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Potential role of endozoochory by cattle and sheep as an important dispersal mechanism of *Zoysia japonica* seeds

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The content of seeds in cattle and sheep dung, the germination of seeds removed from cattle and sheep dung and the establishment of seedling using seeds within cattle and sheep dung were studied in order to describe the potential role of endozoochorous seed dispersal by cattle and sheep. Intact seeds occurred at a mean density of 81760.00 ± 3601.67 and 33146.67 ± 3446.76 seeds/kg in cattle and sheep air-dried dung, respectively. The germination rate was significantly higher in seeds collected from the cattle (65.40 ± 2.36%) and sheep (62.00 ± 3.83%) dung than from the fruiting plants (14.40 ± 2.06%). There were no significant differences in germination between seeds defecated by cattle and sheep. A number of seedlings (134.40 ± 32.83 seedlings/pat) were established and tall (62.86 ± 0.98 mm) from cattle dung pat. A few seedlings (0.17 ± 0.04 seedlings/pellet) were established from sheep pellets despite that they have short height (32.35 ± 0.94 mm). These results suggest that cattle and sheep have the relevant potential of endozoochory as *Zoysia japonica* seed dispersers.

Key words: Seed dispersal, cattle, sheep, *Zoysia japonica*, germination, seedling.

INTRODUCTION

Seed dispersal has been recognized as one of the most important factors affecting plant recruitment, spatial distribution and long term viability of populations (Asquith et al., 1999; Howe, 1984; Janzen, 1970). The seeds of many plant species are dispersed by herbivorous endozoochory because they lack apparent morphological adaptations for ballistic dispersal, exozoochory, endozoochory by birds, or dispersal by wind or water (Myers et al., 2004; Cain et al., 2000). From an evolutionary point of view, herbivorous mammals are important vectors for endozoochorous plant dispersal as a result of co-evolution (Pakeman, 2004; Myers et al., 2004; Vellend et al., 2003; Pakeman, 2001), in which both the plant seed-dispersing animal species and the dispersed plant species benefit. Dispersers directly use the dispersed plant species as a food source and indirectly promote the distribution of their own forage plant species (Steyaert et al., 2009). Benefits of seed dispersal usually include reduction in levels of seed predation, improvement of seed germination, and colonisation of new habitats (Sánchez-Cordeiro and Martínez-Gallardo, 1998).

Many variables can influence germination success of herbivore gut-passed seeds. Two mechanisms could

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determine how herbivores affect germination in dry-fruited plants. One is mechanical and/or chemical scarification of the seed-coat, which may depend upon chewing behavior and gut retention time (Fredrickson et al., 1997) or on the type of food ingested with seeds (Jones and Simao Neto, 1987). The other is the effect of surrounding faecal material on germination and/or future seedling establishment and growth (Quinn et al., 1994; Ocumpaugh et al., 1996). Many studies have also found that ingestion reduces seed hardness, with a greater proportion of seeds capable of germinating after ingestion (Russi et al., 1992; Malo and Suárez, 1996), while other authors have found that this response is not ubiquitous and depends on the dispersed species and the dispersal vector (Traveset et al., 2001a, b; Manzano et al., 2005; Cosyns et al., 2005a).

The impact of herbivores on dispersal and recruitment may depend on herbivore size. Large herbivores are expected to have the strongest effect on plant dispersal. They consume large amounts of seeds (Malo and Suarez, 1995) and move over larger distances, due to their larger home-ranges (Haskell et al., 2002). Also seeds may survive better when passing through larger herbivores compared to smaller sized herbivores (less physical damage), although chemical damage can occur due to a longer retention time in the rumen of large herbivores (Ocumpaugh and Swakon 1993; Olson and Wallander, 2002). In addition, large herbivores can create regeneration niches by trampling and via their dung **(**Bakker and Olff, 2003).

