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Lotus tenuis (Fabaceae) seedling emergence from the soil seed bank covered with cattle dung

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This study examined the *Lotus tenuis* seedling emergence from natural and artificial soil seed banks covered with cattle dung of different thicknesses. We tested the hypothesis that seedling emergence from soil seed banks covered with fresh cattle dung depended on seed weight, its dormancy and dung thickness. Two seed dormancy categories (low and high seed-coat impermeability to water) were obtained from a sample of hard seeds with and without scarification with sandpaper. Natural and artificial soil seed banks of *Lotus tenuis* were covered with 0.5, 2, 4 and 6 cm thick fresh dung pats; control seed banks were not covered. The results of this study showed that increasing dung thickness affected negatively the seedling emergence in different ways: (1) Seeds germinated under dung pats but seedling emergence was impeded with increasing dung thickness. Seedling emergence from heavy seeds was higher than from the light ones. (2) Moisture contained in the fresh dung was enough to trigger the germination of seeds on dry soil. Seedlings died because of dung desiccation. Seed dormancy prevented the germination under fresh dung. The amount of seeds recovered alive decreased with dung thickness. Seeds with high dormancy categories were more numerous than those with low dormancy categories. Cattle dung deposition on the soil seed bank of *L. tenuis* can negatively affect the seed survival and seedling emergence.

Introduction

Seed availability and events that occur during seed germination and seedling establishment are critical for the species that are spread by seeds. Environmental factors that affect the seedling development and survival can severely limit the recruitment of new plants into a population (Harper 1977). At a small scale, cattle dung deposition on the soil creates sites enriched with nutrients and organic matter. These conditions are favourable for seedling recruitment of plants whose seeds are dispersed through endozoochory (Janzen 1984, Haynes & Williams 1993, Malo & Suárez 1995, Dai 2000, Pakeman *et al*. 2002, Gokbulak & Call 2004, Cosyns *et al*. 2006). However, small-scale studies of the effects of cattle dung deposition on seed survival and seedling recruitment from the soil seed bank are so far scarce.

Dung generate micro environmental conditions of light, temperature, moisture, as well is a source of fungi and phytotoxic substances that generally are unfavourable for soil seed banks survival and plant growth of some species (Janzen 1984, Haynes & Williams 1993, Malo & Suárez 1995, Dai 2000, Traveset *et al*. 2001). Seedling recruitment in most plant communities depends on the occurrence of microsites and on seed attributes such as weight and dormancy. Seed weight can determine the success of individuals in the colonization of different microsites and physical dormancy can prevent seed germination and mortality under unpredictable environment (Harper 1977). Therefore, under grazing conditions, plant population dynamics of some species could be better understood if the effects of cattle dung on the soil seed bank were considered.

Lotus tenuis was the species selected to study the effects of cow dung on seedling emergence. This species is an important forage legume in different countries (Blumenthal & McGraw 1999). Its seeds are small (approx. 1 mg), have physical dormancy and produce seedlings with poor vigor (Sevilla *et al*. 1996). They can be dispersed through endozoochory in cow dung. We tested the hypothesis that seedling emergence from the soil seed bank covered with fresh cow dung depends on seed weight, its dormancy and dung thickness.

Materials and methods

Experiments with artificial and natural soil seed banks were conducted using *L. tenuis* seeds (cv. Chaja) at the Unidad Integrada Balcarce (EEA, INTA Balcarce, Facultad de Ciencias Agrarias, UNMdP), Buenos Aires Province, Argentina (37°47´S, 58°17´W). Recently dropped, fresh cow dung free of *L. tenuis* seeds, was gathered from a grassland free of *L. tenuis* plants at the EEA, INTA Balcarce and used in the experiments. The dung pat thickness (mean ± SE = 5.72 ± 0.22 cm, median = 5.50 cm) was the vertical distance from the center of the dung to the soil surface.

