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Full Length Research Paper

Effects of animal's rumen juice on seed germination of *Vicia angustifolia* with different seed size

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To help understand the effects of grazing on seed germination characteristics of *Vicia angustifolia* L., we conducted a laboratory germination experiment of *V. angustifolia* L., which is a main companion species of Leguminosae family in alpine grassland of the Qinghai-Tibetan Plateau, using Yak and Tibetan sheep rumen juices along three seed size categories. Results show that Yak and Tibetan sheep rumen juices significantly restrained germination of seeds for three size categories, decreased seed germination percentage, germination index and prolonged first and mean germination times. Meanwhile, the medium-size seeds presented the maximum geminated percentage, and the larger seeds germinated better than the smaller seeds. Additionally, there were significant interaction effects between rumen juice and seed size on seed germination of *V. angustifolia*. Our results suggest that grazing pressure of animal feed present have significant negatively effects on seed germination for *V. angustifolia* with different seed size in alpine area of the Qinghai-Tibetan Plateau.

Key words: *Vicia angustifolia*, rumen juice, seed mass, germination, herbivory.

INTRODUCTION

Herbivory play an important role in regulating plants population dynamics as it is a central problem in grass-land ecosystems, presenting a major influence on vegetation composition with consequent effects on associated fauna and human livelihoods (Duncan et al., 2006). The major effects of large herbivores on tree-grass interactions have been studied experimentally in savanna systems (Riginos and Young, 2007). Livestock feed forage seeds or reproduction organs selectively and changes the competitiveness of pasture species and community environment, which led to species move in and out in the process of grazing. Many studies have reported the influences of herbivory on plant biomass, reproduction, growth rate and so on. Animals grazing can directly affect plant reproduction and indirectly reduced the population density (Vazquez and Simberloff, 2004). Peco et al. (2006) reported that most species showed lower survival and lower germination rate after simulated sheep ingestion on Mediterranean grassland. According to the stochastic simulations, high-intensity grazing can significantly reduce the stochastic population growth rate (Ramula, 2008) and herbivory may have inhibition effects on seed germination by rumen or gut passage of grazers. Following passage through the gastro-intestinal tract, seeds are spread in dung. Passage through the gastro-intestinal tract exposes seeds to digestive juices which may influence seed germination (Peinetti et al., 1993; Li et al., 2010).

Wu et al. (2009) reported that increased grazing intensity facilitated the increase of functional groups of forbs and species of Leguminosae family. But, it is unknown if the animal feed could affect the germination and recruitment of *Vicia angustifolia* in alpine grassland community. In addition, seed size is a key and relatively stable life history characteristic of plants, which play an important role in seed dispersal and germination (Wu and Du, 2008a, 2009). Many studies reported that seed size was related to species germination, seedling emergence and community regeneration (Rees et al., 2001; Guo et al., 2000; Guo, 2003; Murray and Leishman, 2003; Murray et al., 2005; Wu and Du, 2009; Wu et al., 2010).

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Table 1. Seed mass range and seed vigor percentage for three seed size classes of *V. angustifolia*.

Parameter	Mean seed mass (mg/per seed)	Seed mass range (mg)	Seed vigor (%)
The larger seeds	26.734	24.77~28.53	93.31
Medium seeds	20.394	19.25~22.22	95.74
Smaller seeds	8.117	5.69~13.42	97.10

Therefore, we conducted this experiment to study the effects of rumen juices of Yak and Tibetan sheep on seed germination of *V. angustifolia* with different seed sizes. Our objectives were to evaluate: (1) the effect of rumen juices of Yak and Tibetan sheep on seed germination traits; and (2) the effect of intraspecific variation of seed size on seed germination traits for *V. angustifolia* in alpine grassland community of the Qinghai-Tibetan Plateau.