Zoysia japonica Steud., commonly called Japanese or Korean lawngrass and which is commonly used as a turfgrass, largely dominates the grassland in Liaodong Peninsula, China. During the annual fruiting season, its dormant seeds (Dong et al., 2001) are dispersed primarily by different-sized herbivores, such as cattle and sheep. However, the dispersal ecology of this species is unknown. In this paper, we quantify intact seed content of cattle and sheep dung, the germination of seeds removed from cattle and sheep dung (‘free seeds’), and the establishment of seedling using seeds within cattle and sheep dung (‘enclosed seeds’). We compare the effects of ingestion on the seed germination by cattle and sheep grazed in the same site and to assess the influences of faecal material on the establishment and growth of seedling from cattle and sheep dung under the same conditions. With these results, we produce a description of the potential role of endozoochorous seed dispersal by cattle and sheep.

MATERIALS AND METHODS

Study plant species

Z. japonica is a perennial species of the Gramineae, mainly distributed across Japan, the Korean Peninsula, and the Liaodong and Jiaozhou Peninsulas of China (Dong et al., 2001). It can form a very dense vegetation of 9632.0 ramets/m^2 (Zhang et al., 2006) by clonal propagation via underground rhizomes. Flowering begins in the middle of June and the seeds ripen about the end of July in the study site. The mean production of seeds is about 14.8 g/m^2. On average, the number of seeds produced in a spike is 34 to 46. The length and width of seeds of *Z. japonica* ranged between 2.0-3.5 and 1.0 to 1.5 mm, respectively, and the thousand seed weight is 0.41 to 0.85 g. Dormancy is characteristic for seeds of *Z. japonica* (Dong et al., 2001).

Study site

The present study was undertaken on one of the sun-facing slopes (40°19'21'' N, 123°11'21'' E, 170 m above sea level) in Liaodong Peninsula, Liaoning Province, north-east China. In the study area, the annual average temperature is 7.8°C, the annual precipitation is 877 mm, and the sunshine duration is 2354.7 h. The soil type in the study site is sandy soil. It is a traditional pasture and holds a single dominant *Z. japonica* population. The domestic animals (cattle and sheep) are grazed on the site year round.

Dung sampling and treatment

Fresh dung samples (less than 1 day old) of domestic herbivores (sheep and cattle) were collected opportunistically from their ranches in the study sites during three consecutive days in early August 2010 during the fruiting period. Ten separate ‘whole cattle dung pat’ samples were taken, each approximately 200 g in air-dried mass. When collecting the dung pats care was taken to leave the lower part in the field to avoid collection of seeds that were sticking on the bottom. Thirty separate ‘cattle dung’ samples, each approximately 10 g in air-dried mass, was collected from the internal part of dung. A composite sheep dung sample comprised about 1000 pellets, about 300 g in air-dried mass. Seeds used as control were directly taken from fruiting plants at the same time in the site. All samples were kept in paper bags at room temperature until the following spring after they were sun dried in the greenhouse.

Intact seeds in dung

A sub-sample of 5 g was taken from each of the 30 ‘cattle dung’ samples. Thirty sub-samples (5 g each) were also taken from the composite sheep dung sample. Thus, 30 groups (replicates) of 5 g cattle and sheep dung samples were shredded, and all intact seeds were counted, respectively. Each of 50 sheep pellets was dissected to count the intact seeds in it.

Seed germination

Intact seeds isolated from dung (‘free seeds’) of both cattle and sheep and the control seeds taken directly from fruiting plants were tested for germination. For each treatment (control seeds, and those defecated by cattle and sheep, respectively), five replications of 100 seeds were sown in 9.0 cm Petri dishes covered at the bottom with two layers of filter paper. The appropriate distilled water was added daily to each dish. Germination tests were carried out in a growth chamber under optimal conditions (light/dark cycle conditions of 16/8 h at 30/25°C and 80% relative humidity) in order to detect the potential contribution of endozoochory to seed dispersal (Han et al., 2000). The experiment started on 17 April 2011. Seeds were considered to have germinated when the radicle penetrated the seed coat. Germinating seeds from all tests were counted every week and discarded. The seeds were incubated for 28 days (Han et al., 2000).