Experiment 1

Two seed dormancy categories (high and low seed-coat impermeability to water), two irrigation regimes (irrigated and not irrigated), two dung pat thicknesses (0.5 and 2 cm) plus control with no dung, in four replicates (total of 48 pots) were applied under greenhouse conditions. The average germination of high dormancy category seeds was 4.23% (I.S.T.A. 1985). The low dormancy category seeds (42% germination) were obtained from a sample of hard seeds scarified with sandpaper (Baskin and Baskin 1998). On 24 May 2005, soil cores (10 cm diam. and 5 cm heigh) were taken with a steel cylinder from a grassland at the EEA, INTA Balcarce. The soil was a Typic Natralbol [pH 6.1 (soil H_2O, 1:2.5), organic matter 2.2% [Walkley & Black (1934) method], nitrates 10.2 ppm, phosphorus 15.4 ppm [Bray 1 method; Bray & Kurtz (1945)], electrical conductivity 0.6 dS m^{-1}). Shoot biomass and the first 0.5 cm of the soil were eliminated in order to reduce the possible presence of *L. tenuis* seeds. Each soil core was placed in a 12.80 cm diam. and 11 cm heigh pot to be even with the upper rim of the pot (*see* Fig. 1). The remaining empty space in the pot was filled with a mixture of the same soil and perlite (1:3).

On 13 June 2005, 50 *L. tenuis* seeds of one seed dormancy category were randomly distributed on the surface of each soil core and immediately covered with fresh dung. Dung pats (hereafter dung) were molded using plastic molds (8 cm internal diameter, and 0.5 or 2 cm heigh). Dung was protected with a transparent plastic pot (11 cm diam. and 12 cm heigh) perforated to facilitate ventilation (Fig. 1).

The seeds in the control samples were not covered with dung. They were randomly distributed on the soil-core surface and protected with a plastic circular hoop (8 cm diam. × 0.3 cm heigh) which was fixed to the soil to avoid seed displacement during manipulation of the pots. The seeds were covered with a 0.3-cm-thick layer of river sand.

Pots with irrigation samples were placed on plates which held 0.5 cm of water to keep the samples moist by capillary suction through holes in the bottom of the pot. The samples without irrigation remained dry and the only moisture they had was that derived from the dung.

Seedling emergence and survival in the peripheral and interior regions of the dung were recorded weekly until on two successive occasions no newly emerged seedlings were encountered. On 3 August 2005, shoots of the seedlings were harvested by cutting them carefully with histological scissors. Dry weight of the shoots (to the nearest 0.0001 g) was determined after drying in 60 °C for 72 h. On 17 August 2005, the experiment was terminated. The first 0.5 cm of the soil and the dung being in contact with the soil, were carefully removed with a brush. Remaining, ungerminated *Lotus tenuis* seeds were retrieved and their viability determined (I.S.T.A. 1985). We believe that not recovered seeds died and were degraded by microorganisms or germinated but seedling emergence did not take place. Percentage of not recovered seeds (NRS) was calculated as follows:

NRS = [(Seed number distributed on the soil
– Seedling number emerged
– Seed number recovered)
/(Seed number distributed on the soil)] × 100.

Seedling mortality (SM, %) was calculated as follows:

SM = [1 – (seedling survival at the end of experiment
/maximum number of seedling emerged)] × 100.

Experiment 2

Four dung thicknesses (0.5, 1, 2 and 4 cm) and no-dung control, two seed-weight categories (heavy and light) in five replicates (total of 50 pots) were used. The weight of 1000 heavy (1.1129 ± 0.0184 g) and light (0.8297 ± 0.0077 g) seeds was significantly different ($p < 0.0001$). Heavy seeds were retained in a 16-mesh sieve (1 mm opening) while light seeds passed through this sieve. On 12 August 2005, soil core samples were taken from an Argiudol soil of the experimental garden at the EEA, INTA Balcarce (pH 6.0, organic matter 5.4%, nitrates 5.2 ppm, phosphorus 7.4 ppm, electrical conductivity 0.3 dS m^{-1}).

On 26 September 2005, 30 *L. tenuis* seeds of one weight category were placed on the surface of each soil core and covered with dung as in experiment 1. The pots were left in field conditions, under a black mesh — not touching the pots — that intercepted 50% of solar radiation. Irrigation and protection were as experiment 1. Shoot biomass was harvested on 8 November 2005 and on 3 March 2006. Viability of recovered seeds was evaluated as in experiment 1.

