Ungulate Seed Dispersal

Aspects of endozoochory in a semi-natural landscape

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Aspect of endozoochory in a semi-natural landscape

Zaadverbreiding door Hoefdieren

Aspecten van endozoöchorie in een halfnatuurlijk landschap

door

Eric Cosyns

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1 General introduction

Coastal dune grassland with scattered patches of small *Ligustrum vulgare* and *Salix repens* scrub (Westhoek North).

Introduction

Seed[1] dispersal is an important component of the plant colonisation process [Harper 1977] which may influence many key aspects of plant ecology, including: meta-population survival [Hanski 1998], migration of species both in a paleo-ecological, historical and future context, e.g. under changing climatic conditions [Cain *et al.* 1998; Clark *et al.* 1998; Higgins & Richardson 1999; Higgins *et al.* 2003; Watkinson & Gill 2002], plant recruitment and species diversity [Primack & Miao 1992, Nathan & Mueller-Landau 2000; Willson & Traveset 2000], Plant invasions [Shigesada *et al.* 1995] and the success or failure of restoration and nature management [Bakker & Berendse 1999; Bullock *et al.* 2001].

Animals can play an important role in the seed dispersal cycle through the active or passive uptake of seeds and the subsequent external [epizoochory] or internal transport [endozoochory] of seeds [Wang & Smith 2002]. Depending on the rate of seed passage [endozoochory] or adhesive capacities [epizoochory] and on patterns of animal behaviour, seeds could be distributed over a large area. Selective habitat use will dictate the specificity of sites where seeds could arrive [Stiles 2000]. This potential for directed long-distance[2] seed dispersal may be an important aspect of zoochory as compared to other dispersal mechanisms.

Endozoochory by ungulates in a semi-natural context

Hildebrand [1872] and Hildebrand [1873, cit. in Bonn & Poschlod, 1998] was one of the first authors who systematically derived plausible dispersal modes from seed morphology. Hildebrands' approach can still be found back in studies which document on the major means of seed dispersal [Ridley 1930*;* van der Pijl 1982*;* Grime *et al.* 1988*;* Bouman *et al.* 2000]. In this context plant species bearing fruits or seeds with a fleshy coat or arillus are considered to be dispersed endozoochorically i.e. more specifically by means of birds [ornithochory] and frugivorous mammals and wild carnivores and marsupials [Herrera 1989*;* Willson 1993]. Plant species bearing fruits or seeds which are barbed, hooked, spiny or viscous are thought to be dispersed epizoochorically. A lot of seeds are categorised as 'unassisted'. However, Janzen's [1984] 'the- ' foliage-is-the-fruit' theory suggests that for

[1] Seed is used throughout this work as a synonym of the entity of vascular plants, being or carrying the generative diaspore[s], that which is dispersed [from Gr. diaspeiro: to disseminate]. Hence it can mean either what is morphologically indicated as fruit [fructus: basically, what has developed from the ovary], seed [semen: resulting from the growth of the ovulum, which is situated in the ovary] or spore [in case of ferns].

[2] Long-distance dispersal is difficult to define, because it is case specific. The distinction between long-distance and normal, short-distance dispersal can be made on differences in mechanism [dispersal mode] or differences in consequences [in the simplest case, dispersal distance; Nathan *et al.* [2003]. From a plant species eye of view, long-distance seed dispersal can be considered as each dispersal event that results in distances which are encompassed by the tail of the dispersal curve i.e. where density of deposited seeds per unit area becomes very low. To distinct short-distance and long-distance seed dispersal, we here adopt the approach of Tackenberg *et al.* [2003] who choose a threshold distance of 100m to compare the wind dispersal potential of 335 European vascular plant species.

a number of plant species which have small seeds without obvious external, morphological adaptations to a certain dispersal mode, endozoochory may well be the normal dispersal mode for these unassisted seeds. Moreover plants and seeds are selected for this dispersal mode. At the time of seed set plants may promote herbivory by large ungulates to achieve seed dispersal. Quinn *et al.* [1994] could underpin this hypothesis in the case of Buffalo grass [*Buchloe dactyloides*] but Dinerstein [1989] failed to find evidence in the plant species of South Asian flood-plains.

At the beginning of the 20th century some authors e.g. Kempski [1906], Adams [1907] [cit. in Bonn & Poschlod 1998] and Kerner von Marilaun 1916 [cit. in Lennartz 1957] were already aware from of the potential of endozoochorous seed dispersal by domestic livestock. They ran some experiments with cattle and horses to get insight in the importance of the endozoochorous seed dispersal process within an agricultural context, i.e. to prevent the spread of undesirable 'weeds' into the arable fields and pastures, e.g. by grazing livestock or by spreading of the livestock manure.
After the second world war, further attempts were made by Lennartz [1957] who investigated the survival capacity of gut-passed seeds of a selection of temperate grassland species and by Müller-Schneider [1954] and Müller [1955] who elaborated endozoochory mainly by cattle and red deer in semi-natural grassland.
During the last two decades the scientific study of endozoochory in temperate floras gained momentum. The focus is now on a broad spectrum of aspects of endozoochory.
A major part of the studies emphasize the role of domesticated and wild ruminants and to a lesser extend rabbits as seed dispersing agents for a wide range of plant species, even those with obvious adaptations to other assumed dispersal modes [Welch 1985; Bakker 1989; Malo & Suárez 1995a; Pakeman *et al.* 1998]. Pakeman *et al.* [2002] recently succeeded to identify ecological correlates of endozoochory by sheep and rabbits in a cool temperate flora. The necessity for such studies is related to the almost impossible task to identify all plant species which can take advantage of this dispersal mode.

To get more insight in the significance of endozoochory in the dispersal and regeneration of plant species experimental studies were conducted. Most of the more recent feeding experiments dealt with one or a few species of domesticated ruminants [cattle and sheep] and with plant species that are of importance in an agricultural context [Özer 1979; Russi *et al.* 1992; Gardener *et al.* 1993; Ghassali *et al.* 1998]. These studies showed considerable differences in germination success of gut-passed seeds of different plant species.
In addition these studies documented on passage time which is an important aspect of the potential long-distance seed dispersing capacity of the herbivore species [Pakeman 2001].

Another important step in the seed dispersal cycle is the establishment of plant individuals from endozoochorically dispersed seeds [Wang & Smith 2002]. Only a few studies put attention to this phase. But Welch [1985], Malo & Suárez [1995b] and Pakeman *et al.* [1998] respectively demonstrated that ungulate and rabbit dung could be an important seed source for plant colonisation in grasslands. Dai [2000] and Pakeman *et al.* [1998] furthermore give evidence for an interaction between soil seed bank and dung seed content in relation to germination and establishment of grassland species in gaps.

The significance of endozoochory for vegetation dynamics and diversity is discussed in several studies [e.g. Welch 1985; Malo and Suárez 1995a; Malo and Suárez 1996; Pakeman *et al.* 1998; Bakker & Olff 2003]. Bonn & Poschlod [1998] furthermore stressed the ability of domesticated ungulates to act as dynamic ecological corridors i.e. connecting isolated patches of similar habitats in a landscape. However few studies really showed the positive effect of endozoochorous seed dispersal on restoration management [Russi *et al.* 1992; Traba *et al.* 2003].

Semi-natural landscapes e.g. heathlands, coastal dunes and moorlands are of key interest in Northwest European nature conservation. It are in fact the only remaining sources offering a broad array of spontaneous, wild, native plant and animal species. They are established as a result of historical land use including the closely related dynamics which acted together with still occurring natural processes. These dynamics may have influenced the species diversity and composition of the semi-natural habitats through their effect on e.g. seed dispersal [Bonn & Poschlod 1998; Bruun & FritzbØger 2002]. Adequate preservation and restoration of these semi-natural habitats need knowledge of former land use practice and the closely related dynamics in order to be able to re-establish the same practice or to find suitable surrogates. Most of the semi-natural landscapes exist of open habitats which could be managed by grazing, mowing and cutting. Since the 1970's the use of domesticated ungulates i.e. cattle, horses, sheep has become increasingly popular in nature management [e.g. Piek 1998; Eggermont et al. 1996]. Grazing has received considerable attention from biologists who aimed to determine its impact on plant diversity. It is obvious and frequently proven that grazing influences plant diversity, but the reasons why its effect may differ considerably are less clear [Olff & Ritchie 1998]. One possible component of the answer to this question is the aspect of zoochorous seed dispersal. However, the role of large herbivores in the dispersal and establishment of plant species is poorly understood [Van Wieren & Bakker 1998]. Unravelling zoochorous dispersal mechanisms in a semi-natural environment may therefore offer both fundamental and necessary applicable ecological knowledge.

To understand the potential role of seed dispersal in a semi-natural context, we chose coastal dunes as general environment. The diversity of plant species and plant communities is very high [about 40% of the Flemish plant species occur in our study area] which, together with the recently established grazing management [horses and cattle], offered opportunities to study different aspects of endozoochorous seed dispersal by horses and cattle within the semi-natural coastal landscape. This setting furthermore yields potential to contribute to the increasing demand for reliable autoecological information on dispersal-related topics of the temperate, native European flora [Grime *et al.* 1988; Knevel *et al.* 2003].

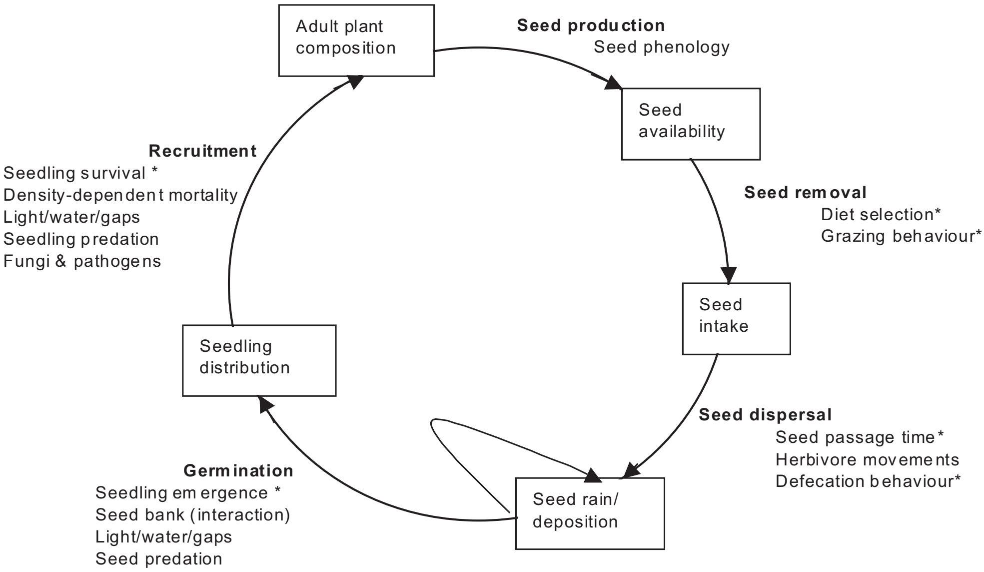


Figure 1.1 The seed dispersal cycle as it happens in grazed temperate habitats, with indication of the main patterns [framed] and processes [bold] including several important aspects [after Wang & Smith 2002]. Aspects which are studied in this Ph.D. are indicated with an asterisk [*].

Aims of the study

Studies on ungulate endozoochory mainly stress the potential importance of this seed dispersal mechanism for far more plant species than previously thought [Pakeman *et al*. 2002]. But even for the rather small and well-studied Northwest European flora we lack reliable information on the kind of dispersal modi plant species really use. Very often the dispersal mode of a plant species is deduced from its morphological adaptations, but very little is known about the entire dispersal spectrum of every species and the relative importance of each of the dispersal modes involved. Above all, there is still a considerable gap in our understanding of the ecological significance of endozoochory as a seed dispersal mode in grazed landscapes. Therefore, there is a need to further elaborate the different constituent steps and processes inherent to this dispersal mode [Fig.1.1], e.g.:

- quantification of seed production and availability of seeds in grazed environments;
- habitat use and diet selection of ungulates;
- germination success after gut passage;
- dung [seed] deposition patterns e.g. the distance that germinable seeds are deposited from the source plant and in what habitats seeds tend to arrive [are their indications of directed dispersal?];
- seedling emergence and plant establishment under natural conditions.

This study contributes on a selection of the mentioned aspects. The aims are: To provide further knowledge on which plant species could be dispersed endozoochorically by mammal species, differing in body size and gastero-intestinal system, i.e. free ranging cattle, different equid breeds, sheep and rabbit;

- To compare the dung germinable seed content to the diet composition of free ranging ungulates;
- To explore the possible ecological correlates of the dung germinable seed content;
- To determine experimentally the germination success and the mean retention time of gut-passed seeds of a selection of plant species;
- To determine plant establishment from the dung seed content under field conditions;
- To integrate this information in conclusions on the importance of endozoochory from a plant ecological point of view and to provide recom-
- mendations for nature conservation.

The Study areas

This Ph.D. study on ungulate endozoochory was conducted at four sites in the Belgian and North French coastal dunes [Fig.1.2]. The Westhoek [335 ha] and Doornpanne dunes [190ha] are both located at close distance from the Belgian coast [0-1 km]. Both dune areas are part of a belt of recently established dunes [c. 500-1000 years B.P., De Ceunynck 1992]. The sandy soil is calcareous, with a varying $CaCO_3$ content [8-4%, Ampe 1996]. In this study the focus was on two sites in the Westhoek, which both are grazed by horses and cattle: Westhoek North [since 1998] and Westhoek South [since 1997]. At the Doornpanne we studied endozoochory in an area which, since 1996, is grazed by Shetland pony's [Table 1.1].
The 'Dunes fossiles de Ghyvelde' [France] are situated 3 km from the coast. These dunes are among the oldest dunes of the Flemish coastal plain, which extends from Cap Blanc Nez to the Scheldt estuary. These dunes are considered as remnants of a Neolitical dune belt [c. 4500 years B.P., De Ceunynck 1992]. The soil is decalcified down to 2-3 m, with a $CaCO_3$ content of less than 2 % [Ampe 1996]. Since 1997, part of these dunes are grazed by Haflinger horses [Table 1.1]

The floristic richness and diversity of plant communities results from the complexity of the underlying, often small scaled, variety in abiotic patterns and processes, which can be summarised as follows:

- the micro climatic conditions which vary along a gradient perpendicular to the coastline;
- the variation in lime content;
- the impact of groundwater fluctuations.

Biotic factors are superimposed on the abiotic conditions. Man, with his domestic grazing stock and rabbits are the most important among them. During the 19th and the beginning of the 20th century grazing by domesticated livestock was a common practice in the coastal dunes [De Smet 1961]. For example in 1828 the dune area of the Western Belgian coast [c. 2500 ha] was grazed by 450 sheep, 240 cows, 112 donkeys and 51 horses. Scrub was regularly cut down and used as firewood. Locally, small fields were created in the dunes for the cultivation of potatoes, rye and vegetables. Dune vegetation then largely consisted of grey dunes, moist slack vegetation and dune grassland [Massart 1908]. After this type of agricultural land use was abandoned the entire dune area showed a strong tendency towards scrub development since the 1970's [De Raeve 1989]. Yet, the diversity of plant communities and species is still high. For our purposes, however, we will generalise to some degree, and in the studies of habitat use by the ungulates we have recognised ten major habitat types [Table 1.2].

Habitat types were distinguished according to vegetation structure and abiotic conditions, and therefore mainly exist from different plant communities. Vegetation data were compiled from different studies: Westhoek South [Goerlandt 1999], Westhoek North [Degezelle 2000, Cosyns unpubl.], Doornpanne [1999, Cosyns unpubl.; Waumans 2001], Ghyvelde [1999, Cosyns unpubl; Hendoux 1997]. The vegetation was mapped using recent aerial photographs [1:2,500 or 1:5,000] and additional field measurements. The proportion habitat in each study area was derived from these vegetation maps using a GIS [Arcview] [Table 1.2]. The species nomenclature in this Ph.D. thesis follows Lambinon *et al.* [1998].

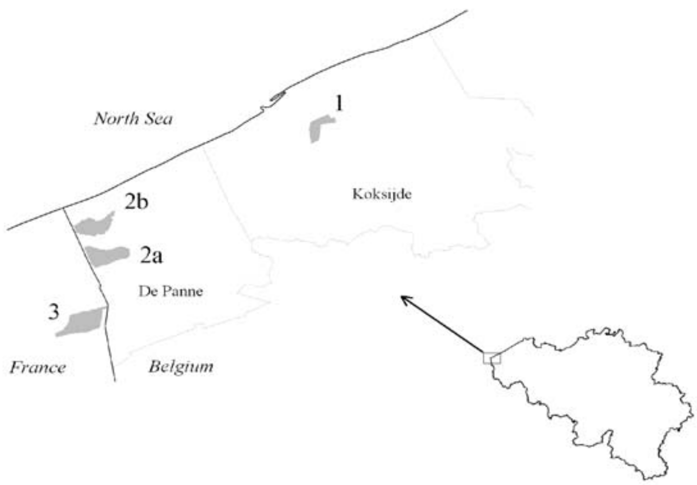


Figure 1.2 Location of the study sites in the Belgian and North French coastal dune area. The numbers on the map indicate the following nature reserves: [1] Doornpanne, [2] De Westhoek [2a Westhoek South, 2b Westhoek North], [3] Dunes Fossiles de Ghyvelde [France]. In each of these nature reserves we studied the process of endo-zoochory.

Table 1.1 Site characteristics: geographical position, horse and cattle breeds and adult herbivore numbers [#] present during the sampling period [July - October 2000].

Site	Geographical position	Horse and cattle breed	Area [ha] grazed	# animals [2000]
Ghyvelde [France]	51° 02' 48'' N 2° 33' 02'' E	Haflinger horse	75	14
Westhoek North [Belgium]	51°05' 12'' N 2° 35' 27'' E	Konik pony Scottish Highland cattle	54	9 4
Westhoek South [Belgium]	51°04' 50'' N 2° 34' 19'' E	Shetland pony Scottish Highland cattle	61	19 4
Doornpanne [Belgium]	51° 07' 14'' N 2° 39' 44'' E	Shetland pony	30	7

Major habitat types

White and Grey dunes [A/T] are mainly found on dry, dune ridges. Due to wind dynamics which cause sand erosion and accumulation, these dunes suffer from a high degree of abiotic stress. White dunes [A; *Ammophilion* communities] are therefore scarcely covered with vegetation. It is poor in vascular species and mainly exist of *Ammophila arenaria*, *Festuca juncifolia* and *Carex arenaria*. As sand mobility decreases and relief is more or less fixed, grey dunes [T] develop. Grey dunes [*Tortulo-Koelerion* communities], on calcareous soils, are covered with a closed moss layer dominated by *Tortula ruralis* ssp. *ruraliformis*. On partly decalcified soils, *Hypnum cupressiforme* and *Cladonia* div. spp. dominate the moss layer. The sparsely covering herb layer exists of several winter annuals [e.g. *Erophila verna*, *Veronica arvensis*, *Arenaria serpyllifolia*, *Phleum arenarium*, *Crepis capillaris*] and some perennial species [e.g. *Carex arenaria*, *Hypochaeris radicata*, *Sedum acre*, *Festuca rubra* ssp. *arenaria*]. Grey dunes appear to be a rather stable successional stage, further succession leads to open, low scrub of *Hippophae rhamnoides*.

Dune grassland on dry soil [Gd], is characterised by the dominance of graminoids and a variety of herb species. Frequent occurring graminoids are *Poa pratensis*, *Festuca rubra* and *Carex arenaria*. Frequently recorded herb species are e.g. *Achillea millefolium*, *Veronica chamaedrys*, *Galium verum*, *Plantago lanceolata* and *Lotus corniculatus*. The variant on lime rich sandy soils as it mainly occurs at the Westhoek North and at the Doornpanne is very rich in species, the concentration of 'chalk grassland' taxa being conspicuous e.g. *Helianthemum nummularium*, *Potentilla neumanniana*, *Asperula cynanchica*, *Thesium humifusum*, *Primula veris* [*Polygalo-Koelerion* communities]. Locally, *Rosa pimpinellifolia* invades these grasslands. At Ghyvelde a variant of decalcified sand, less rich in species compared to the former type, is distinguished. *Jasione montana*, *Carex arenaria*, *Anthoxanthum odoratum*, *Trifolium arvense*, *Ornithopus perpusillus* and *Aira praecox* are among the most abundant species [*Thero-Airion* and *Plantagini-Festucion* communities].

Dune slack vegetations [Gw] are only well developed in the Westhoek. These dune slacks are influenced by seasonally varying groundwater fluctuations close to the soil surface. This habitat type includes several successional phases, i.e. short pioneer vegetation [only Westhoek North] and late successional graminoids and herb dominated plagioclimax vegetation [see also Bossuyt et al. 2003a +b]. Young phases [< 5 years] have a sparse vegetation, mainly dominated by graminoids: *Juncus articulatus*, *Carex arenaria*, *C. flacca*, *C. viridula*, *Agrostis stolonifera*. *Salix repens* is quite abundant from an early stage in succession onwards. Gradually more species establish in the next two phases [5-25 years]: e.g. *Sagina nodosa*, *Epipactis palustris* and *Parnassia palustris*. *Salix repens* becomes the dominant species, unless mown or cutted. The older phases [25-50, +50 years: Westhoek North and South] are dominated by graminoids, such as *Calamagrostis epigejos*, *C. canescens*, *Holcus lanatus*, *Agrostis stolonifera* and/or *Juncus subnodulosus*. Several herb species contribute to a further enrichment of the vegetation: *Lysimachia vulgaris*, *Lythrum salicaria*, *Lycopus europaeus*, *Iris pseudacorus*, *Hydrocotyle vulgaris*, *Galium uliginosum*. If not managed [e.g. mown or grazed], succession in dune slacks give rise to rapid scrub encroachment.

Tall scrub [S] essentially dominated by species like *Hippophae rhamnoides*, *Ligustrum vulgare* and *Salix repens* largely replaced the species rich grassland vegetation and now occupies 25-65 % of the study areas [Table 1.2]. At the Westhoek South *Ligustrum*-scrub is locally replaced by a more open *Crataegus monogyna* dominated vegetation. In the gaps of aging and degrading scrub, *Calamagrostis epigejos* or *C. canescens* give rise to more or less monospecific grassland [C]. When grazed or mown the dominance of both grass species rapidly declines and a less species poor grassland soon develops [see Chapter 6, Table 6.1].

Between 1996 and 1998, at the Westhoek South and North, several hectares of scrub were removed. After scrub removal a tall, herb-dominated vegetation [U] established. The tall herb vegetation on wet soils [Uw], influenced by a high mean groundwater level and periodically inundated, mainly exists of a closed grass layer, dominated by *Poa trivialis* and patches of *Lythrum salicaria*, *Lysimachia vulgaris*, *Lycopus europaeus* and *Eupatorium cannabinum*. Tall herb vegetation on drier soils [Ud], are characterized by an open grass layer of *Holcus lanatus,* sparsely accompanied by *Poa pratensis*, *P. trivialis* and *Calamagrostis epigejos*. In addition some tall herb species occur: *Cirsium arvense*, *Senecio sylvaticus, S. jacobaea* and *Eupatorium cannabinum*.
All forests [F] have once been planted, usually on abandoned arable fields. *Alnus glutinosa*, *Populus tremula*, *P. alba* and *Populus x canadensis* are the most frequent planted tree species; they dominate the present-day forest canopy. In general, the ground flora consists of ruderal annuals, such as *Claytonia perfoliata*, *Anthriscus caucalis* and *Stellaria media* and of perennial species, like *Urtica dioica*, *Glechoma hederacea*, *Galium aparine* and *Poa trivialis*.
Paths can be considered as a kind of 'artificial' habitat occupying a 2-3m wide strip of disturbed ground that is sometimes slightly raised above the surrounding area. It is frequently used by the ungulates, both for grazing and walking. The vegetation is dominated by *Poa trivialis*, *P. annua* and *Juncus bufonius.* Locally, in open patches, ruderal annuals may establish, such as *Claytonia perfoliata*, *Stellaria media* and *Arenaria serpyllifolia*.

Dung collection and treatment

Germination data, discussed in chapters 2, 3 and 4, were obtained from field samples which were all collected during the same sampling period, using the same dung collection and seed germination procedure.

Dung was collected during seven, more or less fortnightly period [18th July-11th October 2000] at four sites: Westhoek North, Westhoek South, Doornpanne and 'Dunes fossiles de Ghyvelde'. At each study site we observed the ungulates defecation behaviour. Freshly deposited dung was immediately collected after defecation occurred, leaving behind the lowermost part of the dung to avoid contamination of seeds on the soil surface. From each type of herbivore 2 times 2.5 L of freshly deposited excrements were collected during each session. Immediately after dung collection, samples were spread out in trays [40 x 40 x 10cm] and put in a greenhouse [< 35 °C] to dry. Horse dung samples then on average weighted 210 g [± 20.6 Stdev, n = 12]. Cattle dung samples weighted 303 g [± 14.6 Stdev, n = 12].

After sun-drying in a greenhouse [2-3 weeks], the samples were kept at 4 °C for at least two weeks. After grinding in a Retsch mill [type SK 100], the samples were spread out over a sterilised sand/peat substrate [40 x 40 x 2 cm, 1:1 ratio] in a layer of about 0.75 cm. To detect possible germination from the potting soil substrate and contamination in the greenhouse, 15 trays with only the sterilised sand/peat substrate were also set up.

To maintain humidity, sample trays were watered twice a day during the whole germination period. Greenhouse conditions were kept at 20-25 °C with a relative humidity of 50-60 % during 16 hours of light [280-410 mmol.$m^{-2}.s^{-1}$] and at 10-15 °C and 80-90 % relative humidity during 8 hours of darkness. Counting of seedlings was conducted as soon as identification was possible and was continued for six months. During the last two months very few seedlings emerged. Counted and identified seedlings were removed to avoid competition between seedlings and to prevent flowering. Only in a few cases, seedlings could not be identified accurately. This was the case for *Erodium cicutarium* and *E. lebelii*, *Sagina apetala* and *S. procum-*

bens and *Veronica chamaedrys* and *V. arvensis*, respectively. They were lumped in three "pseudospecies". We used the genus name for those seedlings that could only be identified at the genus level, e.g. *Epilobium* spp. *Poa* spp., *Rumex* spp. and considered them as 'species' in the data analysis.

Table 1.2 Main habitats [and their abbreviation as used in this thesis] at the different study sites with a brief description of their vegetation characteristics and indication of their area proportion. The total number of plant species recorded during several inventories [1990-2000] at the different sites is also shown.

Study sites		**Westhoek S.**	**Westhoek N.**	**Doornpanne**	**Ghyvelde**
Habitat	Description	Area [%]	Area [%]	Area [%]	Area [%]
White dunes [A]	Open vegetation with *Ammophila arenaria, Carex arenaria, Festuca juncifolia*	1.70	4.11	9.08	
Grey dunes [T]	Mosses and lichens rich dunes with scattered *Carex arenaria* and annuals.	7.13	5.24	4.82	22.20
Vegetation dominated by tall herbs on dry soils [Ud]	Short herb layer [*Holcus lanatus*, *Poa trivialis*] with scattered patches of tall herbs [e.g. *Eupatorium cannabinum, Cirsium arvense, Senecio jacobaea*]	5.62	8.23	4.74	0.91
Vegetation dominated by tall herbs on wet soils [Uw]	Short herb layer [*H. lanatus, P. trivialis*] with scattered patches of tall herbs [e.g. *E. cannabinum, Lythrum salicaria, Lycopus europaeus*]	3.76	5.39	-	-
Dune grassland on dry soils [Gd]	Short grasslands with a high plant species diversity [e.g. dicotyledons] incl. dune grasslands dominated by *Rosa pimpinellifolia*	4.09	5.12	9.02	50.32

Study sites		**Westhoek S.**	**Westhoek N.**	**Doornpanne**	**Ghyvelde**
Habitat	Description	Area [%]	Area [%]	Area [%]	Area [%]
Wet dune slack vegetation [Gw]	Graminoid dominated vegetation type, including short pioneer vegetation with *Carex* spp., *Juncus* spp. ; young *Salix repens* and *Hippophae rhamnoides* scrubs and herb rich later succession phases.	8.30	3.64	-	0.74
Almost monospecific grassland [C]	Tall vegetation dominated by *Calamagrostis epigejos*, *C. canescens* and/ or *Arrhenaterum elatius*. Frequently under deteriorating scrub.	5.36	10.95	1.35	0.85
Scrub [S]	Scrub dominated either by *Ligustrum vulgare*, *Hippophae rhamnoides*, *Salix repens* or mixed with other shrubs.	48.60	56.21	64.05	24.99
Forest [F]	*Populus* spp. or *Alnus glutinosa* dominated wood patches	15.40	0.31	3.93	[incl. in S]
paths	Pioneer vegetation of dry or wet situations	0.50	1.45	2.87	-
	Area [ha]	61	54	30	75
	Total number of plant species	422	402	278	139

Outline of the thesis

The first part of this thesis [Ch. 2-4] focuses on the potential for grassland plants to be dispersed endozoochorically. The study sites offered opportunities to extend the knowledge on plant species that could be dispersed endozoochorically and to analyse the impact of different sites and herbivore species. In chapter 2 these aspects are investigated together with the animals' grazing and defecation behaviour which enabled us to get a first quantified view on seed deposition patterns across habitats during the main fruiting season. In the 3rd chapter a first attempt is made to compare the dung germinable seed content to the diet composition of the studied free ranging horses and cattle.

Horses are frequently used in nature management in Western Europe. Until now very few studies focussed on endozoochory by horses. Horse dung germinable seed content is analysed in detail in the 4th chapter. The analysis further adds to the knowledge on endozoochorically dispersed plant species. Seed characteristics of the species involved were used to elaborate the ecological correlates of horse endozoochory.

The second part of this thesis [Ch. 5-6] focuses on the possible realization of endozoochory in the field, dealing with the critical phases in the seed dispersal cycle [fig. 1.1] and hence emphasizing the endozoochorous dispersal costs for the plant.

One of the most critical phases of an endozoochorically dispersed seed is its passage through the gastro-intestinal system of the herbivore. In chapter 5, this phase is investigated within an experimental set up. In most feeding experiments only ruminant species, mostly cattle, are involved and little attention is given to plant species of semi-natural, cool temperate grasslands. Therefore, seeds of 19 plant species that are important constituents of such grasslands were fed to five different species of herbivores, selected for differences in body size and gastro-intestinal system. The experiment provides information on germination success and mean retention time across animal and plant species.

The next important step in the process of successful endozoochorous seed dispersal by ungulates is the seedling emergence and plant establishment from seeds which were deposited as part of the ungulate dung. Chapter 6 provides information on an experiment in which these aspects of the seed dispersal cycle are addressed.

The synthesis in chapter 7 puts the findings from the entire study within a broader perspective and deals with the possible significance of endozoochory in a semi-natural context. The chapter is concluded with the possible implications for nature conservation and some perspectives for further research.

2 Potential endozoochorous seed dispersal by large ungulate herbivores in a spatially heterogeneous dune landscape

Eric Cosyns , Sofie Claerbout, Indra Lamoot & Maurice Hoffmann

Emerging seedlings from dung samples under greenhouse conditions (Wenduine, 2000).

Abstract

Seed dispersal has become an important issue in plant ecology and restoration management. In this chapter we examined dung seed content and seed deposition patterns of horses [Shetland and Konik breeds] and Scottish Highland cattle grazing two medium scaled coastal dune nature reserves. 2 times 2.5L of fresh dung from each type of herbivore was collected during 7 consecutive sessions in the main fruiting season. Dung samples were placed under greenhouse conditions after drying and cooling. Animal defecation patterns were derived from a study of herbivore activities during 6 hour sessions, 8 times a month.

117 plant species i.e. 27 % of all species occurring in the study area, were recorded as seedlings emerging from the dung samples. In general, dung seed density is high [1158 seedlings/sample]. Most plant species [62 %] were recorded from less than 20 samples [40 %]. A comparable amount of plant species [66 %] emerged with on average less than 1 seedling per sample, only 12 % count more than 10 seedlings per sample. The most abundantly and frequently recorded plant species were *Urtica dioica*, *Juncus* spp. and different *Poaceae* and *Caryophyllaceae* species. Seed density and species richness were further analysed in order to detect possible animal and site related characteristics.

Dung deposition patterns reflect a disproportional use of habitats and hence a non random seed deposition among habitats. Calculated seed deposition per square meter ranged from a few [< 10] to more than 100 in the most frequently selected habitat types in relation to their surface.
From the herbivores' selective habitat use and their estimated mean retention time we can further assume their ability for inter-habitat endozoochorous seed dispersal. This characteristic of large herbivores is further discussed in the light of nature management and restoration.

Key-words: cattle, coastal dunes, defecation behaviour, endozoochory, grassland dynamics, horse

Revised version accepted: Plant Ecology

Introduction

Plant diversity is affected by herbivores through their impact on dominant plant species, plant regeneration opportunities and propagule transport [Olff & Ritchie 1998]. The latter has become an important issue in plant ecology in general [Primack & Miao 1992] and restoration management in particular. The [re-]establishment of characteristic semi-natural plant communities sometimes fails, due to unsuitable abiotic conditions for the target species or because of biotic constraints [Bakker 1998; Bakker & Berendse 1999]. Many plant species cannot rely on a long-term persistent seedbank for regeneration after their disappearance from the relict vegetation. Seed dispersal then becomes a serious bottle-neck in restoration management [Verhagen *et al.* 2001; Pywell *et al.* 2002]. Therefore studies on possible seed dispersal mechanisms are of key interest in the understanding of the colonisation abilities of plants at the landscape scale. Despite the increasing demand for reliable autoecological information on dispersal related topics of the temperate, native European flora, most of this information is still anecdotal [Grime *et al.* 1988]. It certainly can not be derived adequately just from assumed morphological adaptations to specific dispersal mechanisms.
In the framework of nature conservation and restoration, seed dispersal by livestock was examined by Welch [1985], Bakker [1989], Malo & Suárez [1995] and Stender *et al.* [1997], who show the potential of endozoochorous seed dispersal in semi-natural landscapes. Moreover those studies indicate that many more plant species are successfully dispersed through endozoochory than previously thought [Pakeman *et al.* 2002].

Seed dispersal patterns are not simply a function of distance from the parent plant. It can also be affected by animal behaviour [Nathan & Muller-Landau 2000]. Although selective habitat use is a well known feature of mammalian herbivores [e.g. Gordon 1989; Duncan 1983; Putman 1986], there has been almost no attention so far for its potential importance for seed dispersal patterns in West European situations. Only Malo *et al.* [2000] demonstrate non random deposition of endozoochorously dispersed seeds among four different *dehesa* habitats. Malo *et al.* [1995] mentioned rabbits to act as directional seed dispersers in Mediterranean pastures. From the establishment of a year round, low density grazing regime, plant ecologist and nature managers expect that through the process of selective grazing the herbivores will create a mosaic of different vegetation communities varying in structure and plant species composition. It is believed that such a mosaic in the end will guarantee the survival and persistence of a high degree of biodiversity. However the role of herbivores in the dispersal and establishment of plant species is poorly understood [van Wieren & Bakker 1998]. Particularly, field data on endozoochory in a North West European context are still rather scarce. The specific conditions i.e. high degree of plant species richness and plant communities due to a considerable variation in abiotic conditions, of the coastal dune landscape offered unique opportunities to extend the existing knowledge on plant species that can take advantage of endozoochory. Furthermore the heterogeneous distribution of plant communities and formations [further called habitats] across the landscape forced us to think about the possible impact of selective habitat use on seed deposition patterns. With this chapter we try to combine data on seed content and seed deposition patterns to get a first insight into the potential role of endozoochorous seed dispersal within a medium-scaled fragment of the semi-natural coastal landscape. Therefore our aim is to:

- qualify and quantify the dung seed content of herbivore dung during the main fruiting season;
- compare dung seed content characteristics between different animal species grazing the same site [animal effect] and the same animal species grazing different sites [site effect];
- determine seed deposition characteristics among different habitats.

Materials and methods

Study area and species

The data on dung germinable seed content, that are used in this chapter, are obtained from 7 dung collection sessions [18th July-11th October 2000] at Westhoek North and South [see chapter 1] The dune landscape is dominated by *Hippophae rhamnoides* and *Ligustrum vulgare* shrub. Grassland covers at least one third of the area. Part of the grassland area is scattered within the scrub as small and mostly species-poor remnants of dune grassland or as species-poor *Calamagrostis epigejos* dominated patches, which were recently established after scrub degradation. Flowering and fruiting of plant species is concentrated from April to October.

The selected dune nature reserves are stocked with small numbers of two species of domestic livestock [Table 2.1]: Scottish Highland cattle and Konik pony [Westhoek North], Scottish Highland cattle and Shetland pony [Westhoek South].

Table 2.1 Study sites with indication of the animal breed, total number of individuals present during the sampling period, adults' mean body weight [kg, ± SD] and estimated mean retention time [MRT] in hours [h]. Body weights are mean maximum weights [October 2000] or [*] are visually scored estimates of body weight of adult individuals which were used both for dung sampling and to behavioural observations. Mean retention time is estimated according to Illius and Gordon [1992].

Site + Area grazed [ha]	Animals	Bodyweight [kg] Mean [± SD]	# Adults weighed	MRT [h]
Westhoek North [54 ha]	Konik pony [9]	330 [± 38]	7	41
	Scottish Highland [cattle] [4]	530 [± 73]	4	74
Westhoek South [61ha]	Shetland pony [19]	201 [± 24]	13	36
	Scottish Highland [cattle] [4]	+/-500*	4*	+/-74

Flora and Vegetation data

Presence of plant species was based on surveys, which resulted from a compilation of several inventories that took place between 1990-2000. Main vegetation units at each site were sampled in June and July 1998 and 1999 using visual estimates of plant species in plots [1x1, 2x2 or 3x3 m], usually 5 plots per vegetation structural unit. Plant species abundance was considered and noted as percentage cover [Londo decimal scale, Londo 1976]. Overall abundance [cover %] was calculated for each species as the overall sum of mean cover c in vegetation unit i times the relative area a, occupied by vegetation unit i:

$$\sum_{i=1}^{n} \% c_i \times \% a_i$$ (n = number of vegetation units = 10, see table 1.2 - chapter 1)

The relationship between dung germinable seed densities and plant species abundance [cover %] was analysed using Spearman rank correlation.

For the evaluation of the importance of endozoochory for nature management we will focus on the presence of 'Red List' and 'Characteristic species' in dung samples and vegetation. Red List species are considered rare or threatened in Flanders [Biesbrouck *et al.* 2001, see Appendix 2.1]. Characteristic species are species that are indicative for the main plant community [-ies] habitats exist of [see chapter 1] i.e. it are diagnostic species according to Schaminée et al. [1995-1998]. The characteristic and 'Red List' species will further be mentioned as 'target species' [Appendix 2.1]

Dung collection and treatment

Dung collection and treatment was carried out as described in chapter 1. At both sites we collected 2 times fourteen dung samples of each animal species. However, during the germination experiment in the greenhouse five cattle-dung samples were lost due to stagnating water.
Differences in dung germinable seed density and plant species richness, between herbivore species and between sites, were tested using data of

dung seed content of different herbivore species at the same site [animal effect] and of same herbivore species grazing both study sites [site effect]. Because of non normality and lack of homogeneity of variance we used the non-parametric Mann-Whitney test for statistical analysis of these data [Siegel & Castellan 1988].

Animal defecation behaviour

Herbivore behaviour data [e.g. movements, grazing and defecating] were extracted from our nutritional ecology research programme [Cosyns *et al.* 2001]. During 6 hour observation periods [6-12h, 12-18h, 18- 24h, 0-6h], 8 times a month, continuous time budget of herbivore activity was registered by one observer close to the focal animal [within a distance of less than 3m]. This animal was randomly chosen before each session started. During the observations all bitten plant species and plant parts were recorded as well as the plant community, in which activities occurred. Each 15 minutes the individuals' position was recorded on a detailed aerial map [1/2000]. These site locations allowed an estimation of possible seed dispersal distances by taking into account independent pairs of positions, which an animal occupied at the beginning and at the end of the mean retention time period.

To be able to estimate the total volume of dung produced during the sampling period and per site we counted defecation frequency per animal species during the behavioural studies and estimated the mean pile volume per animal species by carefully measuring the volume of 20 freshly deposited piles per animal species. Therefore, each individual dung sample was put in a water-filled measuring jug [5L, ±0.025L]. By measuring the differences of the water level before and after adding the dung sample we obtained the dung volume. Differences in mean dung volume, between animals, were analysed using an independent samples T-test [Sokal & Rohlf 1997].

