Reduced germination success of temperate grassland seeds sown in dung: consequences for post-dispersal seed fate

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Keywords

Dispersal success; endozoochory; germination; grassland species; seed dispersal.

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Editor

R.Bekker

ABSTRACT

* Endozoochory is one of the main drivers shaping temperate grassland communities by maintaining plant populations of its constituents and enabling plants to colonize new

habitats. Successful endozoochorous dispersal implies that seeds not only get con- sumed and survive the digestive tract but are also able to develop into viable seedlings in a dung environment.

* We experimentally assessed the germination probability and timing of 15 annual and

perennial temperate European grassland species in cattle and horse dung and in differ-

ent climatic conditions (greenhouse and outdoor conditions).

* Interspecific variation in germinability and germination timing are found, while life strategy had only an effect on germination timing. We found adverse effects of both

cattle and horse dung on the germination characteristics of all tested grassland species, but the effects of cattle dung were more pronounced. In comparison with the control treatment, fewer seeds emerged in dung and more time was needed to germinate. Also, germination metrics clearly differed between the artificial greenhouse and outdoor conditions, with generally a lower germinability in outdoor conditions.

* According to our results, a large cost seems to be associated with endozoochorous dis-

persal in this stage of the life cycle, as seed dispersal effectiveness strongly depends on

the quality of the deposition site with a lowered survival and germination probability when seeds are deposited in dung.

INTRODUCTION

Seed dispersal has a direct influence on individual plant fitness as the deposition site determines whether a seed will live, and eventually germinate and establish, or die (Wenny 2001). Con- sequently, plant community composition, structure and dynamics are initially shaped by the spatial patterns of seed dis- persal and dispersal success (Schupp & Fuentes 1995; Nathan

& Muller-Landau 2000). Diaspore morphology determines to a large extent whether the most likely dispersal vector is abiotic (*e.g.*, wind, water) or biotic (animals or the plant itself) (Levin *et al.* 2003; Albert *et al.* 2015). One of the animal-mediated dis- persal processes is endozoochory implying that seeds get dis- persed via ingestion by vertebrate animals and consecutive deposition in a dung environment. Endozoochory has been

documented in a wide range of habitats and latitudes from spe- cies carrying fleshy, palatable fruits (Traveset *et al.* 2007) to herbaceous species lacking any apparent morphological adap- tations to dispersal (Pakeman *et al.* 1998; Mouissie *et al.* 2005b). The ‘foliage is the fruit’ hypothesis postulates that graz- ing herbivores are attracted to the palatable foliage of the spe- cies in the latter group and ingest seeds rather inadvertently (Janzen 1984). Endozoochory by large herbivores enables long distance seed dispersal as the gut retention time of seeds is rather long (Cosyns *et al.* 2005b) and animal mobility can be

high (Nathan *et al.* 2008), and is often much higher than other dispersal agents. Furthermore, many germinable grassland spe- cies have been found highly concentrated in herbivore dung which suggests that endozoochory is one of the main drivers, shaping temperate grassland communities (Pakeman *et al.* 2002; Cosyns *et al.* 2005a; Couvreur *et al.* 2005).

In the last decades, zoochorous dispersal of grassland seeds

and the consequences for nature restoration and conservation gained much scientific attention. Nevertheless, most of these studies focus on diet choice, animal behaviour and/or the pres- ence of germinable seeds in dung, and, therefore, provide valid proof for the existence and relevance of this dispersal mode, but few examine the consequences of the quality of depostion sites. Undoubtedly, the first step in successful endozoochorous dispersal includes seed survival in the consecutive digestive processes (mastication, rumination and digestion). Gut passage may result in the breaking of dormancy and thus in enhancing or reducing germination success and timing (Traveset 1998; D’hondt & Hoffmann 2011). Besides that, the deposition of seeds in a highly nutritive environment such as dung may affect germination and the early development of seedlings as well (Deshaies *et al.* 2009).

The effect of dung on post-dispersal germination and growth

is less studied and is subject to rather contrasting results. Fresh dung often suppresses the existing vegetation and creates,

therefore, a beneficial microhabitat for germination by elimi- nating competition with the already developed vegetation (Tra- veset 1998). Dung can also shape plant communities by changing the relative abundance of species in the soil seed bank and by providing extra nutrients for the growth and flowering phase of endozoochorously dispersed seeds (Traveset 1998; Dai 2000). The moist and nutrient-rich dung environment leads in some cases to more and faster germination (*e.g.*, Archer & Pyke

(1991), Carmona *et al.* (2013), Malo & Suarez (1995a), Quinn *et al.* (1994), Traveset *et al.* (2001)), while germination was inhibited in other studies (*e.g.*, Carmona *et al.* (2013), Izhaki & Ne’eman (1997), Par'e *et al.* (1997)). Besides a high concentra-

tion of growth promoting macronutrients (N, K and to a lower extent P, Ca and Mg) and essential trace minerals (*e.g.*, Fe, Mn and Cu) (Haynes & Williams 1993; Lupwayi *et al.* 2000), ani- mal excrements contain growth-inhibiting substances such as phenolic compounds and fatty acids. These phytotoxic com- pounds may alter the activity of enzymes that regulate the ger- mination rate and, therefore, inhibit germination of certain plant species (Marambe *et al.* 1993).