MATERIALS AND METHODS

Study area

This species was sampled in five alpine grassland communities at 3500 m a.s.l. in the eastern Qinghai-Tibetan Plateau at Maqu Wetland Protection Area (33°06′30″~34°30′15″, 100°45′45″~102°29′00″) in Gansu Province, PR China. The mean daily air temperature is 1.2°C, ranging from -10°C in January to 11.7°C in July. Mean annual precipitation is 620 mm, mainly falling during the short, cool summer. The growing season was from April to September of each year. The monthly mean temperature and precipitation, annual average precipitation and annual accumulated temperature of ≥ 0°C from 1969 to 2005 in Maqu County were reviewed in Wu et al. (2009). The annual cloud-free solar radiation is about 2580 h. The vegetation is typical alpine grassland (Wu and Du, 2008b). Ripe seeds were collected randomly from 4 populations and more than 30 individuals in each population for this species to get an adequate representation of the whole community. Ripe seeds of this species were black or bottle green. After collection from the month of August, 2009, seeds were stored at 4°C in the storerooms till they were used (Wu et al., 2006, 2009).

Study species

V. angustifolia is a species of Leguminosae family widely distributed in Europe, Africa and Asia, and also reported in the northwest, east, central and southern areas of China. It belongs to perennial or annual herb, with straight roots and strong nitrogen-fixing ability. It is also a widespread and main companion species in alpine grassland community of the Qinghai-Tibetan Plateau.

Germination experiments

Seeds tested for germination were classed into three different mass categories (Table 1): the larger (24.77 ~ 28.53 mg/per seed), the medium (19.25 ~ 22.22 mg/per seed) and the smaller (5.69 ~ 13.42 mg/per seed), from different plants among sampling communities. Yak and Tibetan sheep are the most important livestock in alpine grassland of the Qinghai-Tibetan Platea. We took fresh rumen juices by rumen fistula method at a temperature of 39°C, which is the *in vivo* temperature for rumens of yak and sheep, preservation by extracting method in healthy yak and Tibetan sheep in local herders. The larger, the medium and the smaller seeds were three seed-size categories, while the rumen juices of Yak and Tibetan sheep and distilled water (control) were used (that is; 3 rumen juices x 3 seed sizes, two factor experiments). They were interactive for two factors. 10 ml rumen juices of yak, sheep and distilled water were irrigated respectively in corresponding treatments. Three replicates were setup in each treatment. Fifty seeds of each treatment were placed on two layers of filter paper in each 9 cm Petri-dish in warm period 25/5°C (day /night) and light cycle 12/12 h (day/night) of the incubator, which was similar to field light and temperature conditions (Wu and Du, 2008b). The whole experiment lasted for 60 days (Li et al., 2010).

Data collection and germination indices

Germinated seeds were counted and removed daily. Radical protrusion was considered to be the criterion for germination. The germination percentage (GP), germination index (GI), weighted germination index (WGI), first germination time (FGT) and mean germination time (MGT) were measured. WGI is a comprehensive assessment effect of different treatments on seed germination.

$$GP (\%) = GN / SN \times 100$$

Where, GP is the final germination percentage; GN is the total number of germinated seeds;
SN is the total number of vigorous seeds (determined by tetrazolium chloride (0.3% TTC) to test);

$$GI = \sum (Gt / Dt)$$

Where, G is the germination number on the day (t) and D is the corresponding days of germination;

$$WGI= [60\times n1 + 59\times n2+\ldots+1\times n60] / (60\times N)$$

Where, n1,n 2, ……,n60 are the number of seeds that germinated on 1st, 2nd, and subsequent days until the 60th day, respectively; 60,59,…,1are the weights given to the seeds germinated on 1st, 2nd, and subsequent days until the 60th day. N is the total number of seeds placed for germination (Bu et al., 2007);

$$MGT = \sum G_i \times i / \sum G_i$$

Where, i is the number of days of germination elapsed since the day of sowing (day 0) and G_i is the number of seeds germinated on day 1 .Seeds that did not germinate at the end of the assay were not considered in this calculation (Figueroa and Armesto, 2001).