Summer defecation frequencies [1998, 1999 or 2001] were used to calculate the animals species' proportional defecation pattern over all habitats. A chi-square analysis, with Yates correction, was used to determine whether herbivores utilised all habitat types in proportion to their availability [Siegel

& Castellan 1988]. If chi-square analysis indicated a significant difference existed between habitat use for defecating and availability, significant disproportional use for individual habitat types was determined using the Bonferroni Z simultaneous confidence interval [CI] approach revised by Byers & Steinhorst [1984]. Grazing and defecation activity data [%], observed in each of the different habitats were subjected to a Spearman rank correlation to indicate whether both activities were correlated. All statistical tests were carried out using Spss software 11.01 [Spss, 2001] except for the computation of the CI approach [Byers & Steinhorst 1984] which was conducted in excel [MS Office, 1997].

Results

Viable seed content of herbivore dung

From the remaining 51 dung samples a total of 59,049 seedlings emerged. The mean number of seedlings per sample was 1158 ± 722.2. These seedlings represent 117 different plant taxa or 27 % of all species ever recorded in both study areas [Fig. 2.1]. The mean number of plant species per sample of 2.5 litres of excrements was 31 ± 5.6.

Urtica dioica, *Juncus bufonius* and *J. articulatus* were among the most abundant germinating species. Seedlings of *Poa trivialis*, *Veronica chamaedrys /arvensis*, *Cerastium fontanum*, *Poa pratensis*, *Agrostis stolonifera* and *Sagina procumbens / apetala* appeared very frequently and were reasonably abundant. Moreover, 65 %-85 % of the dung germinable seed content exist from these species [Fig. 2.2]. Other plant species showed notable numbers of seedlings in part of the samples e.g. *Lycopus europaeus*, *Ranunculus repens* and *Agrostis capillaris* in cattle dung at Westhoek North [Fig. 2.2]. A considerable number of plant species was recorded regularly [> 40 % of all samples] but showed on average low mean seedling densities [< 5] e.g. *Cardamine hirsuta*, *Epilobium*, *Geranium molle*, *Phleum pratense*, *Potentilla reptans*, *Rubus caesius* and *Veronica officinalis* [Appendix 2.1].

Seedling densities of species that are of nature conservation interest i.e. Target species represent 2-5 % of the total number of seedlings recorded from each type of dung samples [Fig. 2.2]. The number of Flemish Red List species occurring in dung samples was low [2-7] compared to the total number of Red List species presented at Westhoek south [63] and North [76] [Table 2.4]. Out of the 112 [Westhoek N.] respectively 109 [Westhoek S.] Target species 33 and 26 occur in dung samples [Table 2.4].The number of target species, that are representative for a particular type of habitat, varied across the different types of habitat as did the relative proportion of Target species recorded from dung samples [Fig. 2.3]

Viable seed content and vegetation abundance

Species [co-] dominant in the vegetation [e.g. *Calamagrostis epigejos*, *Holcus lanatus*] as well as less frequent species [e.g. *Helianthemum nummularium*, *Ranunculus bulbosus*, *Trifolium arvense*] were dispersed endozoochorously. However, the rare species are less well represented in the dung samples than expected. Frequent and codominant species are better represented in the dung samples than expected [Table 2.3].
Seedling density in cattle and horse dung was positively correlated with species cover at Westhoek North but not at Westhoek South [Table 2.2]

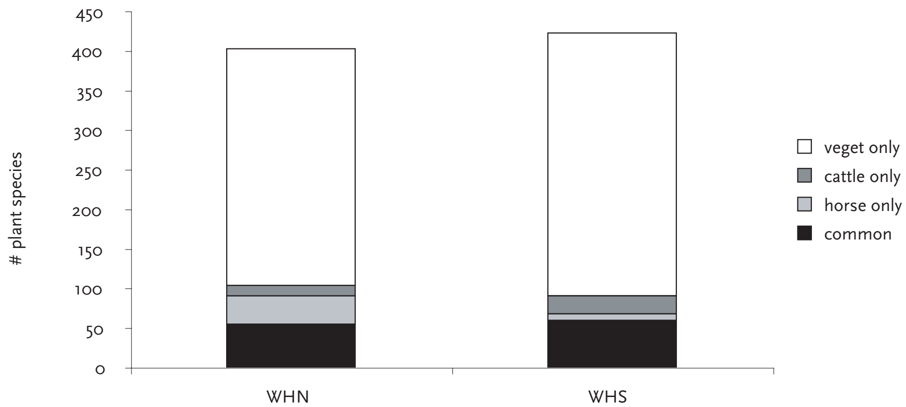


Figure 2.1 The proportional amount of plant species recorded from dung samples, which were collected at Westhoek North [WHN] and Westhoek South [WHS].
At both sites a similar number of species [54 resp. 59] occurred in both types of dung. The other species were exclusively found in dung of either horse [WHN] or cattle [WHS]

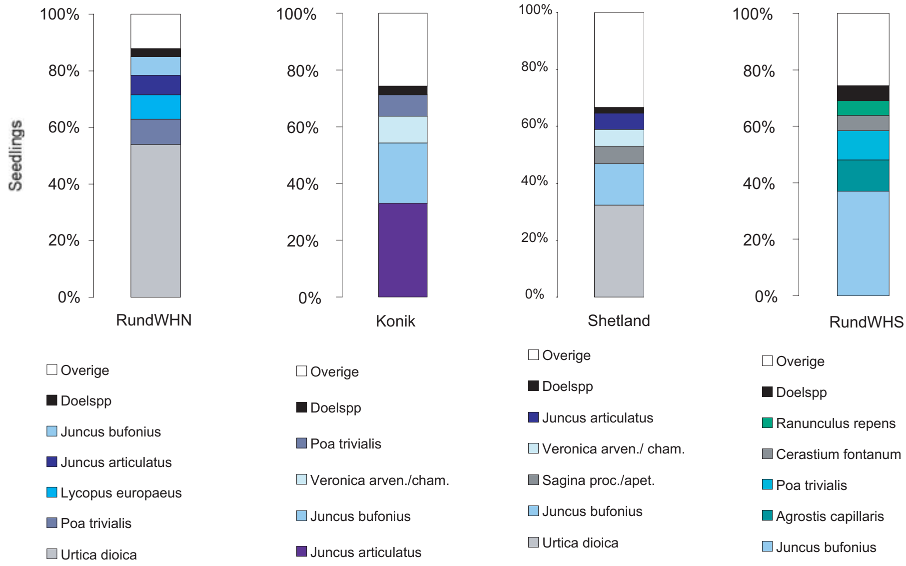


Figure 2.2 The relative abundance of seedlings of plant species in Highland cattle and horse dung samples, that were collected at Westhoek North [WHN] and Westhoek South [WHS]

Table 2.2 Spearman rank correlation [rs] between dung germinable seed density and mean plant species cover in the vegetation of Westhoek South [WHS] and Westhoek North [WHN]. Correlations were calculated only including the plant species present in dung samples.

Animal species			r_s	*P*	n
Konik [WHN]	*seedling density*	*vegetation cover*	0.569	0.002	85
Highland cattle [WHN]	"	"	0.565	0.011	63
Shetland [WHS]	"	"	0.493	NS	60
Highland cattle [WHS]	"	"	0.369	NS	75

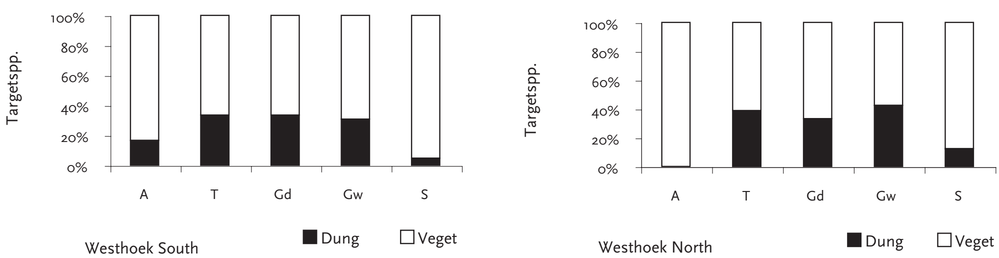


Figure 2.3 Total number of target species across different types of habitat that are of nature conservation interest. The number of target species which were recorded from cattle or horse dung and the numbers of species that were only recorded from vegetation are also shown for each type of habitat. [A: white dunes, T: grey dunes; Gd, Gw: dune grassland on dry respectively wet soils and S: scrub] at Westhoek South and North respectively.

Table 2.3 Observed [Obs] and expected [Exp] number of plant species in dung samples [Dung], the number of plant species that were only recorded from vegetation [Veg] and the total number of plant species [Tot] at both study sites across five classes of abundance in vegetation at Westhoek North and Westhoek South. Chi square values for 4 degrees of freedom and their probability [P] is also shown.

	Westhoek North				Westhoek South			
	Dung		Not in Dung		Dung		Not in Dung	
Abundance	Obs	Exp	Veg	Tot	Obs	Exp	Veg	Tot
present	8 *	31	112	120	9 *	28	123	132
occasional	25	36	116	141	24	32	125	149
frequent	53 *	29	61	114	42 *	24	72	114
codominant	15 *	6	7	22	15 *	5	7	22
dominant	2	1	3	5	0	1	5	5
Total	103		299	402	90		332	422
Chi square [4]	69.114				64.209			
P	<0.001				<0.001			

[*] indicate significant differences between observed and expected values [based on Bonferroni Z simultaneous confidence interval approach, P< 0.05] [Byers & Steinhorst 1984].

Herbivore and site related impact on dung seed content

Within both sites the highest seed densities are recorded from cattle dung [2.4]. But seed density differs only significantly between Highland cattle and Konik at Westhoek North [Mann-Whitney U: 22.0, P<0.003].
Dung of all animal species in general contained considerable amounts of plant species, with maxima in Konik [43] and cattle dung [Westhoek South, 40]. Mean plant species richness varies significantly between animal species at both sites. More plant species are counted from dung of Konik than of cattle at Westhoek North [Mann-Whitney U: 33.0, P<0.016] and from cattle than from Shetland pony at Westhoek South [Mann-Whitney U: 45.5, P 0.012] [Appendix 2.1]. Jaccard similarity of dung seed composition from both animal species is 51.9 and 65.6 at Westhoek North and South respectively, suggesting a considerable amount of plant species common to both sets of dung samples in the latter case [Table 2.4]. Seed density was significantly higher in cattle dung at Westhoek North than in cattle dung at Westhoek South [Mann-Whitney U: 12.0, P<0.001] but species richness of their dung did not differ significantly among sites [Mann-Whitney U: 37.0, P=0.073] [Table 2.4].

Table 2.4 Total number [#] of seedlings and plant species [inclusive Red List and Target species] germinated from all dung samples, mean [± stdev] and maximum seed density and species richness in 2.5L dung samples of ponies and cattle. The Jaccard similarity index [%] is calculated for each set of samples between animal species at both sites. Dung samples resulted from random selected adult individuals of each animal species [9 konik and 19 Shetland ponies, 4 cattle at each site] during 7 sampling periods.

	Westhoek N		**Westhoek S**	
	Konik	**Cattle**	**Shetland**	**Cattle**
Total # germinated seeds	15098	23290	9814	10811
Mean seed density	1078 ±444.1	2118 ±862.3	701 ±243.1	901 ±354.8
Maximum seed density	1782	3622	1308	1507
Minimum seed density	465	665	351	313
Total # plant spp.	91	67	67	81
Total # Red List species	7	4	2	3
Total # Target species	28	24	18	24
Mean species richness	34 ±5.2	28 ±4.9	29 ±4.9	33 ±5.4
Maximum species richness	43	34	38	40
Minimum species richness	27	23	22	19
Jaccard similarity	51.9		65.6	
# samples [2.5L]	14	11	14	12

Defecation frequencies and seed numbers dispersed during the fruiting season

Within sites both herbivore species show very comparable defecation rates [Table 2.5]. Given the mean dung seed content, mean dung volume and the calculated number of defecations during the fruiting season the potential importance of endozoochory becomes obvious [Table 2.5]. Konik and Highland cattle at Westhoek North potentially disperse the highest amounts of seeds per animal during this period, hence contributing potentially to a considerable endozoochorous seed dispersal at this site. However the same cattle breed in both study areas shows a clear difference in the total amount of viable seeds which they potentially disperse, since significant differences appear in mean germination numbers from their dung [see supra]. Taking into account defecation characteristics and dung seed density of Shetland ponies and Highland cattle, endozoochorous seed dispersal at Westhoek South in general will be much lower [Table 2.5].

Table 2.5 Using the mean volume [L, litres] of a dung pile and the mean number [#] of defecations per hour [h], the potential number [#] of germinable seeds, that is on average endozoochorously dispersed by individual Konik, Shetland pony and Scottish Highland cattle, during a whole summer [92 days], in a coastal dune area, was calculated. Means were calculated from the indicated number of samples [n], which were obtained from random selected adult individuals of each animal breed [9 konik, 19 Shetland ponies and 4 Scottish Highland cattle at each site]. A considerable higher mean dung volume of Konik compared to cattle at Westhoek N was observed [Independent T-test]. Shetland ponies and cattle at Westhoek South did not differ for this variable.

	Westhoek N		**Westhoek S**	
	Konik	**Cattle**	**Shetland**	**Cattle**
Mean dung volume [L] ±Stdev	1.6^{a} ± 0.67	0.8^{b} ± 0.19	0.8^{b} ±0 .41	0.8^{b} ± 0.24
[n]	[20]	[20]	[20]	[20]
Mean # defecations / h ±Stdev	0.8 ± 0.26	0.8 ± 0.51	0.6 ± 0.23	0.6 ± 0.22
[n]	[18]	[20]	[20]	[14]
Mean # germinable seeds dispersed/ h	552	542	135	173
Mean # germinable seeds/summer & individual	±1,200,000	±1,200,000	±291,000	±382,000

[Independent samples T-test: T= 4.768, df: 38, values followed by a different small letter significantly differ: $P<0.001$]

Defecation patterns and seed deposition among habitats

At both sites all herbivore species show a disproportional use of habitat types, for defecating, in relation to their area [Table 2.6]. In general, observed defecation frequencies of all herbivores were significantly higher than expected in tall herbs dominated habitats on dry and wet soils [Ud and Uw resp.], except Shetland pony for Uw [Table 2.6]. At Westhoek North, Highland cattle defecated more than expected in dune grasslands on dry soil [Gd] and in the small sized forest [F]. At Westhoek South, cattle deposited more dung than expected in monospecific, *Calamagrostis epigejos* grassland [C1]. In several habitats dung deposition was lower than expected e.g. in Scrub [S], white and grey dunes [AT], except for cattle at Westhoek South. Both horse breeds never defecated in forest [F] [Table 2.6].
A very comparable selective use of habitats was noted for grazing. Each herbivore species shows highly comparable values of defecation frequency [%] and grazing time [%] in a given habitat i.e. the number of defecation is high in those vegetation units which are grazed intensively and vice versa.

Hence, a significant positive relationship between both activities could be derived [Fig. 2.5].
From Fig. 2.5 we can also expect a considerable chance for plant species growing and fruiting in frequently grazed vegetation types to be eaten and dispersed endozoochorically into the same type of habitat. On the other hand plants growing in much less frequently grazed habitats not only have a lower chance to be eaten and hence to be dispersed endozoochorically, but also have a minor chance to be dispersed in the same type of habitat.

Because of the disproportional dung deposition patterns among habitats, one can expect a non random endozoochorous seed dispersal in the study areas. From Fig. 2.4 a disproportional deposition of endozoochorically dispersed viable seeds can be derived for each of the herbivore species at both sites. Moreover, both herbivore species tend to intensify seed deposition in preferred habitats mainly vegetation types dominated by tall herbs on dry [Ud] and wet soils [Uw] , resulting in a calculated mean seed deposition of about 100-200 seeds per square meter at Westhoek North and a clearly lower amount [15-60] at Westhoek South. The relative high seed input in forest habitat [144 seeds/ m^2] at Westhoek North reflects in fact the extreme disproportional use of this very small sized habitat by ruminating and resting cattle, which also regularly defecate at this place.

Figure 2.4 Estimated number of seeds deposited per m2 in each habitat, during summer, and the proportional contribution of all adult individuals of horse breed and Highland cattle respectively at Westhoek N and Westhoek S. Calculations are based on mean dung seed content and defecation frequency in each habitat during summer. AT: white and grey dunes; Ud, Uw: vegetation dominated by tall herbs on dry respectively wet soils; Gd, Gw: dune grassland on dry respectively wet soils; C1 Calamagrostis epigejos dominated grassland, S: scrub; F: forest.

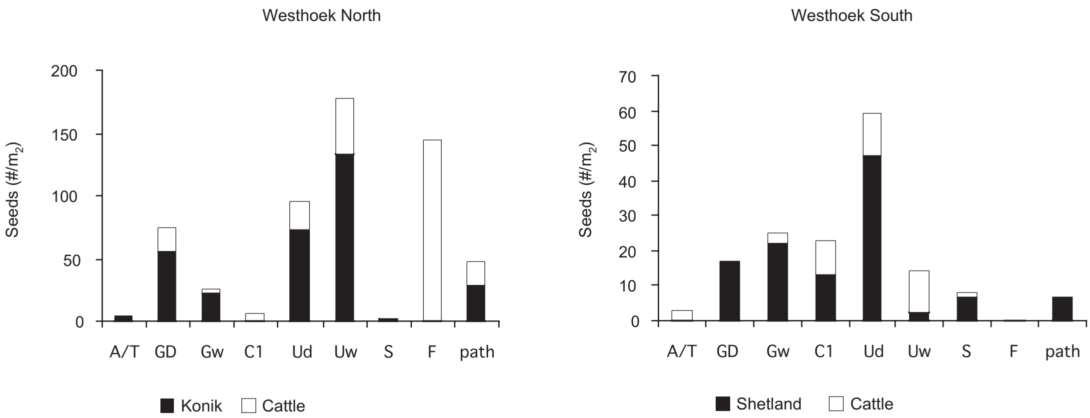
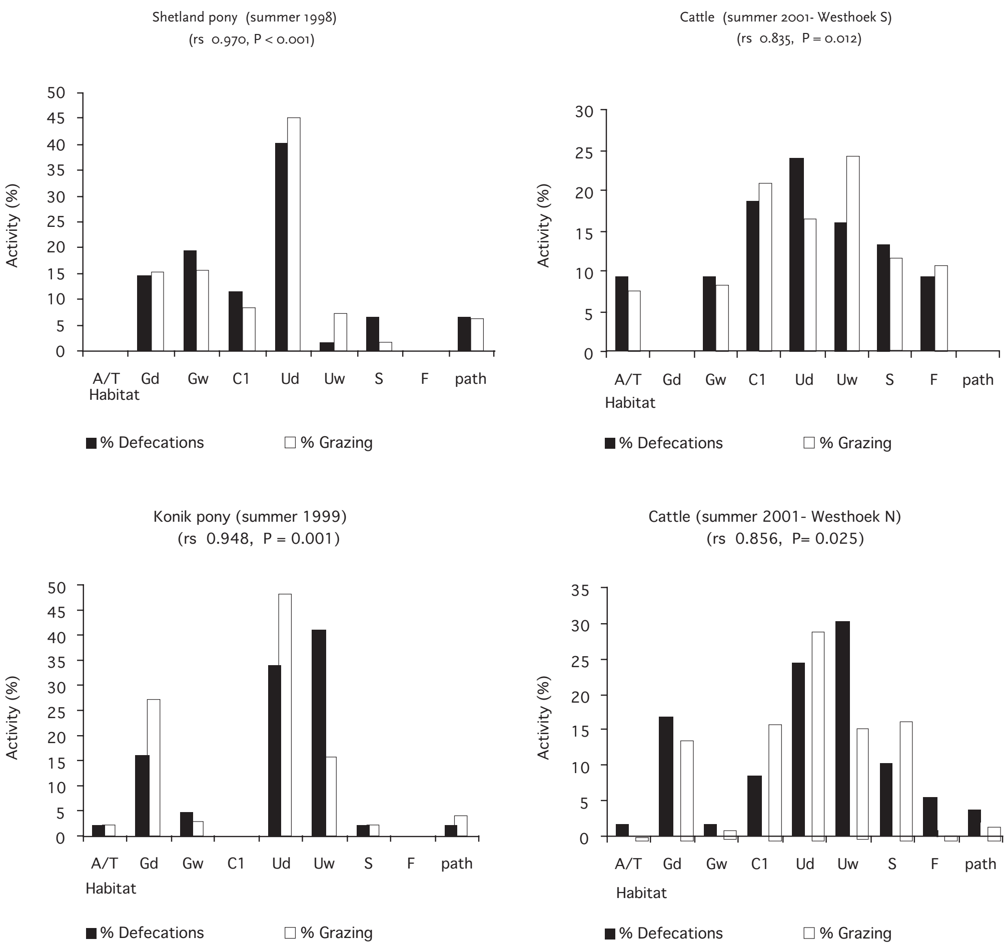


Table 2.6 Observed [obs.] and expected [exp.] defecation frequencies by different herbivore species in 9 habitats at both study sites and calculated chi-square values for 8 degrees of freedom with its probability [P].

Westhoek North			**Westhoek South**					
		Cattle		**Shetland**				
Habitats	**Konik obs**	**exp**	**obs**	**exp**	**obs**	**exp**	**Cattle obs**	**exp**
AT	1*	4.0	2*	9.9	0*	5.5	7	6.6
Gd	7	2.2	16*	5.4	9	2.5	0*	3.1
Gw	2	1.6	2	3.9	12	5.1	7	6.2
C1	0*	4.7	9	11.6	7	3.3	14*	4.0
Ud	14*	3.5	25*	8.7	25*	3.5	18*	4.2
Uw	17*	2.3	31*	5.7	1	2.3	12*	2.8
S	1*	24.2	11*	59.6	4*	30.1	10*	36.5
F	0*	0.1	6*	0.3	0*	9.3	7	11.3
path	1	0.6	4	1.5	4	0.3	0*	0.4
Chi Square [8]		150.9		283.9		219.4		112.9
P		<0.001		<0.001		<0.001		<0.001

A significant difference between expected and observed defecation frequency in a certain habitat, based on Bonferroni Z simultaneous confidence interval approach [Byers & Steinhorst 1984] is indicated with [*]. AT: white and grey dunes; Ud, Uw: vegetation dominated by tall herbs on dry respectively wet soils; Gd, Gw: dune grassland on dry respectively wet soils; C1 *Calamagrostis epigejos* dominated grassland, S: scrub; F: forest.

Figure 2.5 The mean proportional distribution of grazing time and defecation frequency is shown for each herbivore species over the different habitats at each study site. These behavioural variables can be interpreted as a measure for the expected contribution of each habitat to dung plant seed composition and the chance that dung will be dropped in a specific habitat. Spearman Rank correlation [rs]between grazing time [%] and defecation frequency [%] is also shown. AT: white and grey dunes; Ud, Uw: vegetation dominated by tall herbs on dry respectively wet soils; Gd, Gw: dune grassland on dry respectively wet soils; C1 Calamagrostis epigejos dominated grassland, S: scrub; F: forest.



Discussion

Estimating the viable seed content of herbivore dung

Estimations of the dung germinable seed content i.e. its density and composition, are always open to critical questions. Their accuracy depends on the way that dung was collected, processed and tested. Several aspects need to be considered here.

First, dung collection was carried out during summer and early autumn. Hence, seed densities of some early fruiting plant species may be underestimated [e.g. *Cerastium semidecandrum*, *Saxifraga tridactylites*, *Cardamine hirsuta*, *Claytonia perfoliata*] or some plant species, already recorded in other similar studies, may have been missed. However, compared to these studies [Müller-Schneider 1954; Welch 1985; Malo & Suárez 1995; Stender *et al.* 1997; Bonn & Poschlod 1998; Pakeman *et al.* 2002] it appeared that only one species, occurring in minor quantities in both our study areas, has probably been missed; *Cerastium glomeratum* [Malo *et al.* 1995].
Second, dung samples exist from freshly produced dung, which was collected from the soil. The plant species which were covered by dung and those surrounding the dung could have been a potential source of seed-contamination. To avoid possible seed contamination freshly deposited dung was immediately collected, leaving the lowermost part of the dung untouched. Dung collecting bags, which could be a good alternative to the mentioned problem, were not used for practical reasons. More in particular, the need for frequent capturing and handling the free-ranging animals in this case, was considered not feasible.

Third, grinding the samples in order to be able to easily spread them out in a thin layer could have damaged larger sized seeds. This could be a reason why some plant species, although the fruits have been seen consumed, did not emerge from the dung samples e.g. *Rosa pimpinellifolia, R. canina, Ligustrum vulgare*. Further evidence for such damage came from a comparable greenhouse experiment without grinding dung samples in which several *Ligustrum vulgare* seedlings emerged from horse dung.

Fourth, some uncertainties are associated with the experimental set up under greenhouse conditions, which are assumed to be favourable for most of the plant species. But germination conditions can differ between plant species [Grime *et al.* 1988], hence comparison of frequency distribution of different plant species may be biased this way [Malo 2000]. Furthermore, the experiment was stopped after 6 months, because at that moment no significant further germination was detected. Nonetheless, the presence of still viable seeds could not be excluded. For instance, Müller [1955], extracted a further 30% of viable *Urtica dioica*-seeds from a cattle dung after a 9 months germination trial. Because of similar reasons, Malo [2000], recently argued for longer lasted germination experiments. It therefore is very plausible to assume that the data presented here represent to some degree an underestimate of the real amount of potentially germinable seeds in horse and cattle dung.

It should be kept in mind though that we aimed at detecting the *potential* contribution of endozoochory to seed dispersal. Therefore it was justified to follow germination in semi-optimal greenhouse conditions. However, germination should also be tested under field conditions to be able to estimate the true contribution of endozoochory to dispersal, taking into account the less favourable conditions in the field caused by factors such as competition, climate, physical conditions of the dung, herbivory, etc.

The viable seed content of herbivore dung

Most of the plant species, which showed high seed densities or appeared frequently in the dung samples, such as *Urtica dioica*, *Juncus* spp., *Poa* spp. and different species of *Caryophyllaceae,* were also regularly recorded by Pakeman *et al.* [2002], Welch [1985] Dai [2000] and Malo & Suárez [1995] emphasising their great ability to survive passage along the molar mills and through the gastro-intestinal tract. The large difference in numbers of *Lycopus europaeus* seedlings found in dung of Highland cattle [Westhoek N] versus Konik are mainly the result of important differences in diet selection i.e. the observed frequent consumption of *Lycopus* by cattle and avoidance of it by Konik [see Chapter 3]. Because of its scarcity in the species pool of

Westhoek south, *L. europaeus* was almost absent from cattle and pony dung at this site [Appendix 2.1]. So *Lycopus* can be considered a very illustrative species to demonstrate both the possible effects of diet selection and seed availability on dung seed content. Moreover the observed significant differences in dung mean seed density between Konik and cattle is largely due to differences in viable seed density of *Urtica dioica* in their respective dung samples, which is consistent with the observed more frequent consumption of *Urtica dioica* by the latter [Chapter 3].

Besides the above mentioned frequently occurring species, we were able to detect a considerable amount of plant species occurring only in minor densities and frequencies of which several are to a certain level, stress tolerant grassland species e.g. *Galium verum, Veronica officinalis, Luzula campestris, Trifolium spp*. Their scarceness is at least partly due to their low availability in the species pool [Appendix 2.1] though seedlings of e.g. *Helianthemum nummularium* and *Galium mollugo* on dung were also mentioned by Müller-Schneider [1954] and *H. nummularium, G. verum, Plantago lanceolata* and *Trifolium campestre* by Dai [2000] , suggesting their ability to survive the gastro-intestinal tract.

The observed variations in species richness [mean, maxima/sample and total species richness, Table 2.4] between dung samples of different herbivore species mainly resulted from the accidental presence of in most cases rare plant species. Within herbivore species, dung samples in general consist of a core of 'commonly present' plant species [c. 15-20 species] complemented with a varying selection and number of infrequent plant species. Between herbivore species the size of this infrequent plant species pool tend to vary according to the observed total species richness.

All of the studies on large herbivore endozoochory suggest its potentiality as a seed dispersal mechanism with a large number of seeds of a considerable number of plant species dispersed during the fruiting season. The relevance of this mechanism is furthermore stressed in this study in a qualitative way by the fact that at least 27% of all plant species which were recorded from both sites in this study can potentially be dispersed endozoochorically, although only part of them in large amounts. When adding the knowledge on endozoochorically dispersed plant species from other studies [Müller-Schneider 1954; Welch 1985; Malo & Suárez 1995; Stender *et al.* 1997; Bonn & Poschlod 1998; Pakeman *et al.* 2002] a still larger fraction of plant species is candidate to be dispersed in this way [41%]. This is in agreement with Pakeman *et al.* [2002], who found 37% of the species recorded in the vegetation to be capable of dispersal by endozoochory.

Quantitatively the potential of endozoochory becomes obvious when taking into account the animal species' defecation frequencies and mean dung volumes during the fruiting season. Horses and cattle both are capable, depending on site and animal related characteristics, to disperse large amounts of viable seeds. The simple calculation, shown in Table 2.5, gives an estimate of the potential amount of viable seeds [c. 1,200,000] that were dispersed by an individual Konik and Highland cow during the fruiting season.

Defecation patterns and its possible impact on grassland dynamics

In this study plant species growing preferably in dry grassland [habitats Gd and Ud] and to some extent also in wet [Uw] or monospecific grasslands [C1] generally have the best chance to be dispersed into the same habitat [Fig. 2.4], which is expected to be more suitable for germination and recruitment of the grassland specific plant species than other habitats. Plant species related to one of the other habitats [e.g. scrub and forest] have a minor chance ever to be dispersed endozoochorically in an identical habitat. On the other hand, whenever grazed they have far higher chance to be dispersed to grass dominated habitats. It is plausible to suggest that the most preferred habitats receive the largest proportion of viable seeds from plants that grow in other habitats. It can be hypothesised that these habitats therefore are more 'vulnerable' for invasion of new species. Although seed arrival is no guarantee of recruitment, the non-random dispersal to other habitats might induce succession, if late successional species are dispersed into earlier successive phases.

Of course within a potentially suitable habitat it is not a priori sure that deposited seeds will all meet the right germination conditions [Harper 1977; Grime *et al.* 1981]. On the other hand not all plant species are exclusive for only one habitat. Species characteristics can differ and some species 'behave' rather opportunistic, while others depend on narrowly defined environmental conditions [e.g. Grime *et al.* 1988]. For this reason, a species based approach can add further specific information about the significance of endozoochorous seed transport through the landscape, also in relation to other dispersal mechanisms. The hypothesis that non-random dispersal of late-successive species towards earlier successive phases could enhance succession, should be further investigated as well.

Because of the size and scattered distribution of the preferred habitats within the landscape we may assume that large herbivores have to move regularly through the landscape. From the animal behaviour data we found indications for potential movements - measured in a straight line from the origin to a given point after mean retention time [Table 2.1 and Chapter 5] of several hundreds of metres. Moreover such distances are regularly bridged within even relatively short periods of six hours. Taking into account the potential of all herbivore species to cover almost each point within the fenced area after mean retention time, inter-habitat endozoochorous dispersal therefore is perfectly possible.

Furthermore, the long retention period may favour seed dispersal over very long distances [kilometre scale]. Bonn & Poschlod [1998] and Bruun & FritzbØger [2002] rely on strong historical evidence to derive and state a high degree of connectivity between the different units of the traditionally used man-made i.e. semi-natural landscape such as grassland, arable field, heathland, villages as a result of the presence of many dispersal vectors and processes of which several can be attributed to livestock grazing practices inclusive the regular movement of livestock between different parcels, habitats or landscapes as it may occur during shepherding transhumance [Ruiz & Ruiz, 1986, Fischer *et al.*1996].

Conclusions in relation to ecosystem management and rehabilitation

From this study it is clear that mammalian endozoochory may be a relevant dispersal mechanism for a significant part of the plant species, even for some of the Target species presented at the study sites [Fig. 2.3.]. Secondly, there is important evidence from this study that domesticated large herbivores can contribute significantly to the long-distance seed dispersal. The general tendency of the free ranging domesticated herbivores studied here to select preferentially grass dominated habitats to feed [e.g. Putman 1986; Duncan 1983; Gordon 1989], makes it reasonable that not only within the heterogeneous dune landscape but also in other parts of the degraded and highly fragmented semi natural NW European landscape large herbivores can play an important role as seed dispersal agents between isolated patches of comparable habitats by enhancing inter-site endozoochorous seed dispersal through their species-specific grazing and defecation preferences. In other words and as already suggested by Bonn & Poschlod [1998], they can act as important dynamic ecological corridors.

Several authors already mentioned or suggested to [re-]introduce livestock in 'nature reserves' in order to enhance species richness of highly impoverished habitats within a well defined landscape. A large number of examples is concerned with species-poor 'target units' that could be connected with species-rich 'source units' by means of zoochorous seed dispersal from the latter. Examples were found for calcareous grasslands [Hillegers 1993] and heathland [Bülow Olsen 1980; Bakker 1989]. From this study we can derive furthermore important evidence for possible seed input of true grassland species into actually species-poor grassland habitats [C1, Ud and Uw]. A simple calculation, using the data of Table 2.5 & 2.6 and seedling densities in dung [Appendix 2.1], shows that within one summer the amount of germinable seeds of Gd-Target species possibly deposited in e.g. C1 species poor habitat at Westhoek South could range between about 130 [*Lotus corniculatus*] and 4500 [*Carex arenaria*]. Whether this would result in the establishment of species-rich grassland communities needs further study of germination under field conditions [see Chapter 6]. A comparable use of livestock is related to the beneficial use of cattle and sheep as 'sowing machines' to improve grasslands [Lowry 1997; Ghassali *et al.* 1998]. In the same context we would therefore argue to consider the opportunities which arise from necessary livestock movements between parcels, small-scaled nature reserves or from the transport of livestock between summer grazing areas and wintering grounds as part of the seasonal grazing system in Flanders [Couvreur *et al.* subm.]. Knowing their potential as seed dispersing agents one can try to outline a grazing management which can help to maintain or enhance plant species diversity through long-distance seed transport.

Bonn & Poschlod [1998] and Bruun & FritzbØger [2002] argue that the loss and decrease of dispersal processes and vectors in todays' man-made landscape are an important cause of local extinction of species in the remnants of the semi-natural landscape. Therefore, nature conservation and restoration management should regard this fact and include the restoration or a simulation of these dispersal processes [Fischer *et al.* 1996; Zobel *et al.* 1998].

Appendix 2.1 Mean number of seedlings in dung samples [2.5 L] of different horse breeds and Highland cattle at Westhoek North and South. The plant species overall mean number of seedlings [Mean #] and its frequency [Freq] of occurrence across all dung samples [maximum = 51]. The Nature conservation status in Flanders [Red List] and the target habitat a species is characteristic for is indicated. The estimated total cover [%] of the plant species in the whole area is also shown. The total cover is obtained by taking into account the average cover from at least 5 relevés in each of the habitats and the relative surface of each habitat. Plant species with a total frequency < 3 are not shown.

Species			**Westhoek N**			**Westhoek S**			**Mean #**	**Freq.**
	Red List	Target Habitat	Cover %	Konik	Cattle	Cover %	Shetland	Cattle	2,5L	max = 51
Agrostis capillaris		T/Gd	0.03	0.2	18.7	0.53	6.0	99.0	29.0	26
Agrostis stolonifera			0.89	24.0	39.5	2.90	28.4	18.9	27.4	47
Aira praecox			0.02	0.4	1.2	0.72	2.9	1.2	1.4	14
Anthriscus caucalis		S	0.01	0.1	0.0	0.03	0.1	0.4	0.2	3
Arenaria serpyllifolia			0.40	17.2	3.5	0.72	9.6	14.6	11.5	44
Calamagrostis epigejos		Gd	1.20	0.1	0.0	13.54	0.4	0.6	0.3	10
Cardamine hirsuta			0.14	1.3	0.5	0.02	0.5	0.4	0.7	21
Carex arenaria		T/Gd	3.62	3.3	20.0	3.70	1.8	9.2	7.9	39
Carex flacca		Gw	0.27	1.8	1.0	0.90	0.0	0.0	0.7	8
Centaurium erythraea			0.05	5.6	0.0	0.29	0.9	1.3	2.1	15
Centaurium littorale	R	Gw	0.01	0.5	0.1	0.01	0.1	0.3	0.3	9
Cerastium fontanum			0.48	28.9	32.0	0.45	29.1	48.3	34.2	51
Cirsium arvense			5.53	0.1	0.5	2.16	0.0	0.1	0.2	6
Conyza canadensis			0.18	0.9	0.0	0.20	0.1	0.2	0.3	9
Crepis capillaris			1.61	0.3	0.0	0.27	1.1	4.6	1.5	10
Dicotyl spp			NA	0.0	0.9	NA	0.0	0.4	0.3	8
Epilobium ciliatum			0.13	0.9	0.0	0.06	0.0	0.5	0.4	10
Epilobium hirsutum			0.06	0.3	0.3	0.06	0.4	1.1	0.5	9
Epilobium parviflorum			0.01	0.1	0.0	0.01	0.0	0.1	0.1	3
Epilobium roseum			0.97	1.2	6.7	0.09	0.3	4.3	2.9	32
Epilobium sp.			NA	2.1	0.2	NA	2.7	1.0	1.6	31
Erodium cicutarium/ lebelii		T	1.59	0.1	0.2	0.55	0.1	0.0	0.1	5
Eupatorium cannabinum			7.44	3.8	0.0	1.57	0.4	0.3	1.2	19
Festuca rubra			3.58	0.2	0.7	3.62	0.0	1.3	0.5	8
Galium aparine			0.41	1.9	0.5	0.28	0.1	0.8	0.9	13
Galium mollugo		Gd	0.04	0.0	0.2	0.66	1.2	1.0	0.6	6
Galium palustre		Gw	0.01	0.6	0.2	0.07	0.1	0.3	0.3	11

Species			Westhoek N			Westhoek S			Mean #	Freq.
	Red List	Target Habitat	Cover %	Konik	Cattle	Cover %	Shetland	Cattle	2,5L	max = 51
Galium uliginosum			0.37	*14.6*	0.5	1.30	0.5	0.6	4.4	14
Galium verum		Gd	4.67	*5.0*	2.4	2.41	0.1	0.4	2.0	15
Geranium molle			0.03	*0.1*	1.2	0.13	1.6	1.8	1.2	27
Gnaphalium uliginosum			0.01	*3.6*	0.0	0.00	0.0	0.0	1.0	10
Helianthemum nummularium	R	Gd	0.97	*0.0*	0.6	0.00	0.0	0.0	0.2	5
Holcus lanatus			2.07	*6.6*	18.9	7.85	19.9	25.9	17.5	47
Hydrocotyle vulgaris		Gw	1.12	*1.1*	0.1	1.15	0.1	0.3	0.5	14
Hypericum tetrapterum			0.01	*0.0*	0.3	0.01	0.0	0.0	0.1	3
Juncus articulatus		Gw	0.32	*356.6*	148.5	0.03	40.8	28.8	147.9	50
Juncus bufonius			0.08	*228.4*	139.0	0.03	102.1	334.0	199.3	51
Juncus subnodulosus	R	Gw	0.09	*0.0*	0.1	0.55	0.0	0.2	0.1	3
Lotus corniculatus		Gd	0.03	*0.1*	0.8	0.07	0.0	0.3	0.3	3
Luzula campestris		Gd	0.27	*0.4*	1.5	0.56	0.4	0.2	0.6	17
Lychnis flos cuculi			0.00	*0.0*	0.0	0.01	0.0	0.6	0.1	3
Lycopus europaeus			0.28	*4.6*	180.8	0.01	0.5	1.2	40.7	18
Lysimachia vulgaris			1.71	*0.9*	0.0	6.24	0.1	0.1	0.3	4
Lythrum salicaria		Gw	4.66	*10.6*	7.2	1.06	1.6	2.7	5.5	28
Mentha aquatica		Gw	1.16	*2.4*	7.3	1.00	0.7	2.3	3.0	18
Oenothera glazioviana			0.20	*0.2*	0.1	0.09	0.5	2.3	0.7	10
Phleum pratense			0.50	*1.6*	0.5	0.50	0.6	1.0	0.9	23
Plantago coronopus	R	Gw	0.04	*1.9*	0.0	0.01	0.1	0.0	0.5	4
Plantago lanceolata			0.05	*0.9*	3.7	1.58	0.1	0.2	1.1	14
Plantago major			0.04	*15.9*	1.8	0.00	7.3	4.2	7.7	37
Poa annua			0.06	*20.4*	4.9	0.02	22.8	6.9	14.5	46
Poa pratensis			0.75	*28.7*	64.3	1.58	22.1	17.8	32.0	49
Poa trivialis			1.21	*81.5*	188.5	2.00	32.4	93.4	93.9	50

Plant species [continuation]	Red List	Target	Cover Habitat	Konik %	Cattle	Cover	Shetland %	Cattle	2.5L	max = 51
Potentilla reptans			0.02	*1.0*	1.5	0.62	1.3	4.4	2.0	26
Prunella vulgaris			0.06	*1.1*	0.7	0.06	1.6	7.4	2.6	22
Ranunculus repens			0.06	*1.5*	1.5	1.36	6.9	46.8	13.6	39
Ranunculus sceleratus			0.05	*0.2*	0.0	0.01	0.0	0.2	0.1	5
Rubus caesius		Gd	7.58	*1.5*	0.9	3.91	1.0	1.4	1.2	22
Rumex acetosella			0.05	*0.1*	1.8	0.05	0.0	2.9	1.1	5
Rumex conglomeratus			0.10	*0.3*	0.2	0.10	0.9	2.7	1.0	14
Rumex crispus			0.10	*0.1*	0.5	0.10	0.5	0.2	0.3	8
Rumex sp.			NA	*0.1*	0.0	NA	0.0	0.6	0.2	4
Sagina procumbens/ apet.			0.20	*16.1*	1.6	0.07	43.1	28.8	23.4	44
Samolus valerandi	R	Gw	0.03	*1.1*	0.0	0.01	0.0	0.0	0.3	6
Scirpus setaceus			0.01	*0.1*	0.0	0.01	1.9	1.4	0.9	16
Sedum acre			2.62	*0.1*	0.0	0.80	0.0	0.1	0.1	3
Senecio jacobaea			0.33	*1.6*	0.6	0.73	7.9	3.4	3.5	26
Silene latifolia			0.01	*0.0*	0.1	0.01	0.0	0.8	0.2	4
Solanum dulcamara		S	0.14	*0.5*	0.0	0.05	0.0	0.0	0.1	4
Solanum nigrum			0.01	*0.9*	0.0	0.00	0.0	0.1	0.3	5
Sonchus asper			*0.01*	*0.0*	0.0	0.18	0.1	0.3	0.1	4
Sonchus oleraceus			0.09	*0.7*	0.6	0.00	0.0	0.0	0.3	12
Stellaria media			0.10	*11.5*	19.4	0.10	0.9	5.3	8.8	39
Taraxacum sp.			0.03	*0.6*	0.0	0.01	0.0	0.3	0.2	4
Trifolium campestre			0.00	*0.0*	0.0	0.10	0.1	1.6	0.4	6
Trifolium dubium			0.02	*0.2*	0.4	0.06	4.5	1.0	1.6	22
Trifolium repens			0.06	*3.3*	2.4	1.66	19.2	19.2	11.2	39
Urtica dioica			0.62	*42.3*	1144.8	0.14	226.6	16.7	324.7	51
Veronica arvensis/ cham.			0.16	*102.6*	35.1	0.76	41.1	18.6	51.4	51
Veronica officinalis			0.00	*4.8*	4.6	0.10	1.5	1.3	3.0	34
Veronica serpyllifolia			0.01	*0.2*	0.1	0.05	0.2	0.1	0.2	6
Total number of all species				91	67		67	81		117

3 The comparison of diet and dung germinable seed content of free ranging horses and cattle: which plant species are the most probable successful endozoochores in a coastal dune landscape?

