration of forest herbs. *Ecology*, 84: 1067–1072. [doi:10.1890/0012-9658\(2003\)084\[1067:DOTSBD\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2003)084[1067:DOTSBD]2.0.CO;2)
- Vermeulen, M., 2005: Plant adaptations to ungulates, a classification of defensive strategies of vascular plants in West and Central Europe. Unpublished Msc Thesis, Wageningen.
- Von Oheimb, G., Schmidt, M., Kriebitzsch, W.U., Ellenberg, H., 2004: Dispersal of vascular plants by game in northern Germany. Part II: Red deer (*Cervus elaphus*). *European Journal of Forest Research*, 124: 55–65. [doi:10.1007/s10342-005-0053-y](https://doi.org/10.1007/s10342-005-0053-y)