Statistical analysis

The data was normally distributed. The effects of seed sizes and rumen juices treatment on germination traits were analyzed with two-way ANOVA. Significant differences for all statistical tests were evaluated at $P \leq 0.05$. All data analyses were conducted using SPSS 13.0 (Chicago, IL, USA).

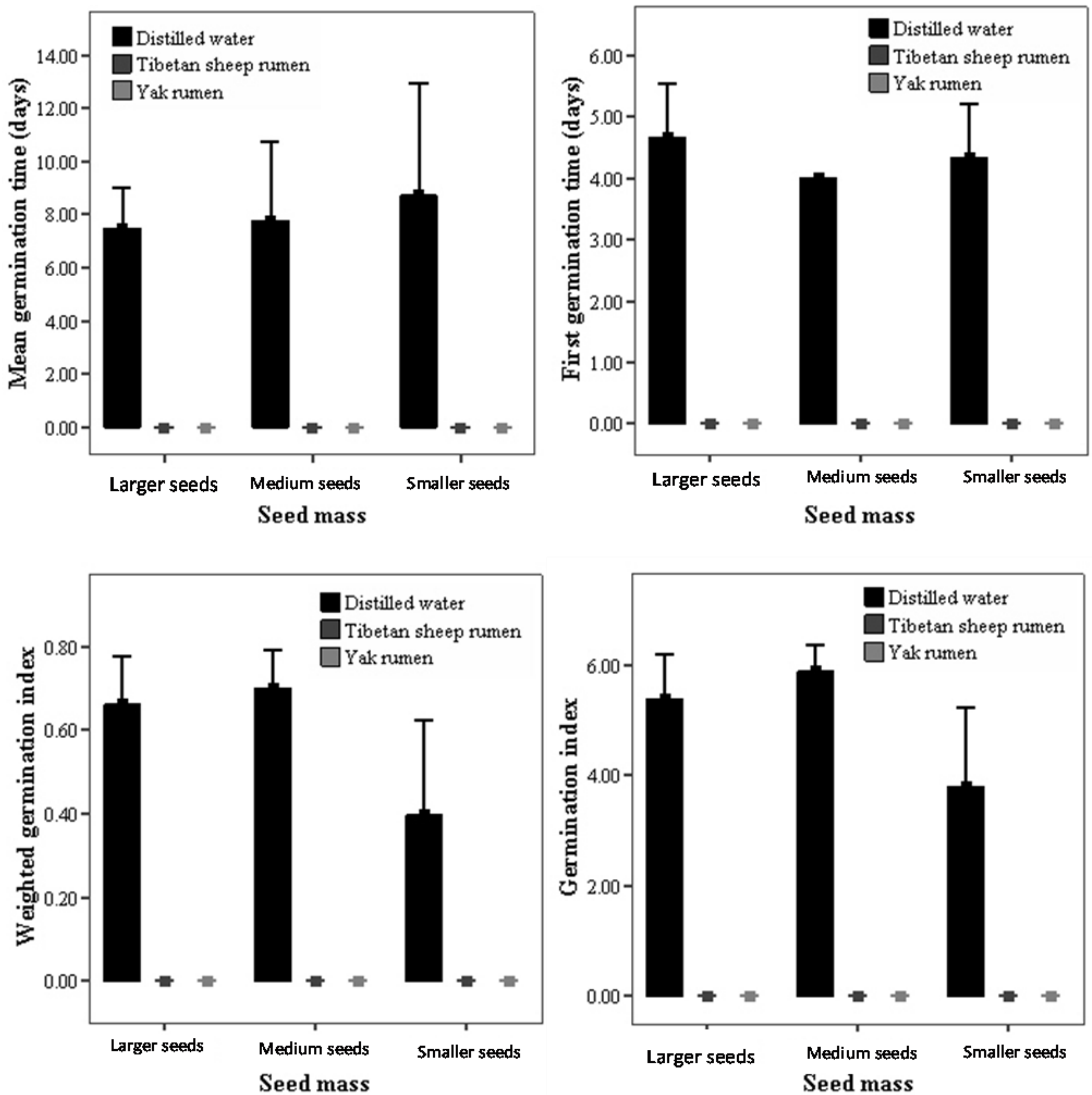

Figure 1. Changes (mean ±SE) of germination percentage, germination index, weighted germination index, first germination time and mean germination time of *Vicia angustifolia* with different seed size under different treatment of Yak and Tibetan sheep rumen juices.