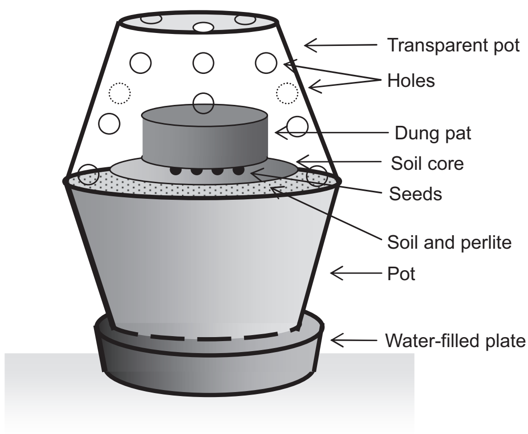

Fig. 1. Experimental appliance used in experiments 1 and 2.

In experiments 1 and 2, eight pots (*see* Fig. 1) were prepared only with soil cores to record the presence of *L. tenuis* seeds in the soil through seedling emergence. In addition, three fresh dung samples were spread out in a layer 0.5–1 cm thick in trays (12 cm wide × 20 cm long × 8 cm deep) on a bed of river sand. Soil cores and dung were watered. *Lotus tenuis* seedling emergence did not take place during the study.

Experiment 3

In summer 2005, *L. tenuis* plants' — grown at the experimental garden EEA, INTA Balcarce — pods dehisced and shattered seeds on the soil. On 1 August 2005 (winter), *Lotus tenuis* and other plants being in the vegetative stage were cut with scissors to the hight of 2 cm. On 3 August 2005, using plastic molds we made dung samples of 17 cm in diam. with respective thicknesses of 0.5, 2, 4 of 6 cm, plus control (six replicates; 30 total) and placed them on the soil where seeds were shattered. The control (also six replicates) was without dung. At the moment of placing the

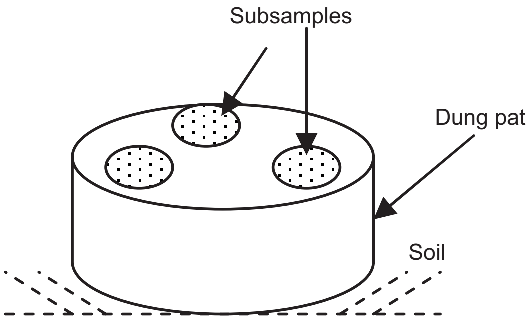

Fig. 2. Schematic illustration of a dung pat with three subsamples in experiment 3. *Lotus tenuis* seedlings were recorded from the 3 subplots (76.51 cm² in total).

dung, recruitment of *L. tenuis* seedlings from soils did not take place. For protection, during the experiment each dung sample and the control were placed under plastic cages.

Seedling emergence was recorded weekly from three subplots delimited with plastic circular hoops (5.70 cm diam. × 0.3 cm height) that were pressed on the surface without modifying the soil and dung surface (Fig. 2). On 26 October 2005, the experiment was finished and shoot biomass was harvested. The three subsamples and the first 2 cm of soil in contact with these were taken with a steel cylinder of 5.70 cm in diam. Seeds were recovered and their viability determined as in Experiment 1. Here, percentage of seeds not recovered from the soil seed bank (NRSB) which was covered with dung was calculated from the area of 76.51 cm² (*see* Fig. 2) as follows:

NRSB = [(SSBC – Seedlings number emerged on dung – recovered seeds under of dung)/SSBC] × 100.

where SSBC = (Seedling emergence + recovered seeds) for the control (no dung) soil seed bank.

Table 1. Experiment 1. Maximum (T_{Max}) and minimum (T_{Min}) air temperatures; maximum (H_{Max}) and minimum (H_{Min}) relative air humidity; and radiation in greenhouse condition.

Month	T_{Max} (°C)	T_{Min} (°C)	Radiation (MJ m⁻²)	H_{Max} (%)	H_{Min} (%)
June	20.39	4.96	4.254	96.4	67.40
July	25.30	3.34	5.043	99.0	55.78
August	28.06	5.36	6.584	99.0	44.41

Climatic data and experiments maintenance

Air temperature, relative humidity and radiation in the greenhouse were recorded with a Hygro-Thermometer and LI-COR, LI-188B instrument (Integrating Quantum Radiometer Photometer) (Table 1). Climatic data for experiments 2 and 3 were provided by INTA Balcarce (Table 2). Precipitation values during experiment 3 were 109, 59 and 60 mm in August, September and October, respectively. The pots in experiments 1 and 2 were randomly arranged. Pots were relocated weekly and maintained free from weeds and insects. Plants other than *L. tenuis* that emerged from the soil seed bank and dung were removed by cutting them with scissors.