The chemical and structural composition of fresh dung highly depends on herbivore diet (*e.g.*, captive *versus* free-ran- ging animals), but dung quality is also linked with the nutritive physiology of mammals as on average dung from non-rumi- nant species is more fibrous and has a lower nitrogen content compared with ruminant dung (Holter 2016). Furthermore, the composition of dung changes while ageing, with decreasing water and nitrogen content (Holter 1991, 2016), and the nature of these changes might also vary between dung types. The rapid desiccation of dung types consisting of small pellets (such as sheep and rabbit excrements) or the appearance of a hard and dry crust on watery dung types (such as cattle dung) may also prevent the imbibition of seeds prior to germination (Welch 1985; Eichberg *et al.* 2007). Depending on the climatic circum- stances and its composition, dung can become a very dry envi- ronment which is suboptimal for germination and seedling growth. This is certainly the case with cow dung and dung of many other ruminants. Dung pad desiccation starts superfi- cially creating a dry more or less impermeable crust, isolating seeds in the dung from the local environment; in the end, the dung pat may dry out completely. Many germination trials have been conducted either in controlled greenhouse condi- tions or in outdoor conditions. In a greenhouse or standard- ized lab environment, the temperatures are likely to be higher and more constant and the water supply is kept optimal for plant growth, which makes it hard to extrapolate greenhouse results to true outdoor conditions. Studies comparing both greenhouse and natural conditions are rare, but generally found more and faster germination in controlled lab or green- house environments compared to outdoor conditions (Welch 1985; Ramos-Font *et al.* 2015).

We conducted a dung addition experiment using 15 herba-

ceous plant species which are commonly found in temperate European grasslands and have a known potential for endozoo- chorous dispersal. Since cattle (used as a model species for a ruminant) and horses (used as a model species for a hindgut fermenter) are commonly introduced in nature management in these habitats, we used dung of both opposing herbivore types in the experiments. Environmental differences were addressed by replicating the experiment in both greenhouse and outdoor conditions. The questions raised in this study are:

1. What is the effect of the presence of dung on germinability and germination timing?
2. Why and to what extent do greenhouse conditions modify the germination process compared to outdoor or natural conditions?
3. Does the germination response differ between cattle (rumi- nant) and horse (hindgut fermenter) dung?

MATERIAL AND METHODS

Plant species

About 15 grassland species belonging to seven plant families were used. In most families both annual and perennial species were selected as species capable of setting seeds the first year have a tendency to germinate sooner and more abundantly (Shipley & Parent 1991). Most species are common in temper- ate European grasslands and were previously shown to germi- nate from cattle and horse dung (Cosyns & Hoffmann 2005; Cosyns *et al.* 2005a,b; Couvreur *et al.* 2005). Cistaceae species are less common but were added as earlier research suggested that endozoochory might be an important dispersal mecha-

nism in this family (Manzano *et al.* 2005; Ramos *et al.* 2006; Mancilla-Leyton *et al.* 2011). For example, the distribution of *Helianthemum nummularium* remarkably increased after the introduction of large herbivores in calcareous dune grasslands in Belgium and the germination rate of this species is known to increase after ingestion (D’hondt & Hoffmann 2011; Provoost *et al.* 2015). *Tuberaria guttata* is a species with a Mediterranean distribution (Herrera 2004) and has been found germinating in dung as well (Malo & Suarez 1995b, 1996). Intact seeds of Cis- taceae species are known to germinate very slowly due to their hard and water-impermeable seed coat (Thanos *et al.* 1992) while mechanical scarification of seeds is commonly used to induce germination (P'erez-Garc'ıa & Gonz'alez-Benito 2006; Miloti'c & Hoffmann 2016). In order to gain a complete over-

view of the germination process of the Cistaceae species, both intact and pre-treated seeds of *H. nummularium* and *T. guttata* were tested. Pre-treated seeds were mechanically scarified using fine grit sandpaper with average particle diameter of 125 lm. The scarification process was standardized by putting the seeds between two pieces of sandpaper which were moved twice.