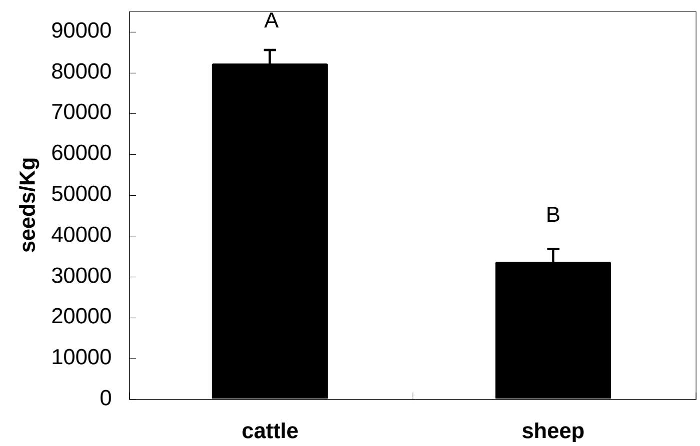

Figure 1. Number of seeds of *Zoysia japonica* per kilogram of air-dried dung of cattle and sheep. The bars represent the standard error of the mean. Vertical bars with different letters indicate differences at P < 0.01.

Seedling establishment and growth

The experiments of seedling establishment and growth were carried out under the same light and temperature conditions as the seed germination experiment. Plastic pots (15 cm diameter × 12 cm height) filled with river sand were used for two treatments: Cattle dung pat and sheep pellets, each treatment with five pots (replicates). Five pots were covered with the appropriate area of cattle dung pat (approximately 170 cm^2 and 200 g). Thirty-five sheep pellets (approximately 8.9 g) were planted just under the surface of sand in each of another five pots. Each pot was moistened daily with distilled water. The pots were relocated randomly once a week to avoid position effects. The number of established seedlings using seeds within cattle and sheep dung ('enclosed seeds') was recorded every week. The seedling height was recorded at five points in the inner pot at the end of experiment. The experiments continued for 7 weeks until the number of established seedlings did not changed.

Statistical analyses

Number of intact seeds in the dung was calculated and then analyzed to determine the statistical differences between treatment means using *t*-tests for independent samples. To analyze the effect of passage through the herbivore on germination, germination rate values were arcsine transformed to approximate normality (Zar, 1984), and all treatments were tested with one-way ANOVA followed by Duncan's multiple comparison test. Values were considered statistically significant at P < 0.01. Data are presented as means ± SE. All the analyses were performed with SPSS 11.5 for windows software.

RESULTS

Seed content

Altogether the 60 dung samples (30 for cattle and 30 for sheep) contained 17236 seeds of *Z. japonica*. The number of seeds per sub-sample of cattle dung was 408.80 ± 18.01, equivalent to 81760.00 ± 3601.67 seeds/kg. Each sub-sample of sheep dung contained 165.73 ± 17.23 intact seeds, equivalent to 33146.67 ± 3446.76 seeds/kg (Figure 1). The mean numbers of seed per kilogram of air-dried cattle and sheep dung were different from each other (P < 0.01). The average seed content per sheep pellet was 8.2 ± 0.63.

Seed germination

The germination rate of the control seeds was 14.40 ± 2.06%, less than those that were obtained from seeds collected from cattle dung (65.40 ± 2.36%) and from sheep dung (62.00 ± 3.83%) (Figure 2). The effect of seed source (control and dispersed by cattle and sheep) was different (P < 0.01). A Duncan's multiple comparison revealed differences between control seeds and those dispersed by cattle and sheep (P < 0.01).

Seedling establishment and growth

Seedlings began to emerge within 7 and 14 days, and continued to emerge and establish for 35 and 49 days from cattle and sheep dung, respectively, after the pots were placed into the chamber and watered (Figures 3 and 4). The rate of establishment was high during 7 to 14 days for seedlings from cattle dung, whereas a slow but almost linear increase in number of seedlings was observed from sheep dung during 7 to 42 days of the experiment. The final mean numbers of established seedlings were 134.40 ± 32.83 seedlings/pat with a height of 62.86 ± 0.98 mm from cattle dung and 0.17 ± 0.04 seedlings/pellet with a height of 32.35 ± 0.94 mm from sheep dung, respectively.