In addition, three fresh dung samples were spread out in a layer 0.5–1 cm thick in trays (12 cm wide × 20 cm long × 8 cm deep) on a bed of river sand and watered. *Lotus tenuis* seedling emergence did not take place during the study.

Statistical analysis

The effects of dung thicknesses on seedling emergence, shoot biomass and seed recovery were analysed using ANOVA. Data expressed as percentages were arcsine square-root transformed before the analysis. A *post-hoc* LSD (least significant difference) analysis was performed to compare treatment means (cf. Steel & Torrie 1980). Differences were considered significant at $p < 0.05$.

Table 2. Experiments 2 and 3. Maximum (T_{max}) and minimum (T_{min}) temperatures, relative air humidity (H), and radiation in field conditions.

Month	T_{max} (°C)	T_{min} (°C)	Radiation (Mj m⁻²)	H (%)
August	13.34	4.65	7.59	85.48
September	15.81	5.29	12.47	82.70
October	19.12	5.95	18.22	74.45
November	23.64	10.36	20.49	70.63
December	23.69	9.80	21.84	70.06
January	25.73	12.96	22.66	74.58
February	25.11	14.14	18.99	78.53
March	24.11	10.91	16.13	73.71
April	21.43	9.57	10.62	79.80

Results

Under irrigation (experiments 1 and 2), seedling emergence decreased significantly with increasing dung thickness (Tables 3–6). Seedling emergence varied with seed weight (Table 5). Seedling mortality (on average 21.25% ± 6.34%) took place only in experiment 1 and did not depend on seed dormancy category or dung thickness. Shoot biomass did not present a clear pattern with increasing dung thickness and seed weight (Tables 3–6).

The number of not recovered seeds increased significantly with the dung thickness (Tables 3–6). Low dormancy category seeds were affected more significantly than those belonging to the high-dormancy category (Table 3).

Without irrigation, the moisture contained in the fresh dung covering the dry soil was enough to promote seed germination and seedling emer-

Table 3. Effects of dung pats of different thickness (diam. 8 cm) on *Lotus tenuis* seedling emergence, shoot biomass and seed recovery under irrigation. Values are means ± SEs recorded during the experiment 1. Same superscipt letter indicates lack of significant ($p < 0.05$) differences. HDCS = high dormancy category seeds, LDCS = low dormancy category seeds.

Dung thickness (cm)	Seedling emergence (*n*)		Shoot biomass (mg pl^{-1})		Not recovered seeds (%)	
	HDCS	LDCS	HDCS	LDCS	HDCS	LDCS
0	25.7 ± 1.8^{c}	30.5 ± 3.4^{c}	3.5 ± 0.8^{bc}	3.7 ± 0.6^{c}	5.0 ± 1.3^{a}	17.0 ± 4.4^{a}
0.5	19.0 ± 1.6^{b}	22.2 ± 2.8^{b}	5.1 ± 0.9^{ab}	3.8 ± 0.6^{bc}	20.5 ± 7.3^{a}	37.0 ± 7.0^{b}
2	9.0 ± 1.5^{a}	7.5 ± 0.6^{a}	2.2 ± 0.3^{c}	7.4 ± 0.8^{a}	46.5 ± 2.6^{bc}	60.5 ± 6.2^{c}

Table 4. Analysis of variance (p) for seedling emergence, shoot biomass and seed recovery.

Variable	Dung thickness	Seed dormancy	Dung thickness × Seed dormancy
Seedling emergence	0.00001	NS	NS
Shoot biomass	0.053	NS	0.002
Not recovered seed	0.00001	0.005	NS

Table 5. Effects of dung pats of different thicknesses (diam. 8 cm) on *Lotus tenuis* seedling emergence, shoot biomass and seed recovery. Values are means ± SEs recorded during the experiment 2. Same superscipt letter indicates lack of significant ($p < 0.05$) differences. HWCS = heavy weight category seeds, LWCS = light weight category seeds.