Seed quality test

All seeds were purchased in specialized web shops. Prior to the dung addition experiments, seed quality was tested in standardized lab conditions. In this trial, 50 seeds per repli- cate were sown at equal distance in Petri dishes filled with 1% water agar. Per species five replicates were used and all Petri dishes were arranged in a complete random design in a germination chamber with day: night fluctuating tempera- tures between 14 and 30 °C with a 12 h day: 12 h night per- iod. Light was provided by white fluorescent tubes (Sylvania, 36W, Germany) with a light intensity (photosynthetic active radiation, PAR) between 10 and 45 lmol·m-²·s depending on the location on the rack. Newly germinated seeds were counted daily during the first 3 weeks of the trial, and after- wards once every 2 days until the 60-day germination trial period ended. Germination was considered to occur when- ever a white radical emerged through the testa. After each

counting round, all germinated seeds were discarded and the Petri dishes were put in new randomized sequence in order to lower the impact of possible unequal abiotic conditions in

MTG ¼

P*k*

*N* ð1Þ

*i*¼1*ti* \* *ni*

the germination chamber.

Dung addition experiment

The experiment was run simultaneously in outdoor and greenhouse conditions. At both locations, three treatments were used: cattle and horse dung treatments and a control treatment in which no dung was added. Round plastic plant pots (diameter: 15 cm, height: 16 cm) with drainage holes were either put on felt fabric in the greenhouse to limit evapo- ration or on a sturdy plastic screen in order to prevent inter- action with the existing vegetation in the outdoor experiment. The outdoor experiment was covered with a net in order to prevent interference of wild rodents or birds with the experi- ment. Pots were filled with a 1:1 mixture of sand and com- post. Dung was collected from stabled cattle and horses in order to keep contamination with wild seeds minimal. Before sowing, the pots were watered until the soil felt moist. 20 seeds were sown per pot with 20 replicates per treatment in both environments, summing to a total of 120 plant pots per species. In the treatments including dung, the entire surface of the pots was covered with dung and the seeds were mixed with dung in order to simulate seed deposition following endozoochory. In the control treatment, seeds were put homogeneously on the substrate surface in order to mimic dung-unassisted dispersal. Blank pots covered by either cattle or horse dung, or no dung (but without added seeds) were installed in both environments to check for seed contamina- tion originating from the sand-compost mix, dung or sur- roundings. All pots were put in a complete randomized

where *ti* is the time from the start of the experiment to the *i*th observation, *ni* is the number of seeds germinated within the period *i*, *N* is the total number of germinated seeds during the experiment and *k* is the last time of germination (Ranal & Gar- cia De Santana 2006).

All analyses were performed with R version 3.2.2 (R Core Team, 2015). Germinability and MTG of the seeds in the seed quality test were compared among species using one-way ANO- VAs and Tukey *posthoc* tests. Nested ANOVA’s were used on the entire dataset of the dung addition experiment with ger- minability and MTG as measured variable and dung type, life strategy, environment and family as nominal variables with a nested family: species term. The initially included life strategy – dung and life strategy – environment terms were discarded from the final models as no significant interactions were found and all nominal variables were kept as main effects. For each individual species, the effects of cattle or horse dung addition and the environment of the pots (greenhouse or outdoors) on germinability were analyzed by two-way ANOVAs and Tukey *posthoc* tests in order to distinguish significantly differing dung types. In order to meet the assumptions of parametric tests, germinability and MTG data were respectively arcsine square- root and log10 transformed.

Survival analysis is increasingly being used to analyse germi-

nation data and is especially valuable in analysing germination timing in case germination is measured in time intervals (Ono- fri *et al.* 2010; McNair *et al.* 2012). The germination probability was estimated non-parametrically using the Kaplan–Meier method:

*s*

design. Pots in the greenhouse were watered manually on a daily basis while outdoor pots were subjected to precipitation and did not receive any additional water. Both experiments were run in natural daylight cycles; no artificial lighting was

Y

ð Þ ¼

*S t*

*j*¼1

*dj* \

1 -

*n*

*j*

ð2Þ

used in the greenhouse. Seeds were sown on April 5th, 2012 at both locations. From then on, seed germination was moni- tored in 3-day intervals during the first 20 days of the experi- ment, while the counting effort was lowered to weekly counts afterwards. As the outdoor experiment got invaded by rabbits at day 65, the experiment was terminated at day 62 when the last rabbit-free count took place. In order to test the germina- tion of slow-germinating species, the greenhouse experiment was monitored longer and was stopped at day 200 after no germination had taken place during the preceding 50 days. During the experiment, temperature and relative humidity of air and soil was measured in 15 min intervals using HOBO U23 Pro v2 Temperature/Relative Humidity Data Loggers (ONSET, USA). In total, four devices were used which either were put on top of a planting pot or dug into the substrate in outdoor and greenhouse conditions.