Eric Cosyns & Maurice Hoffmann

Konik, grazing Tall herb vegetation on wet soils (Westhoek North, Winter 2001)

Abstract

A crucial first step to make endozoochorous seed dispersal by ungulates successful, is the active or passive consumption of seeds by these animals. In this chapter the foraging behaviour of free ranging cattle and horses in two nature reserves was examined in order to compare their diet composition to the germinable seed content of their dung and to determine the most probable successful endozoochores. It was tested, according to 'the foliage is the fruit' theory, whether successful endozoochores did differ from unsuccessful endozoochores in their seed characteristics and forage value of their foliage.

Fresh dung of cattle and horses [2 times 2.5 L] was collected during 7 sessions [July-October]. Dung samples were placed in the greenhouse after sun drying and vernalisation. Diet composition of the ungulate species during different seasons, was obtained from a study of the ungulates grazing behaviour during 6 hour observation sessions, 8 times a month.

During May-October, cattle and horses mainly grazed in tall herb dominated habitats. They consumed a broad spectrum of plant species but graminoid species were by far the most important constituents of their diet. The cattle diet was supplemented with herbs and several woody species, the horse diet was mainly supplemented with herbs. Matching of diet composition and dung germinable seed content of cattle and horses was high at Westhoek North but very low at Westhoek South. Plant species differed in their ability to get endozoochorically dispersed. Eleven plant species were found more frequently dispersed via ungulate dung than could be expected from their contribution to the diet. Woody species with fleshy fruits seem not be able to profit from this type of endozoochory. The observed discrepancy between diet composition and dung viable seed content is further discussed in relation to the differential effect of endozoochory among plant species and the applied methods. This discrepancy suggests the need for further research.

We furthermore found that successful endozoochores had smaller seeds with a higher longevity index than the less successful endozoochores, as was predicted by Janzen [1984] from the 'Foliage is the Fruit' theory. We did not find support for a higher nutritive value of the foliage of the successful endozoochores as compared to not successful endozoochores.

Key-words: diet selection, endozoochory, foliage is the fruit, graminoids

Introduction

To understand about the role of large herbivores in the seed dispersal cycle as it could happen in grazed temperate habitats, it is necessary to examine the different intermediary steps and processes in which these animals are involved [Wang & Smith 2002]. Two main phases are of importance. First, the seed intake phase and second, the seed [dung] deposition itself. The first phase deals with such aspects as diet selection and grazing behaviour. The second phase involving such aspects as herbivore movements and defecation behaviour. In the latter case the amount of seeds that are still germinable after gut passage is of key importance. Without germinable seeds the seed dispersal cycle will get interrupted [see Chapter 1].

Animals may acquire seeds either actively, through the process of selecting different seeds or fruits or passively, as hitchhikers attached to fur or consumed incidentally with other food items [Stiles 2000]. Large herbivores may passively consume considerable amounts of seeds of small seeded herbaceous plant species while they are eating the foliage of the parent plants, which Janzen [1984] proposed to serve as the attractant for large herbivore dispersal of seeds.
Therefore, the plants are assumed to be edible at the time of seed set and the foliage of sufficiently high nutrient value to be attractive to the herbivores. Herbivores will select their food non-randomly and show varying degrees of preference and avoidance [Danell & Bergström 2002].
Knowledge on the foraging behaviour and diet composition of the large herbivores will contribute to a better understanding of the potentiality of endozoochorous seed dispersal.

The amount of germinable seeds in herbivore dung will not only depend on the amount of consumed seeds but also will depend as well on seed traits favouring survival of the gastero-intestinal tract after seed ingestion. Janzen [1984] hypothesised that successful endozoochores most probably would have sufficiently small, tough, hard and inconspicuous seeds to escape the molar mill and spitting response of a large mammal. Seed coats have the ability to resist digestion during a transit period of days to months; this trait is also of evident value in, and selecting for, seed dormancy in the soil and litter.

Most papers dealing with ungulate endozoochory in temperate habitats almost exclusively focus on the characteristics of the dung germinable seed content. In contrast to most studies on tropical frugivory [e.g. Motta-Junior & Martins 2002; Wehncke *et al*. 2003] and as far as we know, no one study on large herbivore endozoochory exist, that considers both diet composition and dung germinable seed content. This paper focusses on the foraging behaviour of free ranging cattle and horses in a structurally heterogeneous coastal dune landscape and their dung germinable seed content. The study had the following objectives: [1] to determine the diet composition of horses and cattle at the plant part and plant species level during the main fruiting season [May-October]; [2] to determine their habitat use for grazing; [3] to study the matching between dung germinable seed content and diet composition [4] to determine which of the consumed plant species are most probable successfully dispersed via dung and [5] to determine whether these successful endozoochores differ from less successful endozoochores in their seed characteristics and forage value of the foliage.

Materials and methods

Study area, management and animals

The Westhoek [335 ha] is located in the most South-western point of Belgium, in the coastal dunes [Fig.1.2]. Two areas were fenced. In the northern part [54 ha] nine Konik horses and four Scottish Highland cattle and in the southern fenced part [61ha] 19 Shetland ponies and four Scottish Highland cattle graze year round without additional feeding [Table 1.1]. Water was available during the whole period at different sites in the study area. *Hippophae rhamnoides*, *Ligustrum vulgare* and to some lesser extent *Salix repens* occupy the largest part of the grazed areas. Before the start of the grazing project about 10% of the original shrub cover was cut down and removed, resulting in an area of tall herbs-vegetation composed of a low, grass-dominated layer and patches of tall herbs [*Eupatorium cannabinum*, *Lythrum salicaria* and *Cirsium arvense]*. Old, deteriorating *Hippophae*-scrubs are generally replaced by *Calamagrostis epigejos* or *C. canescens*. Species rich dune grasslands, moss-rich grey dunes, open sand dune and young dune slacks all together occupy another substantial part of both dune areas [Table 1.2 - Chapter 1].

Feeding observations

Feeding observations were conducted using a method similar to the bite-count method [Vulink & Drost, 1991; Hobbs *et al*. 1983]. Each month we observed herbivore activities during 48 hours, distributed more or less evenly over 6-hourly morning [6-12 h], afternoon [12-18 h] and evening [18-24 h] sessions. Before starting a session, 1 animal was randomly chosen and followed for the next 6 hours. Observations were conducted within a 3-m range; animal behaviour was not visually affected by the presence of the observer. Grazing activities were recorded by one observer in both areas. All plant species and plant parts seen bitten were recorded.
Data used for the different animal species were obtained from the 1999 [Konik, Shetland pony] and the 2001 fruiting season [Konik, Highland cattle and Shetland pony].

Dung germinable seed content

Freshly deposited dung [2 times 2.5L] from the free ranging horses and cattle [randomly chosen individual animals] were collected during 7 sessions in a period of 3 months [18th July-11th October 2000]. Dung samples were than treated as described in chapter 1. The list of plant species that emerged from these dung samples and numbers can be found in chapter 2 [Appendix 2.1].

Habitat use, diet composition and dung germinable seed content

To investigate habitat use for grazing and diet composition we amalgamated the information from all observation sessions. The proportion of a certain plant form [graminoid, herb, woody, mix i.e. mixture of two or more plant forms], plant part [leave, stem, flower +fruit, root] or plant species to the total number of bites was calculated as the amount of bites consumed of the food item divided by the total number of bites. Habitat use for grazing was also based on the proportion of bites taken from that habitat.

Similarity between dung germinable seed content [July-October 2000] and diet species composition during the fruiting season [May-October 1999 / 2001] was calculated using the Jaccard similarity index. In addition Spearman's rank coefficient was used to further quantify the variation between diet composition [% bites] and dung germinable seed content [% seedlings].
To determine among the common species of the diet [i.e. those species contributing at least 0.1% of the bites taken from May-October] those species that were preferentially dispersed by endozoochory and those which were not we calculated a dispersal preference index [D] based on Jacobs' modification [1974] of Ivlev's Electivity Index:

$$D = [r_i - p_i] / [r_i + p_i - 2 r_i p_i]$$

where r_i is the proportion of species *i* in the dung germinable seed content and p_i the proportion of species *i* in the diet. D varied from -1 to 0 for species which proportionally disperse less well compared to their contribution into the diet. D varied from 0 to +1 for species which disperse better than expected from their contribution to the diet.

To confirm statistically differences in seed characteristics and nutritive value of the foliage between successful [0, +1] and not successful endozoochorically dispersed plant species [0, -1]; we applied a two way ANOVA with fixed factors ENDOZOOCHORES [successful, not successful] and PLANT FORM [graminoid or herb]. We tested for possible differences of the following seed traits: seed length and width, shape, seed mass, seed longevity index [see Janzen's hypothesis, 1984] and parameters of forage value i.e. content [%] of crude protein, NDF and ADF in plant tissue [Coleman *et al.* 1999]. To match normality and homogeneity of variance assumptions of ANOVA, before analysis, seed length, width, shape and mass were $\log_{10}$ transformed.
Seed shape was calculated as the seed length / width ratio. Seed longevity, was based on Thompson *et al.* [1997] and calculated according to Thompson *et al.* [1998]. Information on seed length, seed width and seed mass was collected from Zwaenepoel [1993] and Grime *et al*. [1988]. Biochemical analysis of the forage plants was conducted on plant samples that were collected immediately after observation sessions during the fruit-

ing season and subsequently oven-dried for 48 hours at 60°C and ground [1mm sieve]. For the analysis we used 'Near-infrared reflectance analysis [NIRS] [Coleman *et al.* 1999 and references herein]. The necessary calibration was carried out before, on similar plant samples, of which five subsamples were biochemical analysed according to standard procedures. Crude protein content was determined using the micro Kjeldahl method. Cell wall constituents [NDF, ADF, ADL] were determined according to Van Soest [1967] and Goering & Van Soest [1970].

Results

Habitat grazing

With total bites as a criterion a disproportional habitat grazing was observed. During the fruiting season cattle and Konik both grazed most in the tall herb dominated habitat on wet [Uw] and dry soils [Ud] [Fig. 3.1]. At Westhoek South, cattle and Shetland pony grazed most in the tall herb dominated vegetation on dry soil and in dune grasslands on wet soils [Gw] [Fig 3.1]. All animal species grazed only in minor quantities in white and grey dunes [A/T], scrub [S] and forest [F].

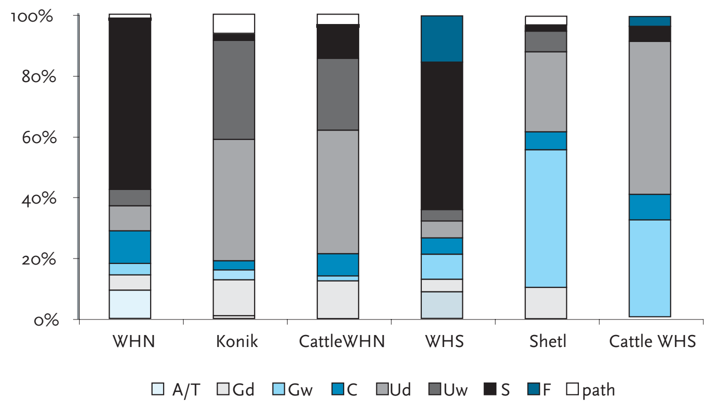


Figure 3.1 Proportional habitat use [%] for grazing by free ranging horses [Konik and Shetland pony] and Scottish Highland cattle at the Westhoek North [WHN] and South [WHS]. The proportion area of each habitat type at both study sites is also shown. The different habitats are white and grey dunes [A/T], dune grassland on dry [Gd] and wet [Gw] soil, tall herb dominated habitats [roughage] on dry [Ud] and wet soils [Uw], monospecific grassland [C], Scrub [S], Forest [F] and paths [see Chapter 1].

Westhoek North

PLANT GROUP

During May-October, graminoids were by far the main component of both Highland cattle and Konik diet. The cattle diet was mainly supplemented by herbs and some woody species. Koniks in addition select mainly herbs [Fig 3.2].

PLANT SPECIES

Both ungulates, however, consumed a wide variety of plant species. During the fruiting season, cattle ate from at least 103 plant species: 23 graminoid species, 62 herb species and 18 woody species. Koniks ate from at least 114 plant species: 33 graminoid species, 74 herb species and 7 woody species. In both cases about one third of all plant species known in the study area were bitten.

Holcus lanatus, *Poa trivialis* and *Calamagrostis epigejos* formed the bulk of the plant species recorded as eaten by cattle and Konik [together c. 50 % of all bites]. Cattle in addition selected on some tall herb species: *Lycopus europaeus*, *Urtica dioica*, *Lythrum salicaria* and the ruderal annual *Claytonia perfoliata*. Two woody species i.e. *Salix cinerea* and *Ligustrum vulgare* furthermore contributed to the varied cattle diet [Appendix 3.1]. Konik additionally mainly selected on some other graminoid species: *Calamagrostis canescens*, *Juncus subnodulosus*, *J. bufonius*, and *Agrostis stolonifera*. The tall herbs *Eupatorium cannabinum*, *Cirsium arvense* and two annuals i.e. *Arenaria serpyllifolia* and *Claytonia perfoliata* were the most frequently selected herbs. *Rubus caesius* was the only woody species that occurred regularly, but in minor quantities in the May-October diet of Koniks [Appendix 3.1]. *vulgare* and Konik ate the inflorescence of *Cirsium arvense* and *Eupatorium cannabinum* and the fruits of *Rosa pimpinellifolia* and *Rubus caesius*.

PLANT PARTS

Leaves were by far the most frequently eaten plant parts by both Ungulates [Fig. 3.3]. This was certainly true for all graminoids, but not always the case for herbaceous or woody plant species. For example cattle selected specifically on the fruits of *Rosa canina* and *Ligustrum vulgare*.

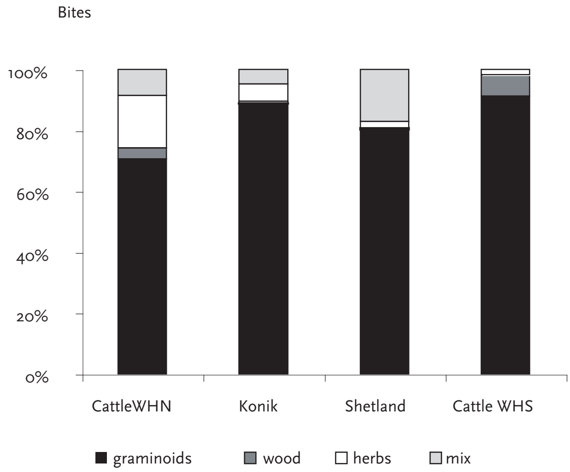


Figure 3.2 Proportion [%] of plant groups, based on total number of bites, contributing to the diet of Highland cattle and horses [Konik and Shetland pony] during May-October in the Westhoek North [WHN] and South [WHS]. Graminoids are by far the most grazed plant group. The composition of a bite was indicated 'mix' when it existed from different plant species belonging to at least two different plant groups

Westhoek South

Plant group

Graminoids were on average the main component of both Highland cattle and Shetland pony diets [Fig. 3.2]. During the fruiting season, the cattle diet was mainly supplemented by woody species, which were browsed frequently [Fig. 3.2]. Shetland ponies additionally mainly select herbs, which were consumed most frequently in combination with graminoids [i.e. mixed bites] [Fig. 3.2].

Plant species [May-October]

Holcus lanatus and *Calamagrostis epigejos* were the most important constituents of both cattle and Shetland pony diet. Cattle in addition selected on two other graminoid species: *Poa trivialis* and *Juncus subnodulosus*. Cattle furthermore mainly browsed woody species such as *Rosa pimpinellifolia* [especially the fruits], *Prunus spinosa*, *Clematis vitalba*, *Salix repens*, *Rubus caesius* and *Crataegus monogyna* [Appendix 3.1]. Shetland pony in addition selected on several graminoid species: *Agrostis stolonifera*, *Poa trivialis*, *Juncus subnodulosus* and *Carex arenaria*. *Cirsium arvense* and *Claytonia perfoliata* were the most frequently selected herbs. *Rubus caesius* was the only woody species that was eaten occasionally by the Shetland pony during the fruiting season [Appendix 3.1]

Both herbivores consumed a wide variety of plant species during the fruiting season. Shetland ponies ate 74 plant species: 29 graminoid species, 43 herb species and 2 woody species. Because of too few observations we could only notice 35 plant species eaten by the cattle: 8 graminoid species, 13 herb species and 14 woody species.

Plant parts

Leaves are by far the most bitten plant parts by Shetland pony [Fig. 3.3]. Flowers and or fruits were consumed from e.g. *Rosa pimpinellifolia*, *Rubus caesius, Cirsium arvense*, *Urtica dioica* and *Epilobium hirsutum*. Consumption of plant parts was not recorded for Highland cattle.

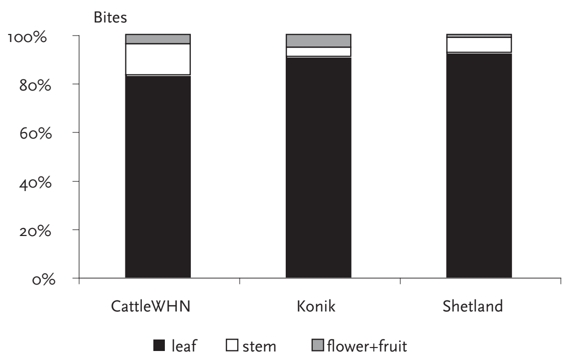


Figure 3.3 Proportion [%] of plant parts, based on total number of bites, contributing to the diet of cattle and horses during May-October in the Westhoek North and South

Diet composition and dung germinable seed content

Both ungulate species at Westhoek North showed a high degree of similarity between diet composition and dung germinable seed content [Table 3.1]. At Westhoek South, Highland cattle and Shetland pony the composition of

Table 3.1 Plant species numbers in diet and of consumed and of the dung germinable seed content of cattle and horses at Westhoek North and South. Matching between the species composition of diet and dung germinable seed content is expressed by Jaccard similarity and Spearman coefficient. Plant species numbers in the diet are obtained from visual observations of grazing activities. Plant species numbers in dung are derived from germination experiments of dung sampled from July-October.

Plant species in	**Westhoek N.**		**Westhoek S.**	
	Konik	Cattle	Shetland	Cattle
Diet	114	103	74	35
Dung seed content	90	67	67	82
Both diet and dung	58	36	36	16
Diet+Dung	145	132	105	99
Jaccard similarity [%]	40	27	34	16
Spearman r_S	0.675	0.645	0.187	-0.212
N	114	103	74	35
P [r_S]	<0.001	<0.001	NS	NS

the whole diet differed considerably from the dung germinable seed content. However, Shetland pony diet showed some qualitative overlap with dung germinable seed content [Table 3.1]. When considering the plant species of which the flowering or fruiting parts were seen bitten, a positive relationship between the proportional number of bites and the proportion germinable seeds in the dung, was only noticed for cattle at Westhoek North [Spearman r_S: 0.556, N =53, $P = 0.024$]. For Konik the significance level was not completely convincing [Spearman r_S: 0.699, N =16, $P = 0.055$].

Plant species from which was regularly eaten [i.e. at least 0.1 % of all bites consumed during the study period] from May until October differed in their ability to get endozoochorically dispersed. Eleven plant species showed a positive D-value, indicating that they were more frequently dispersed via ungulate dung than could be expected from their contribution to the diet [Table 3.2]. We consider these plants as successful endozoochores. Among them no woody species were observed. Proportionally, 38 plant species were better represented in the diet than in the dung germinable seed content. These plants are considered as unsuccessful endozoochores. Except *Rubus caesius*, none of the ten woody species did at all show seedling emergence from the dung samples [Table 3.2]. On the other hand, some well represented plant species in the dung germinable seed content were never recorded as part of the diet [Table 3.3], indicating that they were consumed passively and/or were missed during observations.

Differences in seed characteristics and forage value of successful and unsuccessful endozoochores

Seeds of successful endozoochores on average have a higher seed longevity index, have lower mass and are smaller than seeds of unsuccessful endozoochores [Fig.3.4 a-c]. Seed shape and width did not show any significant differences between successful and unsuccessful endozoochores [shape: $F_{18,1}$: 2.14, NS; width: $F_{18,1}$: 3.25, NS].

Crude protein, NDF and ADF content of the foliage did not show any significant differences between successful and unsuccessful endozoochores [Crude protein: $F_{15,1}$: 0.005, NS; NDF: $F_{14,1}$: 0.615, NS; ADF: $F_{14,1}$: 0.133, NS; ADL: $F_{14,1}$: 1.23, NS - see also Appendix 3.2].

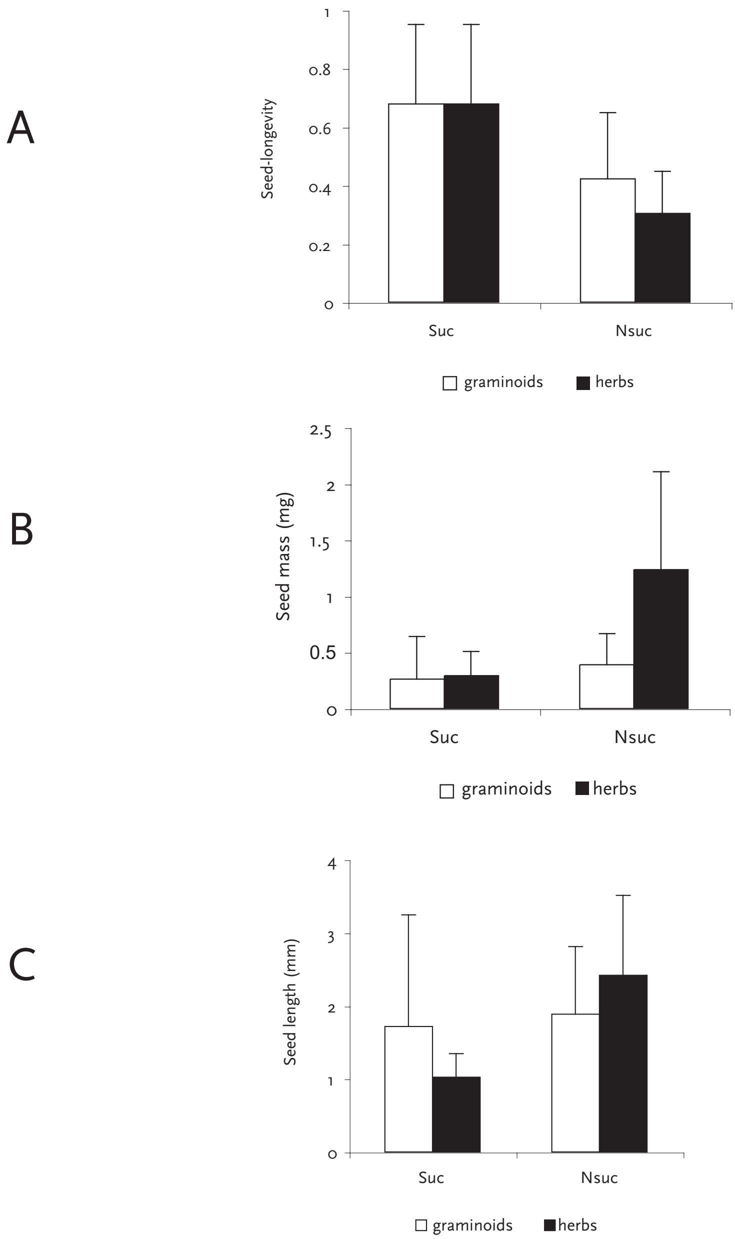


Fig. 3.4 a-c Mean seed longevity index, seed mass and seed length of successful [Suc] and unsuccessful [NSuc] endozoochorous graminoids and herbs [see Table 3.2]. In all three cases seed characteristics were significantly different between both groups of success. [Seed longevity index: $F_{26,1}$: 15.2, $P=0.001$; Seed mass: $F_{19,1}$: 7.9, $P=0.011$; Seed length $F_{26,1}$: 5.9, $P=0.022$]

Table 3.2 Alphabetically ordered plant species list, with indication of the preference index [D]. D varied from -1 to 0 for species which proportionally disperse less well compared to their contribution into the diet i.e. it are less successful endozoochorically dispersed plant species. D varied from 0 to +1 for species which disperse better than expected from their contribution to the diet; it are successful Endozoochores. For each plant species D is also shown for each of the studied ungulate species. The plant group the plant species belongs to is also indicated [g: graminoid, h: herb, w: woody]

Plant species	Plant group	Konik	Cattle Westhoek N	Shetland	Cattle Westhoek S	Mean D
Aegopodium podagraria	h				-1	-1
Agrostis stolonifera	g	0.22	0.22	-0.56	1	0.22
Arenaria serpyllifolia	h	0.33				0.33
Calamagrostis canescens	g	-0.99	-1			-0.99
Calamagrostis epigejos	g	-0.99	-1	-0.99	-1	-0.99
Carex arenaria	g	-0.32	0.76	-0.66	1	0.53
Carex disticha	g			-1		-1
Carex flacca	g	-0.54	-0.38			-0.46
Carex riparia	g	-1		-1	-1	-1
Carex trinervis	g	-0.51				-0.51
Cirsium arvense	h	-0.97	-0.90	-1	-0.93	-0.95
Claytonia perfoliata	h	-0.96	-1	-1		-0.99
Clematis vitalba	w		-1		-1	-1
Crataegus monogyna	w				-1	-1
Dryopteris filix-mas	h		-1			-1
Elymus sp.	g			-1		-1
Eupatorium cannabinum	h	-0.50	-1			-0.75
Festuca juncifolia	g	-1			-1	-1
Galium aparine	h	0.04	-0.93			-0.45
Hippophae rhamnoides	w				-1	-1
Holcus lanatus	g	-0.93	-0.92	0.18	0.19	-0.37

Plant species	Plant group	Konik	Cattle Westhoek N	Shetland	Cattle Westhoek S	Mean D
Juncus articulatus	g	0.99	0.94			0.96
Juncus bufonius	g	0.92	0.85	0.99		0.92
Juncus inflexus	g			-0.94		-0.94
Juncus subnodulosus	g	-1	-0.99	-1	-1	-0.99
Ligustrum vulgare	w		-1		-1	-1
Lolium perenne	g		-1			-1
Lycopus europaeus	h		0.18			0.18
Lysimachia vulgaris	k		-1			-1
Lythrum salicaria	h		-0.62			-0.62
Mentha aquatica	h	-1	0.32			-0.34
Phragmites australis	g	-1		-1		-1
Poa annua	g	0.74	-0.28			0.23
Poa pratensis	g	0.57	0.88			0.72
Poa trivialis	g	-0.22	-0.30	-0.17	0.02	-0.17
Prunus spinosa	w				-1	-1
Ranunculus repens	h		-0.53			-0.53
Rosa pimpinellifolia	w			-1	-1	-1
Rubus caesius	h	-0.69	-0.84	-0.35	-1	-0.72
Salix cinerea	w		-1		-1	-1
Salix repens	w		-1		-1	-1
Sambucus nigra	w		-1			-1
Stellaria media	h	0.42	0.56			0.49
Trifolium campestre	h			-0.96		-0.96
Trifolium repens	h	0.12				0.12
Urtica dioica	h	0.92	0.92	0.39		0.74
Vicia cracca	h			-1		-1

Table 3.3 List of plant species which were well represented in the dung germinable seed content but which were never recorded as part of the diet. The percentage a plant species contributes to the dung germinable seed content is shown in the columns. The total number of seedlings recorded are indicated for each ungulate species

Plant species	**Konik**	**Cattle** Westhoek N	**Shetland**	**Cattle** Westhoek S
Achillea millefolium		0.06		
Agrostis capillaris		0.88		
Arenaria serpyllifolia		0.16	0.14	1.62
Cardamine hirsuta	0.12			
Epilobium roseum	0.11	0.32		0.48
Galium verum		0.11		0.51
Gnaphalium uliginosum	0.33			0.12
Hydrocotyle vulgaris	0.11			
Mentha aquatica	0.23			0.25
Phleum pratense	0.15			0.11
Plantago coronopus	0.17			
Plantago lanceolata		0.18		
Plantago major	1.48		0.10	0.46
Rumex acetosella		0.09		0.32
Sagina procumbens/apetala	1.49	0.08	0.62	3.19
Senecio sylvaticus	0.11			
Veronica officinalis	0.44			0.14
Total number seedlings	15098	18302	9814	9267

Discussion

Although the bulk of the diet consisted of a limited set of graminoids, the diet spectrum of the observed free ranging cattle and horses was remarkably large. The diversity of the ungulate diet may be explained by the fact that the studies were carried out in two species rich areas. The availability of palatable plant species had a great influence on the diet selection. The ungulates seem to select first those graminoid species that are common and widespread [see Appendix 2.1] and which, in contrast with herbaceous and woody species, presumably have less or no unfavourable secondary compounds [Putman *et al*. 1987; Gordon 1989; Duncan 1992; Groot Bruinderink *et al*.1997; Vulink 2001]. Several herbaceous species [e.g. Table 3.3] must have become part of the diet as a result of associational palatability i.e. they presumably were grazed together with other species the grazers select on, hence contributing to mixed bites [Fig. 3.2]. Yet, selective feeding e.g. on the inflorescences and fruits of different herbaceous and woody species also occurred. But still, many common herbs were almost never eaten, presumably because of secondary compounds or structural defences [e.g. *Iris pseudacorus*, *Senecio jacobaea, Lysimachia vulgaris*].

In contrast to Shetland pony and Highland cattle at Westhoek South, Both ungulate species at Westhoek North showed a high degree of similarity between diet composition and dung germinable seed content. The incomplete matching of the diet composition and dung viable seed content may be partly the result of the differential effect of endozoochory among plant species. The effect of gut passage on plant species varies considerably. The seeds of some species are able to survive ingestion and digestion by herbivores, others do not survive consumption at all [Lennartz 1957; Özer 1978; Gardener et al. 1993; Chapter 5]. Secondly, data may be biased by the methods used: [i]Grinding the dung samples in order to be able to easily spread them out in a thin layer could have damaged large sized seeds. This could be the reason why some plant species, although the fruits have been seen bitten, did not emerge from the dung samples e.g. *Crataegus monogyna, Ligustrum vulgare*. The latter species emerged from intact dung samples under similar greenhouse conditions. [ii] Greenhouse conditions may have affected the germination success of plant species differently [Grime, Hodgson & Hunt 1988 and Chapter 2]. [iii] Perhaps, a more prolonged dung sampling period could have resulted in more plant species recorded. For some species the dung sampling period may have started too late [*Salix* spp. and annuals such as *Myosotis* spp, *Cerastium* spp] or stopped to early [*Clematis vitalba*, *Hippophae rhamnoides]*.

On the other hand several plant species, rather common in the area, were recorded frequently from dung samples but not from the diet. For example *Aira praecox, Sagina procumbens, Arenaria serpyllifolia, Plantago coronopus* and *Samolus valerandi* were presumably not observed to be bitten because of their small stature [Table 3.3]. Other species may have been missed because they mainly occur in [very] species rich vegetation with a low sward [e.g. Gw and Gd]. In such situation it was impossible to determine adequately the detailed bite composition. Such bites were indicated as 'mixed' [Fig. 3.2]. This category of food class forms an important part of the diet of the free ranging ungulates and might expectably be composed of such species as *Helianthemum nummularium, Galium verum, Plantago major, Veronica officinalis* which form part of the relatively low sward and were all recorded from dung samples. This at least implies the previous consumption of their seeds during grazing, although possibly in a passive way as seeds deposited in the vegetation and not attached to the mother plant anymore. Above that, the bad matching of the cattle diet with the dung germinable seed content at Westhoek South may be the result of an insufficient number of observations [Appendix 3.1] When comparing the frequently and quantitatively dominant diet species with the dung viable seed content, eleven species could be identified that were more frequently found in the dung as germinating seed than in the diet [D>0]. The low 'dispersability index' found for some grass species may be due to failing seed production as a consequence of high grazing pressure. *Calamagrostis epigejos*, *Holcus lanatus*, *Poa trivialis* and *Phragmites australis* all show low inflorescence production in both study areas and in grazed situation in general. The observed inability for successful endozoochorous seed dispersal of most woody species may be due to methodological problems [see supra]. On this point further research is needed.

The results of this study support two of the expected traits [7 and 8] of successful endozoochorously dispersed plants predicted by Janzen [1984] as part of the ‘Foliage is the Fruit’ theory. Trait 7, which states that seeds of successful endozoochores are small, tough, hard and inconspicuous to escape the molar mills, is supported by the fact that in this study successful endozoochores, compared to the less successful endozoochores, have a shorter seed length and lower seed mass [Fig 3.4 b-c]. The fact that successful endozoochores have a higher seed longevity index than the unsuccessful endozoochores [Fig 3.4 a] supports trait 8: seed coats have the ability to resist digestion during a transit period of days to months. This trait is also of evident value in, and selecting for, seed dormancy in the soil and litter [Janzen 1984].

The analysis of the nutritive value of the foliage of successful and unsuccessful endozoochores did not show a significant differences between both categories of plant species. But the results of this analysis showed that the consumed plant species all were valuable food items. Hence these plant species are to some degree all attractive for large herbivores [trait 3 of Janzen’s theory]. It should however be kept in mind that the nutritive value of plant species is not only determined by the factors mentioned in this study, but also by other possibly important factors such as minerals or the amount of anti-quality factors [e.g. tannins, alkaloids].

The observed discrepancy between diet consumption and dung viable seed content holds arguments for further study of both processes in order to better understand the potential impact of large ungulates on vegetation dynamics i.e. extended sampling periods, studying favourable germination conditions of some species [e.g. several woody species], using additional methods for the determination of the diet composition [indirect observations e.g. Zeevalking & Fresco 1977]. The general lack of information on endozoochory of morphologically not specifically adapted plant species [but see Janzen 1984] in general seed dispersal literature [e.g. van der Pijl 1969; Harper 1977; Bouman et al. 2000; Herrera 2002; Baskin & Baskin 1998] and the findings in this study and other studies [e.g. Pakeman et al. 2002] justify the hypothesis that endozoochory might well be underestimated as compared to other dispersal mechanisms.

Appendix 3.1 The 15 most frequent bitten plant species [May-October] in different habitat types by horses [i.e. Shetland pony and Konik] and Scottish Highland cattle at the Westhoek Nature Reserve. The total amount of bites consumed, during the observation sessions [N], from each plant species in each type of habitat is shown. The habitat types were white and grey dunes [A/T], dune grassland on dry [Gd] and wet [Gw] soil, tall herb dominated habitats [roughage] on dry [Ud] and wet soils [Uw], mono- specific grassland [C], Scrub [S], Forest [F] and paths. The proportion of all bites [Total] taken from a plant species to the total amount of all bites is shown in the right most column [% bites].

Plant species	A/T	C	F	Gd	Gw	Path	S	Ud	Uw	Total	%bites
Shetland [N = 33]											
Holcus lanatus	31	2519	175	0	5777	785	1344	20403	2915	33949	17.09
Calamagrostis epigejos	56	3066	266	0	1716	1709	429	7396	3207	17846	8.98
Cirsium arvense	0	172	0	6	283	46	166	496	2186	3355	1.69
Poa spp.	0	0	0	0	1871	74	0	706	38	2689	1.35
Poa trivialis	41	272	42	0	366	243	163	1316	237	2680	1.35
Agrostis stolonifera	0	0	0	0	2154	0	1	6	83	2243	1.13
Juncus subnodulosus	0	136	0	0	1065	70	96	273	583	2225	1.12
Claytonia perfoliata	40	476	8	0	38	34	357	990	0	1943	0.98
Carex arenaria	722	201	3	22	29	7	4	295	5	1288	0.65
Rubus caesius	0	151	6	5	419	4	73	277	24	958	0.48
Carex disticha	0	0	0	0	783	0	0	0	0	783	0.39
Festuca rubra	0	60	0	0	226	0	0	445	18	748	0.38
Juncus bufonius	0	0	0	8	406	0	0	8	0	421	0.21
Phragmites australis	0	0	0	0	283	0	0	26	0	309	0.16
Juncus inflexus	0	2	9	0	48	0	5	53	192	309	0.16
Urtica dioica	155	45	0	0	0	0	0	0	80	280	0.14
Elymus spp.	1	60	7	0	74	0	20	7	69	237	0.12
Carex riparia	0	26	0	0	166	0	32	0	0	224	0.11
Vicia cracca	0	5	0	0	45	1	1	43	125	219	0.11
Trifolium campestre	0	0	0	218	0	0	0	0	0	218	0.11
Rosa pimpinellifolia	0	0	0	0	0	35	9	152	2	198	0.10
Total of all bites	2019	1338	71494	13894	76032	18988	5597	874	8388	198624	100.00
Konik [N = 38]	A/T	C	F	Gd	Gw	path	S	Ud	Uw	Total	%bites
Calamagrostis epigejos	209	2993	68	10954	1895	2471	4334	27821	24927	75671	20.64
Holcus lanatus	359	758	155	8407	646	2765	801	43418	9032	66341	18.1
Poa trivialis	525	178	68	672	7	2654	19	22751	16844	43718	11.93
Calamagrostis canescens	0	4832	0	0	23	634	0	1761	3165	10415	2.841
Juncus subnodulosus	0	303	0	0	1	443	0	271	6722	7740	2.112
Agrostis stolonifera	0	25	0	1455	77	194	0	727	2780	5258	1.434
Eupatorium cannabinum	0	306	22	228	45	386	135	2021	732	3875	1.057
Claytonia perfoliata	0	0	114	0	2	23	20	1705	1506	3370	0.919
Juncus bufonius	0	0	0	0	0	527	0	415	2404	3346	0.913

Arenaria serpyllifolia	0	0	0	122	0	137	0	2244	477	2980	0.813
Rubus caesius	0	548	28	529	36	134	27	1124	370	2796	0.763
Cirsium arvense	5	96	2	4	50	273	62	1303	944	2739	0.747
Poa pratensis	0	0	0	0	0	764	0	1804	50	2618	0.714
Carex arenaria	378	0	0	700	269	121	95	569	28	2161	0.589
Carex flacca	0	0	0	0	1857	0	5	21	177	2060	0.562

Konik [N = 38]	A/T	C	F	Gd	Gw	path	S	Ud	Uw	Total	%bites
Stellaria med	0	0	32	0	0	452	0	582	527	1593	0,43
Festuca juncifolia	433	0	0	0	105	8	771	0	0	1317	0,36
Phragmites australis	0	202	0	20	44	14	1	634	109	1024	0,28
Poa annua	0	0	0	0	2	808	0	182	0	992	0,27
Trifolium repens	0	0	0	0	446	33	0	328	78	885	0,24
Caexr riparia	0	0	0	0	408	0	0	300	163	871	0,24
Juncus articulatus	0	25	0	0	33	0	0	59	637	754	0,21
Vicia cracca	3	9	0	37	95	6	5	356	233	744	0,2
Galium aparine	0	14	1	0	0	0	296	292	6	609	0,17
Urtica dioica	0	0	117	23	0	5	0	154	304	603	0,16
Carex trinervis	0	0	0	0	446	0	0	0	0	446	0,12
Total of all bites	3307	11073	807	43156	11798	23172	7756	146250	119221	366540	100

Cattle diet: see next page.