RESULTS

Results show that the two kinds of rumen juice both significantly inhibited seed germination of *V. angustifolia*. Seed GP for three seed-size categories were all close to zero, while, germination of the larger, the medium and the smaller seeds reached 76, 87 and 46%, respectively under distilled water (Figure 1). The GI, WGI, FGT and MGT were all close to zero. Meanwhile, seed mass significantly affected seed GP ($F = 5.99$, $P<0.05$) and WGI ($F = 5.98$, $P<0.05$). The highest GP was showed for the medium seeds, which was double to that of the smaller seeds. Moreover, there was an interaction effect on GP between rumen juices treatments and seed mass ($F = 6.33$, $P<0.01$).

In addition, there was significant inter-action effects between seed sizes and rumen juices treatments on weighted WGI ($F = 9.74$, $P< 0.01$) (Table 2).

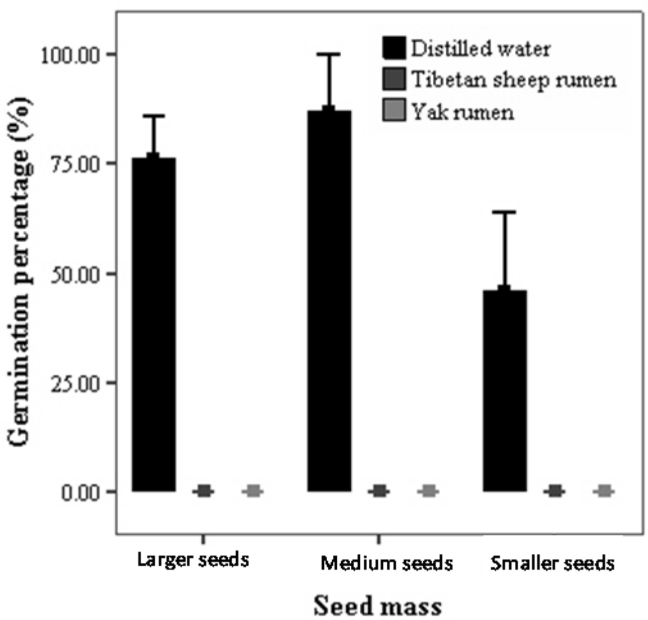

Figure 1. Contd.

The larger seeds showed the higher GP, GI and WGI than the medium and smaller seeds (Figure 1). FGT of the larger seeds was the highest, followed by the smaller and the medium seeds while MGT of the smaller seeds was at maximum (Figure 1).

DISCUSSION

Effects of rumen juices on seed germination of *V. angustifolia*

Contrast with treatment of distilled water, rumen juices of yak and Tibetan sheep significantly decreased seed germination of *V*. *angustifolia* during our test conditions. Previous studies had also reported that rumen juices can significantly inhibited the seed germination (Li et al., 2010). Testing for un-germinated seed, the vitality of these seeds was higher than 75%, which revealed that rumen juices inhibited the germination of the seeds. In a feeding experiment, Toland (1978) reported that there was a lower percentage of grain voided in faces for Avon compared with Swan oats and the breakdown of whole grain in rumination was important for decreased germination. Hashim (1990) held the view that the process of passing through goats' digestive systems can decrease germination of seeds. Results of Lacey et al. (1992) also reported that germination of leafy spurge seeds was reduced by sheep and goats. Interestingly, gut passage from donkeys were proved not to have a positive function on germination. However, this is contrary with the conclusions of Fredrickson et al. (1997), who showed that ruminants were effective disseminating agents for Lehmann love-grass seeds. Maybe livestock only became an effective role of disperser on two legume species, *Adenocarpus decorticans* Boiss and *Retama sphaerocarpa* (L.) Boiss (Robles et al., 2005). In addition, it is reported that between ingested seeds and the control, germination capacity did not have significant functions (Razanamandranto et al., 2004).