Data analysis

At the end of the experiments, germinability and mean time to germination (MTG) were calculated for each individual pot. Germinability was expressed as the cumulative percentage of seeds that had germinated by the end of the experiment while MTG was calculated as:

where *dj* is the number of seeds germinating in a given interval

of time *j* and *nj* is the number of seeds “at risk” of germination

in the same interval which is defined as the number of non-ger- minated seeds entering the interval minus one-half of the num- ber of seeds germinated during that same interval (Venables & Ripley 2002). Germination probability was calculated for each species and dung type using the function *survfit* in the package ‘survival’ version 2.38-3 (Therneau 2015). The resulting germi- nation curves were compared with AFT (accelerated failure time) regressions using the *survreg* function in R. As suggested by (Onofri *et al.* 2010) the problem of clustering seeds within randomization units (plant pots) was overcome by adopting a

frailty approach with plant pots as the clustering (gamma dis- tributed) random effect. Exponential, Weibull, Log-Normal and Log-Logistic distributions were tested per species. The best fitting model for each species was selected using the second order Akaike’s information criterion (AICc) and a graphical comparison of the model outcome with the Kaplan–Meier esti- mator. Time ratios were derived from the AFT-models using the method of (Onofri *et al.* 2010) and imply that the germina- tion timing in each dung type in both greenhouse and outdoor conditions is compared to the control treatment in greenhouse conditions. The control treatment has a fixed time ratio of 1. Treatments with a time ratio higher than 1 have longer

germination times while time ratios smaller than 1 indicate accelerated germination compared to the control treatment.

Environmental conditions were expressed as cumulative

Table 1. Results of the seed quality tests of the seeds used in the dung addition experiments. Tests were performed in standardized lab conditions.

growing degree-days (GDD) and the daily relative humidity

plant family and

species name

life

strategy

germinability

(%) MTG (d)

which was calculated as the average value of all relative humid-

ity measurements of each experimental day. GDD was calcu-

Caryophyllaceae

|  |  |  |  |
| --- | --- | --- | --- |
| lated using the method recommended by (McMaster &Wilhelm 1997): | *Stellaria media* (L.) Annual Vill. | 28.0 ± 11.3 ABC | 133.5 ± 54.2 ab |
| Cistaceae |  |  |
|  | *n* f*T*max;*i* - *T*min;*i* Þl | *Helianthemum nummularium* (L.) Mill. |  |  |
| GDD | X¼ 2*i*¼1 | - *T*base ð3Þ | intact seeds Perennial scarified seeds | 10.4 ± 2.5 CD 90.4 ± 2.0 E | 152.6 ± 30.8 a24.2 ± 7.2 bc |

where *T*max*,i* and *T*min*,i* are respectively the maximum and min- imum daily temperature recorded on day *i*, and *T*base is set to a fixed 10 °C which is an often used threshold value in temperate climates. If [(*T*max*,i*-*T*min*,i*)/2] is smaller than *T*base then *T*base equals [(*T*max,*i*-*T*min,*i*)/2] (McMaster & Wilhelm 1997).

*Tuberaria guttata* (L.) Fourr.

intact seeds Annual 39.2 ± 8.2 ABF 203.5 ± 48.6 a

scarified seeds 44 ± 4.3 ABF 83.3 ± 27.1 ab Fabaceae

*Trifolium pratense* L. Perennial 88.8 ± 2.6 E 1.9 ± 0.2 de

*Trifolium repens* L. Perennial 97.6 ± 0.4 E 1.5 ± 0.0 e Gentianaceae

RESULTS

Seed quality test

Interspecific variation in germinability and mean time to ger-

*Centaurium erythraea*

Rafn Juncaceae

|  |  |  |  |
| --- | --- | --- | --- |
| *Juncus bufonius* L. | Annual | 29.6 ± 4.6 ABC | 12.8 ± 0.5 bc |
| *Juncus effusus* L. Perennial 90.8 ± 2.5 E 10.9 ± 0.8 bcdoaceae |

P

Perennial 57.2 ± 3.3 F 9.7 ± 0.4 bcd

mination (MTG) was found (Table 1). The germinability of

the *Trifolium* species, *Juncus effusus*, *Poa annua* and the scari-

*Agrostis capillaris* L. Perennial 46.8 ± 3.0 AF 22.7 ± 8.6 bc

*Agrostis stolonifera* L. Perennial 22.0 ± 3.6 ABC 10.1 ± 1.8 bcde

fied seeds of *Helianthemum nummularium* was near to 100%

while very few seeds of both *Urtica* species germinated in the

*Alopecurus myosuroides* Huds.

Annual 21.2 ± 4.8 BC 44.6 ± 14.3 ab

lab. MTG was high for *Stellaria media* and the Cistaceae species

except the scarified seeds of *H. nummularium* while the *Tri- folium* species had the fastest germination rate (Table 1).

*Poa annua* L. Annual 95.6 ± 1.0 E 4.6 ± 1.3 cde

*Poa pratensis* L. Perennial 33.2 ± 2.9 ABF 12.4 ± 0.7 bc Urticaceae

*Urtica dioica* L. Perennial 2.0 ± 0.6 D 22.1 ± 8.3 bc

*Urtica urens* L. Annual 1.2 ± 1.1 D 23.0 ± 9.5 abc

Environmental conditions in the dung addition experiments

Environmental conditions clearly differed between the outdoor and greenhouse situation (Fig. 1). Air measurements of GDD rose rapidly in the greenhouse at the start of the experiment but reached a steady level after 42 days once the difference between the minimum and maximum temperatures became minimal. In outdoor conditions, a higher cumulative GDD was reached at the end of the experiment and the difference between air and soil GDD values was smaller than in greenhouse conditions. Relative humidity varied between 65 and 100%, except for the soil measurements in outdoor conditions where a nearly con- stant humidity of 100% was measured (Fig. 1).