DISCUSSION

Seed content and germination

Disperser effectiveness is a complex and multi-factor trait that also incorporates aspects related to the quantity of seeds dispersed (Schupp, 1993; Loiselle and Blake 1999). There are large numbers of seeds in cattle (81760.00 ± 3601.67 seeds/kg) and sheep (33146.67 ± 3446.76 seeds/kg) dung. In addition, the germination rates of *Z. japonica* seeds ingested by cattle (65.40 ± 2.36%) and sheep (62.00 ± 3.83%) are significantly higher than that collected directly from fruiting plants (14.40 ± 2.06%), suggesting that ingestion by both cattle and sheep can markedly promote *Z. japonica* seed germination. *Z. japonica* seeds are characteristic of dormancy mainly due to its hard seed coats (Dong et al., 2001). The promotion of germination by ingestion of cattle and sheep may result from scarification during chewing and the action of gastric juices on seed coats (Barnea et al., 1991). One may expect that seeds of *Z. japonica*

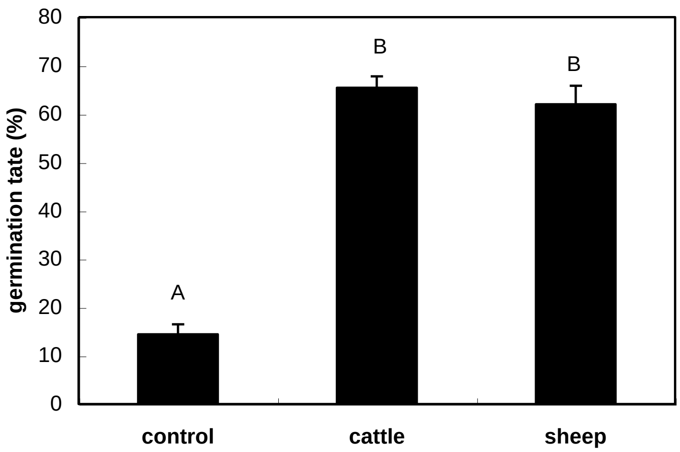

Figure 2. Germination rate of seeds of *Z. japonica* isolated from cattle and sheep dung and seeds directly taken from fruiting plants. The bars represent the standard error of the mean. Vertical bars with different letters indicate differences at P < 0.01.

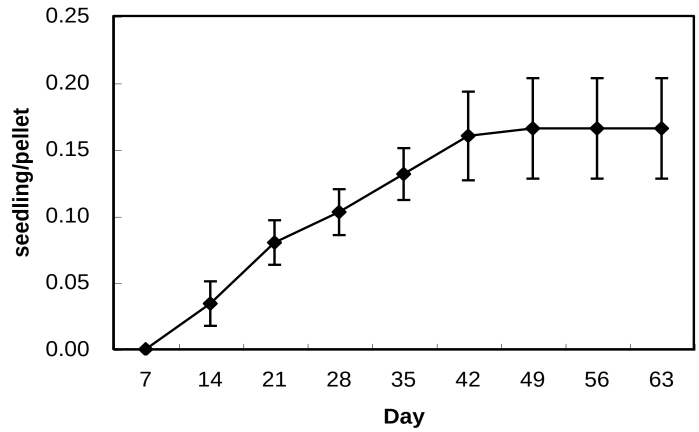

Figure 4. Number of *Z. japonica* seedlings established using seeds within sheep pellets. The bars represent the standard error of the mean.