Highland cattle Westhoek N [N = 38]	A/T	C	F	Gd	Gw	path	S	Ud	Uw	Totaal	%bites
Holcus lanatus	0	1062	33	2599	304	1841	3830	27684	4244	41597	20.99
Poa trivialis	0	2001	104	1602	36,5	789,5	2138	22291	3719	32680	16.49
Calamagrostis epigejos	0	1314	28	1460	622	777	1993	8992	3190	18374	9.27
Lycopus europaeus	0	191	0	0	0	66	245	1220	9945	11667	5.89
Claytonia perfoliata	12	3573	192	407	0	114	4272	1221	206	9997	5.05
Urtica dioica	0	696	255	0	0	12	920	1868	471	4222	2.13
Lythrum salicaria	0	29	0	0	12	2	443	230	2142	2858	1.44
Calamagrostis canescens	0	1357	0	0	0	0	203	394	707	2661	1.34
Agrostis stolonifera	0	41	48	0	18	108	0	292	1877	2384	1.20
Salix cinerea	0	896	0	0	0	74	178	708	292	2148	1.08
Ligustrum vulgare	128	734	0	283	0	372	326	101	2	1946	0.98
Galium aparine	0	521	14	1	0	8	743	80	0	1367	0.69
Juncus subnodulosus	0	0	0	0	0	0	0	3	1050	1053	0.53
Juncus bufonius	0	0	0	0	51	45	40	119	765	1020	0.51
Rubus caesius	0	326	13	17	1	8	217	113	305	1000	0.50
Cirsium arvense	0	27	2	0	1	3	212	367	340	952	0.48
Lysimachia vulgaris	0	92	0	5	0	0	230	60	533	920	0.46
Salix repens	46	8	0	47	15	0	46	68	635	865	0.44
Poa annua	0	0	15	106	165	3	131	0	388	808	0.41
Lolium perenne	0	255	0	0	0	11	0	413	0	679	0.34
Dryopteris filix-mas	0	241	0	209	0	0	81	48	0	579	0.29
Stellaria media	0	165	41	0	0	0	29	223	54	512	0.26
Ranunculus repens	0	0	0	0	0	0	0	30	444	474	0.24
Juncus articulatus	0	0	0	0	11	0	0	0	427	438	0.22
Eupatorium cannabinum	0	31	0	0	0	0	144	247	8	430	0.22
Poa pratensis	0	10	0	40	0	0	16	331	0	397	0.20
Mentha aquatica	0	23	0	0	0	3	0	1	320	347	0.18
Carex arenaria	0	14	0	24	0	0	162	60	0	260	0.13
Sambucus nigra	2	27	9	23	0	2	106	51	0	220	0.11
Carex flacca	0	0	0	119	0	14	29	0	46	208	0.10
Glechoma hederacea	0	0	0	0	0	0	0	174	27	201	0.10
Clematis vitalba	0	0	0	0	0	94	101	0	0	195	0.10
Total of all bites	624	14853	904	24522	3222	7333	21457	83053	47798	198129	100,00

Cattle Westhoek S [N =15]	A/T	C	F	Gd	Gw	path	S	Ud	Uw	Total	%bites
grasmix	90	0	515	0	7667	0	736	14472	197	23677	39.3
Holcus lanatus	0	1100	400	0	2566	0	390	9964	162	14582	24.2
Poa trivialis	0	890	265	0	5111	0	406	4824	131	11627	19.3
Juncus subnodulosus	0	0	0	0	2488	0	0	0	0	2488	4.1
Calamagrostis epigejos	0	1990	0	0	4	0	0	35	0	2029	3.4
Rosa pimpinellifolia	0	32	69	0	9	0	471	376	0	957	1.6
Prunus spinosa	0	38	0	0	231	0	203	281	15	768	1.3
Clematis vitalba	0	332	209	0	0	0	135	0	0	676	1.1
Salix repens	0	0	0	0	611	0	40	12	0	663	1.1
Rubus caesius	50	298	107	0	36	0	48	114	7	660	1.1
Crataegus monogyna	0	241	12	0	7	0	177	19	3	459	0.76
Carex riparia	0	0	0	0	326	0	0	19	0	345	0.57
Salix cinerea	0	0	0	0	0	0	224	0	0	224	0.37
Ligustrum vulgare	13	78	0	0	40	0	16	32	0	179	0.30
Betula pendula	0	13	142	0	0	0	0	0	0	155	0.26
Aegopodium podagraria	0	0	134	0	0	0	0	0	0	134	0.22
Cattle Westhoek S	A/T	C	F	Gd	Gw	S	path	Ud	Uw	Total	%bites
Carex arenaria	130	0	0	0	0	0	0	0	0	130	0.22
Cirsium arvense	0	45	0	0	6	0	0	77	1	129	0.21
Festuca juncifolia	100	0	0	0	0	0	0	0	0	100	0.17
Hippophae rhamnoides	0	17	0	0	17	34	0	9	0	77	0.13
Total of all bites	303	5125	1936	0	19195	2899	0	30274	517	60249	100

Appendix 3.2. Alphabetically ordered plant species list, with indication of their preference index D [see table 3.2] their seed characteristics and the nutritive value of the foliage expressed as % crude protein [CP], % neutral detergent fibre [NDF], % acid detergent fibre [ADF] and % acid detergent lignin [ADL], which were all used in the Anova to detect possible differences between successful [D>=0] or unsuccessful [D<0] endozoochores.

Plantspecies	D-index	Seed characteristics					Nutritive value [% content]			
		Length[mm]	Width[mm]	Mass[mg]	shape	longevity	CP	NDF	ADF	ADL
Aegopodium podagraria	-1	4.00				0.33				
Agrostis stolonifera	0.22	1.00	0.50	0.02	2.00	0.38	10.72	61.06	31.16	4.33
Arenaria serpyllifolia	0.33	0.50	0.50	0.06	1.00	0.76				
Calamagrostis canescens	-0.99	1.50				0.30	11.60	67.36	35.75	5.08
Calamagrostis epigejos	-0.99	2.90	0.50		5.80	0.43	10.80	71.09	38.33	5.80
Carex arenaria	0.53	4.70	1.70	1.00	2.76		8.72	65.97	32.63	6.55
Carex disticha	-1					0.22				
Carex flacca	-0.46	1.60	1.30	0.37	1.23	0.46				
Carex riparia	-1									
Carex trinervis	-0.51									
Cirsium arvense	-0.95	3.50	1.60	1.17	2.19	0.34	18.51	41.93	27.84	7.72
Claytonia perfoliata	-0.99	1.50					18.05	30.69	18.13	5.78
Clematis vitalba	-1									
Crataegus monogyna	-1					0.00				
Dryopteris filix-mas	-1									
Elymus sp.	-1									
Eupatorium cannabinum	-0.75	3.00	0.50		6.00	0.33	9.51	46.51	32.49	8.88
Festuca juncifolia	-1						5.51	71.65	39.00	6.24
Festuca rubra	-1	3.50	1.10	0.79	3.18	0.14				
Galium aparine	-0.59	2.30	2.00	7.25	1.15	0.24	11.86	42.42	28.75	6.38
Glechoma hederacea	-1			0.69		0.17				
Hippophae rhamnoides	-1									
Holcus lanatus	-0.37	1.80	0.80	0.32	2.25	0.55	17.39	58.90	24.24	6.08
Juncus articulatus	0.96	0.70	0.30	0.02	2.33	0.88	13.36	56.95	28.28	5.75
Juncus bufonius	0.86	0.60	0.40	0.02	1.50	0.86	15.14	47.78	24.10	4.40
Juncus inflexus	-0.94	0.50				0.81	14.27	51.89	24.20	2.30
Juncus subnodulosus	-0.99						7.22	73.62	36.50	3.11
Ligustrum vulgare	-1						7.50	40.98	28.21	12.55

Plantspecies	D-index	Seed characteristics					Nutritive value [% content]			
		Length[mm]	Width[mm]	Mass[mg]	shape	longevity	CP	NDF	ADF	ADL
Lolium perenne	-1					0.20				
Luzula campestris	-0.36	1.70	1.10	0.64	1.55	0.37				
Lycopus europaeus	0.18	1.00					9.63	40.86	21.32	9.88
Lysimachia vulgaris	-1	2.00				0.32				
Lythrum salicaria	-0.62	1.00				0.24	12.05	31.94	18.46	5.40
Mentha aquatica	-0.34	1.10	0.60	0.14	1.83	0.39				
Phragmites australis	-1			0.12						
Poa annua	0.23	1.70	0.70	0.26	2.43	0.89				
Poa pratensis	0.72	1.60	0.60	0.25	2.67	0.38	16.29			
Poa trivialis	-0.17	1.60	0.60	0.09	2.67	0.75	15.28	46.52	18.73	2.59
Prunus spinosa	-1					0.00				
Ranunculus repens	-0.53	3.90	2.30	2.32	1.70	0.64				
Rosa pimpinellifolia	-1						6.47	46.33	35.42	15.03
Rubus caesius	-0.72	3.60	3.10				10.85	42.66	26.04	7.22
Salix cinerea	-1									
Salix repens	-1					0.00	7.67	56.27	42.27	15.72
Sambucus nigra	-1			3.40			20.06	28.65	15.89	6.01
Stellaria media	0.49	1.30	1.00	0.35	1.30	0.75				
Trifolium campestre	-0.83	1.50		1.85		0.28				
Trifolium repens	0.12	1.00	1.00	0.56	1.00	0.42				
Urtica dioica	0.74	1.30	0.90	0.19	1.44	0.78	15.58	32.15	20.02	5.98
Vicia cracca	-1	2.80	2.60	14.29	1.08	0.07				

4 Horse dung germinable seed-content in relation to plant species abundance, diet composition and seed characteristics in summer in a dune ecosystem

Eric Cosyns & Maurice Hoffmann

Counting of identified seedlings which emerged from ungulate dung sample (Wenduine, 2000).

Abstract

The abundance and species richness of the germinable seed content of dung of free ranging horses was quantified and related to eight variables: plant species abundance in the field [cover] and in the horse diet, seed sizes [length, width and mass], seed longevity index, seed shape which was expressed as variance in seed dimensions and assumed dispersal strategy.

From 56 dung samples [2.5L] 53 493 seedlings emerged, representing 106 different plant species, i.e. 21.4 % of all species recorded in the established vegetation of the study sites. A majority of plant species [50 %] was only found in less than 10 % of the dung samples while 68 % of the germinating species emerged with on average less than 1 seedling per dung sample. Overall dung seed density ranged on average between 280.4 - 525.2 seedlings per litre. Mean species richness of dung samples varied between 19 and 34 plant species. *Urtica dioica*, *Veronica* spp. and several graminoid species were among the most abundant and frequently out of dung emerging plant taxa. Dung seed content was positively correlated with plant species abundance in the field and in the diet. There was no clear impact from the morphological seed characteristics to an assumed dispersal strategy on species presence/absence in dung samples. Relative dung seed density [i.e. per unit cover] was positively correlated with seed longevity index and negatively correlated with all three seed size variables. There was no significant relationship with seed shape. As far as the possible role of endozoochory for conservation of plant diversity in grassland is concerned, the results emphasizes on the importance of large herbivores as potentially strong seed dispersal vectors.

Key-words: coastal dunes, endozoochory, grassland management, seed longevity, seed dispersal.

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Introduction

Seed dispersal has become an important issue in plant ecology and in nature management in particular [Primack & Miao 1992; Bakker 1998; Bakker & Berendse 1999], especially in the fragmented landscape of Western Europe. Despite the increasing demand for reliable autoecological information on dispersal-related topics of the temperate, native European flora, most of this information is still anecdotal [Grime *et al.* 1988; Pakeman *et al.* 2002; Knevel *et al.* 2003]. Knowledge on this particular issue has so far mainly been based on the assumed morphological or biochemical adaptations [e.g. van der Pijl 1982; Grime *et al.* 1988; Bouman *et al.* 2000] rather than on the knowledge of the mechanism itself. It certainly can not be derived adequately just from assumed morphological seed adaptations to specific dispersal mechanisms, such as seeds with elaiosomes that would be adapted to myrmecochory or fleshy fruit that would be an adaptation to ornithochory or other types of endozoochory. There is no a priori reason to assume that these seeds could not be dispersed in alternative ways.

Welch [1985], Malo & Suárez [1995] and Pakeman *et al.* [2002] all mention large and medium sized herbivores as potentially important seed dispersing agents in a variety of graminoid rich habitats. Germination experiments indicate that many more plant species may be successfully dispersed through endozoochory than only those with obvious morphological adaptations to this dispersal mechanism [Pakeman *et al.* 2002]. These results may indicate that many grassland species that were assumed to show no specific dispersal adaptations, may well be adapted one way or the other to endozoochory [Janzen 1984]. That way, grazers would be indispensable vectors for seed dispersal and establishment of many plant species in temperate grass-dominated habitats. The latter generally show a rather closed canopy and can be considered as rather hostile to seed invasion and germination. Dung deposition may help to create gaps for the seeds present in the dung. If endozoochory proves to be important as a dispersal mechanism, this would be very important for nature management. Since grazers are frequently used now in nature reserve management, their introduction could be helpful in recolonization. However, extensive surveys on endozoochory by large herbivores, in which a broad spectrum of plant species is involved, are scarce, certainly in the temperate region of Western Europe [Welch 1985;

Stender *et al* 1997; Dai 2000; Pakeman *et al.* 2002].

In order to be able to extend the existing knowledge on plant species that can take advantage of endozoochory this chapter focusses on the diversity and abundance of germinable seed content of dung of free ranging horses [*Equus cabbalus* L.].

In their study, Pakeman *et al.* [2002] found that germination from dung of rabbits and sheep was best predicted by seed mass and seed longevity index i.e. species that did germinate from dung were characterised by small seed size and a high capability of forming a persistent seedbank. These characters formed an additional set to seed hardness that was previously identified in other studies as a good predictor of survival [Simao Neto & Jones 1987; Russi *et al.* 1992; Gardener *et al.* 1993]. Pakeman *et al.* [2002] relied on a rather small number of plant species. In this chapter we will use a more extensive set of plant species, from a heterogeneous landscape, and we will compare the quantitative and qualitative aspects of the dung germinable seed content with: [i] the abundance of plant species in vegetation and their seed production; [ii] the diet composition of the horses; [iii] the presence of external morphological adaptations of the seeds to assumed dispersal mechanisms [iv] seed characteristics that may affect the ability to be endozoochorically dispersed e.g. seed size and shape, seed mass and seed longevity.
We hypothesise that the density of germinable seeds in the dung will be positively related to the abundance of these plant species at the study sites and in the diet respectively [see Pakeman *et al* 2002; Chapter 2 & 3]. We furthermore assume a negative relationship between dung seed density and seed 'sizes' [seed mass and shape variables] [see Pakeman *et al.* 2002] and a positive relationship with the ability to form a persistent seed bank [seed longevity index] [see Pakeman *et al.* 2002].

Materials and Methods

Study area

Dung was collected during seven sessions in a period of three months [18th July - 11th October 2000] at four sites along the Northern French and Belgian coast: Ghyvelde [France], Westhoek North, Westhoek South and Doornpanne [see Chapter 1, Table 1.1]. The dune landscape is dominated by scrub of *Hippophae rhamnoides* and *Ligustrum vulgare*. At all sites though, grassland covers at least one third of the area [see Chapter 1, Table 1.2]. Flowering and fruiting is concentrated from April to October. The selected dune nature reserves are all grazed by small numbers of one or two species of domestic livestock [see Chapter 1, Table 1.1].

Dung collection and treatment

Dung collection and treatment was carried out as described in chapter 1. At all sites we collected a total of 14 horse dung samples.

Flora and vegetation data

Presence of plant species was based on surveys, which resulted from a compilation of several inventories that took place between 1990-2000. Main vegetation units at each site were sampled in June and July 1998 and 1999 using visual estimates of plant species in plots [1x1, 2x2 or 3x3 m], usually 5 plots per vegetation structural unit. Plant species abundance was considered and noted as percentage cover [Londo decimal scale, Londo 1976].

Overall abundance [%] was calculated for each species as the overall sum of mean cover c in vegetation unit i times the relative area a, occupied by vegetation unit i :

$$\sum_{i=1}^{n} \% c_i \times \% a_i$$ (n = number of vegetation units = 10, see table 1.2 - chapter 1)

Diet composition

Data on summer diet composition of Shetland pony [Westhoek S, 1998 & 1999] and Konik diet [Westhoek N, 1999] were obtained from 6 hourly observation sessions, which were conducted 8 times a month during which one observer within close distance from the animal [< 3m] recorded all bitten plant species and plant parts as well as the plant community, in which grazing occurred [see also Cosyns *et al.* 2001]. The diet composition data were used to calculate the proportion bites [%] that were consumed from each plant species compared to the total number of bites in summer.

Dispersal and seed characteristics

Information on seed mass, seed length, seed width and dispersal modi of plant species known to be present at the different study sites, were collected from Zwaenepoel [1993] and Grime *et al.* [1988]. Additional information on seed bank biology was obtained from Thompson *et al.* [1997] and on seed production from Salisbury [1942] and Eriksson & Jakobsson [1998]. Seed longevity, was based on Thompson *et al.* [1997] and calculated according to Thompson *et al.* [1998]. Seed shape was expressed as variance in seed dimensions and taken from Thompson *et al.* [1993].

Data analysis

Dung seed densities were Pearson correlated with plant species cover [abundance] respectively % bites [diet composition] ,for each site, after $\log_{10}$- $\log_{10}$ transformation. To test for possible differences in mean seed densities and number of species in horse dung between study sites, we used One-way ANOVA, after $\log_{10}$ transformation. When differences were significant a post hoc Tukey test was run.

A chi-square goodness-of-fit test was used to compare the categorical data on dispersal mode and seed bank [persistent or not] of plant species in the dung and of the other species present in the vegetation. Following Grime *et al.* [1988], we distinguished between 6 dispersal modi: epizoochory, endozoochory, unspecified, hydrochory, anemochory and myrmecochory. To enhance reliability only plant species which were at least represented in 2 dung samples [on a total of 56 dung samples] were considered 'as dispersed endozoochorically'.

Dung germinable seed content was divided by plant species mean cover to give a relative measure of dung seed density per site. This procedure allowed to compare data between sites, and rules out seed production at the plant scale. An overall dung relative seed density variable was than created by summation of the dung relative seed densities of each horse breed divided by the times the plant species was present at the four study sites. Dung relative seed density was used as the dependent variable in the linear regression analysis with all continuous seed characteristics [seed length, width, mass, seed longevity index and variance in seed dimensions]. Relative seed density and seed size and mass were $\log_{10}$ transformed. All analyses were carried out using SPSS 10.0 for Windows [SPSS, Chicago, IL, USA].

Results

Dung seed content, vegetation composition and diet selection

Dung germinable seed content of horses that graze at different sites is affected by site characteristics. The highest plant species richness is recorded from Konik dung [Westhoek North]. Dung of Shetland pony [Westhoek South] count significantly more plant species than dung of Shetland ponies at 'Doornpanne' or of Haflingers at Ghyvelde [Table 4.1]. This is concordant with the pattern in plant species richness among sites. Dung germinable seed density differs significantly between Haflinger and Shetland pony [Table 4.1].

A total of 53 493 seedlings emerged from 56 dung samples [on average 382.1 seedlings per L dung] representing 106 different plant species or 21.4 % of all species recently [1990-2000] recorded in the study areas [Table 1.1]. 50% of all germinating plant species is represented in only a few dung samples [< 10%]. On the other hand a minority of the germinating plant

species [18 %] occurs in more then 50 % of all dung samples. Compared with the distribution pattern of herbs, a much larger part of the graminoid species [50 %] occur frequently in dung samples of horses [Fig. 4.1].
A similar pattern is observed for seedling densities, with 68 % of the plant species emerging with on average less than 1 seedling per dung sample. Only 15 % is present with on average more than 10 seedlings per sample. Compared with the frequency distribution pattern of herb species, a much larger part of graminoids [50 %] emerged with on average 10 or more seedlings [Fig. 4.2]. *Urtica dioica* was by far the most abundant species. Seedlings of *Juncus articulatus, J. bufonius, Veronica chamaedrys + arvensis*, *Poa pratensis, P. trivialis, P. annua, Agrostis capillaris* and *Aira praecox* appeared very frequently and were reasonably abundant. Other plant species showed notable numbers of seedlings in part of the samples e.g. *Carex arenaria*, *Rumex acetosella*, *Sagina procumbens + apetala*, *Galium uliginosum* and *G. verum*. A considerable number of plant species was recorded regularly [> 30 % of all samples] but showed on average low seedling densities e.g. *Veronica officinalis, Potentilla reptans, Phleum pratense, Geranium molle, Trifolium dubium, Senecio jacobaea, Rubus caesius, Luzula campestris, Eupatorium cannabinum* and *Cardamine hirsuta* [Appendix 4.1].

The linear regression revealed a positive relationship between the dung germinable seed content of most horse breeds [except Shetland pony] and plant species cover [Table 4.2]. The abundance of the plant species in the dung is also positively related to the portion of the species in the diet of Konik and Shetland pony [Table 4.2]. The relative germinable seed density was not significantly correlated with the estimated seed production per capita plant.

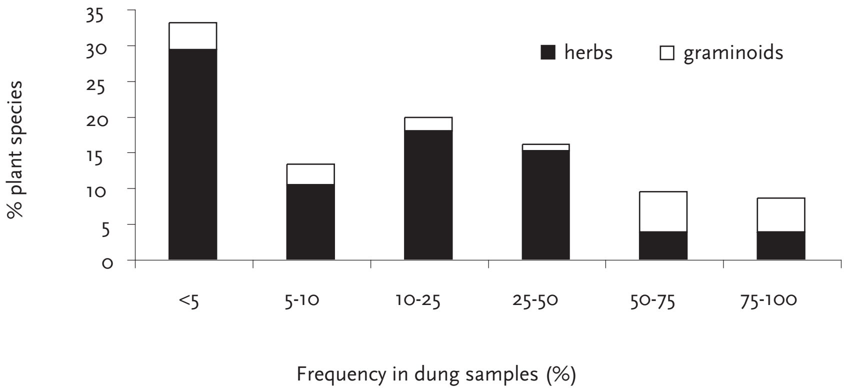

Figure 4.1 Distribution of plant species over six classes according to their frequency of emerging from dung samples [n =56]. The only woody species of which seedlings emerged from dung samples [Rubus caesius] is included in the herb-group.

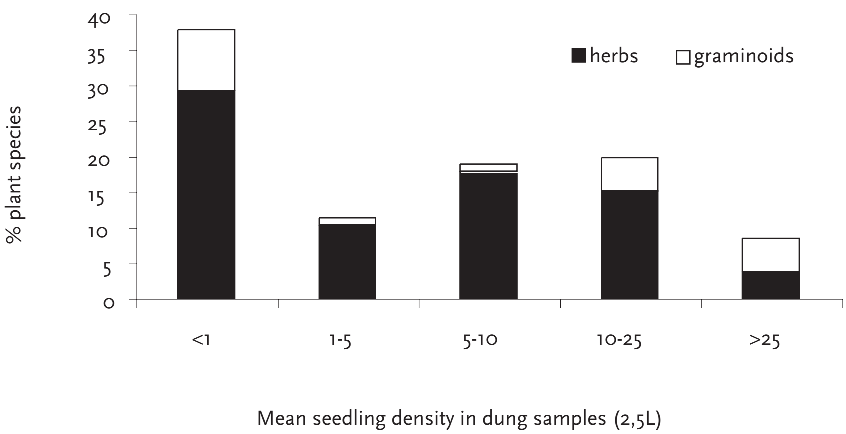

Figure 4.2 Distribution of plant species over five classes according to their seedling density in horse dung samples [n =56]. The only woody species of which seedlings emerged from dung samples [Rubus caesius] is included in the herb-group.

Table 4.1 Main characteristics of the viable seed content of horse dung which was sampled at different sites. Total number of seeds and species germinated from all dung samples, mean [± stdev], median and maximum seed density and plant species richness. Means of seed density and number of species that are not followed by the same letter differ at P > 0.05 [Tukey test].

Horse breed + site	Haflinger Ghyvelde	Konik Westhoek N	Shetland Westhoek S	Shetland Doornpanne
Number of samples	14	14	14	14
Mean seed density [± stdev]	1313 [± 886.1] a	1078 [± 444.1] ab	701 [±243.1] b	728 [±420.2] b
Median seed density	1319	1265	708	728
Maximum seed density	2325	1566	1039	1179
Mean number of species [±stdev]	19 [±3.1] a	34 [±5.2] b	29 [±4.9] c	23 [±6.1] a
Median number of species	20.5	32.0	27.0	25.0
Max number of species	22.0	43.0	34.5	30.0
Recorded plant species from dung	45	91	67	52

Table 4.2 Dung seed density and vegetation cover degree [%] [both log10 transformed], are positively correlated except at Westhoek South. Dung seed density and the portion of each plant species in the diet of Konik and Shetland pony, both log10 transformed, show also a positive correlation [Pearson correlation coefficient].

Horse breed			*r*	*P*	*n*
Haflinger Ghyvelde	*seed density*	*vegetation cover*	0.517	<0.001	42
Konik Westhoek N	"	"	0.266	0.013	85
Shetland Westhoek S	"	"	0.173	NS	60
Shetland Doornpanne	"	"	0.505	<0.001	51
Konik Westhoek N	*seed density*	*portion in diet*	0.370	0.007	51
Shetland Westhoek S	"	"	0.344	0.042	34

Seed attributes and seed density in dung

A crosstable comparing the dispersal modi of the plant species recorded from the dung samples to those of the other species present in the different dune areas shows no significant differences between the two species groups [Table 4.3a] i.e. there is no clear impact from the observed external morphological seed characteristics on species presence/absence in horse dung samples. Both in the study areas and in dung samples plant species showing adaptations to anemochory, epizoochory or lacking any obvious adaptation to a specific dispersal modus were well represented [Table 4.3a].

The same analysis for plant species with and without a persistent seed bank [type 2 and 3 according to Thompson *et al.* [1997] showed a high significant difference [P< 0.001]; species with a persistent seed bank tend to have a greater chance to be present in germinable form in dung than those lacking this feature [Table 4.3b]. Moreover, the linear regression analyses revealed a positive relationship between horse dung relative seed density and seed longevity index according to Thompson *et al.* [1998] [Fig. 4.3A]. Seed length, width and mass are significantly negative correlated with relative seed density in dung [Fig. 4.3B-D] whereas variance in seed dimension [seed shape] did not show a significant linear relationship [Fig. 4.3E].

Table 4.3a Observed and expected number of plant species distributed among six categories of dispersal modi [based on Grime et al. 1988] and either present [n=78] or absent [n=190] from horse dung samples. Pearson Chi Square statistics shows no significant difference in dispersal modus between species which are present [in at least two samples] or absent from dung samples [Chi-Square: 6.94, df: 5, P = 0.225]. Expected numbers assume an equal chance of being present in the samples.

	Number of species present in dung samples		Number of species absent from dung samples	
	observed	expected	observed	expected
Anemochory	22	25	64	61
Endozoochory	4	7	21	18
Epizoochory	17	13	28	32
Hydrochory	5	4	9	10
Myrmecochory	1	3	9	7
Unspecialised	29	26	59	62
Total number	78	78	190	190

Table 4.3b Observed and expected number of plant species according to the ability of their seeds to form a persistent seed bank or not [Thompson et al. 1997] and either present [n=78] or absent [n=190] from horse dung samples. Pearson Chi Square statistics shows that seeds of plant species emerging from dung samples have a greater chance than expected to be found as part of a persistent soil seed bank compared to plant species which did not emerge from the dung samples [Chi-Square: 19.62, df: 1, $P < 0.001$].

	Number of species present in dung samples		Number of species absent from dung samples	
Seed bank	observed	expected	observed	expected
Persistent	46	30	57	73
Not persistent	32	48	133	117
Total number	78	78	190	190

Discussion

Dung seed content

Graminoids were well represented in the dung samples which is at least partly related to the selective habitat and food selection of the horses i.e. graminoid dominated habitats are preferred grazing sites and several graminoid species are important constituents of the horse diet e.g. *Poa trivialis*, *Holcus lanatus*, *Carex arenaria* [Cosyns *et al.* 2001, Chapter 3]. *Urtica dioica* was the most common plant species in the dung samples, however, it was not at all common in the vegetation. The consumption of withered fruiting stems of *Urtica dioica* by Koniks and Shetland pony [Westhoek] was observed several times [Cosyns unpubl.] but the removal of the seeds with foliage can not be excluded either. Most of the plant species which showed high seed densities or appeared frequently in the dung samples such as *Urtica dioica*, *Juncus* spp., *Poa* spp. and different species of *Caryophyllaceae,* were also regularly recorded by Welch [1985], Malo & Suárez [1995], Pakeman *et al.* [1998], Dai [2000] and Pakeman *et al.*[2002], emphasising their great ability to survive passage along the molar mills and through the gastro-intestinal tract.

With the applied sampling technique using large volumes of dung, it was possible to detect a considerable amount of plant species occurring only in minor quantities and frequencies. Their scarceness is at least partly due to their low availability in the species pool at the moment of sampling. Some plant species may show a relatively low seed density because their main fruiting period had passed already when dung samples were taken, e.g. *Cardamine hirsuta, Cardamine pratensis* and *Claytonia perfoliata*. Other species have clearly the potential to be dispersed endozoochorically but the pattern shown in this study reflects site related characteristics, i.e. cover degree and hence site-seed production. *Aira praecox* and *Rumex acetosella* are very abundant in the acid grasslands of Ghyvelde but are scarce at the other sites, both species are very well represented in dung sampled from Ghyvelde. On the other hand plant species from wet habitats e.g. *Juncus articulatus, Agrostis stolonifera, Ranunculus repens,* are only recorded in considerable amounts from horse dung of Westhoek North and South. Only at these sites wet dune slack communities are well represented. Some plant species, rare at all sites, and only occurring in minor quantities from dung e.g. *Helianthemum nummularium* and *Trifolium campestre* were also mentioned by Müller [1954] and Dai [2000], suggesting their ability to survive the gastro-intestinal tract. It should also be stressed that the applied greenhouse conditions could have hampered seed germination of several plant species [Grime *et al.* 1988]. Therefore the results of this study underestimate the real quantity of germinable seeds in dung. Hence, there is strong evidence that endozoochory is a widespread seed dispersal mechanism within the grazed system under consideration.

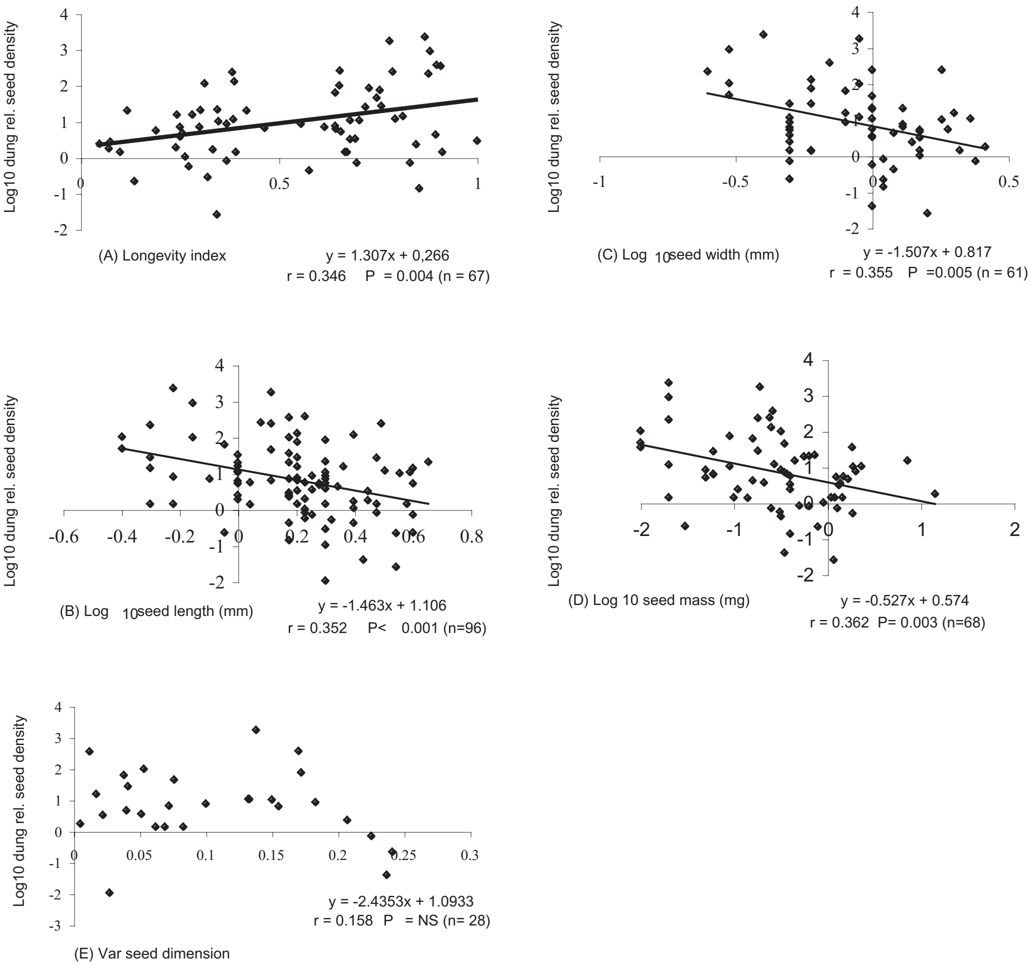

Figure 4.3 [A-E]. Results of linear regression analyses showing the relationship respectively between seed sizes [length, width and mass], longevity index, variance in seed dimensions and horse dung relative seed density [calculated across all study areas]. Seed size correlates negatively with relative seed density in dung whereas seed longevity index shows a significantly positive relationship with this variable.

Seed characteristics and dung seed density

The ability to be dispersed endozoochorically certainly could not be derived adequately just from [assumed] morphological seed adaptations to specific dispersal mechanisms [Table 4.2a].
The lack of a difference in dispersal modi of species that emerged from dung to those who did not, suggests that there is no reason to use morphological attributes or so called 'adaptations' to predict the way seeds will be dispersed. It's even more likely that seeds can be dispersed by more than one dispersal mechanism [e.g. Johansson *et al.*1996; Honnay *et al.*2001; Bruun & Fritzbøger 2002;]. Adaptations to a particular dispersal modus does not exclude other mechanisms to occur, e.g. seeds of plant species adapted to epizoochory such as those with a mucilaginous coat, also tend to be found more in dung of large herbivores than those who lack this adaptation, e.g. *Juncus* spp. However, the relative significance of each of these modi i.e. amount of seeds that are dispersed by each of them, the frequency of occurrence and their effectiveness needs further investigation.

One of the most obvious shared characteristics of the seeds represented in dung samples of this survey is their capacity to form a persistent seedbank. Moreover, there is a positive linear relationship between seed longevity index and relative seed density [Fig.4.3A], which is broadly in accordance with Pakeman *et al.* [2002]. This relationship may have important ecological consequences because of the spatial and temporal component associated with it [see further].
The seed longevity index in general is negatively correlated with seed size and seed mass [Thompson *et al.* 1998; Hodkinson *et al.* 1998]. In this study a convincing negative linear relationship between those seed characteristics and relative seed density was shown, which is concordant with the results obtained by Bruun & Fritzbøger [2002] and Pakeman *et al.* [2002]. Thus seed size seems to influence both the capacity to be successfully dispersed by means of endozoochory and to form a persistent seed bank. Seed shape, expressed as variance in seed dimensions, had no impact on dung seed density [Fig. 4.3E]. This indicates that within the already selected group of rather small seeds surviving horse gut passage [incl. molar mills], seed

shape is of no additional importance.

Implications for conservation of grassland plant diversity

Grazing by livestock has become an important nature management tool for conservation and restoration of many habitats in northern temperate regions. Hence, the impact of large herbivores on plant communities has become a critical issue in conservation and restoration ecology [van Wieren 1991; Wallis De Vries 1995; Bakker & Londo 1998]. Until now much attention was drawn on the contribution of livestock to plant diversity patterns, both at the local and landscape scale, through their activities of selective grazing, trampling and defecating, which influence processes that enhance local extinction rates. But herbivores may also influence plant diversity through processes that affect colonisation rates [Olff & Ritchie 1998].

From this survey it can be revealed that large herbivores are also useful management tools [or in case of wild herbivores an important management goal] because of their potential long-distance seed dispersal capacity for a broad range of plant species both via endo- and epizoochory [Fisher *et al.* 1996]. Moreover, due to their habitat preferences [cf. Chapter 2 and 3] herbivores may connect suitable habitats across different spatial scales. However, this does *a priori* not guarantee successful establishment of a population [Primack & Miao 1992]. An important part of the viable seeds in horse dung are from less common or even rare plant species that can contribute significantly to the diversity of the local species pool. Examples from this study are *Galium verum, Luzula campestris, Veronica officinalis, Centaurium erythraea, Helianthemum nummularium, Scirpus setaceus.*
It further became clear that seeds of a considerable number of plant species represented in dung are able to form a persistent seed bank [Table 4.3b, Fig. 4.3A], which can be seen as a trait promoting colonisation success by adjusting seeds to their environment [Ehrlén & van Groenendael 1998]. Such a mechanism seems plausible because there is evidence that seeds in dung mainly contribute in a quantitative and qualitative way to seed bank build up [Malo *et al.* 1995; Pakeman *et al.* 1999; Dai 2000].

Moreover, activation of the seed bank generally depends on disturbance of the local situation for instance by herbivore activities such as trampling, digging, rooting up etc. Hence, the interaction of the dung seed content with the soil seed bank may be important in determining the final chance of establishment, but this needs further attention [van Wieren & Bakker 1998]. The growing evidence of the role large herbivores could play in the seed dispersal process urge for a well-considered nature management policy that not only focusses on amelioration of habitat conditions, but also considers the spatial arrangement of suitable but still unoccupied patches for critical plant species in order to enable plants to bridge gaps in space and time, which may favour a sustainable conservation of critical plant populations.

Appendix 4.1 Mean germinable seed density of plant species [i.e. number of seedlings [#] / litre dung] in dung of different breeds of free ranging horses [Shetland pony: Westhoek S and Doornpanne; Konik: Westhoek N; Haflinger: Ghyvelde]. Total number of occurrence across all horse dung samples [Freq. Tot. per 2.5L, n max = 56] and estimated overall cover [%] of the plant species at the different sites are also shown as well as their status in Flanders [Red List species] and the habitat they are a target species of. The total cover is obtained by taking into account the average cover from usually 5 relevés in each of the habitats and the relative surface of each habitat. Only species which were present in at least 3 dung samples [5%] are shown [total number of species = 106].