Seed germination characteristics in dung environment

None of the selected test species emerged in the blank pots nei- ther in cattle or horse dung or the sand-compost mix in the greenhouse and outdoor environment. Both germinability and MTG differed between species and families and were affected by dung type and environment (Table 2). Life strategy had only a significant impact on MTG, with annuals generally germinat- ing faster than perennials. No seeds of *Urtica urens* germinated during the experimental period; therefore, this species was dis- carded from further analyses. The presence of dung decreased the germinability of seeds in greenhouse conditions, as signifi- cantly more seeds of all tested species germinated in the control pots compared to the pots where cattle or horse dung was added (Fig. 2). In most cases, dung types did not affect

Average germinability and mean time to germination (MTG) with standard errors are shown for the studied plant species and families with their respec- tive life strategies. Different letters in the same column indicate significant differences between species after applying ANOVA and Tukey posthoc tests.

germinability, but in case differences were found more seeds germinated in horse dung compared to cattle dung (*H. num- mularium* (scarified seeds), *Tuberaria guttata* (both categories), *Trifolium pratense*, *Trifolium repens*, *Juncus effusus*, *Agrostis sto- lonifera* and *Poa annua* in greenhouse conditions and

*A. stolonifera*, *T. repens* and *T. guttata* (both categories) in out-

door conditions). In the outdoor experiment, significantly more seeds of *T. guttata* (scarified seeds), *T. repens*, *J. effusus*, *Agrostis capillaris*, *P. annua* and *Urtica dioica* germinated in the control treatment compared to cattle and/or horse dung addi- tion treatments. Most species germinated more abundantly in greenhouse conditions, except for *A. stolonifera* where the opposite effect was found while no effect was found for *Alopecurus myosuroides*, *H. nummularium* (scarified seeds) and

*P. annua* (Fig. 2). Survival analysis pointed out that seeds sown

in the control treatment had a higher probability to germinate than seeds sown in dung covered pots (Fig. 3). Intact seeds of

*H. nummularium* and *A. myosuroides* were less likely to germi- nate in cattle dung while seeds of all other tested species had a lower germination probability in both horse and cattle dung.

Compared to the control treatment in greenhouse conditions, the germination rate was significantly slower for most species

100

**Location**

Greenhouse, air

 Greenhouse, soil Outdoors, air

 Outdoors, soil

60

90

**Cumulative GDD (°C)**

**Relative humidity (%)**

40

80

20

70

Fig. 1. Cumulative growing degree-day (GDD, °C) and relative humidity (%) of air and soil during the experi- ments in greenhouse and outdoor conditions.

0

0 20 40 60

**Time (day)**

60

0 20 40 60

**Time (day)**

Table 2. Results of nested ANOVAs with consecutively germinability and mean time to germination (MTG) as measured variable and dung type, life strategy, environment and family as nominal variables and a nested family: species term.

measured variable factor Df *F* value *P*

with the findings of Shipley & Parent (1991) although we did not find different germinability.

For most species the greenhouse conditions were more favourable for germination as more seeds germinated and the time ratio for the outdoor control treatment was significantly

 higher in most cases. *Agrostis stolonifera* was a clear exception

as for this species significantly more seeds germinated at a much faster pace in the outdoor treatment. The environmental conditions differed between both experimental locations and may have triggered different germination responses. In the first weeks of the experiment, the cumulative growing degree-day (GDD) in the greenhouse experiment was higher, which was caused by the higher maximum temperatures, while night tem- peratures in the unheated greenhouse were comparable with the outdoor conditions. Remarkably, GDD of the soil was much lower in the greenhouse and therefore, the difference between air and soil GDD was smaller in the outdoor experi- ment. Relative air humidity was highly variable in both loca-

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| germinability | Dung type | 2 | 155.697 | <0.001 |
|  | Life strategy | 1 | 0.362 | 0.548 |
|  | Environment | 1 | 71.210 | <0.001 |
|  | Family | 6 | 239.492 | <0.001 |
|  | Family: species | 6 | 115.569 | <0.001 |
| MTG | Dung type | 2 | 24.790 | <0.001 |
|  | Life strategy | 1 | 32.201 | <0.001 |
|  | Environment | 1 | 426.897 | <0.001 |
|  | Family | 6 | 88.397 | <0.001 |
|  | Family: species | 6 | 13.610 | <0.001 |

when sown in dung or in outdoor conditions (Fig. 4). One exception was found with *A. stolonifera*. This species germi- nated significantly faster when sown in outdoor conditions irrespective of the addition of dung. In most cases, germination was hindered most when cattle dung was added to the pots (Fig. 4).