Dung thickness (cm)	Seedling emergence (*n*)		Shoot biomass (mg pl^{-1})		Not recovered seeds (%)	
	HWCS	LWCS	HWCS	LWCS	HWCS	LWCS
0	15.8 ± 2.2^{e}	14.0 ± 1.5^{de}	15.0 ± 1.6^{ab}	13.9 ± 1.0^{ab}	21.3 ± 5.1^{a}	26.6 ± 3.7^{a}
0.5	11.2 ± 1.7^{d}	7.4 ± 1.8^{c}	18.6 ± 3.4^{ab}	10.8 ± 1.0^{a}	31.3 ± 5.0^{a}	31.3 ± 7.1^{a}
1	5.6 ± 0.7^{bc}	3.2 ± 0.3^{ab}	20.5 ± 1.5^{ab}	23.4 ± 6.6^{b}	55.9 ± 3.7^{bc}	47.9 ± 5.6^{b}
2	1.2 ± 0.5^{a}	1.4 ± 0.6^{a}	19.0 ± 5.6^{ab}	10.8 ± 1.5^{a}	69.3 ± 7.5^{d}	56.6 ± 3.6^{bc}
4	1.6 ± 0.5^{a}	0.4 ± 0.2^{a}	21.2 ± 6.9^{ab}	13.3 ± 0.005^{ab}	64.6 ± 5.2^{d}	59.3 ± 4.1^{bc}

Table 6. Analysis of variance (p) for seedling emergence, shoot biomass and seed recovery.

Variable	Dung thickness	Seed weight	Dung thickness × Seed weight
Seedling emergence	0.00001	0.028	NS
Shoot biomass	0.09	NS	NS
Not recovered seed	0.0001	NS	NS

gence. Seedling emergence took place 14 days after the beginning of the experiment. One and four seedlings per replicate were recorded only in two replicates with low dormancy seeds. The seedlings survived during two weeks. The number of not recovered seeds increased significantly with dung thickness and for low dormancy category seeds (Tables 7 and 8).

The *Lotus tenuis* soil seed bank in control conditions of experiment 3 was 177 ± 28 seeds. Approximately 57.70% ± 3.40% of the seeds under the dung were not recovered. The percentage of not recovered seeds varied significantly with the dung thickness (Tables 9 and 10). Seedling emergence started 29 days after placing the dung on the soil. Maximum seedling emergence was recorded in the control conditions and decreased significantly with increasing dung thickness (Tables 9 and 10). Seedling emergence did not take place in case of the 4.0- and 6.0-cm thick dung pats. Shoot biomass per plant varied significantly with dung thickness (Tables 9 and 10).

Viability of recovered seeds in the three experiments was approximately 98%. The seeds were hard and had to be scarified to facilitate their germination. During seed recovery some dead seeds were with their radicle exposed and with their cotyledons chlorotic.

Table 7. Effects of dung pats of different thickness (diam. 8 cm) and no irrigation on *Lotus tenuis* seed recovery. Data are means ± SEs recorded during the experiment 1. Same superscipt letter indicates lack of significant ($p < 0.05$) differences. HDCS = high dormancy category seeds, LDCS = low dormancy category seeds.

Dung thickness (cm)	Not recovered seeds (%)	
	HDCS	LDCS
0	8.0 ± 3.0^{a}	6.6 ± 3.7^{a}
0.5	31.5 ± 1.7^{c}	56.5 ± 4.5^{d}
2	49.5 ± 5.9^{d}	76.0 ± 2.5^{c}

Discussion

At a small scale, through dung deposition cattle can generate detrimental conditions for seed survival in the soil bank and a physical impediment for seedling recruitment of some species (Dai 2000). *Lotus glaber* seedling emergence varied with dung pat thickness, seed hardness and weight. *Lotus glaber* heavy seeds produced seedlings with more biomass than light ones. A positive relationship between seed weight, seedling weight and recruitment in different ground-cover was reported for different species (Gross 1984, Rowarth & Sanders 1996, Jakobosson & Eriksson 2000). Seedling vigor of *L. tenuis* is related to seed weight (Rowarth & Sanders 1996). With increasing dung thickness, seed reserves may be exhausted before seedlings reach the surface. For example, seeds of *Poa secunda* (0.5 mg seed^{-1}) and *Agropyron desertorum* (2.1 mg seed^{-1}) spread through endozoochory differed in the seedling emergence. On a 4-cm think dung pat only *Agropyron desertorum* seedlings emerged (Gokbulak & Call 2004). Physical impediment may prevent seedling emergence. Larger seeds have greater energy reserves, being less affected than the small ones. In this case, seedlings from small seeds have higher probability of establishing if the seeds are near the dung surface (Gokbulak & Call 2004).