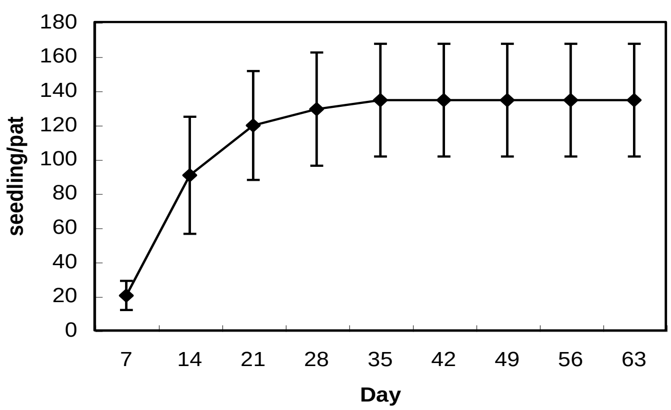

Figure 3. Number of *Z. japonica* seedlings established using seeds within cattle dung pat. The bars represent the standard error of the mean.

probably favor sheep gut passage due to their small size. However, there were no significant differences in germination between seeds defecated by cattle and sheep, in spite of their differences in gut retention time, that is, 24 to 40 h for sheep (Manzano et al., 2005) and 74 h for cattle (Cosyns et al., 2005b), and factors potentially related to the degree of mechanical and/or chemical abrasion of seeds (Traveset, 1998; Traveset et al., 2001a, b). Similarly, a previous study has shown that the effect on germination of seeds ingested by gulls, blackbird and rabbits is not significantly different (Calviño-Cancela, 2004).

Seedling establishment and growth

The seed deposition pattern created by a seed disperser is one of the components of the efficiency of a species as seed disperser (Schupp, 1993). Seed deposition patterns influence the fate of seeds and the establishment and development of seedlings, ultimately determine the recruitment of the plant (Howe, 1989). The study results have shown that the established seedlings using seeds within cattle dung were 134.40 ± 32.83 seedlings/pat with an average height of 62.86 ± 0.98 mm, which means that the turf could be successfully established by spreading cattle pat in an area. Although seedlings developed from clumped seeds may be at a disadvantage in relation to isolated seedlings because of intra- or inter-specific competition (Howe, 1989; Loiselle, 1990), seedling establishment and growth was not affected by the clumped *Z. japonica* seeds in cattle dung. This is characteristic of *Z. japonica* because its natural populations can reach high densities such as 9632.0 ramets/m^2 (Zhang et al., 2006). On the other hand, it had been observed, just after the experiment, that the seedlings mainly developed from seeds in the upper part of cattle dung, and the seeds in the deeper layer of cattle dung could not germinate, or could germinate but not penetrate the dung pat and died, which reduced the effects of clumped deposition pattern in some degree. Faecal material surrounding deposited seeds is generally considered to enhance germination by providing fertilizer (Traveset et al., 2007), but in some cases, its presence may be detrimental (Meyer and Witmer, 1998). Cattle dung, as a fertilizer, may be helpful to successful establishment and development of seedlings. In addition, a cattle dung pat can act as a bare patch to create recruitment opportunities in a grazed grassland **(**Bakker and Olff, 2003). The establishment of 0.17 ± 0.04 seedlings per sheep pellet with a height of 32.35 ± 0.94 mm is low because the seedling emergence rate was only 2.07% (the quotient of 0.17seedlings divided by 8.2 seeds per pellet on average), much lower than germination rate of seeds removed from pellets (62.00%). This clearly indicates that the material of sheep pellets surrounding deposited seeds is detrimental for the germination of *Z. japonica* seeds. However, the large number of pellets defecated by sheep maybe compensate for the generally low establishment success of seedling.

This study stresses the potential of endozoochory by cattle and sheep as an important dispersal mechanism of *Z. japonica* seeds during the fruiting season by controlled experiment. Our results indicated that there were large quantities of intact seeds in both cattle and sheep dung, and ingestion by both cattle and sheep markedly promoted *Z. japonica* seed germination. A high number of seedlings established and grew well from cattle dung pat. Fewer seedlings established from sheep pellets and they had shorter height. These revealed that the endozoochory by cattle and sheep would favor the recruitment of this plant. Compared to the detrimental effect of sheep pellets, cattle dung pat, functioning as a bare patch and as a fertilizer, can create recruitment opportunities and may be helpful to establishment and development of *Z. japonica* seedlings. Hence, cattle can provide a more effective service for *Z. japonica* seed dispersal.

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