Plant species	Red List	Target Habitat	Westhoek S cover %	Westhoek S density #/L	Westhoek N cover %	Westhoek N density #/L	Doornpanne cover %	Doornpanne density #/L	Ghyvelde cover %	Ghyvelde density #/L	Overall Avgdens #/L	Freq Tot n
Agrostis capillaris		T/Gd	0.53	2.40	0.03	0.09	6.98	2.97	20.45	48.14	13.40	37
Agrostis stolonifera			2.90	11.37	0.89	9.60	2.57	0.71	1.00	3.83	6.38	46
Aira praecox			0.72	1.14	0.02	0.14	0.43	8.86	3.32	45.86	14.00	36
Anthriscus caucalis		S	0.03	0.06	0.00	0.03	0.20	0.37	0.00	0.00	0.11	8
Arenaria serpyllifolia			0.72	3.83	0.40	6.89	0.52	9.83	0.50	1.29	5.46	49
Calamagrostis epigejos		Gd	0.05	0.00	0.13	0.20	0.01	0.03	0.00	0.00	0.06	5
Cardamine hirsuta			0.02	0.20	0.14	0.51	0.14	0.06	0.10	0.06	0.21	18
Carex arenaria		T/Gd	3.70	0.71	3.62	1.31	5.15	3.11	12.34	24.80	7.49	48
Carex flacca		Gw	0.90	0.00	0.27	0.71	0.43	0.00	0.00	0.00	0.18	4
Centaurium erythraea			0.29	0.34	0.05	2.23	0.02	0.63	0.01	0.03	0.81	14
Centaurium littorale	R	Gw	13.54	0.17	1.20	0.09	1.26	0.00	0.11	0.00	0.06	6
Cerastium fontanum			0.45	11.63	0.48	11.54	0.13	1.37	0.01	0.40	6.24	45
Conyza canadensis			0.20	0.06	0.18	0.34	0.10	0.06	0.00	0.00	0.11	9
Crepis capillaris			0.27	0.43	1.61	0.11	2.35	0.14	0.11	0.46	0.29	16
Epilobium ciliatum			0.06	0.00	0.13	0.34	0.00	0.00	0.00	0.00	0.09	5
Epilobium hirsutum			0.00	0.06	0.00	0.20	0.00	0.00	0.00	0.00	0.06	5
Epilobium roseum			0.09	0.11	0.97	0.49	0.10	0.09	0.00	0.00	0.17	14

Plant species			Westhoek S		Westhoek N		Doornpanne		Ghyvelde		Overall	Freq
			cover	density	cover	density	cover	density	cover	density	Avgdens	Tot
			%	n/L	%	n/L	%	n/L	%	n/L	n/L	n
Epilobium spp.			NA	1.08	NA	0.84	NA	0.00	NA	0.00	0.47	34
Erodium cicutarium+ lebelii		T	0.55	0.06	1.59	0.03	0.96	0.09	0.03	0.03	0.05	7
Eupatorium cannabinum			1.57	0.14	7.44	1.51	0.00	0.00	0.10	0.17	0.46	21
Festuca filiformis			0.00	0.00	0.16	0.00	6.94	0.11	2.89	0.00	0.03	3
Festuca rubra			3.62	0.00	3.58	0.09	10.57	1.06	1.61	0.23	0.34	13
Galium aparine			0.28	0.03	0.41	0.77	0.02	0.40	0.03	0.03	0.31	10
Galium mollugo		Gd	0.50	0.23	0.50	0.66	0.05	0.29	0.02	0.34	0.38	9
Galium palustre		Gw	0.06	0.14	0.06	0.11	0.00	0.00	0.00	0.00	0.06	7
Galium uliginosum			1.30	0.20	0.37	5.86	0.00	0.00	0.00	0.00	1.51	10
Galium verum		Gd	2.41	0.06	4.67	2.00	8.96	3.37	1.63	4.91	2.59	22
Geranium molle			0.13	0.66	0.03	0.06	0.76	0.71	0.32	0.29	0.43	26
Gnaphalium uliginosum			0.00	0.00	0.01	1.43	0.00	0.00	0.00	0.00	0.36	10
Holcus lanatus			7.85	7.97	2.07	2.66	1.00	8.89	0.10	0.26	4.94	38
Hydrocotyle vulgaris			1.15	0.06	1.12	0.46	0.00	0.00	0.00	0.00	0.13	11
Juncus articulatus		Gw	0.03	16.31	0.32	142.66	0.00	0.03	0.00	0.09	39.77	32
Juncus bufonius			0.03	40.83	0.08	91.34	0.00	0.06	0.00	1.06	33.32	37
Luzula campestris		Gd	0.56	0.14	0.27	0.14	2.04	0.77	6.32	0.91	0.49	28
Lycopus europaeus			0.01	0.20	0.28	1.86	0.00	0.00	0.00	0.00	0.51	7
Lysimachia vulgaris			5.72	0.03	1.66	0.37	0.00	0.00	0.00	0.00	0.10	3
Lythrum salicaria		Gw	1.06	0.66	4.66	4.26	0.00	0.00	0.00	0.00	1.23	15
Medicago lupulina			0.10	0.03	0.10	0.00	0.01	0.09	0.10	0.03	0.04	5
Mentha aquatica		Gw	1.00	0.29	1.16	0.97	0.13	0.00	0.00	0.00	0.31	10
Oenothera glazioviana			0.09	0.20	0.20	0.09	0.53	1.03	0.00	0.00	0.33	13
Phleum pratense			0.66	0.49	0.04	0.00	0.70	1.03	0.10	0.00	0.38	26
Plantago coronopus	R	Gw	0.01	0.03	0.04	0.74	0.19	0.97	0.00	0.00	0.44	12

Plantago lanceolata			1.58	0.06	0.05	0.34	1.28	0.71	0.01	0.09	0.30	17
Plantago major			0.00	2.91	0.04	6.37	0.00	0.00	0.00	0.03	2.33	25
Poa annua			0.02	9.11	0.06	8.17	1.00	5.63	0.02	0.46	5.84	50
Poa pratensis			1.58	8.86	0.75	11.49	4.43	33.94	0.16	28.46	20.69	54
Poa trivialis			2.00	12.97	1.21	32.60	1.00	5.89	0.03	2.14	13.40	50
Potentilla reptans			0.62	0.51	0.02	0.40	0.02	2.09	0.00	0.00	0.75	22
Prunella vulgaris			0.06	0.63	0.06	0.43	0.15	0.00	0.00	0.00	0.26	13
Ranunculus repens			1.36	2.74	0.06	0.60	0.17	0.09	0.00	0.00	0.86	23
Ranunculus sceleratus			0.05	0.00	0.05	0.00	0.07	0.09	0.00	0.00	0.02	3
Rubus caesius		Gd	3.91	0.40	7.58	0.60	0.19	1.14	0.07	0.00	0.54	19
Rumex acetosella			0.05	0.00	0.05	0.03	0.83	0.00	11.74	24.91	6.24	15
Rumex conglomeratus			0.10	0.34	0.10	0.11	0.01	0.00	0.00	0.00	0.11	7
Rumex crispus			0.10	0.03	0.10	0.23	0.00	0.00	0.00	0.00	0.06	4
Sagina procumbens + apetala			0.07	17.26	0.20	6.43	0.02	0.91	0.01	0.14	6.19	37
Samolus valerandi	R	Gw	0.01	0.00	0.03	0.43	0.00	0.00	0.00	0.00	0.11	6
Scirpus setaceus			0.01	0.77	0.01	0.03	0.00	0.00	0.00	0.00	0.20	10
Sedum acre			0.80	0.00	2.62	0.06	0.89	0.14	0.55	0.00	0.05	4
Senecio jacobaea			0.73	3.17	0.33	0.63	1.37	2.03	0.66	3.94	2.44	29
Senecio sylvaticus			0.59	0.00	0.65	0.46	0.01	0.00	0.53	0.09	0.14	5
Solanum dulcamara		S	0.10	0.20	0.10	0.06	0.00	0.00	0.00	0.00	0.06	5
Solanum nigrum			0.00	0.00	0.01	0.34	0.00	0.00	0.10	0.00	0.09	4
Sonchus oleraceus			0.00	0.00	0.09	0.29	0.01	0.06	0.01	0.06	0.10	10
Stellaria media			0.10	0.37	0.10	4.60	0.07	1.51	0.05	0.09	1.64	27
Trifolium campestre			0.10	0.06	0.00	0.00	0.02	0.11	0.01	0.37	0.14	7
Trifolium dubium			0.06	1.80	0.02	0.09	0.02	0.46	0.02	2.00	1.09	32
Trifolium repens			1.66	7.69	0.06	1.31	0.57	0.40	0.01	0.06	2.36	25
Urtica dioica			0.14	90.66	0.62	16.91	0.19	168.71	0.26	315.11	147.85	56
Veronica chamaedrys + arvensis			0.76	16.43	0.16	41.06	0.22	19.37	0.63	12.49	22.34	56
Veronica officinalis			0.10	0.60	0.00	1.91	0.02	0.14	0.10	1.09	0.94	32
Veronica serpyllifolia			0.05	0.09	0.01	0.09	0.00	0.00	0.01	0.11	0.07	5

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

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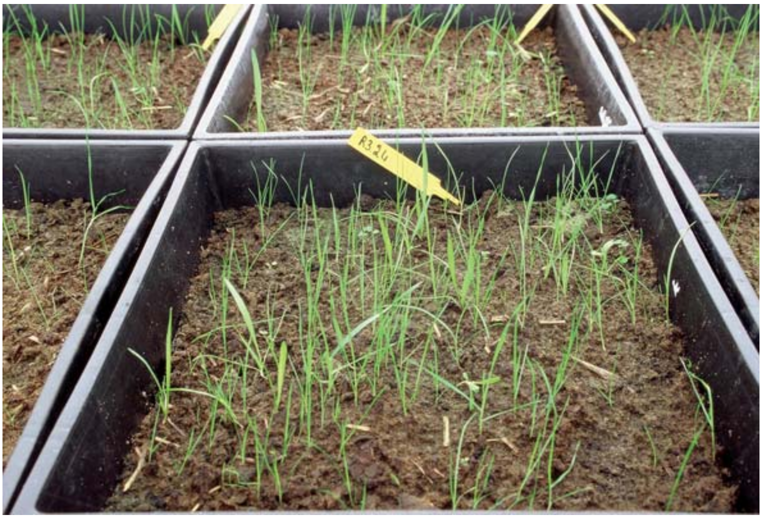

Seedling emerging from cattle dung sample which was collected 24 hours after seed feeding (Wenduine, 2002)

Abstract

One of the critical life phases of an endozoochorically dispersed plant seed is its uptake by the herbivore and its exposure to several digestive fluids during passage through the gastero-intestinal track. To assess adequately the ecological significance of endozoochory as compared to other dispersal mechanisms, it is therefore necessary to examine the survival rate of seeds during this phase.

A seed feeding experiment was conducted to determine the impact of passage through the digestive system on germination success of seeds of 19 potentially endozoochorically dispersed plant species. They are important constituents of temperate semi-natural grasslands most of which do not show obvious morphological adaptations to other dispersal mechanisms. Germination success of gut-passed seeds may depend on the kind of digestive strategy involved. Therefore two foregut fermenters i.e. ruminants [cattle, sheep], two colon fermenters [horse, donkey] and a caecum fermenter [rabbit] were included. Mean retention time of germinable seeds was also determined and possible predictors of germination success amongst different seed characteristics were searched for.

We aimed to examine the Plant species selection was based on their importance in temperate West European grassland communities and their importance for their nature management, on general morphological seed characteristics, possibly related to germination success, on taxonomic differentiation and on the absence or presence of specific morphological adaptations to certain dispersal mechanisms. Animal species were selected for differentiation in gastro-intestinal characteristics [ruminants, hindgut fermenters and a caecum fermenter], importance for nature management [use as grazers in semi-natural grassland management] and differentiation in expected retenGut-passed seeds had a much lower germination success [0-26%] than non gut-passed seeds sown directly on dung [2-79%].
Relative germination success also differed considerably between plant species. It also varied individually between animal species. This may result from herbivore specific, complex interactions of animal [chewing, digestion] and seed characteristics. However, the combination of plant species with sheep tend to result in a lower germination success at least compared to

the equid species and rabbits.

Within this experiment, seed shape and seed longevity affect germination success but their effect did not vary between animal species. Germination success was better for long-lived than for short-lived seeds and for lanceolate seeds compared to other seed shapes.
Retention time of germinable seeds varied between c. 12h [rabbit]- 72h [ungulates], allowing long-distance seed dispersal.

Herbivore seed intake and passage through the herbivore gut seem to represent a very high cost for the endozoochorically dispersing plant seed, since large amounts of viable seeds are lost during this process. At least some grassland species show high survival rates, such as *Helianthemum nummularium*, *Trifolium arvense*, *Agrostis capillaris*, and can be assumed to be dispersed efficiently through endozoochory.

Key-words: endozoochory, grassland ecology, retention time, seed ecology

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Introduction

Several studies show the potential of endozoochorous seed dispersal by ungulates and rabbits in the temperate and Mediterranean regions of Europe [e.g. Welch 1985; Malo & Suárez 1995a; Dai 2000; Pakeman et al. 2002]. However, interpretation of the results from those studies remains difficult. First, it is almost impossible to get an adequate estimate of the available amount of viable seeds in the respective study areas. Second, the amount of seeds consumed by the potential dispersers and the effects of their digestive system on germination success of seeds remains largely unclear. Hence, the ecological significance of the viable seeds from dung is difficult to assess. To fully understand the significance of endozoochory in the dispersal and regeneration of plant species, Pakeman et al. [2002] have already suggested that a more experimental approach is needed. One of the most critical phases of an endozoochorically dispersed plant seed is its passage through the digestive system of the herbivore. It is therefore necessary to conduct experiments to determine the survival rate of excreted seeds.

Such seed feeding experiments have already been conducted in the context of rangeland management to select appropriate tools and methods for reseeding of rangeland with preferred plant species [Russi et al.1992; Lowry 1996; Ghassali et al. 1998; Gökbulak 1998]. So far, the germination success of gut-passed seeds of temperate grassland species has only been investigated in a historic agricultural context [Kempski 1906; Kerner von Marilaun 1916 cit. in; and Lennartz 1957] but an adequate replication of animal individuals is lacking. More recently, Özer [1978] used ten young sheep in a feeding experiment with seeds of a selection of grassland species of East Anatolia to determine their retention time and germination capacity. A few temperate grassland species were also used by Russi et al. [1992] and Ghassali et al. [1998] and by Gardener et al. [1993a] in their experiments with sheep and cattle respectively. However, most seed feeding experiments do not allow discrimination between different herbivore species or include only domestic ruminant species [Simao Neto et al.1987].

Comparing different herbivore species with a different digestive physiology and morphology, kept under similar conditions, will allow us to study the effects of their specific gastro-intestinal system and will offer a first insight in the relative importance of those effects compared to diet selection and seed availability. Such experiments are vital to determine what seed characteristics affect survival after gut passage and hence also determine colonisation and dispersal capacities. Ultimately it should be possible to make a cost-benefit analysis for individual plant species as far as their successful dispersal is concerned.

This paper focusses on the seed germination success of plant species that occur regularly in semi-natural grassland communities in Western Europe and which have been shown to germinate from herbivore dung samples. The aims of the experiment were to determine: [1] the combined impact of seed intake and passage through the guts on germination success of seeds of a selection of grassland species fed to herbivores with different gastero-intestinal systems and that are important in grassland management. Germination success of gut-passed seeds was compared to uningested seeds of the same plant species which were sown either on bare soil or dung+soil substrate [control]; [2] the impact of different functional digestive systems: foregut fermenters i.e. ruminants [sheep and cattle] and hindgut fermenters i.e. colon fermenters [donkey and horse] and caecum fermenters [rabbits], on the germination success of the fed seeds; [3] in addition, because of its importance for possible long-distance seed dispersal, the mean retention time of germinable seeds within each of the studied animal species; [4] the possible relationship between seed characteristics [e.g. seed mass, seed shape and seed longevity index] and observed patterns in germination success.

Materials and methods

Plant species, seed collection and treatment, seed sizes and weight

Our choice of study species was based on four criteria: [1] Their importance in temperate West European grassland communities being a substantial part of those communities [e.g. Poaceae, Juncaceae, Cyperaceae] or their importance for nature management, e.g. contributing to the specific floristic diversity: *Helianthemum nummularium* [L.] Mill., *Thymus pulegioides L., Trifolium arvense L.*; [2] Results of previous field studies [Dai 2000; Claerbout 2001; Pakeman et al. 2002] were taken into account to include a broad range of possible germination responses. These results also indicate plant species which may have a realistic chance to be consumed which is a crucial first step in endozoochory e.g. *Poa pratensis L.* [high frequency and abundance]; *T. pulegioides* [very rare]; [3]Morphological seed characteristics, possibly related to germination success i.e. differentiation in seed size, shape and mass e.g. *Plantago lanceolata L.* [large seed + epizoochory adaptation], *Centaurium erythraea* Rafn [very small seed]. [4] Practical aspects [e.g. availability of large enough amounts of seeds, its costs [financially] or workload to collect them]. The seeds of the 19 plant species used in this study were either bought from a seed commercial [Ecoflora, Halle-Belgium] or were collected from the wild from a large number of individuals during the summer of 2001 [Table 5.1]. 'Seed' is used in this paper either to indicate what is morphological a seed or a fruit i.e. the main dispersule unit.

Mean seed mass was determined by weighing five replicates of 250 air dried seeds each [accuracy ± 0.1 mg]. Seed width and length were derived from Grime et al. [1988]. Seed longevity index was calculated according to Thompson et al. [1998] and the necessary data were obtained from Thompson et al. [1997]. Previous to the seed feeding experiment seeds were kept dry and in darkness at room temperature.

Animal species and housing

Seeds were fed to five different animal species which belong to three different groups according to their gastero-intestinal system: domesticated rabbits [*Oryctolagus cuniculus L.*] as caecum fermenters and a selection of domesticated ungulates that are regularly used in grassland management i.e. cattle [*Bos taurus L.*, breed; Blonde d'Acquitaine], sheep [*Ovis aries L.*, Texel breed], both ruminants, donkey [*Equus asinus L.*] and horse [*Equus cabbalus L.*, Shetland pony], both colon fermenters. The animal species were assumed to have a different mean retention time according to their variation in body mass and digestive system [Illius and Gordon, 1992] [Appendix 5.1]. Five individuals of each animal species were kept under similar indoor conditions. Rabbits were housed in individual cages [0.8m x 0.8m x 0.5m] , sheep were housed in individual pens [c. 3 x 3m] while the other animal species were kept in stables. Cages, pens and stables had a flat, concrete floor that allowed accurate dung collection. During the entire experiment the basal diet of all animal species was a highly nutritious and digestible commercial pellet food with additional barley straw, except for cattle, which received additional ensiled maize [Appendix 5.2]. This food regime was applicated from ten days before seeds were fed, until six days after the seed feeding event. All animals were fed twice a day [9.00 and 19.00 h] with equal proportions of the experimental diet: 1 kg of pellets/day/animal and 0.7 kg of straw/day/animal [sheep, horse and donkey], 0.1 kg of pellets/day/rabbit and 5 kg of pellets/day/cow + ensiled maize ad libitum. All animals had free access to fresh water.

Feeding experiment

In the morning of day 11, a known amount of seeds of 19 plant species was mixed with the commercial food and offered to the animals in a bucket [Table 5.1]. Each individual animal was observed carefully to ensure that all seeds were consumed. If necessary the remaining seeds were mixed in the bucket with a small piece of bread which was then offered to the animal. In all cases less than 0.1% of the total amount of seeds was not consumed by the animals.

Immediately before seed feeding, all faeces in cages, pens and stables was removed and of each individual animal a sample was taken as a control for possible 'external' seed contamination [sample at t = 0].

Table 5.1 List of 19 plant species which were used in the feeding experiment ordered within families [ordered alphabetically], with indication of the mean mass [Mean, ± Stdev], mean seed size [length x width] origin of seed collection and the amount of seeds of each plant species fed to the indicated animal species. The mean seed mass is based on 5 weightings of 250 air dried seeds each. * indicates which animal species seeds were fed to.

Plant family and species	Seed mass [mg]	Length x width [mm]	Origin of seed collection	Sheep	Cattle	Rabbit	Horse	Donkey
Asteraceae								
Crepis capillaris	0.29 ± 0.004	2.0 x 0.5	Commercial	*	*	*	*	*
Cystaceae								
Helianthemum nummularium	1.1 ± 0.105	1.8 x 1.0	Commercial	*		*	*	*
Cyperaceae								
Carex arenaria	1.0 ± 0.075	4.7 x 1.8	2001 [De Panne, Belgium]	*	*	*	*	*
Fabaceae								
Trifolium arvense	0.36 ± 0.008		Commercial [NW Germany]	*	*	*	*	*
Trifolium campestre	1.86 ± 0.057		Commercial	*		*	*	*
Trifolium pratense	2.76 ± 0.08	2.1 x 1.5	Commercial	*		*	*	*
Trifolium repens	0.75 ± 0.01	1.0 x 1.0	Commercial	*	*	*	*	*
Gentianaceae								
Centaurium erythraea	0.01 ± 0.001	0.4 x 0.3	Commercial	*	*	*	*	*
Juncaceae								
Luzula campestris	0.7 ± 0.026	1.7 x 1.1	Commercial	*		*	*	*
Lamiaceae								
Prunella vulgaris	0.96 ± 0.029	2.0 x 1.0	Commercial [Belgium]	*	*	*	*	*
Thymus pulegioides	0.14 ± 0.008		Commercial [NW Germany]	*	*	*	*	*
Poaceae								
Agrostis capillaris	0.12 ± 0.003	1.0 x 0.5	Commercial [NW Germany]	*	*	*	*	*
Anthoxanthum odoratum	0.67 ± 0.025	2.1 x 1.0	Commercial [NW Germany]	*	*	*	*	*
Poa pratensis	0.35 ± 0.008	1.6 x 0.6	Commercial [NW Germany]	*	*	*	*	*
Plantaginaceae								
Plantago lanceolata	1.36 ± 0.09	3.6 x 1.8	Commercial [Belgium]	*	*	*	*	*
Rubiaceae								
Galium mollugo	0.53 ± 0.039	1.3 x 1.2	Commercial [Belgium]	*	*		*	*
Galium verum	0.272 ± 0.025	1.5 x 1.4	Commercial	*	*	*	*	*
Scrophulariaceae								
Veronica arvensis	0.11 ± 0.011	1.1 x 0.8	2001 [De Panne, Belgium]	*	*	*	*	*
Veronica chamaedrys	0.27 ± 0.013	1.3 x 1.0	Commercial [NW Germany]	*	*	*	*	*
Number of seeds fed of each plant species / animal				1500	5000	250	2500	2500

Dung collection and treatment

From the seed feeding event onwards all dung from each individual animal was collected regularly and put together in trays according to an a priori fixed time schedule. After air-drying in a greenhouse [2-3 weeks, < 35 °C], the samples were kept at 2-4 °C for two weeks. Air dried faeces was weighed, slightly crumbled and homogenised. From each dung sample a 150 g sub-sample was taken [not always possible for rabbits and sheep], and spread out over a sand/commercial potting soil substrate [40 x 40 x 2cm] 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 seed-free layer of crumbled cattle dung [taken from t = 0] which was spread out over the sand/commercial potting soil. These were considered 'control' pots. Additionally, five 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 gut passage. All seeds were kept together with the dung samples at 2-4 °C, for two weeks and were sown in separate pots in the same greenhouse. To detect possible germination from the potting soil substrate and contamination in the greenhouse, twelve trays without dung or seed addition were also set up.
All samples were watered twice a day during the whole germination period. Greenhouse conditions were kept at 20-25 °C with a relative humidity of 50-60 % during 16 hours of light [range: 280-410 mol m-2 s-1] and at 10-15 °C and 80-90 % relative humidity during 8 hours of darkness. Seedlings were counted as soon as identification was possible [within two months] after which they were removed to avoid competition and to prevent flowering. Seed emergence was monitored over a period of six months. During the last two months very few seedlings emerged

Data analysis

Absolute germination success was calculated as:

$[_{i=t6}\Sigma^{t96}\, n_i * WD_i * [W_{ssi}]^{-1}] * N^{-1}$.

n_i is the number of seedlings in the subsample [ss] from time period i [+6h, +12h...96h]. WDi is the weight of all dung [D] produced during the mentioned time period and W_{ssi} is the weight of the dung subsample for that time period [usually 150 g]. N is the total number of seeds fed of a particular plant species. When comparing gut-passed seeds with non ingested seeds absolute germination success was used. 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]. These estimates will further be referred to as 'relative germination success'. When comparing germination success of the same plant species between animal species, the cumulative germination percentage after 96 hours 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 which had an absolute germination success of at least 1 %. Therefore only 9 plant species allowed a between animal species comparison.

Statistical analysis

Estimates of absolute germination success were analysed using a two-way ANOVA with SEED TREATMENT [ingested versus uningested seeds] and PLANT SPECIES as fixed factors. Possible significant differences in germination success between ingested and uningested seeds on dung+soil [control] and of the latter with the uningested seeds sown on bare soil were analysed with LSD-test [SPSS 11.0, Chicago, IL, USA]. Differences in relative germination success were analysed with a mixed model ANOVA with repeated structure with PLANT SPECIES and ANIMAL SPECIES as fixed factors and plant x 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 [SPSS, Chicago, IL, USA]. 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 [i.e. seed length * seed width^{-1}] 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 Log10 transformed to get a more adequate description of the relationship and hence a better model [Neter et al. 1996]. ANCOVA was run under the univariate SPSS routine [SPSS 11.0, Chicago, IL, USA].

Results

No seedlings of the fed plant species were found in any of the trays set up to detect possible contamination from the soil or greenhouse environment or from the t_0 - dung samples which were collected just before seeds were fed. In both cases only a few seedlings of *Juncus bufonius*, *J. effusus*, *Epilobium* sp. and *Oxalis corniculata*. were recorded.

Absolute germination success of uningestedcontrol seeds and ingested seeds

Uningested seeds of ten plant species had a substantially higher germination success when sown on a bare soil substrate compared with the control treatment [Table 5.2]. Uningested seeds of most other plant species did not differ significantly in germination success when sown on the dung+soil or

bare soil substrate. *Trifolium campestre* and *Veronica chamaedrys* both had a significant lower germination success when sown on bare soil.
In almost all cases [except *H. nummularium*] gut-passed seeds showed a significant lower germination success than seeds which were not ingested and sown on a dung+soil substrate [LSD-test, $P < 0.05$; Table 5.2]. However, absolute germination success varied according to treatment and plant species [Table 5.3], which was mainly related to the different germination response of uningested seeds sown either on the bare soil or dung+soil substrate.

Relative germination success of gut-passed seeds

Relative germination success differed between plant species. Above that, their germination success varied individually between animal species which is reflected in the significant plant x animal species interaction [Table 5.3]. However half the number of plant species - in general 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 significantly between animal species but no clear pattern across animal species was observed [Table 5.4]. Despite this, seeds of some plant species, which 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, $P < 0.05$] Table 5.4].

Although relative germination success varied considerably between plant species for each of the herbivore species under study, some general patterns emerged. Seeds of *H. nummularium* and *T. arvense* had a significantly higher germination success than seeds of most other plant species, except *T. campestre* and several graminoids i.e. *Agrostis capillaris*, *P. pratensis*, *L. campestris* and *Carex arenaria* L. Gut-passed seeds of those species differ significantly from *Galium verum* L., *Galium mollugo* L., *C. capillaris*, *Trifolium pratense* L. and *T. pulegioides* which had among the lowest germination success [[Tukey HSD Test, $P < 0.05$] Table 5.4].

Sheep. *H. nummularium* clearly had the highest relative germination success of all plant species fed. Furthermore *T. arvense* showed a significantly higher germination success than all other species, except *T. campestre*, which in its turn differed significantly from *Veronica arvensis* L., *C. capillaris*, *T. pulegioides* and *G. mollugo*, which all showed among the lowest germination percentages [Table 5.4].

Table 5.2 Mean [± Stdev] absolute germination success under greenhouse conditions of seeds from pots either sown on bare soil or soil +dung layer [control] that did not pass through a gastro-intestinal tract and seeds from dung [after passage through the gastero-intestinal tract of different herbivore species]. Means that are not followed by the same small letter in a row differ at P < 0.05 [LSD-test]. Animal species are ordered in relation to their overall mean effect on germination percentage with sheep having the most profound negative effect. 'NA' indicates that seeds were not fed to that animal species. Plant species were ordered alphabetically.

Plant species	Bare soil Avg [%] ±Stdv	Dung+soil	Sheep	Cattle	Rabbit	Horse	Donkey
replicates	5	5	5	5	5	5	5
number of seeds/plantsp.	100	100	1000	5000	250	2500	2500
Agrostis capillaris	87.6 ±8.26b	73.6±2.7a	3.3 ±2.61c	11.6 ±1.9c	14.8 ±4.43c	16.3 ±9.01c	18.0 ±6.79c
Anthoxanthum odoratum	51.2 ±6.69a	53.2±9.73a	1.0 ±1.02c	1.9 ±0.94c	3.8 ±1.56c	0.8 ±0.61c	1.6 ±0.97c
Carex arenaria	78.0 ±6.04b	61.6±13.43a	2.0 ±1.16c	8.2 ±1.64c	4.0 ±1.74c	7.2 ±1.53c	26.3 ±6.04c
Centaurium erythraea	83.2 ±5.72b	37.6±21.27a	0.2 ±0.33c	0.7 ±0.28c	1.4 ±1.54c	0.8 ±0.57c	0.5 ±0.24c
Galium mollugo	13.0 ±7.71a	16.2±4.66a	0 ± 0c	0.0 ±0.02c	NA	0.1 ±0.08c	0.1 ±0.09c
Galium verum	6.8 ±3.56a	2.8±1.48a	0.0 ±0.07c	0 ±0.00c	0.1 ±0.18c	0.1 ±0.08c	0.0 ±0.04c
Crepis capillaris	85.8 ±2.59b	62.2±25.11a	0 ± 0c	0 ±0.00c	0.7 ±0.82c	0.2 ±0.16c	0.0 ±0.00c
Helianthemum nummularium	24.0 ±2.55b	12.6±3.29a	6.1 ±1.58a	NA	3.8 ±1.82c	3.0 ±1.55c	5.5 ±1.73a
Luzula campestris	45.2 ±20.79b	32.8±15.29a	0.7 ±0.46c	NA	5.0 ±2.89c	5.3 ±2.80c	4.5 ±1.52c
Plantago lanceolata	26.0 ±6.67a	26.8±3.93a	0.1 ±0.08c	1.1 ±0.68c	1.4 ±0.92c	1.0 ±0.85c	1.0 ±0.81c
Poa pratensis	89.4 ±8.38b	79.4±5.03a	1.5 ±0.53c	7.5 ±1.91c	10.6 ±4.38c	12.4 ±6.01c	12.5 ±3.48c
Prunella vulgaris	11.6 ±6.11a	8.8±5.07a	0.1 ±0.16c	0.0 ±0.03c	1.2 ±0.85c	0.3 ±0.13c	0.3 ±0.28c
Thymus pulegioides	40.0 ±17.48b	2.2±1.924a	0.0 ±0.00c	0.0 ±0.04c	0±0c	0.1 ±0.10c	0.0 ±0.04c
Trifolium arvense	13.2 ±4.44a	13.2 ±6.54a	3.0 ±1.66c	1.9 ±0.22c	4.4 ±2.33c	5.2 ±3.41c	2.9 ±1.62c
Trifolium campestre	36.0 ±6.04b	57.8 ±4.55a	5.0 ±3.16c	NA	9.0 ±8.99c	4.6 ±2.81c	NA
Trifolium pratense	66.2 ±12.03b	27.4±14.64a	0.1 ±0.15c	NA	0.4 ±0.40c	0.5 ±0.32c	0.2 ±0.27c
Trifolium repens	78.0 ±8.22b	64.6±7.37a	0.7 ±0.51c	0.8 ±0.28c	1.5 ±1.48c	0.3 ±0.24c	1.1 ±0.34c
Veronica arvensis	72.4 ±37.83a	77.4±9.92a	0.1 ±0.08c	NA	3.8 ±2.17c	1.6 ±1.36c	NA
Veronica chamaedrys	15.6 ±10.26b	42.4±6.54a	0.2 ±0.25c	0.2 ±0.06c	2.3 ±1.95c	1.2 ±0.77c	0.7 ±0.47c

Table 5.3. 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	**df**	**F**	**P**
Seed treatment	6	976.26	<0.001
Plant species	18	89.77	<0.001
Treatment x plant	100	16.36	<0.001
Error	500		
Relative germination success	**df**	**F**	**P**
Plant species	18	100.63	<0.001
Animal species	4	5.45	0.004
Animal x plant	64	5.04	<0.001
	df	**χ^2**	**P**
Individual animal [R]	1	53.78	0.001
Error	328		
Mean retention time	**df**	**F**	**P**
Plant species	8	5.382	<0.001
Animal species	4	133.03	<0.001
Animal x plant	27	1.781	0.016
Error	139		

Cattle. Seeds of *A. capillaris, T. arvense, C. arenaria* showed a significantly higher germination success than seeds of most other plant species fed, except *P. pratensis*, *P. lanceolata* and *Anthoxanthum odoratum* L. which all showed a moderate germination success. Nevertheless, The effect on seed germinability of passage through the guts on seed germinability however varied according to plant and animal species [plant x animal interaction significant, $P < 0.001$, $F_{4,12} = 4.209$]. Final seed germinabilityGermination success of the same plant species may vary considerably between animal species.germination success of *P. pratensis* was still considerably higher than of *C. capillaris*, *G. verum, G. mollugo* and *P. vulgaris*, which had among the lowest germination success [Table 5.4].

In general, pPassage through the gastro- intestinal tract of sheep in general tends to cause a more substantial decrease in germinability germination success than in the case ofcompared to the all other animal species [Fig. 1]. **Rabbit**. The germination success of *T. arvense*, *H. nummularium*, *A. capillaris*, *L. campestris*, *T. campestre* and *P. pratensis* was significantly higher than of *V. arvensis*, *T. pratense*, *G. verum*, *T. pulegioides* and *C. capillaris* which all showed a strongly reduced germination success. Furthermore germination success of *C. erythraea* and *Trifolium repens* L. seeds was significantly lower than of *A. capillaris*, *T. arvense* and *H. nummularium* seeds. In addition seeds of the latter both species had a significantly higher germination success than seeds of *V. arvensis*, *V. chamaedrys*, *P. lanceolata*, *C. arenaria* and *A. odoratum*. *T. arvense* seeds furthermore had a significant higher germination success than *P. vulgaris*. The latter species, *A. odoratum* and *C. arenaria* still showed a substantially higher germination success than *G. verum*, *T. pulegioides* and *C. capillaris* [Table 5.4].

Horse. Seeds of *T. arvense*, *H. nummularium* and *A. capillaris* which passed through horse guts again showed a significantly higher germination success than seeds of all other plant species except *L. campestris*, *P. pratensis* and *C. arenaria*. These three species still had a significant higher germination success than *G. mollugo*, *T. repens* and *C. capillaris*. In addition *P. pratensis* and *L. campestris* had a substantially higher germination success than *C. erythraea*, *G. verum*, *V. arvensis*, *A. odoratum*, *T. pulegioides* and *T. pratense* [Table 5.4].

Donkey. *H. nummularium*, *C. arenaria*, *A. capillaris*, *T. arvensis*, *P. pratensis* and *L. campestris* had a high germination success that substantially differed from: *V. chamaedrys*, *C. erythraea*, *G. mollugo*, *T. pratense*, *G. verum*, *T. pulegioides* and *C. capillaris* which all had among the lowest germination success. In addition the first four mentioned plant species had a significant higher germination success than *P. lanceolata*, *A. odoratum*, *P. vulgaris* and *T. repens* which all had a moderate low germination success [Table 5.4].

At the plant species level tThis was clearly the case for the seeds of *Luzula campestris*, *Plantago lanceolata, Agrostis capillaris* and *Poa pratensis.* But Remarkable exception to the rule show the seeds of *Helianthemum nummularium*, which tend to germinate best after passage through sheep, donkey or rabbit guts [Table 4 and Table 5]. *Carex arenaria* seeds survived much better passage through donkey guts much better than any other guts passage, moreover Sand Sedge seeds that passed through rabbit and sheep guts had the lowest germination successbility [Table 4]. Seeds of *Galium mollugo* lost their germinability almost completely entirely [Table 4], yet some germination occurred from seeds which passed through donkey, horse and cattle. No germination was recorded from Hedge Bedstraw seeds which were ingested by sheep. Final gGermination success bility of *Veronica chamaedrys*, *Prunella vulgaris*, *Crepis capillaris* and *Anthoxanthum odoratum* seeds that which passed through rabbit guts was significantly higher than of those fed to sheep, cattle [but not *A. odoratum*], to donkey [only for *C. capillaris]* and horse [only *A. odoratum*] [Table 4 and Table 5]. Finally, ingested seeds of six plant species did not show any significant difference in final germination percentagegermination success between animal species: *Trifolium arvense*, *T. pratense* , *T. campestre*, *Thymus pulegioides*, *Galium verum* and *Centaurium erythraea* [Tukey HSD Test, P > 0.05].

Figure 5.1 The cumulative absolute germination success [expressed as the percentage of the total number of germinable seeds initially fed] based on 19 plant species which were fed to each animal species. The different animal species are indicated by: [-♦-] sheep, [- - o - -] Donkey, [■——] Cattle, [▲-.-^-.-] Horse and [_ _X_ _] Rabbit. Error bar lines indicate standard deviation. Seeds that passed through sheep guts tend to have a lower absolute germination success.

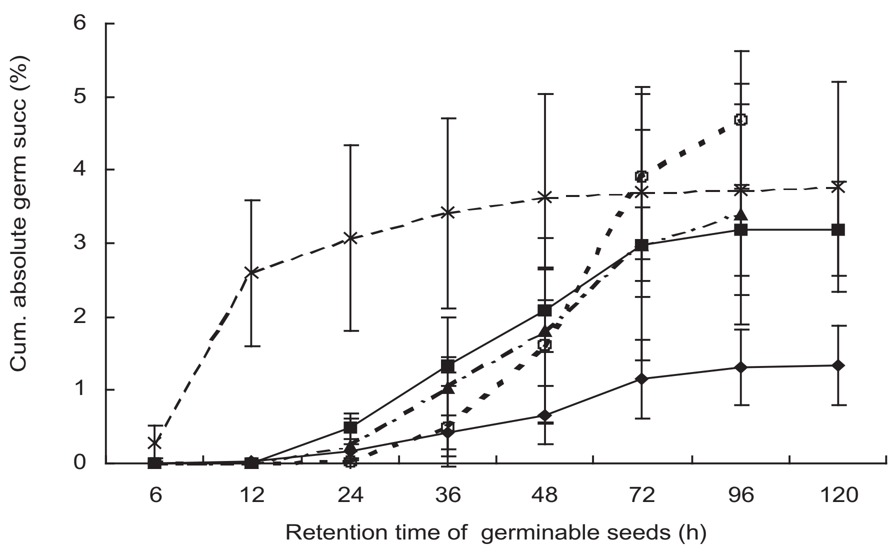


Table 5.4. Relative germination success of gut-passed seeds of different plant species within and between animal species. Frames are homogeneous subsets of plant species which do not differ significantly in relative germination success within each animal species. Subsets differ at the 5% level [Tukey HSD]. The upper row always represents the homogeneous subsets with the highest germination success. Germination success of the subsequent ones decrease successively. Letters represent comparisons of the relative germination success of each plant species between animal species [column]. Values followed by the same letter do not differ significantly at P = 0.05 [Tukey HSD test]. Columns without letters indicate no differences at all, NA means that seeds of the indicated plant species were not fed to that specific animal species. Plant species were ordered descending from left to right according to their overall mean relative germination success.

Plant species	Heli num	Trif arv	Agro cap	Trif cam	Luzu cam	Poa pra	Care are	Prun vul	Plan lan	Anth odo	Vero arv	Ver cham	Cent ery	Gali ver	Thym pu	Trif rep	Trif pra	Crep cap	Gali mol
Sheep																			
Avg	48.2	22.7	4.4	8.6	2.2	1.9	3.3	0.8	0.2	1.9	0.5	0.1	0.6	1.1	0.0	1.1	0,4	0,0	0,0
Stdev	12.6	12.6	3.5	5.5	1.4	0.7	1.9	1.8	0.3	1.9	0.1	0.6	0.9	2.5	0.0	0.8	0,6	0,0	0,0
	a	a	b		a	a	a	a											
Cattle																			
Avg	NA	14.5	15.7	NA	NA	9.4	13.2	0.3	4.1	3.6	NA	0.4	1.7	0.0	1.6	1.3	NA	0,0	0,1
Stdev		1.7	3.7			2.4	2.7	0.3	2.5	1.8		0.1	0.7	0.0	1.6	0.4		0,0	0,1
		b	ab			ab	a	a											
Rabbit																			
Avg	30.5	33.3	20.1	15.5	15.4	13.3	6.5	13.6	5.4	7.2	4.9	5.5	3.8	2.9	0.0	2.4	1,5	1,2	NA
Stdev	14.5	17.7	6.0	15.5	8.8	5.5	2.8	9.6	3.4	2.9	2.8	4.6	4.1	6.4	0.0	2.3	1,5	1,3	
	ab	a	a		ab	ab	a	b											
Horse																			
Avg	23.6	39.3	22.1	7.8	16.3	15.6	11.7	3.4	3.8	1.6	2.1	2.9	2.2	3.0	3.3	0.5	1,7	0,4	0,6
Stdev	12.3	25.8	12.2	5.1	8.5	7.6	2.5	1.5	3.2	1.15	1.8	1.8	1.5	2.8	4.6	0.37	1,2	0,3	0,5
	b	a	a		b	b	a	ab											
Donkey																			
Avg	43.9	22.0	24.4	NA	13.7	15.8	42.7	3.1	3.6	3.1	NA	1.6	1.4	1.1	0.8	1.6	0,8	0,0	0,8
Stdev	13.8	12.3	9.2		4.6	4.4	9.8	3.2	3.0	1.8		1.1	0.6	1.5	1.7	0.5	1,0	0,0	0,5
	ab	ab	a		ab	b	b	ab											

Mean retention time of germinable seeds

Table 5.5 shows the estimates of MRT of plant species with an absolute germination success greater than 1%. MRT of these plant species differed individually between animal species [significant animal x plant species interaction, Table 5.3]. Rabbits defecated germinable seeds significantly faster than any of the other animal species [Fig.5.1]. However deviations from this general pattern were observed for *A. capillaris*, *P. pratensis*, *P. lanceolata* and *T. arvense* [Table 5.5].