The number of not recovered seeds varied with dung pat thickness, seed hardness, weight and irrigation conditions. Other factors that can affect seed survival and seedling emergence are toxic substances present in the dung (Malo & Suárez 1995, Traveset *et al*. 2001).

In the experiments, we neither encountered seed consumption by animals nor worm casts that could be indicative of changes in the seed distribution in the soil. Recovered *Lotus tenuis* seeds were principally hard, which can prevent seed mortality in cattle dung exposed to different environmental conditions in the grasslands (Simao Neto & Jones 1986, Gardener *et al*. 1993, Dai 2000, Pakeman *et al*. 2002).

Table 8. Analysis of variance (p) for seed recovery.

Variable	Dung thickness	Seed dormancy	Dung thickness × Seed dormancy
Not recovered seed	0.0005	0.00001	0.01

Table 9. Effects of dung pats of different thickness (diam. 17 cm) on *Lotus tenuis* seedling emergence, shoot biomass and seed recovery. Data are means ± SEs recorded on a surface of 76.51 cm² during experiment 3. Same superscipt letter indicates lack of significant ($p < 0.05$) differences.

Dung thickness (cm)	Seedling emergence (*n*)	Shoot biomass (mg pl^{-1})	Not recovered seeds (%)
0	102.8 ± 15.1^{a}	3.7 ± 0.2^{a}	0
0.5	9.8 ± 1.9^{b}	4.2 ± 0.7^{a}	67.3 ± 4.5^{a}
2	2.0 ± 0.8^{b}	7.0 ± 1.2^{b}	$49.5 \pm 7.7b^{c}$
4	0	0	47.3 ± 7.6^{c}
6	0	0	66.5 ± 2.2^{ab}

Without irrigation, moisture in the fresh dung covering the dry soil was enough to promote seed germination and seedling emergence principally from soft seeds. In these conditions, all seedlings and a considerable percentage of seeds died as the dung dried out. Seed and seedling mortality were also recorded when the cattle dung moisture fell from 87% to 27% (Gardener *et al*. 1993). Effects of cattle dung on the soil seed bank may be more relevant when the soil surface is dry and if it coincides with high air temperatures (Simao Neto & Jones 1986), for example during summer in the Flooding Pampa grasslands.

Availability of seeds and safe sites for seedling recruitment are the major constraints on species diversity in plant communities of grasslands (Oerterheld & Sala 1990, Zobel *et al*. 2000). The amount of dung pats in grasslands depends on different factors, such as grazing intensity, stocking animal, plant communities and dung decomposition rate. Coffin and Lauenroth (1988) estimated that the dung pat deposition rate was between 67 and 1138 pats ha^{-1} yr^{-1}, according to grazing intensity and topographic position. Besides, these authors reported that the mortality of *Bouteloua gracilis* — a perennial grass — in grassland increased with the dung pat deposition rate. Under old, decomposing cattle dung, the persistent seed bank type was more numerous than the transient one (Dai 2000). A better understanding of the plant populations dynamics in grasslands requires knowledge of the rate of seed input and output of the soil seed bank through germination, consumption by animals, and death (Fenner 1985). According to our results, the *Lotus tenuis* — and possibly others species (Dai 2000) — soil seed bank dynamics under grazing conditions, could be better understood if the effects of dung on seed survival were considered.

Table 10. Analysis of variance (*p*) for seed recovery.

Variable	Dung thickness
Seedling emergence	0.00001
Shoot biomass	0.031
Not recovered seed	0.048

Cattle dung deposition on he soil seed bank affected the seedling emergence in different ways: (1) Seed germinated under dung pats but seedling emergence was impeded with increasing dung thickness. Seedling emergence from heavy seeds was higher than from the light ones. (2) The moisture contained in the fresh dung was enough to trigger the germination of seeds on dry soil. Seedlings died because of dung desiccation. Seed dormancy prevented the germination under fresh dung. The amount of seeds recovered alive decreased with dung thickness. Seeds with high dormancy were more numerous than with low dormancy.

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