Additionally, the size of seed may determine the time it remains in an animal's digestive tract (Levey and Grajal, 1990; Izhaki et al., 1994). The longer the retention time, the deeper the seed coat will be irrigated by chemical reaction and mechanical abrasion, and the higher the germination reached (Razanamandranto et al., 2004). During gut passage, seeds can be scarified by herbivores thus, enhancing the germination capacity (Traveset et al., 2007). But, we supported that rumen juices can reduce seeds germination and harsh treatment of the seed coats can also increase seed mortality through the digestive tract (Hedding and Mullett, 1970; Peinetti et al., 1993; Miller, 1994). We conducted this experiment under a single condition of rumen juices soaking, excluding mechanical frictions. Presumably, within prolonged retention time, acidic substances spread to seed organizations through the skin, causing embryonic death. Another possible explanation is also due to the complex microbial environment of rumen which could inhibit seed germination.

Ruminants spread the seeds and affect the ability of seed germination in the process of feeding, digestion and excretion of plant seeds (Calviño-Cancela, 2004). Through ingestion by ruminants, the seed germination capacity is important for the population dynamics and significant for the evolution of plant-ruminants interactions. The ruminant influence upon the population dynamics of a species has to be appraised relative to other factors that influence germination, whether seed ingestion by dispersers is really disadvantageous to *V. angustifolia.* This can only be evaluated on the fate of the ingested seeds under natural conditions as compared to the un-ingested condition (Traveset, 1998). Therefore, further studies of ruminant effects on germination should integrate chew, digestion and drainage as a whole feed process.

Effects of seed size on germination of *V. angustifolia*

This study reveals that intraspecific seed size presented a significant effect on germination and other study also showed a significant effect on seedling recruitment on *V*. *angustifolia* (Wu et al., 2006). The larger and the medium seed had high numbers of germination, almost double the smaller seeds, which is similar to the findings of Galindo (2008) on *Cecropia obtusifolia Bertol.* The GI, WGI were also markedly higher than smaller seeds. This result was also reported in many other studies (Stanton, 1984; Greipsson and Davy, 1995; Eriksson, 1999; Chacon et al., 1998; Nidhi and Saxena, 2009). Our results also

Table 2. Two-way ANOVA results of rumen juices, seed mass and their interaction on seed germination of *V. angustifolia*.

Variable	Rumen juice			Seed mass			Rumen juice ×seed mass		
	df	F ratio	*P* value	df	F ratio	*P* value	df	F ratio	*P* value
Germination percentage	2	191.66	<0.001	2	5.99	0.010	4	6.33	0.003
First germination time	2	760.50	<0.001	2	1.50	0.250	4	1.45	0.244
Mean germination time	2	371.53	<0.001	2	0.79	0.471	4	0.54	0.549
Germination index	2	122.71	<0.001	2	1.94	0.172	4	1.54	0.147
Weighted germination index	2	224.77	<0.001	2	5.98	0.010	4	9.74	0.003

show that the larger seeds presented an earlier germination than the smaller seeds, in consistent with the report on soybean *(Glycine max)* which showed that large seeds germinated earlier than smaller and the medium seeds (Khandagale et al., 2007). The larger and the medium seeds showed germination advantage, which may be ascribed to bigger storage reserves of these seeds (Gross, 1984; Arista et al., 1992) and some higher relative oil content determined by some gene (Adamski et al., 2009).

Conclusion

In summary, this study supports that livestock grazing significantly decreased seed germination of *V. angustifolia* and the larger seeds presented advantageous germination potential than the smaller seeds within species.

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