Within each of the animal species, except cattle, MRT did not differ significantly between plant species. Within cattle germinable seeds of *P. lanceolata* were defecated significantly faster than the seeds of *C. arenaria* [Table 5.5].

Table 5.5. Mean Retention Times, in hours, of germinated seeds [MRT, ± Stdev] are shown if their absolute germination success >1% from faecal samples of the indicated animal species. Values followed by the same letter do not differ significantly at P = 0.05 [Tukey HSD test]. Capitals represent comparisons of MRT within animal species [column] and small letters between animal species [row].

Plant species	**Sheep**	**Cattle**	**Rabbit**	**Horse**	**Donkey**
	MRT	MRT	MRT	MRT	MRT
replicates	5	5	5	5	5
number of seeds/plantsp.	1000	5000	250	2500	2500
Agrostis capillaris	46.8 ±7.25ab	41.4 ±14.03 ABbc	18.3 ±3.433c	55 ±10.65ab	69 ±9.06a
Anthoxanthum odoratum	45.8 ±18.89a	50.7 ±19.8 ABa	16.8 ±5.48b		64.8 ±19.3a
Carex arenaria	66.2 ±10.41a	63 9 ±3.45 Ba	20.4 ±10.06b	55.7 ±11.43a	68.7 ±9.16a
Centaurium erythraea			26.1 ±14.64		
Helianthemum nummularium	63.8 ±7.56a		16.4 ±5.14b	71.1 ±13.77a	80.4 ±4.6a
Luzula campestris			18.2 ±5.86b	57.1 ±14.26a	67.2 ±5.09a
Plantago lanceolata		34.8±5.63 Aab	13 ±3.03b	47.3 ±13.43ac	64.2 ±6.61c
Poa pratensis	55.9 ±10.45a	45.9 ±7.98 ABab	21.6 ±5.07b	56.2 ±9.35a	66 ±8.2a
Prunella vulgaris			27.2 ±6.92		
Trifolium arvense	52.1 ±7.13ab	57.9 ±4.08 ABa	26.7 ±12.43b	64.8 ±19.3a	71.7 ±4.42a
Trifolium campestre	60. 2 ±14.03a		19.4 ±9.97b	50.4 ±6.24a	
Trifolium repens			17 ±5.41		79.5 ±10.57
Veronica arvensis			13.1 ±4.17	41.5 ±9.28	
Veronica chamaedrys			27.8 ±13.09	47.5 ±15.38	
Calculated MRT					
[Illius & Gordon 1992]	39.2-46.7	71.6		31.2-36.8	29.6-32.8

Seed characteristics and germination success

Within this experiment, seed shape and seed longevity index affected germination success but their effect did not vary between animal species. [No significant interaction term observed, Table 5.6]. Germination success was better for long-lived than for short-lived seeds and for lanceolate seeds compared to other seed shapes [Fig.5.2]. Seed mass did not affect germination success significantly.

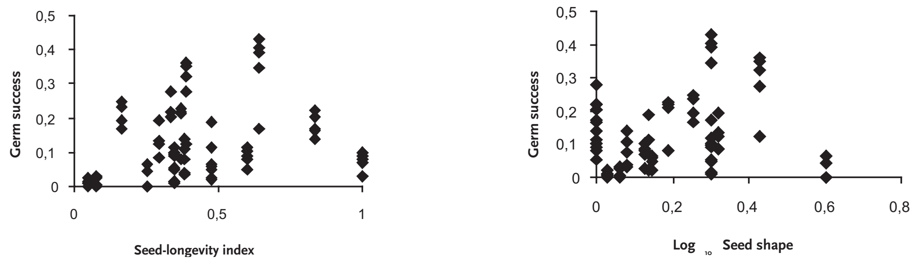


Fig. 5.2 Scatterplots showing the significant positive relationship between both Seed-longevity index [P =0.001] respectively Log10 Seed shape [P =0.008] and germination success of gut-passed seeds [see also Table 5.6]. The points show the average germination success of each plant species used in the experiment for each specific animal species.

Table 5.6. Ancova results, showing the impact of seed mass, seed shape [i.e. seed length * seed width -1] and seed longevity index [covariates] on germination success across animal species [Fixed factor]. Seed shape and Seed longevity significantly affect germination success, their effects did not vary between animal species [no significant interactions].

Relative germination success	df	F	*P*
Animal	4	2.49	0.043
Log_{10}Seed mass	1	2.41	0.122
Log_{10}Seed shape	1	7.16	0.008
Seed longevity	1	11.88	0.001
Animal * Log_{10}Seed mass	4	0.32	0.864
Animal * Log_{10}Seed shape	4	1.34	0.255
Animal * Seed longevity	4	1.00	0.410
Error	325		

Discussion

Methodological considerations

This study aimed to determine the effect of gut passage on seed germinability. Based on Quinn et al. [1994] and Malo & Suárez [1995b] we decided to compare germination success of gut-passed seeds with non gut-passed seeds sown on cattle dung + soil substrate [control]. However, these pots are not really control pots for non-cattle species but this treatment controlled for possible 'crumbled dung effects' and hence to obtain the best possible estimate of gut passage effects under the given conditions. Using this approach we were at least able to control for physical effects which could have occurred in each dung sample i.e. crust forming and separation of this crust from the soil causing a fast mortality of not well established seedlings due to desiccation.

We decided to use five individual animals from each animal species which is within the range of individuals used in previous studies i.e. two [Gardener et al., 1993b]; four [Simao Neto et al., 1987]; five [Russi et al., 1992], seven [Staniforth & Cavers, 1977] and ten by Ghassali et al. [1998]. In none of these studies the effect of individual variation on germination results was evaluated. In our study a small but significant part of the variability in germination success was caused by the effect of individual animals [4 %].

It should be stressed that germination success of control seeds varied considerably between the different plant species which may have resulted from initial differences in seed viability as well as from a different response to the established greenhouse conditions. This urges to interpret germination results without reference germination tests cautiously e.g. as is usually the case with dung samples obtained from free ranging or wild animals [see also Figuerola &Green, 2002].

Germination success after gut passage

Passage through the gastero-intestinal track significantly reduced germination success of all studied plant species [Table 5.2], which is in agreement with results from most other feeding experiments [e.g. Lennartz 1957; Özer 1979; Gardener et al. 1993; but see Ghassali et al. [1998] and Russi et al. [1992] for opposing conclusions]. Yet, in contrast to other feeding experiments, five animal species with different gastero-intestinal systems were involved in this study to test for possible differences in impact on germination success. Based on Illius and Gordon [1992] an inverse relationship between germination success of plant species and the estimates of mean retention time of digesta was expected. This was calculated to be lowest in rabbit, moderate in equid species and sheep, and highest in cattle [Table 5.5]. 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. Moreover the observed variation in germination success amongst the animal species in relation to plant species does not indicate any simple relations 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 both vary independently between animal species [Simao Neto et al. 1987, Staniforth & Cavers 1977] 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 [Table 5.2 & 5.4]. Similar results were found by Shayo & Uden [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. Simao Neto et al. [1987] in addition mentioned similar seed passage rates with sheep, goats and cattle and hence attributed the observed large differences in seed recovery to initial mastication and rumination differences between the animal species. In our experiment mean retention time in sheep also did not differ significantly from the much larger animal species cattle, horse and donkey [Table 5.5]. Compared with the equid species it seems therefore very plausible to relate the differences in germination success to initial mastication and rumination effects. 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 tend to be longer in donkey [66h] than in any other animal species. 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 and Tisdale [1956] who found a consistently ten 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. The in general low germination success obtained in their study, could partly be related to the large seed sizes of the fed species e.g. Bromus tectorum [5-8 mm], Salsola kali [1-2mm]. Whether this means that some 'threshold of seed size' was exceeded above which rabbit mastication causes much more damage than sheep is difficult to assess. Pakeman et al. [2002] showed that smaller seeds [<1 mm] were more likely to germinate from rabbit dung then larger ones whereas other seed related variables i.e. 'dispersed as seed' and a small seed mass [< 0.8 mg] were found as good predictors of germination from sheep dung. Although an intense initial chewing behaviour of rabbits could be expected, we assume that both the ruminating process in combination with the significant longer retention time of sheep could have caused more seed damage than, the 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 [Table 5.4]. This parallelism suggests an underlying overall effect of certain seed characteristics [Table 5.6]. It appeared that lanceolate 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 with germination success must be interpreted within the context of this experiment i.e. a limited set of plant species of which seed sizes were within a limited range of small measures. The results reflect the high relative germination success of most graminoids which all 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 seed 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 endozoochorous long-distance dispersal depends on the amount of viable seeds consumed by the possible dispersers and the effects of their digestive system on germination success and mean retention time of germinable seeds. Most field studies report on the germination of many seedlings of a wide variety of grassland species from ungulate and rabbit dung [Malo & Suárez 1995 a; Pakeman et al. 2002; Dai 2000; Claerbout 2001]. This implies the preceding consumption of a large number of seeds to compensate the generally low germination success after gut passage. However, taking into account the overall low absolute germination success of gut-passed seeds one may question about the efficiency of endozoochory for most temperate grassland species. The process of mastication and gut passage appears to cause a high cost for the plant species. On the other hand the trade-off of other dispersal mechanisms might be high as well but little is known about the cost-benefit effects of different dispersal mechanisms. A first insight is offered by Pakeman et al. [1998] who calculated the relative contribution of different seed sources to the developing vascular plant cover in a cool temperate, acidic grassland. It appeared that rabbit endozoochory contributed for 15 %, other means of dispersal for 40 % and regeneration from the seed bank for 45 %.

On the other hand the observed mean retention times of germinable seeds are favourable for long-distance seed dispersal. Observations on movements of cattle, horses and donkey in medium-sized coastal dune landscapes[60-120 ha] revealed that after mean retention time of germinable seeds each of those animal species could cover the whole area. On average, they are several of hundreds meters [150-500 m] away from where seed consumption may have occurred [Cosyns, unpubl.]. Within more extended nature reserves or the natural home ranges of wild herbivores those distances may even be larger. Rabbits have a smaller home range but still may be important seed dispersers at the medium scale [<150 m], certainly if taken into account the observed germination success [this experiment] and their sometimes high population density. Because grasslands are amongst the most preferred grazing habitats and because of the strong positive relationship between grazing and defecating [Cosyns et al. unpublished data] one can additionally expect some direction in endozoochorous seed dispersal.

To understand, from a plant's-eye-view, the role and relative importance of endozoochory, compared to other dispersal modes occurring in West European semi-natural landscapes further studies are needed which are able to quantify the relative contribution to later generations of the plants from endozoochorously dispersed seeds as compared to the contribution of the plants from seed dispersed by other dispersal modes. This will require an integrating approach combining information from field observations on the frequency of occurrence of the different dispersal modes, on the places where seeds are deposited and information with some specific aspects related to endozoochory e.g. its assumed directed nature [between favoured grazing sites] and the specific conditions of seed deposition i.e. arriving as part of a dung pat [see Gökbulak 1998] opposed to seeds arriving directly by other dispersal modes should get our attention when designing such studies.

Appendix 5.1 List of animal species [+ breed] used in the seed feeding experiment with a typology of their digestive strategy, indication of age [+number of individuals], gender [number of males [M] and females [F]] and body weight [average for each age category].

Animal species	Digestive strategy	Age	Gender	Weight [kg]
Sheep [Texel]	Foregut fermenter	3 years [2]	1 M + 4F	c.85 [3y]
		+ 6 months [3]		c.40 [6 m]
Cattle [Blonde d'Aquitaine]	Foregut fermenter	1.5 year [5]	5 F	c. 425
Rabbit [breed ?]	Caecum fermenter	4 months [5]	3 M + 2 F	3.5
Donkey	Colon fermenter	2 years [2]	5 M	135
		+ 1 year [3]		90
Horse [Shetland pony]	Colon fermenter	5 years [1]	5 M	190
		+ 1 year [4]		110

Appendix 5.2 Main characteristics of the commercial pellets which were fed to the mentioned animal species. Minerals and vitamins are not shown. Values are based on information provided by the producer of the pellet feed.

Component [commercial pellets]	Rabbit	Sheep + Cattle	Horse+Donkey
Crude protein	15 %	17 %	13.5 %
Crude lipids	4 %	3 %	3 %
Crude ash	10 %	8 %	8 %
Crude fibre	15 %	13 %	12.5%
Total carbohydrates and starch			28.5%

6

Plant establishment after-dung deposition: does endozoo-chory contribute to plant species enrichment?

Eric Cosyns, Hadewich Vervaet, Luc Lens & Maurice Hoffmann

Experimental set up -Westhoek South- 2001

Abstract

An important step in the process of successful endozoochorous seed dispersal is the establishment of seedlings from the dung viable seed content. To determine the importance of dung deposition for plant establishment, an experiment was carried out in a grassland. The effect of dung deposition, i.e. the combined effect of being a potential seed source, a regeneration site and a fertiliser, was estimated by comparing seedling emergence and [juvenile] plant appearance in plots with cattle or horse dung deposition and plots without. The possible interacting effect of soil disturbance and dung deposition on plant establishment was evaluated by comparing sod-cut [disturbed] and vegetated plots. To control for small vertebrate granivory and herbivory caged and uncaged plots were used.

Compared to controlled greenhouse germination, field germination success of herbs on dung was higher than that of graminoids and field germination success did not depend on the kind of dung. More seedlings emerged on dung that was deposited on vegetated plots compared with sod-cut plots. From plots protected against granivory/herbivory by small mammals more seedlings emerged than from uncaged plots

After one year, on average more species [2-3spp] were recorded from plots with dung deposition. Generalist plant species were well represented in the dung germinable seed content, and established significantly better in plots with dung deposition [more individuals, higher cover degree] e.g. *Juncus bufonius*, *Cerastium fontanum*, *Trifolium repens, Veronica arvensis+chamaedrys*. These results stress the role of dung deposition by large herbivores as a seed supplying mechanism. The problematic establishment in vegetated plots compared to the enhanced establishment of some plant species [e.g. *Juncus bufonius, Arenaria serpyllifolia*] in sod-cut plots further suggests that dung pats of large herbivores are less powerful in creating regeneration sites ['gaps'] than other mechanisms which are able to cause more profound soil disturbance in grasslands.

Key-words: cattle, disturbance, horse, seedling herbivory, seed bank.

Introduction

Large numbers of plant seeds are potentially dispersed via animal dung [e.g. Welch 1985; Malo & Suárez 1995a; Pakeman *et al.* 2002; Cosyns *et al.* Chapter 2]. However, very little is known about the relative importance of endozoochory in plant establishment. Pakeman *et al.* [1998] estimated that rabbit-dispersed seeds accounted for 15 % of the developing vascular plant cover in a temperate acidic grassland. Dung deposition by cattle influences plant dynamics through the combined effect of seed input and gap formation i.e. dung itself as a potential regeneration site or as a precursor of gaps by suppressing vegetation. Welch [1985] and Malo & Suárez [1995b] demonstrated that several plant species had a greater cover on cattle dung than in the surrounding vegetation as a result of germination from seeds in the dung. Malo & Suárez [1995b] and Dai [2000] showed that seeds in cattle dung were the main seed source for colonisation of the gaps that remained after dung decomposition. Pakeman *et al.* [1998] and Dai [2000] furthermore put forward evidence of an interaction between soil seed bank and dung seed content in relation to germination and establishment of grassland species in gaps. From these studies, it remains unclear though whether dung deposition in a perennial-grass-dominated, closed canopy is able to considerably contribute to the establishment of new plant individuals. It is also not clear whether the establishment of plants is affected by the initial conditions of the microsite where dung is deposited e.g. a closed canopy, hostile to seed invasion and germination versus bare soil, which offers an initially competition free regeneration site. Furthermore little is known about the effects of the kind of dung from which seeds are germinating, e.g. does it make any difference whether seeds are part of a flat, initially undivided, cattle dung pat or from a pelleted horse dung pile.

From a previous study it appeared that seed intake and passage through herbivore guts represent a high cost for the endozoochorically dispersing plant species, since large amounts of viable seeds are lost during the process [Cosyns *et al.*, Chapter 5]. Another important step in the plant dispersal process is the establishment of plant individuals after seed dispersal [Wang & Smith 2002]. To examine the relative importance of cattle and horse dung deposition in the plant establishment process, an experiment was conducted in a species poor, perennial-grass-dominated vegetation.

Three questions were addressed. [1], does the input of dung of large herbivores result in an increase of individuals of plant species already present in the vegetation and of new species in a seedling hostile environment of grassland, dominated by perennial grasses that form a closed canopy? [2], if so, do microsite conditions [closed grassland vegetation versus gaps created by sod-cutting] influence the relative success of dung-induced plant establishment? [3], is seedling emergence from dung affected by the kind of dung [i.e. cattle or horse dung]?

Materials and Methods

Study area

The experiment ran from August 2001 until July 2002 in a dune slack in the southern part of the Westhoek [340 ha], a nature reserve along the Belgian coast [51°04'50'' N - 2°34'19'' E]. Since 1997 this area [61 ha] is grazed year round by on average four Scottish Highland cattle and 19 Shetland ponies. The experiment itself was carried out in a perennial-grass-dominated site. From the start of the experiment onwards part of this site was exclosed [10 x 50m] from large herbivore grazing. Before the start of the experiment it was assured that no dung of large herbivores was present.

Experimental design and treatments

The experiment had a randomised complete block design [sensu Neter *et al.* 1996] with three fixed factors: disturbance ['vegetated' versus 'sod-cut'] and dung deposition ['no dung' versus 'dung added']. Since granivory and herbivory by small mammals [see e.g. Edwards & Crawley [1999a, b]] can be important limiting factors for seedling establishment, we controlled for small vertebrate mammal [i.e. rabbit and mice] interaction within the experimental design by placement of cages. The third factor is therefore called cage ['protected' against small mammal granivory/herbivory versus 'not protected'] [Fig. 6.1].

Twelve blocks, were laid out along a possible ground water gradient. [Mean maximum ground water levels ranged from 120-100 cm in the east to 80-60 cm below surface in the west of the site]. Disturbance is either intact vegetation cover [C] or sod-cut [P]. Sod-cutting implied the removal of vegetation and the upper 5 cm of soil before the start of the experiment. To prevent the plots to be contaminated by clonal growth from the surrounding vegetation sod-cutting was carried out over 0.7 x 0.7 m. Dung deposition consisted of the input of 2.5 L of fresh dung of Shetland pony [S] or Scottish Highland cattle [H] making it also possible to discriminate for 'kind of dung'. To avoid a possible confusing impact of seedling regeneration from the nearby, actual seed rain we prevented seed input by monthly clipping of all vegetation within the blocks at less than 10 cm vegetation height from May to October and by mowing twice between blocks during summer 2001 and 2002.

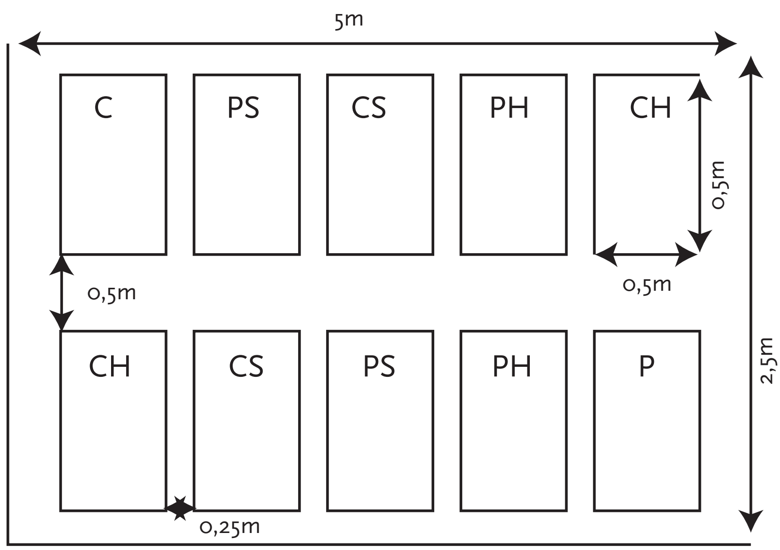


Figure 6.1 Example of a block unit from the experimental randomised block design with indication of sizes and the randomly allocated treatments to each of the 10 plots. Plots either had a closed vegetation [C] or were sod-cut [P]. Dung deposition consisted from the input of 2.5 L of fresh Shetland pony [S] or Highland cattle [H] dung or no dung input as control. To control for possible effects of small vertebrate granivory or herbivory half of the dung plots were randomly caged 0.5x0.5x0.25m wire mesh 0.01m [CH- PH- CS- or PS-]. In total 12 such blocks were constructed.

Freshly deposited dung from the free ranging Shetland ponies and Scottish Highland cattle [randomly chosen individual animals] was collected on several days in August 2001. At that time dung counts the highest seed densities and a broad spectrum of plant species which was favourable for this experiment [Claerbout 2001; Cosyns *et al.* Chapter 2]. The collected dung was aggregated and homogenised per herbivore species. Four 2.5 L subsample were than spread out in the experimental 'dung' plots of one single block. One 2.5 L subsample of the same homogenised dung sample was taken to the greenhouse [see below].

Vegetation survey

Vegetation surveys were made of 60 plots before treatment in July 2001 and of all 120 plots, one year after treatment. The percentage cover of the total vegetation, the grass, the moss, the litter layer, bare soil and of all plant species was estimated, using a decimal scale [Londo 1976].
Before treatments were applied, the vegetation of the experimental site was dominated by perennial grasses. *Holcus lanatus* was the most abundant species. *Calamagrostis epigejos*, *Poa pratensis* and *Poa trivialis* were also abundant or at least frequent. *Senecio jacobaea*, *Galium mollugo* and to some lesser extent *Cerastium fontanum* made up the majority of the herb cover [each species > 1 %]. The cover of the moss layer was low [on average 6.4 %] and bare ground took an average of 12 % of the plot surface. In total 34 vascular plant species were recorded but only six species were recorded from more than 50 % of the plots. The mean number of vascular plants per plot was 9.2 [Table 6.1].

Table 6.1 Mean cover percentage [± standard deviation] and relative frequency [Freq %] of the plant species present in the vegetation plots and mean species richness per plot before treatment [July 2001 Nmax= 60]. Only species present in at least 10% of the plots are shown.

Plant species	**Cover [%]**		
	Avg.	± Stdev	Freq%
Holcus lanatus	63.2	5.49	100
Calamagrostis epigejos	6	1.40	96.7
Senecio jacobaea	3.7	0.67	96.7
Cerastium fontanum	1.7	0.40	73.3
Poa trivialis	3.4	2.21	58.3
Galium mollugo	2.8	0.77	56.7
Juncus bufonius	1.4	0.77	48.3
Poa pratensis	10.3	3.71	48.3
Veronica arvensis	0.5	0.22	43.3
Rubus spp.	0.4	0.07	31.7
Cardamine hirsuta	1.4	1.29	30
Sagina procumbens	0.5	0.29	25
Arenaria serpyllifolia	0.3	0.11	23.3
Poa annua	1.5	1.22	20
Carex arenaria	1	0.78	18.3
Prunella vulgaris	0.3	0.18	13.3
Trifolium repens	0.3	0.35	11.7
Centaurium erythraea	0.1	0.10	10
Cirsium arvense	0.4	0.41	10
Ranunculus repens	0.3	0.26	10
Total	87.7	1.45	
Herblayer	87.1	1.36	
Mosslayer	6.4	1.20	
Bare soil	12.0	1.61	
species richness [vascular plants]	9.2	0.55	

Seedlings and recruits in the field

Seedlings were counted in October 2001, when their density on dung was at its maximum. In March 2002 most dung was decomposed and seedlings could not be distinguished anymore from juvenile plants. Therefore, individuals of a selection of 12 plant species were counted in the experimental plots [March and July 2002]. The species were selected according to the following criteria: they germinated in > 75 % of the samples and generally in considerable amounts from dung samples kept under greenhouse conditions and in the field they were recognisable and countable as individual plants. The following species or species aggregates were counted: *Arenaria serpyllifolia, Centaurium erythraea, Cerastium fontanum, Epilobium spp., Galium spp., Juncus articulatus, Juncus bufonius, Poa annua, Sagina apetala+ S. procumbens, Trifolium repens, Urtica dioica* and *Veronica arvensis + V. chamaedrys*.

Potential species from the soil seed bank and dung seed content under greenhouse conditions

The initial persistent seed bank composition was estimated from soil samples taken in July 2001. A soil core [8 cm deep, 5.8 cm in diameter] was taken at two diagonally opposing corners of each plot. The upper 0.5 cm of each soil core was removed to ensure measurement of the persistent seed bank. Soil cores of each plot were aggregated, based on the treatment 'disturbance', forming two mixed samples of 2 L per block. The soil samples were sieved [4 mm] to remove living plant material and stones and stored at 4°C for three weeks.

Dung and soil samples were than treated as described in chapter 1. The following species and numbers were recorded from the ten control trays with only the sand/commercial potting ground substrate: *Epilobium* sp. [1], *E. hirsutum* [1] and *Juncus bufonius* [12]. Because of the negligible numbers compared to those found in soil and dung samples we did not take them into consideration and assumed no contamination to appear. Seedlings in the greenhouse were identified, counted and removed at regular intervals during one year.

The results of this analysis were used to determine the potentiality of the dung and soil seed content to act as a seed source. Calculation of the relative germination success of seedlings on dung was also based on the greenhouse germination experimental results. Therefore, we treated these 'results' here.

A total of 6776 seedlings of 51 plant species was found in the soil samples. *Urtica dioica* and *Centaurium erythraea* made up the largest part of the total number of seedlings i.e. 45.2 % and 20.3 % respectively. Eight other species together added another 28 % each making up between 1.4 and 6.5 %. Among the frequently emerging plant species several annuals were present [*Juncus bufonius*, *Poa annua*, *Veronica arvensis*, *Stellaria media*, *Cardamine hirsuta* and *Centaurium erythraea*], but in all, perennial species made up the largest group [Table 6.2].
A total of 18974 seedlings of 50 plant species emerged from cattle dung, while 10808 seedlings of 49 plant species germinated from horse dung. Eight plant species emerged from every dung sample [Table 6.2]. The most abundant species was by far *Juncus bufonius* that made up 78.2 % and 69.5 % of all seedlings emerging from cattle and horse dung respectively. A relatively small number of other species each made up more than 1 % of all seedlings: *Sagina* spp., *Poa annua*, *Juncus articulatus*, *Trifolium repens*, *Cerastium fontanum* and *Urtica dioica*. Mean species richness and germinating seed density both were significantly higher in cattle dung than in horse dung [T-test, $t_{spprich}$ = -3.135, df=22, P= 0.005; $t_{density}$= -3.586, df=22, P= 0.002 ; Table 6.2].
Almost all plant species [31] that were present in the vegetation at the experimental site were also recorded from the dung samples. However, 30 plant species were only recorded from the dung samples. Compared to the soil seed bank, 38 species were in common, 23 species emerged only from dung samples and 13 plant species only germinated from the soil seed bank.

Statistical analysis

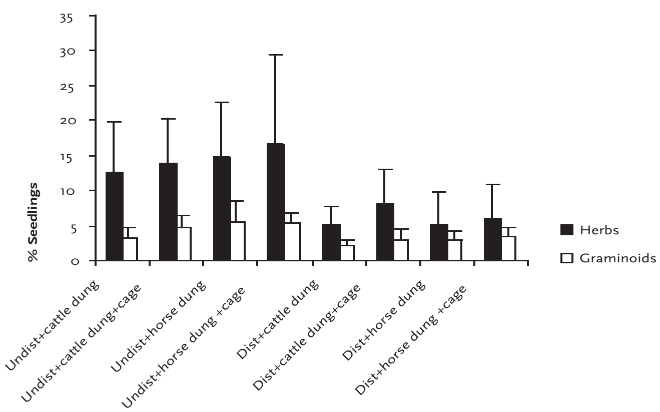
Differences in seedling densities on dung [October 2001] were analysed using a mixed model ANOVA with fixed factors PLANT GROUP [herbs or graminoids], DUNG [horse or cattle], CAGE [with or without exclusion of small vertebrate herbivores] and DISTURBANCE [vegetated or sod-cut] and as a random factor BLOCK. A similar mixed model ANOVA was used to analyse possible differences in the plant establishment and cover degree data that were recorded in July 2002. PLANT GROUP was then omitted from the model because the analysis was carried out for each of the selected plant species separately. Plant cover degree [%], the number of established plants to the total number of plants ratio, and the seedling density to dung germinable seed content ratio were Arcsin[Sqrt] transformed to approach normality and homogeneity of variance.
All statistical analyses were carried out in SPSS 11.1 for Windows [SPSS 2001].

Table 6.2 Mean germinable seed density [Avg. 2.5L-1] and frequency of emergence [Freq. %] of plant species in soil [0.5-8 cm depth n = 24] horse and cattle dung samples [august 2001 n = 12 each] in the greenhouse. Only species present in at least 25 % of soil or all dung samples are shown.

Seed content of	**Soil**		**Horse dung**		**Cattle dung**	
Plant species	Avg # $2.5L^{-1}$	Freq %	Avg	Freq	Avg	Freq
Juncus bufonius	2.4	88	625.9	100	1237.2	100
Poa annua	7.5	71	84.4	100	25.7	100
Juncus articulatus	1.4	63	24.7	100	28.6	100
Cerastium fontanum	1.7	46	12	100	44.3	100
Epilobium spp.	0.3	42	12.1	100	30.2	100
Holcus lanatus	4.8	38	8.5	100	41.9	100
Sagina procumbens+apetala	0.9	21	46.6	100	12.0	100
Poa trivialis	0.05	4	8.7	100	36.8	100
Veronica chamaedrys+arvensis	2.19	92	3.8	92	17.1	100
Arenaria serpyllifolia	8.5	71	2.8	100	3.8	92
Urtica dioica	152.1	100	28.5	100	1.6	83
Agrostis stolonifera	0.05	4	4.3	83	23.3	100
Trifolium repens			5.6	83	24.3	100
Poa pratensis	0.3	25	5.4	83	5	92
Potentilla reptans	0.2	21	1.3	67	6.3	100
Plantago major		0	3.6	83	2.3	83

Seed content of	**Soil**		**Horse dung**		**Cattle dung**	
Plant species	Avg # $2.5L^{-1}$	Freq %	Avg	Freq	Avg	Freq
Stellaria media	11.4	92	0.7	42	4	92
Calamagrostis epigejos	1.9	42	2.7	50	1.9	67
Senecio jacobaea	0.7	38	0.7	42	1.1	75
Galium uliginosum			1.5	42	0.8	58
Ranunculus repens			0.5	33	0.8	67
Rumex conglomeratus			0.3	8	3.0	92
Agrostis capillaris			0.8	42	1.2	50
Galium aparine	0.04	4	0.6	33	2.8	58
Veronica serpyllifolia	0.04	4	0.7	50	1.5	33
Scirpus setaceus	0.04	4	0.8	42	0.6	33
Centaurium erythraea	68.3	96	0.4	42		
Cardamine hirsuta	7.2	92	0.3	25	0.2	17
Carex arenaria	1.0	50	1.0	17	0.3	25
Juncus effusus	1.1	58				
Geranium molle	0.8	42	0.1	8		
Claytonia perfoliata	13.5	38			0.6	33
Carex flacca	0.5	33				
Senecio sylvatica	0.5	33			0.1	8
Mean seed density [± stdev]	**282 [±167.7]**		**900 [±389.2]**		**1581 [±529.7]**	
Mean species number [± stdev]	**16 [±2.8]**		**24 [±3.2]**		**28 [±3.4]**	
Species richness [all]	**51**		**49**		**50**	
Number of samples	**24**		**12**		**12**	

Figure 6.2 Mean percentages of the initial germinable seed content that gave rise to seedlings on cattle and horse dung in the experiment [October 2001]. Relative germination success of herb seeds was significantly higher than graminoid seeds. Germination success of both plant classes was significantly better on dung which was deposited on vegetated plots compared to dung on bare soil. Protection from small mammal herbivory [cage] generally lead to significantly higher numbers of seedlings.



Results

Effect of treatments on seedling emergence on dung

Seedling emergence was measured three months after the experiment started. Compared to the initial dung germinable seed content, which was determined in the greenhouse and which can be considered as an estimate of the potential dung seed bank, germination success of herbs was significantly higher than that of graminoids and germination success of plants did not depend on the kind of dung [Table 6.3]. Significantly more seedlings emerged on dung that was deposited on vegetated compared to sod-cut plots. From plots protected against small mammal granivory/herbivory significantly more seedlings emerged than from uncaged plots [Table 6.3 and Fig.6.2].

Table 6.3 Results of the mixed model ANOVA-analysis [F: fixed, R: random factor]. The mean number of emerging seedlings was significantly higher on dung which was deposited on vegetated plots [disturbance] and when it was protected from small mammal herbivory [cage]. In relation to the greenhouse germination numbers [modelling the potential dung seed bank] herb seedling numbers on dung in the field were significantly higher than that of graminoids [plant group]. There was no differences between cattle or horse dung type of dung [dung].

Factor	df	F	Sig.
Cage [F]	1	5.8	0.017
Disturbance [F]	1	82.4	<0.001
Dung [F]	1	0.7	0413
Plant group [F]	1	134.6	<0.001
Block [R]	11	3.8	<0.001
Error	169		

Effect of treatments on establishment of a selection of plant species

Each of the twelve examined plant species responded differently to the applied treatments. After one year all plant species established better in the sod-cut plots, except *Trifolium repens,* which did not differentiate in establishment for the treatment 'disturbance' [Table 6.4]. Dung deposition led to a significant increase of the number of establishing individuals, e.g. *Cerastium fontanum*, *Trifolium repens*, *V. arvensis+chamaedrys* and *Juncus bufonius.* Yet, the latter species only showed a significant increase of individuals in the sod-cut plots with dung deposition compared to the control sod-cut plots and not in the vegetated plots [significant disturbance*dung interaction, Table 6.4]. Establishment for the remaining species was not significantly influenced by dung deposition [Table 6.4]. Granivory/herbivory protection increased the number of individuals of *Urtica dioica* significantly but surprisingly decreased numbers of *Cerastium fontanum*, *Juncus bufonius* and *Sagina procumbens*.

Effect of treatments on plant cover and vegetation composition

Disturbance. After one year total vegetation cover was significantly lower and the amount of bare ground was significantly higher in sod-cut plots compared to the non-disturbed plots. This was due to the lower cover of grasses and herbs whereas cover of mosses was higher in the sod-cut plots. Mean species richness, with and without mosses was significantly higher [+ 4 spp] in the sod-cut plots than in the undisturbed plots [Table 6.5]. Cover degree of the most abundant species decreased significantly in the sod-cut plots e.g. *Calamagrostis epigejos* and *Holcus lanatus*. On the other hand, *Cerastium fontanum*, *Sagina* spp., *Juncus bufonius* and *Epilobium* spp had a significantly higher cover degree and frequency in sod-cut plots than in undisturbed plots [Table 6.1 and 6.5]. *Poa annua* and *Centaurium erythraea* both showed a significantly higher cover degree in sod-cut plots and occurred also significantly more frequent in disturbed plots than in undisturbed plots [$n = 60$, $C_{Poa.} = 15.2$, df=1, $P<0.001$; $C_{Cent.} = 16.7$, df=1, $P<0.001$].

Dung deposition. Dung deposition led to a higher mean species richness [+2-3 spp] in plots irrespective the other treatments applied. Dung deposition did not affect total vegetation cover nor did it affect the cover of the grass or moss layer [Table 6.5]. Some plant species, including *Ranunculus repens*, *Rubus* spp., *Trifolium repens*, *Cerastium fontanum*, *Juncus bufonius* and *Veronica arvensis* + *V. chamaedrys*, showed a significantly higher cover degree in plots with dung deposition. For the last four taxa this appears to be attributable to regeneration from dung seed content [Table 6.4]. *Juncus bufonius* and *Urtica dioica* showed a pronounced cover increase in sod-cut plots with dung deposition whereas cover in undisturbed plots was hardly affected [significant dung x disturbance interaction, Table 6.5]. Cover degree of *Senecio jacobaea* showed a contrasting response to dung deposition: it was significantly higher in the sod-cut plots as compared to undisturbed plots and decreased in the undisturbed plots compared to the control plots [significant dung*disturbance and dung effect, Table 6.5]. *Rubus* spp. was recorded more frequently from plots in 2002 than in 2001 [n=60, $C_{Rubus.}$= 5.1, df=1, P=0.023] However, it was only recorded in very small amounts from soil and dung samples under greenhouse conditions and no extensive *Rubus* vegetation is present in the neighbouring vegetation, that could have served as a potential external seed source.

Granivory/herbivory protection. Caging of the plots lead to a higher total vegetation cover and higher cover degree of the grass layer compared to unprotected plots [Table 6.5]. This is mainly attributable to a higher cover degree of *Holcus lanatus*. A similar trend was observed for *Calamagrostis epigejos* cover although the differences were not significant. On the other hand a significantly lower cover degree was recorded under protection for *Senecio jacobaea, Juncus bufonius*, *Sagina procumbens* and *Cerastium fontanum* For the latter three species this is concordant to the already mentioned lower numbers in established individuals [Table 6.4].

Table 6.4 Mean absolute number of individuals of a selection of 12 plant species that were established after one year (July 2002) in sod-cut and intact vegetation plots (12 replica's per treatment). with our without cattle or horse dung addition. Some of the plots were protected with a cage against small mammal herbivory. The significance of the differences between the means of each factor is shown at the right; P ≤ 0.001 (***). 0.001 < P ≤ 0.01 (**). 0.01< P ≤ 0.05. P > 0.05 (NS) (Dist. = disturbance i.e. sod-cut or not. Dung = with or without dung deposition. Cage: with or without cage).

	Mean number of individuals										Anova (P)				
	Intact vegetation				Sod cutted										
	Cattle dung		Horse dung			Cattle dung		Horse dung							
Plant species	Cage:	Without	With	Without	With		Without	With	Without	With	Dist.	Dung	Cage	Block	Dist.*Dung
Arenaria serpyllifolia	0.58	0.25	0.00	0.25	0.00	2.17	4.25	1.75	5.50	2.92	***	NS	NS	***	NS
Centaurium erythraea	0.58	0.08	0.33	0.42	0.33	5.42	7.67	8.75	5.67	3.50	***	NS	NS	***	NS
Cerastium fontanum	1.58	3.75	1.92	2.58	2.42	3.42	7.50	6.75	7.08	4.42	***	***	*	NS	NS
Epilobium spp.	0.08	0.25	1.08	0.75	0.33	1.25	1.75	2.50	1.83	1.67	***	NS	NS	NS	NS
Galium spp.	0.00	0.50	0.17	0.75	2.25	0.00	0.67	2.25	1.50	3.17					
Juncus articulatus	0.08	0.08	0.08	0.00	0.08	0.17	0.08	0.08	0.25	0.08					
Juncus bufonius	0.25	0.92	0.17	0.33	0	1.42	12.75	7.17	8.42	6.67	***	***	**	**	***
Poa annua	/	/	/	/	/	1.83	1.67	1.42	2.17	3.67					
Sagina procumbens															
+apetala	1.83	0.58	1.58	1.58	0.33	2.33	4.00	1.50	4.92	3.17	***	NS	*	*	NS
Trifolium repens	0.17	0.92	0.83	1.00	0.17	0.42	0.67	1.17	0.42	1.00	NS	**	NS	NS	NS
Urtica dioica	0.00	0.00	0.25	0.08	0.58	3.75	9.17	18.83	6.92	14.17	***	NS	**	***	NS
Veronica chamaedrys															
+arvensis	0.33	1.33	1.00	1.00	0.58	0.67	1.17	2.50	2.50	1.17	*	**	NS	***	NS

Table 6.5 Mean percentage cover, mean species richness and frequency of occurrence (Freq %) of the plant species present in the non-disturbed, intact (Nmax=60) and sod-cut vegetation plots (Nmax=60, 12 replica's for each treatment). Species were ranked according to their overall frequency in the plots. Only species present in at least 10% of the plots are shown. The significance of the differences between the means of each factor is shown at the right; P £ 0.001 (***), 0.001 < P £ 0.01 (**), 0.01< P £ 0.05, P > 0.05 (NS) (Dist. = disturbance i.e. sod-cut or not, Dung = with or without dung deposition, Cage: with or without cage).