DISCUSSION

The germination metrics are clearly species-dependent as germinability ranged between 1 and 98% and seeds on aver- age needed between 1.5 and 203 days to germinate in the lab test. Except for *Stellaria media*, *Agrostis capillaris* and *Urtica dioica*, more seeds germinated in the lab test com- pared to the control treatments in the greenhouse and out- door environments although for all species seeds from the

same seed lots were used. Similar results were previously found by Ramos-Font *et al.* (2015) and are likely to be linked to the more favourable combination of light quality, day-night cycle length, substrate and constant temperature and humidity in the lab. Additionally, in the lab tests in Petri dishes, it was far more easy to detect germination (ap- pearance of a radicle) than in sand-compost filled pots in the greenhouse or outdoor experiments (appearance of cotyledons). Subtle differences in germination timing were found between life strategies. Annual species generally ger- minated faster than perennial species which is comparable

tions while in the outdoor location soil humidity had a constant value of nearly 100%. The constantly waterlogged substrate in the outdoor experiment might have triggered ger- mination of *A. stolonifera* which is a native species in disturbed grassland communities with temporarily waterlogged soil and is known to increase its biomass, stolon length and intern- odium length in flooded situations (Rozema & Blom 1977). Alternating temperatures have been linked to several germina- tion metrics (Murdoch *et al.* 1989; Probert 2000). Most plant species have an optimal temperature at which maximal germi- nation occurs, with a linear increase towards this optimum. At *supra*-optimal temperatures, alternating temperatures have lit- tle effect while at sub-optimal temperatures germination increases linearly with amplitude (Murdoch *et al.* 1989). The larger daily fluctuations in temperature in greenhouse condi- tions might have increased germination in most of the tested species.

Dung clearly has a negative effect on germination. For all tested species, the germination probability is lower in the dung treatments compared to the control treatments. This effect is even more pronounced in the pots covered with cat- tle dung. In addition to high concentrations of growth-pro- moting macro- and micro-nutrients, dung may also contain toxic compounds that can have adverse effects on seed sur- vival and germination (Welch 1985; Marambe *et al.* 1993;

Malo & Suarez 1995b; Cosyns *et al.* 2005b; Ramos-Font *et al.*

2015). Although many seeds survive the intestinal tract and

***Agrostis capillaris***

a **#**

***Agrostis stolonifera***

40 **#** y

***Alopecurus myosuroides***

20

a

***Centaurium erythraea***

a **#**

60

**Germinability (%)**

40 b **#**

b **#**

20

x

**#** xy

30

**#** x

20

a

10 b

x ab 30

15 x

x

20

10 b

5 10

**Location**

Greenhouse Outdoors

b

b

x x

y xy b x

0 0 0 0

Control Cattle Horse

Control Cattle Horse

Control Cattle Horse

Control Cattle Horse

***Helianthemum nummularium***

***Helianthemum nummularium °***

***Juncus bufonius***

***Juncus effusus***

a **#**

6

**Germinability (%)**

4

x x

b

2 x b

0

40 a

30

x x

c

20 x

b

10

0

a **#**

10

x

5

x b x

b

0

80

**#**

a

60

40 c **#**

x

20 b xy

 y

0

Control Cattle Horse

Control Cattle Horse

Control Cattle Horse

Control Cattle Horse

120

a

***Poa annua***

x

30 a **#**

***Poa pratensis***

***Stellaria media***

a **#**

75

***Trifolium pratense***

a **#**

75

90

**Germinability (%)**

c y 20

60 b y

10

30

b

50

x b x

x 25

a

x

x b x 50 x

x b x

b

25

0

Control Cattle Horse

0

Control Cattle Horse

0

Control Cattle Horse

0

Control Cattle Horse

100

***Trifolium repens***

a **#**

10.0

***Tuberaria guttata***

a **#**

40 a **#**

***Tuberaria guttata °***

100

***Urtica dioica***

75

**Germinability (%)**

x x

c

50 y

b

7.5 75

a

**#**

x

b **#**

b

xy

y

30

y

a

5.0 x 50

xy 20

c z

25

2.5 b x

10 y 25

b

0

Control Cattle Horse

**Dung type**

0.0

Control Cattle Horse

**Dung type**

0

Control Cattle Horse

**Dung type**

0

Control Cattle Horse

**Dung type**

Fig. 2. Germinability by dung type and species. Grey bars indicate greenhouse conditions, white bars indicate outdoor conditions. Different letters above bars indicate significant differences between dung types and the control treatment after applying two-way ANOVAs and Tukey posthoc tests on germination data in either greenhouse (a–c) or outdoor (x–z) conditions. Significant differences in germinability between the greenhouse and outdoor environment are marked with # for each particular dung type. Seeds of species indicated with ° were pre-treated by scarification with sandpaper.