	2002 Plant cover (%)					Freq%	2002 Plant cover (%)					Freq%	Anova (P)				
	Intact vegetation						Sod cutted										
		Cattle dung		Horse dung					Cattle dung		Horse dung						
Plant species	Cage:	without	with	without	with		Cage:	without	with	without	with		Dist.	Dung	Cage	Block	Dist.*Dung
Holcus lanatus	58.3	59.6	69.6	58.3	72.5	100	32.7	35.9	44.6	35.7	50.5	100	***	NS	***	***	NS
Calamagrostis epigejos	26.8	22.3	28.3	28.5	26.3	95	16.5	14.8	19.8	15.2	16.1	100	***	NS	NS	***	NS
Senecio jacobaea	4.0	2.3	3.1	2.7	2.3	92	2.9	5.4	3.7	8.6	4.3	97	*	*	**	***	***
Cerastium fontanum	0.9	1.5	1.1	1.4	1.3	85	3.0	3.8	2.4	4.5	1.6	97	***	*	***	NS	NS
Poa trivialis	0.4	1.8	2.1	1.9	1.2	38	0.5	1.5	1.1	0.1	0.8	28	NS	NS	NS	NS	NS
Galium mollugo	0.0	0.3	0.2	0.3	0.6	23	0.0	0.3	0.8	0.5	1.0	37					
Juncus bufonius	0.2	0.6	0.2	0.3	0.0	15	3.1	9.3	7.8	9.3	4.4	90	***	***	*	**	*
Poa pratensis	19.3	13.6	5.8	9.6	7.3	43	0.4	0.6	1.5	1.0	0.6	30					
Veronica arvensis	0.2	0.3	0.1	0.3	0.3	25	0.2	0.3	0.4	0.6	0.3	30					
Rubus spp.	0.8	1.0	0.8	1.9	1.7	78	0.8	0.9	0.8	1.5	1.3	73	NS	***	NS	NS	NS
Cardamine hirsuta	0.1	0.2	0.1	0.4	0.0	12	0.8	0.5	0.3	0.3	0.2	13					
Sagina procumbens	0.9	0.3	0.5	1.7	0.2	23	1.6	1.1	0.7	1.8	1.3	60	***	NS	*	*	NS
Arenaria serpyllifolia	0.3	0.1	0.0	0.2	0.0	8	0.4	1.3	0.4	1.3	0.9	35					
Poa annua	0.3	0.0	0.0	1.4	0.6	12	2.2	2.0	1.0	2.5	3.1	52					
Carex arenaria	0.5	0.2	0.7	0.6	0.3	17	1.7	1.1	3.0	2.9	0.4	30					
Prunella vulgaris	0.3	5.4	0.4	0.9	1.3	28	0.8	0.6	0.1	0.2	0.0	12					
Trifolium repens	0.3	0.9	0.9	0.7	0.3	47	0.3	0.7	1.1	0.7	1.0	57	NS	***	NS	NS	NS
Centaurium erythraea	0.3	0.1	0.3	0.3	0.3	20	3.1	1.9	3.2	1.8	1.3	70	***	NS	NS	***	NS
Cirsium arvensis	0.5	0.3	0.5	0.2	0.4	20	0.4	0.4	0.5	0.4	0.4	20					
Ranunculus repens	1.5	1.2	1.7	0.8	0.5	53	0.3	2.1	1.9	0.9	1.1	52	NS	***	NS	*	NS
Veronica chamaedrys	0.2	0.5	0.3	0.4	0.1	27	0.1	0.4	0.8	0.1	0.3	32					
Urtica dioica	0.0	0.0	0.2	0.1	0.4	13	1.0	2.0	3.3	1.6	2.1	80	***	NS	**	**	*
Epilobium spp	0.1	0.2	0.7	0.7	0.4	37	0.8	1.2	1.3	1.2	1.3	75	***	NS	NS	NS	NS
Geranium molle	0.7	0.6	0.8	0.5	0.3	45	0.2	0.3	0.3	0.3	0.6	28					
Total	**94.9**	**92.3**	**95.0**	**92.9**	**96.0**		**66.0**	**68.3**	**78.8**	**72.1**	**81.1**		***	**NS**	***	***	**NS**
Herb layer	**93.9**	**92.1**	**94.6**	**92.1**	**96.0**		**58.9**	**64.0**	**73.1**	**68.2**	**75.3**		***	**NS**	***	***	**NS**
Moss layer	**9.1**	**1.5**	**2.1**	**2.6**	**1.8**		**12.1**	**14.6**	**16.4**	**9.6**	**8.3**		***	**NS**	**NS**	***	**
Bare soil	**8.9**	**16.0**	**11.9**	**15.0**	**9.8**		**39.2**	**37.5**	**27.1**	**32.5**	**24.2**		***	**NS**	***	***	**NS**
species richness (all)	**10.5**	**12.3**	**11.7**	**12.6**	**12.0**		**13.9**	**16.9**	**17.6**	**16.5**	**16.2**		***	***	**NS**	***	**NS**
species richness (vasc.)	**8.8**	**11.2**	**10.6**	**11.0**	**10.5**		**12.2**	**15.2**	**16.0**	**14.8**	**14.8**		***	***	**NS**	***	**NS**

Discussion

Seedling emergence on dung

In contrast to later observations on juvenile and adult plants, significantly more seedlings emerged on dung which was deposited in undisturbed grassland [Fig. 6.2]. May be, this was due to a higher relative humidity in the grassland canopy compared to an unvegetated situation. Alternatively more proneness to herbivory in sod-cut plots appeared not to have been the case, since the number of seedlings that survived under cages in sod-cut plots was not significantly higher than in the intact grassland plots. From this part of the experiment it appears that dung of large herbivores could be a seed source and could represent a safe site for germination. However its role as safe site in itself seems limited in time and appears not very efficient for successful establishment.

Establishment from dung seed content

Dung deposition clearly resulted in an increased number of species per plot, more individuals and a higher cover degree of several plant species as compared to plots without dung deposition. Effects of dung deposition were most pronounced in the sod-cut plots.
Dung deposition may have contributed to this result in three possible ways, which can mutually interact.
Firstly, dung as a seed source could lead to the establishment of new plant species and new cohorts of individuals of species already present in the vegetation. Unfortunately, it was not always possible to attribute the establishment of new species or individuals to a specific seed source. More individuals of *Cerastium fontanum* and *Juncus bufonius* established in plots with dung deposition [Table 6.4]. Because of their low seed density in the soil seed bank and the contrasting [very] high density in dung samples, regeneration from the dung seed content is very plausible [Table 6.2]. Germination from the dung seed content was also very likely in the case of *Ranunculus repens* and *Trifolium repens* since both species were not recorded from the soil seed bank [Table 6.2], but nonetheless established in significantly more plots with dung deposition than without.

Secondly, after one year, most pronounced plant establishment occurred in initially disturbed [i.e. sod-cut] grassland. This suggests that the presence of gaps remains an important seed establishment factor. Since the addition of a complete dung cover on the vegetated plots did not create noticeable gaps, we conclude that the gap-forming ability of large herbivore dung in the quantities applied in the experiment, is rather limited as compared to other biological events, such as disturbances through mole or ant activities or biotic disturbance as uprooting and rolling activities of ungulates or burrowing rabbits [Bakker & Olff 2003]. For instance, *Juncus bufonius* that germinated far more frequently from dung than from the soil seed bank, established significantly better in sod-cut [frequent establishment] than in intact grassland plots with dung deposition [almost no establishment] [Table 6.4]. Some other early successional species such as *Arenaria serpyllifolia* and *Poa annua* showed a comparable behaviour. Moreover, plant species which were important constituents of the soil seed bank e.g. *Centaurium erythraea* and *Urtica dioica* did not show frequent establishment in intact grassland plots with dung deposition compared to the control plots but, in contrast, established very well in sod-cut plots.
Thirdly, dung deposition may have influenced vegetation composition also indirectly through its fertilisation effect [Bonis *et al.* 1997; Dai 2000]. Yet, in our study area Ampe *et al.* [2002] only found significantly more exchangeable K^+ and K^+-saturation in the upper 5 cm soil layer under medium aged and old horse and cattle dung. Nonetheless, within the experiment we can not exclude the possible relation between the observed higher cover of several plant species in 'dung treated plots' and some fertilisation effect.

Placement of cages resulted in a significant increase of seedlings on dung [Fig.6.2]. This suggests the presence of a certain degree of small mammal herbivory on these seedlings at the experimental site. At the stage of plant maturity a cage effect was sometimes observed [Table 6.4]. Inferior competitors such as *Cerastium fontanum*, *Juncus bufonius* and *Sagina procumbens* counted significantly less individuals and had a lower cover under cages. This striking result appeared indicative for other interacting mechanisms i.e. competition between some dominant grasses and the mentioned small herbs. The overall increase of *Calamagrostis epigejos* could result from reduced large herbivore grazing i.e. the insufficient simulation of grazing activity through clipping. The increase in cover of *Holcus lanatus* and *Calamagrostis epigejos* under the cages may well have resulted from small herbivore exclusion. As a consequence it is very probable that these two grass species negatively influenced the establishment of the inferior competitors.

Implications for vegetation dynamics

The establishment of plant species is of key importance in maintaining and restoring species richness in temperate, semi-natural grasslands. Several studies [Collins 1987; Burke & Grime 1996; Carson & Pickett 1999] already emphasized the role of disturbance in enhancing plant establishment. Our results are in concordance with these general findings. However, our results furthermore highlight on the interaction between disturbance and endozoochorous seed dispersal which clearly may affect species abundance and distribution patterns in grazed grassland. It appears that the grassland under study had a limited seed source, even for several generalist plant species, although they are present in small numbers in the soil seed bank and the above ground vegetation. The additional seed input through cattle or horse dung deposition led to higher establishment of e.g. *Trifolium repens* and *Cerastium fontanum*, both in sod-cut and intact grassland plots, stressing the role of dung deposition by large herbivores as a seed supplying mechanism [Bakker & Olff 2003]. The pronounced establishment only in sod-cut plots of some plant species [e.g. *Juncus bufonius*] furthermore underpins the idea that dung pats of large herbivores are less powerful in creating regeneration sites than other biologically induced mechanisms which cause more profound soil disturbance in grassland communities. It also indicates that suitable regeneration sites for some plant species were limited in the closed canopy of this grassland.
Our findings have practical implications for the conservation and restoration of species richness in temperate, semi-natural grasslands. We already found indications that large herbivores are able to disperse seeds by the process of endozoochory [Cosyns *et al.* Chapter 2]. The results of this study suggest that plant establishment from the dung germinable seed content

could affect the actual species composition whereas the remaining viable seeds [estimated at c. 90 %] might serve to replenish the soil seed bank, yielding a potential for future regeneration [Willems & Huijsmans 1994; Malo *et al.* 1998; Pakeman *et al.* 1998; Dai 2000].

In this study only common grassland species were involved, but succession towards more species-rich dune grassland will need the input and germination of seeds of more critical [specialist] species of which at least some can be dispersed endozoochorously, e.g. *Galium verum* and *Helianthemum nummularium* [Dai 2000; Cosyns *et al.* Chapter 2]. From a nature management point of view, this holds an argument to connect species-rich with species-poor sites by means of grazing ungulates, that can move freely from one site to the other. Several other techniques could be thought of, essentially all consisting of the collection of dung of free grazing large herbivores in species-rich areas and the input of the collected dung in species-poor areas [Traba *et al.* 2003].

7 Synthesis and conclusions

Helianthemum nummularium in Dry coastal grassland (Gd) - Westhoek North.

Introduction

In this thesis several aspects of the first phases of endozoochorous seed dispersal were examined. In this concluding chapter a synthesis is given of the information that might contribute to our knowledge of the significance of endozoochory as a seed dispersal mode i.e. aspects of its cost for the disperser and its effectiveness for the reproduction of plant species. Furthermore, the importance of endozoochory in relation to nature management and restoration is discussed. Possible avenues for future research are indicated.

Two major components are inherent to the [cost-]effectiveness of a seed dispersal agent, the quantity and quality of seed dispersal. The quantity is the number of seeds dispersed and its quality is the probability that a dispersed seed will produce a new reproducing adult [Schupp 1993]. Some aspects involved in these two major components of disperser effectiveness were treated in this work [Table 7.1].

Table 7.1 A hierarchical outline of the major components which determine the effectiveness of endozoochorous seed dispersal by ungulates [after Schupp, 1993]. Aspects treated in this work are indicated by the Chapter number [C.].

I. Quantity of seed dispersal
- A. Number of seeds transported
  - 1. abundance of the disperser
  - 2. diet [C.2, C.3, C.4]

II Quality of seed dispersal
- A. Quality of seed treatment
  - 1. destroy or pass seeds intact [C.5]
  - 2. alter percentage or rate of germination
- B. Quality of deposition
  - 1. movement patterns
    - a. habitat and patch selection [C.2]
    - b. rate and directionality of movement
  - 2. deposition patterns
    - a. rate and pattern of deposition [C.2]
    - b. seed [diet] mixing

The dung germinable seed content

Seed consumption in relation to diet selection

To make endozoochory happen, ungulates first need to consume seeds. This may occur as the result of selective feeding on fruits and inflorescences. It was shown in chapter 3 that the consumption of these plant parts contributed only to a minor extent to the diet composition of the free ranging horses and cattle. Yet, for some plant species this active seed acquisition through selective feeding may well be relevant as a first important step towards successful seed dispersal e.g. fleshy fruit bearing woody species [e.g. *Rosa* spp., *Crataegus* spp., *Ligustrum vulgare*, *Rubus* spp.]. However, interpreting the large discrepancy between true germination from dung and observed inflorescence consumption, allows to assume passive seed consumption to be the rule. Most seeds are ingested as an incidental part of some other food the ungulates are eating [Janzen 1984]. Horses and cattle are mainly graminoid-feeders although some differentiation in diet composition was observed between both animal species by Putman *et al.* [1987], Gordon [1989], Vulink [2001], Menard *et al.* [2002] and in chapter 3 of this study. When compared to the overall number of plant species, graminoids were well represented in the dung germinable seed content. Some dicotyledonous species were also an important part of the dung germinable seed content e.g. *Urtica dioica* [chapter 2 and 4]. Most of the plant species that showed high seed densities and appeared frequently in the dung samples were also regularly mentioned from other studies i.e. different species of *Caryophyllaceae*, *Poaceae* and *Juncaceae* [Appendix 7.1] This may be related to their commonness within several types of habitats across Europe, their regular consumption and their survival capacity after gut passage e.g. several graminoid species [chapter 5].

Plant species richness and seed densities

During the fruiting season, large ungulates are able to transport considerable amounts of germinable seeds of a wide variety of plant species [chapter 2, 3 and 4]. At least 23 % of all plant species which were recorded from

the study sites were dispersed endozoochorically, although only part of them in large amounts [chapter 2 and 4]. From our own data on endozoochory by sheep, donkey and rabbits [Claerbout, 2001] we could add a further 4 %. When taking into account the information from other European studies, 50 % of the plant species [i.e. 271], which were recorded from the coastal dune area are potentially dispersed via internal transport by ungulates or rabbits [Appendix 7.1].
The potential importance of endozoochory is furthermore stressed quantitatively by taking into account the defecation frequencies and mean dung volumes of the herbivores. In this dissertation [chapter 2] and by Malo & Suárez [1995] it was shown that domestic livestock is capable, depending on site and animal related characteristics, to disperse large amounts of viable seeds during the fruiting season i.e. about 300.000 - 1.200.000 seeds per individual animal.

The qualitative aspect of endozoochory

Passage through the gastero-intestinal system

Passage through the gastero-intestinal system is a critical phase in the endozoochorous seed dispersal process. In chapter 5 it was shown that the germinability of gut-passed seeds was drastically reduced for a considerable number of plant species. A comparable reduction in germination success was also reported by Lennartz [1957], Özer [1979] and Gardener *et al.* [1993]. However, Russi *et al.* [1992] and Ghassali *et al.* [1998] reported a higher germination success for some legume species which passed through sheep guts as compared to untreated seeds. However, for a considerable number of plant species internal seed transport represents a high cost i.e. loss of germinable seeds. This aspect represents an important difference with all other dispersal modes and a possible negative aspect as compared to the effectiveness of seed dispersal.

Our feeding experiments furthermore stressed the complex interaction between animal and seed characteristics and the considerable differences in germination success that exist between plant species. Because variation in germination success seems to be the rule, and the still large gap in our knowledge on relative germination success of most plant species justifies the execution of more feeding experiments. To advance our knowledge on the different parameters which may affect germination success of gut-passed seeds, further experimental research separating possible interacting animal characteristics is needed e.g. separation of chewing and digestion effect. the incorporation of more measurements of seed characteristics e.g. seed coat characteristics [thickness, hardness, physical structure, chemical composition] and the use of different sets of plant species each sharing similar seed characteristics may further help to unravel the determinants of the dung germinable seed content.

Directed seed dispersal and plant establishment

Directed dispersal is defined as non-random seed dispersal into particular habitats on the expectation of local success. There are two aspects to this definition; first, seeds must be deposited disproportionately in a subset of habitats and secondly, in order to have 'adaptive' directed dispersal these habitat types must be a favourable subset [Venable & Brown 1993; Wenney 2001]. In chapter 2 it was argued that directed endozoochorous seed dispersal might well be possible as a consequence of selective habitat use both for grazing and dunging. If both do not differ, plant species growing in grassland generally have the best chance to be dispersed into the same habitat, which is expected to be more suitable for germination and recruitment of the grassland specific plant species then other habitats. Plant species related to one of the other habitats [e.g. scrub and forest] have a minor chance ever to be dispersed endozoochorically in an identical habitat. On the other hand, whenever grazed they have far higher chance to be dispersed to grass dominated habitats. It is plausible to suggest that the most preferred habitats receive the largest proportion of viable seeds from plants that grow in other habitats. It can be hypothesised that these habitats therefore are more 'vulnerable' for invasion of new species. Although seed arrival is no guarantee of recruitment, the non-random dispersal to other habitats might induce directional succession, if late successional species are dispersed into earlier successive phases. However, several of these plausible assumptions need further and ample investigation.

Of course within a potentially suitable habitat it is not a priori sure that deposited seeds will all meet the right germination conditions. In chapter 6 it was shown that some generalist species could establish from the germinable seed content of horse and cattle dung. Dung deposition furthermore contributed to the increase of plant diversity of the studied grassland. Taking into account the observed defecation pattern that showed the likely deposition of dung in the studied habitat [C-grassland, chapter 2] the importance of endozoochory as a possible [long-distance] seed dispersal mode for a selection of plant species is indirectly shown.

[Directed] long-distance seed dispersal

Because of the size and scattered distribution of the preferred habitats within the landscape we may assume that large herbivores regularly move throughout the whole landscape. The potentiality of long-distance seed transport which revealed from several observations of animal movements in relation to calculated passage rates was mentioned in chapter 2. Distances of several hundreds of metres were regularly bridged within the studied sites. The mean retention times measured during the feeding trials [chapter 5] furthermore underpin this potentiality.
However, until now, to our knowledge, there exists no study that empirically has determined dispersal distances between parental plants and deposited seeds in cool temperate landscapes [but see e.g. Fragoso [1997] and Wehncke *et al.* [2003] for tropical situations]. The lack of such studies may be mainly due to the difficulties associated with these attempts i.e. the need for intensive observations on animal movement and behaviour and the high costs associated with attempts that will use highly sophisticated equipment [e.g. GPS-collar and equipment to record at least grazing and defecating activity].

The combination of long mean retention time [and hence the possible long-distance seed dispersal i.e. in fact seed dispersal within the whole home range or nature reserve area] and some directness in seed dispersal may be one of the important differences with other less directed seed dispersal modes i.e. resulting in different seed shadows. The ecological significance of such differences remains to be elucidated. Through such directed long-distance seed dispersal, plant species may move within and between habitats even in structurally highly heterogeneous semi-natural landscapes such as coastal dunes, semi-open forests [e.g. the New forest, Borkener Paradies, ...]. An other aspect associated with the disproportional use of highly heterogeneous landscapes for grazing and defecating is the alteration of nutrient dynamics which in the end may affect vegetation dynamics. Bokdam [2003] noted the removal of nitrogen, phosphorus and potassium from grass patches preferred for grazing by free ranging cattle and of an expected input of these nutrients in forest that was used as shelter for resting. Nutrients play an important role in the resource-mediated successional grazing cycles: nutrient accumulation may induce gaps and favour the establishment of lawn grasses [note: which can be part of the dung germinable seed content - this thesis]. Nutrient depletion may limit the longevity of lawn grasses by inducing wood encroachment.

Nature conservation implications

Since the legal protection of all remaining coastal dunes in Flanders [Decree of 14.07.1993[1]], nature management of this semi-natural landscape has gained momentum. Following the development of an ecosystem approach for the entire coastal dune area [Provoost & Hoffmann 1996] grazing of domestic livestock [horses, cattle, donkeys, sheep] has become a commonly used nature management tool [Cosyns et al. 2001]. It was expected that these herbivores would positively affect the diversity of vegetation structure and plant and animal diversity through a variety of mechanisms: direct damage by biomass removal, and ability of plants to avoid or tolerate this; damage by trampling and excretion; nutrient pulses in excreta; creation of vegetation gaps; and alteration of competitive interactions [Bullock & Marriot 2000]. But herbivores will also influence plant diversity through processes that affect colonisation rates [Olff & Ritchie 1998]. Until now, less attention was given to the latter. However, the importance of seed availability was already stressed by Bonn & Poschlod [1998] and Poschlod *et al*. [1998] who stressed the role and value of domestic livestock and other dispersal vectors and processes in the historic semi natural landscape as a source of plant species richness in different habitats.

The results of this study furthermore emphasise the potential role of cattle and horses as highly relevant dispersal agents for a wide variety of plant species actually present in the coastal dunes, inclusive germinable seeds of plant species that are of conservation interest [Appendix 2.1 and 4.1]. However, in relation to their low abundance in vegetation, the amount of germinable seeds of these species, recorded from dung was low [Fig. 2.3]. Out of the 107 Flemish Red List species, that were found in all four study sites, only 11 plant species occurred from dung samples. A higher proportion i.e. 36 out of the 143 target species [Red List and characteristic species, Chapter 2] which were recorded from all four study sites, were recorded from horse or cattle dung. The proportion species of which seedlings emerged from dung samples varied between habitat types. The proportion was lowest for the target species of white dunes [A] and scrub [S]. From a much higher percentage of dry and wet dune grassland [Gd, Gw] and grey dunes [T] target species, seedlings emerged from horse and cattle dung, suggesting their greater ability to get endozoochorically dispersed [Fig.7.1].

Although only few seedlings of target species were recorded from horse and cattle dung, the total amount of germinable seeds which got endozoochorically dispersed in the area, during summer, may be considerably high due to the number of grazers and their defecation frequency [Table 2.5]. It is also very plausible to assume that the amount of germinable seeds in dung was underestimated [see discussion Chapter 2]. On the other hand the establishment rate under natural conditions may be fairly low. The proportion of established plants in the field experiment at Westhoek South [Chapter 6] ranged between 8% [*Cerastium fontanum*] and 0.003% [*Urtica dioica*]. But part of the remaining seeds may have become part of the soil seed bank, waiting for suitable germination conditions.

Seed dispersal is necessary, both for the preservation of existing habitats and the development of new ones. The combination of on average long mean retention time [Table 5.5] and of selective habitat use for grazing and defecating [Fig. 2.5, Table 2.6] may result in perhaps the most characteristic pattern of endozoochorous seed dispersal, as compared to other seed dispersal modes such as anemochory or hydrochory, i.e. the disproportional dispersal of seeds throughout the grazed area, with concentrations towards habitats which the herbivores most use for grazing and defecating [Fig. 2.4.]

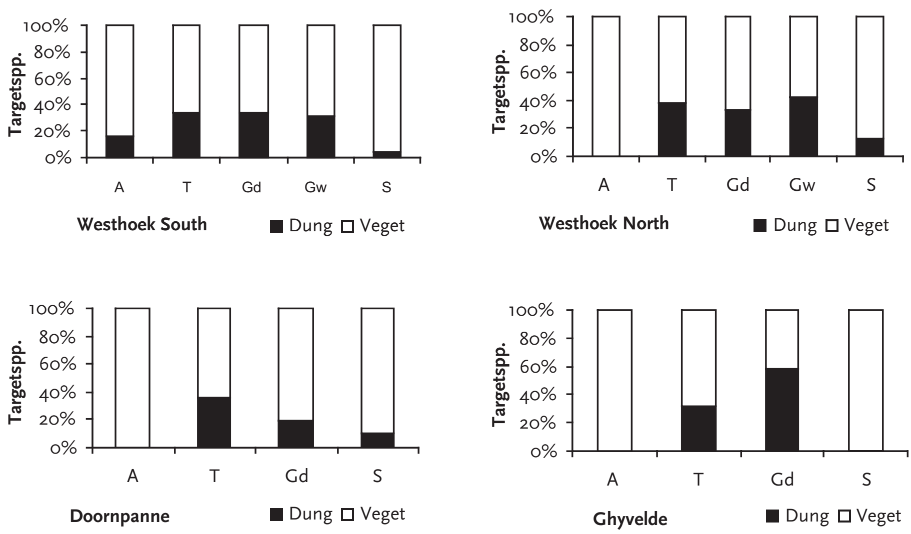


Figure 7.1. The proportional [%] amount of target species of five different coastal dune habitats of which germinable seeds were recorded from horse and cattle dung samples at the four mentioned study sites [A: white dunes, T: grey dunes, Gd, Gw: dune grassland on dry respectively wet soils and S: scrub - see also Chapter 1, Table 1.2].

The preservation or restoration of semi natural habitats is of key interest for nature conservationists and nature managers. Both, suitable environmental conditions and the availability of germinable seeds, are of major importance for successful nature management [Bakker & Berendse 1999]. Within the strongly fragmented semi-natural landscapes of Western-Europe it is important to realise the potential role of grazing ungulates as dynamic ecological corridors at different spatial scales. Knowledge of the potential seed dispersal capacities of e.g. target species is one important aspect that should be considered when setting realistic targets for nature management. It will also add evidence to nature management policy when deciding on the appropriate spatial arrangement of nature reserves and their management when aiming for a large scaled ecological network, that will maintain and restore biodiversity.

To illustrate the potential importance of having insight in aspects of the seed dispersal cycle we will rely on an example from the Westhoek. It was aimed by the nature managers to use domestic livestock to control for among others the dominance of coarse graminoids such as *Calamagrostis epigejos* which dominated species poor vegetations that established after scrub degradation [C1-habitat]. Four year of grazing did rapidly decline the dominance of *C. epigejos* and a less species poor grassland developed [Vervaet 2002, Table 6.1]. From hydrological data and soil conditions it can be expected that, under the current grazing regime, C1 habitat would most probably result in Gd habitat [Provoost *et al.* 2002]. However, the establishment of target species of dune grassland on dry soils [Gd] may take a long time, even under assumed, suitable abiotic conditions. Six Gd-target species were found in permanent plots in C1 habitat in 1998 and seven in 2001, after four years of horse and cattle grazing. A low number of seedlings of four Gd-target species, that were already established in the 2001-vegetation plots, were recorded nearby from the 2001-soil seed bank [Table 6.2]. Thus, the number of target species is still far away from the expected number of 30 species, that possibly ever could establish in the C1 area, from the local species pool [Fig. 2.3, Westhoek S.]. The still lacking target species can possibly been dispersed from the surrounding area [Westhoek South] or from elsewhere e.g. through connection of Westhoek

South and Westhoek North by grazing horses and cattle [Table 7.2].
We will now evaluate the potential contribution of endozoochory to further vegetation succession in C1-habitat for both cases. Considering the number of grazing animals, their defecation frequency and habitat use [chapter 2] and the amount of germinable seeds of target species found in dung, an estimate can be given of the amount of seed of target species which possibly could become deposited in the C1-habitat [Table 7.2.]. Within Westhoek south endozoochory could contribute to potential seed dispersal of 9 out of the 30 target species [Table 7.2]. Interspecific differences in dung germinable seed densities will influence the time needed for establishment, and its impact increases with a decreasing proportion of successful establishment.
In case of one large grazed nature reserve, 2 more endozoochorically dispersed plant species may become deposited in the C1 habitat [*Helianthemum nummularium* and *Ononis repens*, Table 7.2.]. Whether the remaining target species will ever arrive within the target area and by which means remains highly speculative. Wind, could be very well a plausible dispersal agent for e.g. *Hypochaeris radicata*, *Erigeron acer* or *Orobanche* spp. but it may not been overestimated as a potential long-distance seed dispersal modus which can bridge gaps between source and target areas [Soons 2003].

An important part of the dung germinable seed content exist from only a few species which on general are of low conservation interest [Fig. 2.2]. Moreover, these species such as *Urtica dioica*, *Agrostis capillaris* or *Poa trivialis* may determine vegetation succession and hamper other species to get established in the vegetation. But the results of the field experiment, obtained after one year [Table 6.4 and 6.5] did not held strong evidence for such scenario after dung deposition.

However grazing animals can play an important role in seed dispersal of a large variety of plant species [Appendix 7.1] for several other plant species they may not. Therefore, one should not only consider zoochory as the only one dispersal mechanism when trying to enhance the connectivity between sites for a spectrum of target species. Enhancing the impact of other seed dispersal processes e.g. flooding, or using alternative techniques e.g. transport of hay from one site to another and spreading it, or input of ungulate dung on a regular basis [see Traba, 2003]. Many aspects on seed availability such as dispersal modes, dispersal distances and longevity of seeds, still need further research. Reliable knowledge of dispersal mechanisms and their impact will contribute to evidence based nature management which will be characterised among others by realistic formulated targets for vegetation development and by designing the appropriate set of measures to enhance seed dispersal of target species.

Table 7.2 Hypothetical example of an estimate of the time needed before successful establishment of endozoochorically dispersed target species of dry calcareous dune grassland, will occur in a species poor C1-habitat at Westhoek South [WHS]. The estimate is based on seed numbers deposited by both grazer species in the C1-habitat [Fig. 2.4] and the number of individuals that will establish under three possible establishment rates [derived from chapter 6]. Besides the 9 species recorded from summer 2000 dung samples [Chapter 2], several other plant species potentially could rely on endozoochory [Endo] [Appendix 7.1].

Plant species	Actual presence		Endozoochory			Seed deposition					Proportion established		Time expect Establ.
	WHS	WHN	WHS	WHN	Endo	C1-habitat		10%	1%	0,1%	10%	1%	0,1%
	Veg	Veg	Dung	Dung		Horse	Cattle	Both			Years		
Aira praecox	*	*	*	*	*	255	36	29,09	2,91	0,29	1	1	3
Anthyllis vulneraria		*			*								
Arabis hirsuta	*	*											
Asperula cynanchica		*											
Avenula pubescens	*	*			*								
Briza media	*	*			*								
Bromus thominei	*	*			?								
Calamagrostis epigejos	*	*	*	*	*	38	18	5,63	0,56	0,06	1	2	18
Carex arenaria	*	*	*	*	*	159	285	44,37	4,44	0,44	1	1	2
Carlina vulgaris	*	*											
Cerastium arvense	*	*											
Cerastium semidecandrum	*	*			*								
Cirsium acaule		*											
Corynephorus canescens	*	*											
Erigeron acer	*	*											
Euphrasia stricta	*	*			?								
Festuca ovina		*			*								
Galium mollugo	*	*	*	*	*	108	31	13,93	1,39	0,14	1	1	7
Galium verum	*	*	*	*	*	13	13	2,57	0,26	0,03	1	4	39
Helianthemum nummularium		*		*	*	0	0	0,00	0,00	0,00			
Hypochoeris radicata	*	*			*								
Koeleria albescens	*	*			*								
Lotus corniculatus	*	*	*	*	*	0	10	1,03	0,10	0,01	1	10	97
Luzula campestris	*	*	*	*	*	32	5	3,70	0,37	0,04	1	3	27
Myosotis ramosissima	*	*			*								
Ononis repens		*		*	*								
Orobanche caryophyllacea		*											
Phleum arenarium	*	*			*								
Polygala vulgaris	*	*											

Plant species	Actual presence			Endozoochory				Seed deposition			Proportion established		Time expect Establ.
	WHS	WHN	WHS	WHN	Endo	C1-habitat		10%	1%	0,1%	10%	1%	0,1%
	Veg	Veg	Dung	Dung		Horse	Cattle	Both			Years		
Potentilla neumanniana		*			*								
Primula veris	*	*											
Ranunculus bulbosus	*		*		*	0	3	0,26	0,03	0,00	4	39	387
Rosa pimpinellifolia	*	*			?								
Rubus caesius	*	*	*	*	*	89	44	13,31	1,33	0,13	1	1	8
Rumex acetosella	*	*			*								
Sherardia arvensis		*			*								
Silene nutans		*			*								
Thalictrum minus	*	*											
Thesium humifusum		*											
Thymus pulegioides	*	*			*								
Vicia lathyroides	*				?								
Total # of plant species	**30**	**39**	**9**	**10**	**29**								

Perspectives for further research

Endozoochory by large ungulates is a common, but probably underestimated mode of seed dispersal in European semi-natural habitats. The potential for endozoochorous seed dispersal can not be derived adequately just from assumed morphological seed adaptation to specific dispersal mechanisms [chapter 4]. It is even more likely that seeds can be dispersed by more than one dispersal mechanism [[Bruun & Fritzbøger 2002, Johansson et al.1996]. Documentation on all kinds of seed dispersal modes is still needed and holds a basic step towards a further understanding of the possible consequences of seed dispersal limitations and to understand the relative importance of specific dispersal agencies [e.g. the presence or absence of certain animal species or specific processes such as flooding]. However, the relative significance of each of these mechanisms i.e. the amount of seeds that are successfully dispersed by each of them, the frequency of occurrence and their effectiveness certainly needs further investigation.

Appendix 7.1 Alphabetically ordered list of plant species of which germinable seeds were recorded from fresh dung of different Ungulate and Lagomorph species. The on average proportion seeds [%] to the total amount of germinable seeds [= 100] recorded from the indicated amount of dung is shown for each of the mentioned animal species. The on average proportion is also calculated across all animal species [= Total], P indicates presence without indication of seed density. Plant species that were mentioned in this Ph.D. study and by Claerbout [2001] i.e. data on rabbit, sheep and donkey dung in the Belgian and North French coastal dunes are shown in bold. Plant species that were only mentioned from these studies are followed by an asterisk [*] i.e. 59 species. The data are furthermore compiled from: Müller-Schneider [1954]; Müller [1955]; Welch [1985]; Malo & Suárez [1995]; Stender et al. [1997]; Bonn & Poschlod [1998]; Dai [2000]; Digneffe [2000].