are therefore potentially successfully endozoochorous species, seeds deposited in dung can be destroyed by a wide range of biotic and abiotic factors, including insects, fungi, rodents and desiccation (Estrada & Coates-Estrada 2002; Vander Wall

& Longland 2004). Besides being a growth substrate for seed- lings, dung can also promote the growth of fungi and bacteria which in some cases are detrimental for germination and seedling survival (Clark & Wilson 2003; Traveset *et al.* 2007). In our experiments, we observed the rapid development of a hard and dry crust on cattle dung within a few hours after depositing the dung-seed mixture in the pots. According to

Holter (2016), both structural and chemical differences between ruminant and non-ruminant dung can be found. Although the water content of both dung types is more or less the same, non-ruminant dung tends to be more fibrous and has larger particles. On the other hand, ruminant dung on average has higher nitrogen content and a lower C: N ratio (Holter 2016). The more loose structure of horse dung might facilitate the emergence of seedlings. The impact of the higher nitrogen content of fresh ruminant dung is likely to be less important as only a small fraction of the nutrients pre- sent in fresh dung is inorganic and readily available for plant

1.00

***Agrostis capillaris***

1.00

**# #**

***P* < 0.001**

***Agrostis stolonifera***

**# #**

1.00

***Alopecurus myosuroides***

1.00

**#**

***P* = 0.035**

***Centaurium erythraea***

**# #**

**Dung type** Control Cattle

Horse

***P* < 0.001**

0.75

**Probability of not germinating**

0.75

0.75

0.75

0.50

0.50

0.50

0.50

0.25

0.25

0.25

0.25

0.00

0 50 100 150 200

0.00

***P* < 0.001**

0 50 100 150 200

0.00

0 50 100 150 200

0.00

0 50 100 150 200

1.00

***Helianthemum nummularium***

**#**

1.00

***Helianthemum nummularium °***

1.00

**#**

**#**

***P* < 0.001**

***Juncus bufonius***

**# #**

1.00

***Juncus effusus***

**#**

**#**

***P* < 0.001**

0.75

**Probability of not germinating**

0.75

0.75

0.75

0.50

0.50

0.50

0.50

0.25

0.25

0.25

0.25

0.00

***P* = 0.014**

0 50 100 150 200

0.00

0 50 100 150 200

0.00

***P* < 0.001**

0 50 100 150 200

0.00

0 50 100 150 200

1.00

***Poa annua***

1.00

***Poa pratensis***

1.00

**#**

**#**

***P* < 0.001**

***Stellaria media***

1.00

***Trifolium pratense***

0.75

**Probability of not germinating**

0.75

**#**

0.75

**#**

**#** 0.75

**#**

**#**

***P* < 0.001**

0.50

**#**

0.50

0.50

0.50

0.25

0.25

0.25

0.25

0.00

***P* < 0.001**

0 50 100 150 200

0.00

0 50 100 150 200

0.00

***P* < 0.001**

0 50 100 150 200

0.00

0 50 100 150 200

1.00

**Probability of not germinating**

0.75

***Trifolium repens***

**#**

**#**

1.00

0.75

***Tuberaria guttata***

**# #**

1.00

0.75

***Tuberaria guttata °***

**#**

**#**

1.00

0.75

***Urtica dioica***

0.50

0.50

0.50

0.50

**# #**

***P* < 0.001**

0.25

0.25

0.25

0.25

0.00

***P* < 0.001**

0 50 100 150 200

**Time (day)**

0.00

***P* < 0.001**

0 50 100 150 200

**Time (day)**

0.00

***P* < 0.001**

0 50 100 150 200

**Time (day)**

0.00

0 50 100 150 200

**Time (day)**

Fig. 3. Probability of not germinating (Kaplan–Meier estimates) of germination functions by species sown in greenhouse conditions. Treatment curves which differ significantly from the control treatment after applying AFT-models are indicated with #. Confidence bands are indicated in grey. Seeds of species indi- cated with ° were pre-treated by scarification with sandpaper.

uptake (Jørgensen & Jensen 1997; Sitters *et al.* 2014). During the experiment, coprophilous fungi appeared on both dung types. Although fungi are known to increase the decomposi- tion rate of dung (Harper & Webster 1964), little dung was degraded by the end of the experiment which was probably due to the absence of soil fauna in the plant pots.