	Animal species	Cattle	Deer	Donkey	Goat	Hare	Horse	Rabbit	Sheep	Total
	N [dung samples]	277	258	14	3	50	68	195	112	977
	Total weight dung [g]	30947	1301	2940	1245	150	14280	5206	6890	62959
	Proportion germinable seeds	%	%	%	%	%	%	%	%	%
N°	Plant species									
1	***Achillea millefolium***	0.09	0	0.09	0	0	0	0	0.00	0.05
2	*Achillea ptarmica*	0.00	0	0	0	0	0	0	0.03	0.00
3	*Agrostis canina*	0.00	0	0	0	0	0	0.20	0	0.02
4	***Agrostis capillaris***	13.38	4.34	0.66	0	2.04	2.56	4.91	0.53	7.78
5	*Agrostis gigantea*	0	0	0	0	0	0	0	0	P
6	***Agrostis stolonifera***	1.30	0.04	0.94	0	0	1.58	2.99	7.45	2.08
7	*Agrostis vinealis*	0.14	0	0	0	0	0	0	0	0.07
8	***Aira praecox***	0.11	0	0.07	0	0	3.66	10.28	0.74	1.82
9	*Alchemilla vulgaris*	0	0	0	0	0	0	0	0	P
10	*Alopecurus geniculatus*	0.01	0	0	0	0	0	0	0	0.00
11	*Alopecurus pratensis*	0	0	0	0	0	0	0	0	P
12	***Anagallis arvensis***	0	0	0	0	0	0.00	0.00	0.01	0.00
13	***Anchusa officinalis**** *	0	0	0	0	0	0.00	0	0	0.00
14	***Anthoxanthum odoratum***	0.01	0	0	0	0	0.01	0.04	1.45	0.16
15	***Anthriscus caucalis***	0.01	0.04	0.02	0	0	0.07	0.17	0.04	0.04
16	*Anthyllis vulneraria*	0	0	0	0.13	0	0	0	0	0.00
17	*Apera interupta*	0.00	0.12	0	0	0	0	0.02	0	0.00
18	*Aphanes arvensis*	0	0	0	0	0	0	0.00	0	0.00
19	***Aphanes inexpectata***	0.08	0	0	0	0	0.00	0.02	0.24	0.07
20	***Arabidopsis thaliana***	0.00	0.07	0	0	0	0.00	0.03	0	0.01
21	***Arenaria serpyllifolia***	0.62	0	0.30	0	0	2.69	8.35	6.37	2.30
22	*Arrhenaterum elatius*	0	0	0	0	0	0	0	0.03	0.00
23	***Artemisia vulgaris****	0	0	1.56	0	0	0	0	0.05	0.08
24	*Atriplex patula*	0	0	0	2.01	0	0	0	0.47	0.09
25	*Avenula pratensis*	0.00	0	0	0	0	0	0	0	0.00
26	*Avenula pubescens*	0	0	0	0	0	0	0	0.14	0.01
27	*Bellis perennis*	0.08	0	0	0	0	0	0	0	0.04
28	*Berberis vulgaris*	0	0	0	0.19	0	0	0	0	0.00
29	*Betula pendula*	0	0	0	0	0	0	0.00	0	0.00
30	*Betula pubescens*	0.00	0	0	0	0	0	0	0	0.00

		Cattle	Deer	Donkey	Goat	Hare	Horse	Rabbit	Sheep	Total
31	*Briza media*	0.04	0	0	0	0	0	0	0	0.02
32	*Bromus hordeaceus*	0.01	0	0	0	0	0	0	0	0.00
33	***Calamagrostis epigejos ****	0.02	0	0.60	0	0	0.08	0.01	0.00	0.06
34	***Calamagrostris canescens****	0	0	0	0	0	0.00	0	0	0.00
35	*Calluna vulgaris*	0.81	8.68	0	0	61.22	0	0.10	0	0.73
36	*Campanula rotundifolia*	0.08	0	0	0	0	0	0	0.06	0.05
37	**Capsella bursa-pastoris**	0.11	0.45	0.02	0	0	0.00	0.08	0.21	0.10
38	***Cardamine hirsuta***	0.01	0.13	0	0	0	0.06	0.06	0.01	0.03
39	*Cardamine pratensis*	0.08	0	0	0	0	0	0	0	0.04
40	***Carex arenaria****	0.38	0	4.29	0	0	1.03	0.11	4.28	1.08
41	***Carex flacca****	0.01	0	0	0	0	0.03	0	0.01	0.01
42	*Carex flava*	0	0	0	0	0	0	0	0	P
43	*Carex fusca [?]*	0.00	0	0	0	0	0	0	0	0.00
44	*Carex nigra*	0.08	0	0	0	0	0	0	0	0.04
45	*Carex panicea*	0.08	0	0	0	0	0	0	0	0.04
46	*Carex pilulifera*	0.81	0.43	0	0	2.04	0	0	0	0.41
47	***Carex trinervis****	0	0	0	0	0	0.01	0	0	0.00
48	*Carex verna*	1.08	0	0	0	0	0	0	0	0.53
49	***Carex viridula****	0	0	0	0	0	0.00	0	0	0.00
50	*Carum carvi*	0.12	0	0	0	0	0	0	0	0.06
51	*centaurea jacea*	0	0	0	0	0	0	0	0	P
52	*centaurea scabiosa*	0	0	0	0	0	0	0	0	P
53	***Centaurium erythraea****	0.02	0	0	0	0	0.16	0	0.12	0.06
54	***Centaurium littorale****	0.00	0	0	0	0	0.01	0	0.03	0.01
55	***Cerastium fontanum***	2.07	0.43	0.07	0	10.20	2.41	4.48	0.84	2.07
56	*Cerastium glomeratum*	0.20	4.42	0	0	0	0	0.10	0	0.20
57	***Cerastium semidecandrum***	0.05	2.34	0	0	0	0.03	0.54	0	0.12
58	*Chamerion angustifolium*	0	0	0	0	0	0	0.24	0	0.02
59	***Chelidonium majus ****	0	0	0.05	0	0	0	0	0	0.00
60	***Chenopodium album***	0.00	0	0.02	75.82	0	0.00	0.02	0.02	1.51
61	*Chenopodium bonus henricus*	0.00	0	0	1.11	0	0	0	0	0.02
62	**Chenopodium murale***	0	0	0	0	0	0.00	0	0.09	0.01
63	***Chenopodium rubrum***	0	0	0	0	0	0.00	0.00	0.02	0.00
64	*Chrysanthemum leucanthemum*	0	0	0	0	0	0	0	0	P
65	***Cirsium arvense***	0.01	0	0	0	0	0.00	0	0.01	0.00
66	*Cirsium palustre*	0	0	0	0	0	0	0	0	P
67	***Claytonia perfoliata****	0.01	0	0	0	0	0.01	0.01	0	0.00
68	**Cochlearia danica***	0	0	0	0	0	0.00	0.05	0.00	0.01
69	Colchicum autumnale	0	0	0	0	0	0	0	0	P
70	***Conyza canadensis****	0.00	0	0.05	0	0	0.03	0.01	0.24	0.04
71	*Cornus sanguinea*	0	0	0	0.11	0	0	0	0	0.00
72	*Cotoneaster integerrima*	0	0	0	0.19	0	0	0	0	0.00
73	*Crataegus monogyna*	0	0	0	0.11	0	0	0	0	0.00
74	***Crepis capillaris***	0.05	0	0.05	0	0	0.09	0.39	0.29	0.11
75	**Cynoglossum officinale***	0.00	0	0	0	0	0.00	0.00	0	0.00
76	Cynosurus cristatus	0.02	0	0	0	0	0	0	0	0.01
77	Dactylis glomerata	0.00	0	0	0	0	0	0	0	0.00

		Cattle	Deer	Donkey	Goat	Hare	Horse	Rabbit	Sheep	Total
78	Deschampsia cespitosa	0	0.43	0	0	0	0	0	0	0.01
79	Deschampsia flexuosa	0.08	0	0	0	0	0	0.04	0	0.04
80	***Diplotaxis tenuifolia****	0	0	0	0	0	0	0.02	1.26	0.13
81	Eleusine indica	0	0	0	0	0	0	0.00	0	0.00
82	Elymus repens	0.00	0	0	0	0	0	0	0	0.00
83	***Epilobium ciliatum****	0.01	0	0.02	0	0	0.02	0	0	0.01
84	***Epilobium hirsutum***	0.03	0	0.05	0	0	0.02	0	0	0.02
85	***Epilobium obscurum***	0.08	0.43	0	0	0	0	0.09	0	0.06
86	***Epilobium parviflorum****	0.00	0	0	0	0	0.01	0.00	0.03	0.01
87	***Epilobium roseum****	0.15	0	0	0	0	0.13	0.01	0.07	0.11
88	***Epilobium sp.***	0.15	0.01	0.02	0	0	0.29	0.02	0.13	0.15
89	*Erica cinerea*	0	0	0	0	0	0	0.01	0	0.00
90	***Erodium cicutarium/lebelii***	0.00	0.06	0	0	0	0.03	0.17	0.55	0.08
91	***Erophila verna***	0.00	0.04	0	0	0	0	0.29	0.29	0.05
92	***Eupatorium cannabinum***	0.00	0	0.09	0	0	0.09	0.01	0.45	0.07
93	*Euphorbia cyparissias*	0	0	0	0	0	0	0	0	P
94	*Euphrasia sp*	0	0.43	0	0	0	0	0	0	0.01
95	***Fallopia convolvulus****	0	0	0	0	0	0.00	0	0.02	0.00
96	***Festuca arundinacea****	0.00	0	0	0	0	0	0	0	0.00
97	**Festuca filiformis***	0	0	0	0	0	0.01	0	0.00	0.00
98	Festuca ovina	0.68	0	0	0	0	0	0.01	0	0.33
99	Festuca pratensis	0.00	0	0	0	0	0	0	0	0.00
100	***Festuca rubra***	0.14	0	0.05	0	0	0.06	0.12	0.31	0.13
101	*Filipendula ulmaria*	0	0	0	0	0	0	0.09	0	0.01
102	***Galium aparine****	0.04	0	1.37	0	0	0.07	0.02	0	0.10
103	***Galium mollugo***	0.02	0	1.24	0.33	0	0.17	0.05	0.01	0.12
104	***Galium palustre***	0.00	0	0	0	0	0.01	0	0	0.01
105	*Galium saxatile*	0.08	0.43	0	0	2.04	0	0	0	0.05
106	***Galium uliginosum****	0.02	0	0	0	0	0.33	0	0	0.08
107	***Galium verum***	0.07	0	1.37	0	0	0.41	0.14	2.10	0.43
108	*Genista sagittalis*	0	0	0	0	0	0	0	0	P
109	*Gentiana campestris*	0	16.02	0	0	0	0	0	0	0.33
110	*Gentiana cruciata*	0	1.34	0	0	0	0	0	0.03	0.03
111	***Geranium molle***	0.06	0.20	0.11	0	0	0.16	0.43	0.17	0.13
112	***Geranium robertianum****	0.00	0	0	0	0	0	0	0	0.00
113	*Glyceria fluitans*	0.00	0	0	0	0	0	0	0	0.00
114	***Gnaphalium uliginosum***	0.08	0	0	0	0	0.07	0	0.01	0.06
115	***Helianthemum nummularium***	0.10	0	0	4.90	0	0.01	0	0.25	0.18
116	*Heracleum sphondilium*	0	0	0	0	0	0	0	0	P
117	*Herniaria hirsuta*	0.01	0.04	0	0	0	0	1.81	0.03	0.16
118	***Hieracium umbellatum****	0	0	0	0	0	0	0	0.01	0.00
119	***Holcus lanatus***	1.22	0.43	2.04	0	2.04	2.64	5.28	0.29	1.78
120	***Hydrocotyle vulgaris****	0.01	0	0	0	0	0.03	0	0	0.01
121	***Hypericum perforatum****	0.00	0	0	0	0	0	0	0.09	0.01
122	***Hypericum tetrapterum****	0.00	0	0	0	0	0	0	1.23	0.13
123	***Hypochaeris radicata****	0	0	0.05	0	0	0	0.00	0	0.00
124	*Juncus acutiflorus*	7.28	0.38	0	0	2.04	0	0.09	0	3.62
125	***Juncus articulatus***	1.42	2.17	0.39	0	2.04	8.19	0.52	0.56	2.74
126	***Juncus bufonius***	21.83	9.21	0.37	0	0	19.84	0.41	0.08	15.55
127	*Juncus effusus*	1.62	2.17	0	0	10.20	0	0.27	0	0.89
128	*Juncus gerardii*	0	0	0	0	0	0	0	0	P

		Cattle	Deer	Donkey	Goat	Hare	Horse	Rabbit	Sheep	Total
129	***Juncus inflexus***	0.11	4.85	0	0	0	0.01	0.25	0	0.18
130	*Juncus squarosus*	0.08	2.17	0	0	2.04	0	0.06	0	0.09
131	***Juncus subnodulosus****	0.00	0	0	0	0	0	0	0.01	0.00
132	**Koeleria albescens***	0	0	0.07	0	0	0.00	0.03	0	0.01
133	Lathyrus pratensis	0.00	0	0	0	0	0	0	0	0.00
134	*Leontodon autumnalis*	0.00	0	0	0	0	0	0	0	0.00
135	*Leontodon hispidus*	0	0	0	0	0	0	0	0.03	0.00
136	***Leontodon saxatilis****	0	0	0.02	0	0	0.00	0.14	0.13	0.03
137	*Ligustrum vulgare*	0	0	0	0.22	0	0	0	0	0.00
138	*Linum catharcticum*	0.17	0	0	0	0	0	0	0	0.08
139	*Lithospermum officinale*	0	0	0	0.11	0	0	0	0	0.00
140	*Lolium perenne*	0.51	0	0	0	0	0	0.00	0.78	0.33
141	***Lotus corniculatus***	0.01	0	0	0.67	0	0.00	0	0.39	0.06
142	***Lotus pedunculatus***	0.00	0	0	0	0	0.01	0	0	0.00
143	***Luzula campestris***	0.01	0	0	0	0	0.08	0.01	0.03	0.03
144	*Luzula multiflora*	0.40	0.43	0	0	2.04	0	0.09	0	0.22
145	*Luzula sylvatica*	0	0.43	0	0	0	0	0	0	0.01
146	***Lychnis flos cuculi****	0.01	0	0	0	0	0	0	0	0.00
147	***Lycopus europaeus***	1.02	0	0	0	0	0.10	0	0.35	0.56
148	***Lysimachia vulgaris****	0.00	0	0	0	0	0.02	0	0	0.01
149	***Lythrum salicaria****	0.17	0	0.57	0	0	0.25	0	0.08	0.18
150	*Matricaria discoidea*	0.00	0	0	0	0	0	0	0	0.00
151	***Matricaria matricarioides***	0.08	0	0	0	0	0	0.00	0	0.04
152	***Medicago arabica****	0	0	0	0	0	0.00	0	0.02	0.00
153	***Medicago lupulina***	0.00	0	0.34	0.11	0	0.01	0	0.33	0.06
154	***Medicago minima***	0.00	0.01	0	0	0	0	0	0.01	0.00
155	*Melampyrum pratense*	0.00	0	0	0	0	0	0	0	0.00
156	***Mentha aquatica****	0.07	0	0	0	0	0.07	0	0.01	0.05
157	*Mentha arvensis*	0.00	0	0	0	0	0	0	0.02	0.00
158	*Mentha pulegium*	0	0.15	0	0	0	0	0	0	0.00
159	*Mentha suaveolens*	0	0.48	0	0	0	0	0	0	0.01
160	***Mercurialis annua***	0	0	0	0	0	0	0	1.80	0.19
161	*Moenchia erecta*	0.00	0	0	0	0	0	0	0	0.00
162	*Molinia caerulea*	0.08	0	0	0	0	0	0	0	0.04
163	*Montia fontana*	0	0	0	0	0	0	0.01	0	0.00
164	**Myosotis arvensis**	0	0	0	0	0	0.00	0.02	0	0.00
165	Myosotis discolor	0	0	0	0	0	0	0.01	0	0.00
166	**Myosotis ramosissima***	0.00	0	0	0	0	0.00	0.01	0.61	0.07
167	Myosotis scorpioides	0	0	0	0	0	0	0.09	0	0.01
168	Myosotis stricta	0	0.12	0	0	0	0	0.12	0	0.01
169	Nardus stricta	0.08	0	0	0	0	0	0	0	0.04
170	***Oenothera glazioviana****	0.03	0	0.44	0	0	0.06	0.00	0.22	0.07
171	***Ononis repens****	0.00	0	0	0	0	0.00	0	0.10	0.01
172	***Ornithopus perpusillus***	0.00	0	0	0	0	0	0	0.17	0.02
173	*Orobanche caryophyllacea*	0	0	0	0	0	0	0	0	P
174	*Papaver rhoeas*	0	0.01	0	0	0	0	0	0	0.00
175	***Phleum arenaria***	0	0	0	0	0	0	0.00	0.24	0.03
176	***Phleum pratense****	0.02	0	1.44	0	0	0.15	14.47	7.57	2.11
177	*Pimpinella major*	0	0	0	0	0	0	0	0	P
178	***Plantago coronopus***	0.01	0.01	0.02	0	0	0.15	2.01	0.59	0.27
179	***Plantago lanceolata***	0.51	0	0.37	0	0	0.06	0.17	1.32	0.43

		Cattle	Deer	Donkey	Goat	Hare	Horse	Rabbit	Sheep	Total
180	***Plantago major***	1.94	0	0.99	1.13	0	0.54	0.02	0.01	1.15
181	*Poa angustifolia*	0.04	0	0	0	0	0	0	0	0.02
182	***Poa annua***	0.74	4.59	0.55	0	0	3.83	2.40	0.24	1.58
183	***Poa pratensis***	9.11	0	3.41	0	0	3.73	0.52	5.52	6.13
184	***Poa trivialis***	3.86	0.07	3.85	0	0	3.11	0.25	0.35	2.85
185	***Poaceae spp***	0.01	0	0	0	0	0.00	0	0	0.00
186	***Polygonum aviculare***	0.08	0	0	0.33	0	0.00	0	1.45	0.20
187	*Polygonum convolvulus*	0	0	0	0.99	0	0	0	0	0.02
188	*Polygonum hydropiper*	0	0.09	0	0	0	0	0	0	0.00
189	*Polygonum lapathifolium*	0	0	0	0.63	0	0	0	0	0.01
190	*Polygonum persicaria*	0.00	0	0	0	0	0	0	0	0.00
191	***Potentilla anserina****	0.00	0	0	0	0	0.00	0	0	0.00
192	*Potentilla erecta*	0.08	0	0	0	0	0	0	0	0.04
193	***Potentilla reptans****	0.09	0	0	0	0	0.16	0.03	0.01	0.09
194	*Potentilla tabernaemontani*	0.00	0	0	0	0	0	0	0	0.00
195	*Prunella grandiflora*	0.00	0	0	0	0	0	0	0	0.00
196	***Prunella vulgaris***	0.26	0.43	0	0	0	0.07	0	0	0.15
197	*Pyrus communis*	0	0	0	0.11	0	0	0	0	0.00
198	*Ranunculus acris*	0.08	0	0	0	0	0	0	0	0.04
199	***Ranunculus bulbosus****	0.00	0	0	0	0	0	0	0	0.00
200	*Ranunculus flammula*	0.08	0	0	0	0	0	0	0	0.04
201	*Ranunculus parviflorus*	0.05	0.12	0	0	0	0	0	0	0.03
202	***Ranunculus repens***	0.98	0	0.02	0	0	0.24	0	0.00	0.54
203	***Ranunculus sceleratus****	0.00	0	0	0	0	0.00	0	0	0.00
204	***Ranunculus trichophyllus****	0	0	0	0	0	0.00	0	0.03	0.00
205	*Rhinanthus minor*	0.08	0	0	0	0	0	0	0	0.04
206	*Rosa spp.*	0	0	0	1.26	0	0	0	0	0.03
207	***Rubus caesius***	0.03	0	0.07	0.78	0	0.14	0.37	0.79	0.18
208	*Rubus idaeus*	0	0	0	0.86	0	0	0	0	0.02
209	*Rumex acetosa*	0.00	0	0	0	0	0	0	0	0.00
210	***Rumex acetosella***	0.16	0	0	0	0	2.37	9.90	0.05	1.45
211	***Rumex conglomeratus****	0.06	0	0	0	0	0.04	0.02	0.01	0.04
212	***Rumex crispus****	0.00	0	0.02	0	0	0.02	0	0.04	0.01
213	***Rumex obtusifolius***	1.25	0	0	0	0	0.00	0.01	0.01	0.62
214	***Rumex sp.***	0.01	0	0	0	0	0.01	0	0.07	0.01
215	***Sagina nodosa****	0.00	0	0	0	0	0	0	0	0.00
216	***Sagina procumbens / apetala***	7.86	5.14	0.02	0	2.04	3.39	9.03	0.22	5.54
217	*Salix repens*	0.08	0	0	0	0	0	0	0	0.04
218	*Salix sp.*	0	0	0	0	0	0	0.00	0	0.00
219	***Samolus valerandi****	0	0	0	0	0	0.02	0	0.13	0.02
220	*Sarothamnus scoparius*	0	0	0	0	0	0	0	0	P
221	***Saxifraga tridactylites***	0	0	0	0	0	0	0.01	0.01	0.00
222	*Scirpus holoschoenus*	0.01	0.07	0	0	0	0	0	0	0.00
223	***Scirpus setaceus***	0.10	0	0	0	0	0.06	0	0	0.06
224	***Sedum acre***	0.02	0	0	0	0	0.07	0.43	0.27	0.09
225	***Senecio jacobaea***	0.06	0	0.21	0	0	0.60	0.76	0.51	0.29
226	***Senecio sylvaticus****	0.00	0	0	0	0	0.06	0.10	0	0.02
227	***Senecio vulgaris***	0.00	0	0	0	0	0.03	0.18	0.01	0.02
228	*Sherardia arvensis*	0.00	0.01	0	0	0	0	0	0	0.00
229	*Sieglingia decumbens*	0.45	0	0	0	0	0	0	0	0.22
230	*Silene dioica*	0	0	0	0	0	0	0	0	P

		Cattle	Deer	Donkey	Goat	Hare	Horse	Rabbit	Sheep	Total
231	***Silene latifolia****	0.01	0	0.07	0	0	0.01	0.01	0.10	0.02
232	*Silene nutans*	0.08	0	0	0	0	0	0	0	0.04
233	***Solanum dulcamara****	0	0	0	0	0	0.01	0	0	0.00
234	***Solanum nigrum****	0.00	0	0.02	0	0	0.03	0.10	0.14	0.03
235	***Sonchus asper***	0.00	0	0	0	0	0.00	0.02	0.50	0.06
236	***Sonchus oleraceus***	0.00	0	0.21	0	0	0.02	0.02	0.06	0.02
237	*Sorbus aucuparia*	0	0	0	0.22	0	0	0	0	0.00
238	*Sparganium erectum*	0	0	0	0	0	0	0.00	0	0.00
239	*Spergularia rubra*	0	0	0	0	0	0	0	0	P
240	*Spergularia salina*	0	0	0	0	0	0	0	0	P
241	*Stellaria alsine*	0	0	0	0	0	0	0.00	0	0.00
242	***Stellaria graminea***	0.01	0	0	0	0	0	0	0	0.00
243	***Stellaria media***	0.40	14.31	0.16	0.13	0	0.66	2.15	0.09	0.84
244	*Stellaria nemorum*	0	0	0	0	0	0	0	0	P
245	*Stellaria uliginosa*	0.00	0	0	0	0	0	0	0	0.00
246	***Taraxacum sp.***	0.01	0	0	0	0	0.01	0	0.01	0.01
247	*Teucrium chamaedrys*	0	0	0	6.13	0	0	0	0	0.12
248	*Thymus pulegioides*	0	0	0	0	0	0	0	0.01	0.00
249	***Trifolium arvense***	0.00	0	0	0	0	0.00	0	0.25	0.03
250	***Trifolium campestre***	0.07	0.05	0	0	0	0.02	0.01	0.22	0.06
251	***Trifolium dubium****	0.02	0	0.25	0	0	0.23	0.06	0.63	0.15
252	*Trifolium pratense*	0.23	0	0	0	0	0	0	0	0.11
253	***Trifolium repens***	2.30	8.46	0.46	0.11	0	0.79	0.09	0.68	1.59
254	***Trifolium scabrum***	0	0.04	0	0	0	0	0	0.04	0.01
255	*Trifolium striatum*	0.00	0	0	0	0	0	0	0	0.00
256	*Trifolium subterraneum*	0.00	0	0	0	0	0	0	0	0.00
257	***Urtica dioica***	7.20	0	68.97	0	0	25.71	4.53	39.23	17.16
258	*Urtica urens*	0.02	0.62	0	0	0	0	0.03	0	0.02
259	*Vaccinium myrtillus*	0	0	0	0	0	0	0	0	P
260	*Vaccinium vitis-idaea*	0	0	0	0	0	0	0	0	P
261	*Verbascum thapsus*	0	1.56	0	0	0	0	0	0	0.03
262	*Verbena officinalis*	0.08	0.50	0	0	0	0	0.09	0	0.06
263	*Veronica arvensis*	0.00	0	0	0	0	0	0.54	0	0.05
264	*Veronica beccabunga*	0.01	0	0	0	0	0	0	0	0.00
265	***Veronica chamaedrys+arven***	0.79	0	1.47	1.31	0	5.71	7.48	1.73	2.59
266	***Veronica officinalis***	0.08	0	0	0	0	0.18	0.00	0	0.08
267	***Veronica serpyllifolia***	0.42	0	0	0	0	0.02	0.15	0.02	0.23
268	*Vicia angustifolia*	0.00	0	0	0	0	0	0	0	0.00
269	**Vicia cracca***	0	0	0.02	0	0	0.00	0	0.01	0.00
270	Vicia lathyroides	0	0	0	0	0	0	0.00	0	0.00
271	*Viola arvensis*	0.08	0	0	0	0	0	0.00	0	0.04
272	***Viola curtisii****	0	0	0	0	0	0.00	0	0	0.00
		100	100	100	100	100	100	100	100	100

[1] Vlaamse Regering [1993] Decreet houdende maatregelen tot bescherming van de kustduinen van 14.07.1993. Belgisch Staatsblad, 30.08.1993.

SAMENVATTING

Achtergrond en doelstellingen

Begrazing met hoefdieren in casu paard en rund wordt steeds vaker toegepast als natuurbeheermiddel, zo ook in de Vlaamse en Noordfranse kustduin-natuurreservaten [Fig. 1.2 en Tabel1.1]. Van grote herbivoren kan worden verwacht dat ze naargelang de milieuomstandigheden en de samenstelling van de herbivorengemeenschap, de plantendiversiteit significant zullen beïnvloeden. Minstens drie belangrijke factoren spelen hierbij een rol: [1] de invloed die door begrazing op de biomassa en reproductie van de dominante plantensoorten wordt uitgeoefend, [2] de regeneratieplekken die door de activiteiten van de dieren kunnen gecreëerd worden en tenslotte [3] de verbreiding van diasporen van planten door het uitwendig [epi-] of inwendig [endo-] transport ervan door de herbivoren [zoöchorie].
In dit doctoraatsproefschrift worden verschillende deelprocessen onderzocht van de verbreidingscyclus van plantenzaden[1] en dan met name endozoöchoor verbreide zaden [zie Fig. 1.1]

Ondanks de vroege belangstelling voor endozoöchorie zijn uitgebreide studies over deze manier van zaadverbreiding in het ondertussen sterk versnipperde, Noordwest-Europese, halfnatuurlijke landschap eerder schaars [hoofdstuk 1]. De resultaten van deze studies tonen nochtans aan dat veel méér plantensoorten, dan verwacht kan worden op basis van de morfologische aanpassing die hun zaden vertonen aan veronderstelde verbreidingsmechanismen, via inwendig transport door grote herbivoren kunnen verbreid worden. Om de ecologische betekenis van endozoöchorie te kunnen begrijpen is kennis nodig van de verschillende aspecten van de zaadverbreidingscyclus. Onderzoek hiernaar vereist een experimentele aanpak bvb. het bepalen van het relatieve kiemsucces van zaden na de doorgang door het spijsverteringskanaal of het onderzoek naar het kiem- en vestigingssucces van de in de mest aanwezige zaden onder veldomstandigheden en de mogelijke interactie tussen bodem- en mestzaadvoorraad. Onderzoek naar elk van deze aspecten is eerder schaars zeker voor wat betreft de Noordwest-Europese flora. In de weinige tot nu toe uitgevoerde zaadvoederexperimenten waarin Noordwest-Europese soorten zijn gebruikt werd vaak slechts één diersoort gebruikt [rund of schaap] wat vergelijking van

[1] Zaad wordt in deze samenvatting gebruikt om de generatieve verbreidingseenheid van de plant aan te duiden. De term wordt dus zowel gebruikt voor vruchten of schijnvruchten als voor zaden.

interspecifieke diereffecten quasi onmogelijk maakt.
Het doel van dit doctoraatsonderzoek is om het mogelijk belang van endozoöchorie door vrij grazende hoefdieren in een halfnatuurlijke context te bestuderen. Daartoe is er nood aan onderzoek dat focusseert op de verschillende stappen en deelprocessen die inherent verbonden zijn met deze manier van zaadverbreiding [Fig. 1.1]. Met dit proefschrift werd beoogd om:
bij te dragen tot de kennis van, voornamelijk door vrijlevende paarden en runderen, endozoöchoor verbreide plantensoorten;
de kiemkrachtige zaadvoorraad in de mest van vrijlevende paarden en runderen te vergelijken met hun dieetsamenstelling, ondermeer om het belang van passieve versus actieve zaadopname vast te stellen;
te onderzoeken welke zaadkenmerken aan het zaad de meeste kansen biedt op succesvolle endozoöchore verbreiding;
experimenteel het kiemsucces en de gemiddelde retentietijd van zaden van verschillende plantensoorten te bepalen na doorgang door het spijsverteringskanaal van verschillende diersoorten;
het experimenteel onderzoeken van het kiem- en vestigingssucces van in de mest aanwezige kiemkrachtige zaden onder veldomstandigheden om een eerste inschatting te maken van het belang van endozoöchorie voor plantverbreiding in de reële wereld.

Kwantitatieve en kwalitatieve aspecten van de kiemkrachtige mestzaadvoorraad

Het onderzoek is uitgevoerd in enkele natuurreservaten in de Belgische en Noordfranse kustduinen [Fig. 1.1]. De door paard en rund [Westhoek Noord en Zuid] of door paard alleen [Dunes Fossiles de Ghyvelde en Doornpanne] jaarrond begraasde, omheinde reservaatgedeelten zijn 30-75 ha groot en zeer soortenrijk [c. 500 spp zijn in deze gebieden waargenomen, dit is bijna de helft van de Vlaamse flora]. Deze omstandigheden boden daarom interessante perspectieven om te onderzoeken in welke mate zaden van de verschillende plantensoorten in dit gevarieerde halfnatuurlijke landschap door de vrij grazende hoefdieren endozoöchoor kunnen verbreid worden. In hoofdstuk 2 en hoofdstuk 4 zijn de voornaamste resultaten van dit beschrijvend onderzoeksluik weergegeven [Appendix 2.1 en 4.1]. In hoofdstuk 7 is door compilatie van onze gegevens met vergelijkbare, voornamelijk Noordwest-Europese onderzoeksgegevens een voorlopig overzicht gegeven van plantensoorten die endozoöchoor verbreid kunnen worden door gedomesticeerde en wilde hoefdieren, konijn en haas. Deze hoofdstukken bevestigen dat endozoöchorie een mogelijk onderschatte verbreidingsmodus is, waarvan meer plantensoorten zouden kunnen gebruik maken dan te verwachten op basis van de uitwendige zaadkenmerken .

De kiemkrachtige mestzaadvoorraad bestaat over het algemeen uit zaden van een groot aantal soorten [Tabel 2.4 en 4.3]. Van een beperkt aantal soorten komen de zaden zeer frequent en in hoge dichtheden voor. In vergelijking tot de dicotyle soorten komen er onder de 'graminoiden' [een verzamelterm voor Cyperaceae, Juncaceae en Poaceae] meer soorten voor die vaker en meer in de mest gevonden werden [Fig. 4.1 en 4.2]. Dit zou verband kunnen houden met de graasvoorkeur van paarden en runderen die in de zomer hoofdzakelijk 'graminoiden' consumeren [Tabel 3.2 en Appendix 3.1]. In hoofdstuk 3 werd vastgesteld dat de dieetsamenstelling en de respectievelijke kiemkrachtige zaadvoorraad in de mest van paard en rund [Westhoek Noord] een zekere mate van overeenkomst vertonen, enerzijds kwalitatief [Sörensen index resp. 40 % en 27 %] anderzijds beide kwantitatief en kwalitatief [Spearman Rank coëfficiënt]. De verschillen die tussen de dieetsamenstelling en de respectievelijke kiemkrachtige zaadvoorraad bestaan, zijn mogelijk te wijten aan een samenspel van verschillende factoren in het bijzonder de intrinsieke verschillen tussen plantensoorten om hun zaden endozoöchoor te laten verbreiden en onderzoektechnische aspecten bvb. de accuraatheid waarmee de dieetsamenstelling kan bepaald worden, de gehanteerde behandelingsmethoden van de mest en de toegepaste kiemingsomstandigheden die waarschijnlijk niet voor alle plantensoorten even ideaal zijn [zie hoofdstuk 3]. Anderzijds maakt deze scheve verhouding tussen waargenomen dieet en waargenomen zaadkieming uit mest het meer dan waarschijnlijk dat veel zaden passief [ongewild] geconsumeerd worden als onderdeel van vegetatieve delen van de voedselplanten. Door voor elke plantensoort de verhouding te bepalen tussen het aandeel in de kiemkrachtige zaadvoorraad in de mest [op basis van het aantal kiemplanten] en in de dieetsamenstelling [op basis van het aantal happen] kon een lijst opgesteld worden van plantensoorten die meer dan waarschijnlijk via endozoöchorie succesvol kunnen verbreid worden. Binnen de onderzochte set van gegevens konden elf soorten alsdusdanig gecatalogeerd worden o.a. Zomprus [*Juncus articulatus*], Greppelrus [*Juncus bufonius*], Grote brandnetel [*Urtica dioica*] en Veldbeemdgras [*Poa pratensis*] [Tabel 3.6]. Op basis van zijn 'Foliage is the Fruit' hypothese voorziet Janzen [1984] voor planten die endozoöchoor succesvol verbreid worden, tien mogelijke kenmerken, waarvan een aantal betrekking hebben op de voedingswaarde en eetbaarheid van de adulte, vruchtdragende planten en andere op de zaden. Voor de elf endozoöchoor goed verbreidende plantensoorten werd vastgesteld dat zij duidelijk langer kunnen [over-]leven in de bodemzaadvoorraad [hoge 'seed-longevity index'] en gemiddeld genomen kleiner zijn dan de minder succesvolle endozoöchoren. Er werd geen verschil vastgesteld tussen beide groepen voor wat de voedingswaarde van de bladeren betreft [uitgedrukt in functie van de gehaltes [%] celwandbestanddelen [NDF,ADF, ADL] en Ruw eiwit gehalte]. De andere zeven kenmerken konden niet getest worden.

In hoofdstuk 4 is onderzocht welke factoren de samenstelling van de kiemkrachtige zaadvoorraad in paardenmest kunnen helpen verklaren. Naast een duidelijk positief verband met de abundantie van de betrokken plantensoorten in de vegetatie en in het dieet blijkt er tevens een verband met een aantal zaadkenmerken. De relatieve densiteit aan kiemkrachtige zaden in de mest [i.e. rekening houdend met de abundantie in de vegetatie] is negatief gecorreleerd met het zaadgewicht, -lengte en -breedte en positief met de 'longevity index' [mate waarin een plantensoort in staat is een persistente bodemzaadvoorraad op te bouwen]. Er werd geen significant verband gevonden met de zaadvorm [uitgedrukt als variantie in zaadafmetingen] [Fig. 4.3 A-E] . De aan- of afwezigheid van een soort in de mest wordt niet beïnvloed door de aanwezigheid van bepaalde morfologische aanpassingen van het zaad aan veronderstelde verbreidingsmechanismen. De zaden van plantensoorten met een hoge 'seed longevity' index hebben meer kans dan verwacht kan worden volgens het toeval, om in de mestzaadvoorraad aangetroffen te worden [Tabel 4.5 a+b].

Gerichte zaadverbreiding over lange afstand?

In hoofdstuk 2 werd geargumenteerd dat via endozoöchorie door grote hoefdieren zaden gericht en over lange afstand kunnen verbreid worden. De gerichtheid houdt in dat de zaden niet willekeurig in een gebied verbreid worden. In de bestudeerde duingebieden blijkt meer mest [en dus endozoöchoor verbreide zaden], dan volgens het toeval kan verwacht worden, in ruigten en in grasland gedeponeerd te worden. Het omgekeerde is waar voor struweel, bos en mosduin waar precies veel minder gedefeceerd wordt [Tabel 2.5 en Fig. 2.3].

Uit het terreingebruik van de dieren gedurende de retentieperiode van de zaden kan afgeleid worden dat de dieren in staat zijn om zaden over het gehele natuurreservaat te verbreiden [verschillende honderden meters]. Dit houdt in dat de hoefdieren als potentiële lange-afstands-verbreiders kunnen beschouwd worden. Verder empirisch onderzoek is vereist om deze potentialiteit te bevestigen en om een preciezer beeld te krijgen van de zaadschaduwen. Ook de mate van gerichtheid en de betekenis ervan verdient verder onderzocht te worden.

Een hoge kost om endozoöchoor verbreid te worden?

Wellicht één van de meest kritische fasen voor een endozoöchoor verbreidend zaad is de doorgang door het spijsverteringskanaal van de herbivoor. In hoofdstuk 5 is het kiemsucces voor de zaden van 19 plantensoorten na voedering aan en passage doorheen het maag-darmstelsel van vijf diersoorten [paard, ezel, rund, schaap en konijn] onderzocht door middel van een voederexperiment met gekende hoeveelheden zaad [Tabel 5.1]. Het aantal zaden[2] van de 19 plantensoorten dat na doorgang door het spijsverteringskanaal nog kiemkrachtig bleek was veel geringer [0-26 %] dan in het geval van niet geconsumeerde zaden van dezelfde soorten [2-79 %] [Tabel 5.2]. Het kiemsucces van de zaden is specifiek voor elke unieke combinatie van plant- en diersoort [significante plant x diersoort interactie, Tabel 5.3]. Dit resultaat kan verklaard worden wanneer aangenomen wordt dat verschillen-

[2] Dit werd getest door de kieming uit meststalen na te gaan, waardoor geen onderscheid mogelijk is tussen zaden die geheel of gedeeltelijk verteerden tijdens de maag-darmpassage en het deel van de gepasseerde zaden dat nog kiemkrachtig is.

de plantensoort en diersoort gerelateerde factoren samen een rol spelen o.a. kauwgedrag [manier, duur...], fysiologie van de vertering [tijdsduur, biochemische samenstelling spijsverteringssappen,...] en zaadkenmerken b.v. dikte en structuur van de zaadhuid, afmetingen van de zaden. Een covariantie-analyse met zaadgewicht, zaadvorm [uitgedrukt als lengte/breedte ratio] en de 'seed longevity-index' als covariablen, wees in dit experiment op een duidelijke invloed van zaadvorm en 'seed longevity-index' op het kiemsucces van de zaden na doorgang door het spijsverteringskanaal. Er werd evenwel geen interactie tussen diersoort en één van deze covariabelen vastgesteld wat er op wijst dat deze zaadkenmerken in alle gevallen een vergelijkbare invloed hadden op het kiemsucces van de zaden [en overlevingskansen na passage van het spijsverteringsstelsel].
Lang-levende zaden of zaden met een ovale of lancetvorm [vooral grasachtigen]hadden in dit experiment een beter kiemsucces dan kort-levende [lage seed longevity index] zaden of zaden met een eerder ronde vorm. De zwak positieve, doch significante relatie tussen zaadvorm en kiemsucces was niet verwacht. Deze is waarschijnlijk inherent aan het experiment [plantensoortensamenstelling].

Vesting van planten vanuit de mestzaadvoorraad onder veldomstandigheden

Een volgende belangrijke stap in de endozoöchore zaadverbreidingscyclus is de vestiging van planten vanuit de mestzaadvoorraad. In hoofdstuk 6 is het gecombineerd effect onderzocht van in een grasland gedeponeerde uitwerpselen als een mogelijke zaadbron, als regeneratieplek en als bron van plantenvoeding [bemesting]. Daartoe is in een experiment met een factoriële opstelling, paarden- en rundermest uitgespreid in geplagde en niet geplagde proefvlakjes. Een deel van de proefvlakjes werd daarenboven afgeschermd van eventuele zaadpredatie en herbivorie door knaagdieren [Fig. 6.1]. Kieming op de mest gebeurde onder alle omstandigheden het best in de niet geplagde proefvlakjes [Fig. 6.2]. Afscherming van granivorie en herbivorie resulteerde eveneens in een beduidend hoger aantal kiemplanten. De vestiging van planten werd geëvalueerd na één jaar. In vergelijking tot de proefvlakjes zonder mest, werden gemiddeld 2 tot 3 meer plantensoorten waargenomen in de met mest behandelde proefvlakken, ongeacht de andere toegepaste behandelingen. Generalisten o.a. ruderale éénjarigen, waren sterk vertegenwoordigd in de kiemkrachtige mestzaadvoorraad en bleken ook met meer individuen en met een hogere bedekking voor te komen in de met mest behandelde proefvlakjes. De vestiging verliep voor een aantal soorten, ondermeer Greppelrus [*Juncus bufonius*] en Zandmuur [*Arenaria serpyllifolia*] beduidend beter in de geplagde proefvlakjes wat een aanwijzing is dat mest op zich een minder geschikte regeneratieplek kan zijn dan naakte bodem [Tabel 6.4 en 6.5]. Het belang van de mestdepositie in halfnatuurlijke Noordwest-Europese graslanden lijkt daarom eerder gelegen te zijn in de aanvoer van zaden dan in een positieve invloed op het kiemingsmilieu via aanvoer van plantenvoeding of creatie van open ruimte.

Conclusies

In hoofdstuk 7 is gepoogd om de ecologische betekenis van endozoöchorie te evalueren op basis van de resultaten van de eraan voorafgaande hoofdstukken en door vergelijking met reeds eerder uitgevoerd onderzoek. Een cruciale eerste stap in het endozoöchorieproces is de opname van zaden in het spijsverteringskanaal. Daartoe werd aangetoond dat tijdens het grazen, gewild of ongewild, van een aanzienlijk aantal plantensoorten grote hoeveelheden zaden geconsumeerd worden. De resultaten bevestigen verder dat endozoöchorie voor een groter aantal plantensoorten dan verwacht kan worden op basis van de morfologische zaadkenmerken, een valabel zaadverbreidingsmechanisme kan zijn. Toch gaat met endozoöchorie blijkbaar een grote kost gepaard, het totale aantal kiemende zaden wordt door maagdarmpassage immers sterk gereduceerd.

Tegenover deze hoge kost staan vermoedelijk een aantal voordelen ondermeer de gerichtheid waarmee zaden worden verbreid als gevolg van het selectieve habitatgebruik voor grazen en defeceren van de grote grazers. Dit doet veronderstellen dat met name aan grasland gebonden plantensoorten van deze gerichtheid kunnen profiteren nl. ze maken meer dan elders kans om hun zaden door de hoefdieren te laten opnemen en in een vergelijkbaar habitat opnieuw te laten uitscheiden. Daarenboven is er veel kans dat de zaden op een aanzienlijke afstand van de moederplant terecht komen wat doorgaans gunstig is voor de verdere ontwikkeling tot een zich voortplantend individu. Met name rond deze aspecten van endozoöchorie door hoefdieren is nog extra en gericht empirisch en modelmatig onderzoek noodzakelijk. De plantensoorten waarvan de zaden aldus met mest in grasland gedeponeerd worden hebben wel degelijk kans om minstens een aantal individuen zich te laten vestigen vanuit de mestzaadvoorraad. Hiermee wordt de effectiviteit van het verbreidingsmiddel benadrukt.

De ecologische betekenis van endozoöchorie is verder af te leiden uit de hoeveelheid zaden die effectief door de hoefdieren kunnen verbreid worden. Globaliserende berekeningen wijzen uit dat in de bestudeerde natuurgebieden al gauw enkele honderdduizenden, na maag-darmpassage nog steeds kiemkrachtige zaden per zomer door één enkel individu van paard of rund worden getransporteerd. In het kader van natuurherstel en het behoud van lokale en regionale plantensoortendiversiteit mag het fenomeen van endozoöchorie bijgevolg niet onderschat worden. In Tabel 7.2 is een overzicht gegeven van de verschillende situaties waarin endozoöchorie [en vermoedelijk ook epizoöchorie] een meer of mindere rol van betekenis kan spelen.

Tenslotte is uit voorgaande hoofdstukken duidelijk geworden dat endozoöchorie door hoefdieren een mogelijk onderschatte manier van zaadverbreiding is temeer daar het optreden ervan niet uit de aan- of afwezigheid van morfologische zaadkenmerken adequaat kan afgeleid worden. De relatieve betekenis ervan ten opzichte van andere verbreidingsmechanismen moet echter nog verder onderzocht worden en dit bij voorkeur onder verschillende ruimtelijke omstandigheden in meer of minder gefragmenteerde halfnatuurlijke landschappen.

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The use of domesticated ungulates i.e. cattle, horses, sheep has become increasingly popular in NW-European nature management of semi-natural landscapes. Grazing has received considerable attention from biologists who aimed to determine its impact on plant diversity. It is obvious and frequently proven that grazing influences plant diversity, but the reasons why its effect may differ considerably are less clear. One possible component of the answer to this question is the aspect of ungulate seed dispersal. However, the role of these animals in the dispersal and establishment of plant species is still poorly understood. Unravelling zoochorous dispersal mechanisms in a semi-natural environment may therefore offer both fundamental and necessary applicable ecological knowledge. In this thesis we contribute on a selection of aspects related to endozoochory in a semi natural context.

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