Germinability and MTG clearly differed between plant species and families. The tested species in the Urticaceae, Fabaceae and Caryophyllaceae germinated typically fast and abundantly, while few seeds of the Cistaceae and Gen- tianaceae germinated in any of the experimental treatments. Successful endozoochorous dispersal has been especially linked with species carrying persistent, small, rounded

diaspores with a hard seed coat (Albert *et al.* 2015). Many species in the Cistaceae and Fabaceae have a high incidence of dormant seeds due to their hard and water impermeable seed coats (Thanos *et al.* 1992; Gresta *et al.* 2007; D’hondt *et al.* 2010). Mechanical scarification of the hard seed coat induces germination (P'erez-Garc'ıa & Gonz'alez-Benito 2006; Delgado *et al.* 2008). This observation is confirmed in our experiment with higher germinability of scarified *H. nummu-*

*larium* seeds compared to intact seeds. Physical dormancy also occurs in *Trifolium* species. D’hondt *et al.* (2010) found that up to 35% of all viable seeds are water-impermeable in wild populations of *T. repens*. In our experiment, the seeds of both tested *Trifolium* species were not pre-treated but

*Urtica dioica*

*Tuberaria guttata °*

*Tuberaria guttata*

*Trifolium repens*

*Trifolium pratense*

*Stellaria media*

*Poa pratensis*

*Poa annua*

*Juncus effusus*

*Juncus bufonius*

*Helianthemum nummularium °*

*Helianthemum nummularium*

*Centaurium erythraea*

*Alopecurus myosuroides*

*Agrostis stolonifera*

*Agrostis capillaris*

–2 0 2 4

**Time ratio (log)**

Greenhouse

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 ● Control Cattle Horse

Outdoors

* Control Cattle Horse

dormancy is a hereditary trait in this species (Baskin & Baskin 2001). We should also keep in mind that we used intact, undigested seeds. Germination success decreases in many species after gut passage (*e.g.*, Manzano *et al.* (2005); Mouissie *et al.* (2005a); Cosyns *et al.* (2005b), Grande *et al.* (2013), Miloti'c & Hoffmann (2016)), so we can safely assume that an even lower germination success would have

been found when seeds would have been precedingly digested.

We were unable to estimate whether the seeds that remained ungerminated at the end of the experiment were dormant or dead due to the used substrates, given the fact that the substrate did not allow to retrieve the tiny seeds. However, as Cistaceae species are known to spread germi- nation over several seasons (Russi *et al.* 1992), we can assume that a reasonable fraction of the seeds remains dor- mant, even after mild scarification. Endozoochorous disper- sal is known to enrich and alter the species composition of soil seed banks by incorporating seeds that remain dormant following dispersal (Dai 2000; Nathan & Muller-Landau 2000). Early emergence and establishment in a dung envi- ronment may be an advantage for certain species and even a difference in germination timing of a few days may decrease the growth rate and survival probability of later emerged seedlings (Loiselle 1990; Traveset 1998). On the other hand, early germinated seeds might be more suscepti- ble to pathogens, seed predators and the adverse environ- mental conditions in dung. Therefore, selection is not always in favour of early germination or high germination percentages (Janzen 1984; Traveset 1998). Seeds germinating late are able to escape the scramble competition for space and nutrients with other seedlings in the same dung pat. The activity of dung inhabiting fauna whose dung remov- ing and mixing actions may kill or harm vulnerable seed- lings decreases with ageing dung (Janzen 1984). Furthermore, the nutritive content is more readily available for plant uptake in older dung (Jørgensen & Jensen 1997; Sitters *et al.* 2014) which might benefit the growth of seed- lings that germinated later. The large dung pats produced by large herbivores such as cattle and horses, are able to kill or suppress the vegetation underneath the dung pat and, therefore, create gaps with relatively low competition between seedlings and the established vegetation (Brown & Archer 1988). Therefore, the ideal germination timing after endozoochory is a trade-off between the reduced competi- tion early after deposition and the lowered concentration of phytotoxic compounds and the higher availability of nutri- ents in a later stage. We should keep in mind that germi- nation is just the first of many steps in plant regeneration. The high nutrient content in dung might alter plant fitness

Fig. 4. Log-transformed time ratio (TR) and standard errors by species and

dung type after applying AFT models. log(TR) > 0 indicates decelerated ger- mination and log(TR) < 0 indicates accelerated germination compared to the control treatment (log (TR) = 0) in greenhouse conditions. Asterisks indicate significance levels with \*\*\*: *P* < 0.001, \*\*: 0.001 < *P* < 0.01 and

\*: 0.01 < *P* < 0.05. Seeds of species indicated with ° were pre-treated by scarification with sandpaper.

nevertheless had a high germinability. This in line with other experiments using cultivated weed seeds (Miloti'c & Hoffmann 2016) and is probably due to the fact that seed

in a later stage and enhance growth and/or reproductive success.

CONCLUSION

We found adverse effects of both cattle and horse dung on the germinability of all tested grassland species, but the effects of cattle dung were more pronounced. Also, germination metrics clearly differed between the artificial greenhouse and outdoor conditions, with in general lower germinability in outdoor con- ditions. A large cost seems to be related to endozoochorous

dispersal, at least in the first stages of seed survival and germi- nation.

ACKNOWLEDGMENTS

We would like to express our gratitude to the Flanders Mar- ine Institute (VLIZ) for the permission to use their

greenhouse facilities and to Frank Broucke, Michiel T’Jamp- ens and Andr'e Cattrijsse for technical assistance. We would also like to thank Bjorn Jansseune for providing dung and an anonymous reviewer for improving the first version of this paper.

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