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Full Length Research Paper

Yak and Tibet sheep grazing ingestion restrain seed germination of two *Saussurea* species in Tibetan meadow

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Grazing disturbance had been defined as an important mechanism that allows the maintenance of species diversities in plant communities. To help understand effect of grazing on seed germination characteristics, we conducted a laboratory germination experiment with two *Saussurea* species, which were dominant species in alpine meadow communities of the Qinghai-Tibetan Plateau, and yak and Tibet sheep rumen juices. Results showed that yak rumen juices completely restrained seed germination for two species. Tibet sheep rumen juices significantly decreased seed germination percentage, germination index and weighted germination index, prolonged first germination time and mean germination time for two species. *Saussurea japonica* showed a significantly higher germination percentage, germination index and weighted germination index, but a significantly shorter first germination time and mean germination time than *Saussurea iodostegia*. Our study suggests that yak and Tibet sheep grazing ingestion may affect species population dynamic by significantly restraining seed germination in alpine area of the Qinghai-Tibetan Plateau.

Key words: Rumen juices, seed germination, *Saussurea iodostegia*, *Saussurea japonica.*

INTRODUCTION

Seed is the primary carrier of diversity maintenance and provides an important component of species diversity for grassland community (Rees et al., 2001). Seed germination response are central components of plant life-history strategy to disturbance (García-Fayos and Gasque, 2006; Bu et al., 2007a,b; Wu and Du, 2008). Grazing disturbance also had been defined as an important mechanism that allows the maintenance of species diversities in plant communities (Connell, 1978; Kadmon and Benjamini, 2006). Ongoing grazing expansion driven by human population growth in the Qinghai-Tibetan Plateau threatens biodiversity. High-intensity grazing disturbance have brought about the loss of species diversity and multiple degradation of alpine meadow of the Qinghai-Tibetan Plateau (Hao, 2008). Former studies on grazing in grassland community mainly focused on effects of grazing on aboveground biomass and covers (Yagil et al., 2002; Ren et al., 2009), species composition, community structure (Wu et al., 2009), species richness and diversity (Gallacher and Hill, 2008; Wu et al., 2009), forage nutrient cycling (Semmartin et al., 2008), propa-gule availability (Buckley et al., 2007), reproduction (Devoto and Medan, 2004), soil seed bank densities (Sternbergad et al., 2003; Aboling et al., 2008), seedling establishment (Oesterheld and Sala, 1990) and offspring recruitment (Gallacher and Hill, 2008). Community variation was

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Abbreviations: GP, Germination percentage; **CK,** distilled water; **FGT,** first germination time; **MGT,** mean germination time; **GI,** germination index; **WGI,** weighted germination index.

related to seed dispersal which was affected by animal predation traits and feeding habits (Malo and Suárez, 1995). Fraser and Madson (2008) had reported that animal herbivory controlled wet meadow community composition and diversity by seed and seedling predation. Herbivore grazing can adjust plant competition and species coexistence by herbivoring seedling (Hanley and Sykes, 2009).

However, it is also an important ecological process that herbivore eat seeds when they graze. Many herbaceous seeds are eaten by animals. The seeds cannot be digested and are passed out in the process. To date, although some studies on seed germination have been carried out on the Qinghai-Tibetan Plateau (Bu et al., 2007a,b, 2008, 2009; Wu and Du, 2008), the effect of animals grazing ingestion on seed germination had not been reported.

In this study, two dominant *Saussurea* species in alpine meadow communities of the Qinghai-Tibetan Plateau were selected to study seed germination response to rumen juices, which was simulated to be ingested by yak and Tibet sheep. The specific objective of this study is to assess the effect of rumen juices on seed germination characteristics for these two *Saussurea* species and discuss the effect of grazing ingestion on seed germination of two *Saussurea* species in the Qinghai-Tibetan Plateau.

MATERIALS AND METHODS

Study area

The region of species distribution for this study is located in alpine meadow at mean 3500m a.s.l. at Maqu (N33°42'21", E102°07'02") in Gansu Province, P. R China which is located at the eastern Qinghai-Tibetan Plateau. The mean annual temperature is 1.2°C, ranging from –10°C in January to 11.7°C in July, with about 270 frost days. The mean annual precipitation is 620 mm, with a main rain period during a short, cool summer. The annual cloud-free solar radiation was about 2580 h. The vegetation is a typical alpine meadow and is dominated by clonal grasses (*Kobresia* sp., grasses and sedges) and forbs (*Saussurea* sp. and other species of Asteraceae) (Wu et al., 2009).

Study species

Saussurea DC. is one of the largest genera comprising of over 300 species in the Asteraceae and is mainly distributed in temperate regions with the greatest concentration of species being found in subarctic regions of Eurasia (Lipschitz, 1979). The Qinghai-Tibetan Plateau is a major distribution centre of species of *Saussurea* DC (Shi and Jin, 1999), the *Saussurea* species are dominant species in alpine meadow (Wang and Liu, 2004). We selected *Saussurea iodostegia* and *Saussurea japonica* which are two perennial herbaceous broad-leaf species and dominant species in alpine meadow of the Qinghai-Tibetan Plateau.

Seeds were collected randomly from more than 20 individuals for each species to get an adequate representation of the whole community in the alpine meadow around the Maqu country of Gannan County of Gansu Province of China, which is on the east of the Qinghai-Tibetan Plateau in August to September 2009. Seeds of each fixed individual were collected and counted. Ripe seeds were cleaned and conducted with room conditions (about 15°C) in kraft-envelope until use (Wu and Du, 2008), in the laboratory of Northwest A & F University, China.

Germination experimental design

In this study, we compared three treatments: Distilled water (CK), yak rumen juices and Tibet sheep rumen juices. Three yaks and three Tibet sheep were selected for collecting suction of rumen juices in local herder, and fresh rumen juices of three yaks and three Tibet sheep were mixed, respectively. We marinated seeds of the two species into rumen juices of yaks and Tibet sheep, respectively, and maintained the temperature to 39°C to simulate the temperature condition inside the animal rumen. It was sustained for 48 h which was the approximate ruminating time of forages in the body for ruminant animals. Then, seeds were taken out and used for germination experiment. The germination experiment was started on the tenth day of November in 2009 and finished in the mid of January, 2010. The experiment of seed germination lasted for 60 days. Fifty seeds were placed in covered Petri dishes (9 cm diameter) on a double layer of moistened filter paper, then placed in temperature chambers at a diurnal fluctuation (25°C and full light intensity per 12 h day and 5°C and darkness per 12 h night) with a relative humidity of about 50%. The temperature and light of the germination trial resembled natural conditions: It approximated to the daily maximum and minimum temperature in topsoil. Each species in each treatment had three replicates of 50 seeds. Every day, the percentage of seeds that had germinated was recorded. Newly emerged seedlings were removed from the Petri dishes, and seeds were regularly watered with distilled water. A seed was considered as germinated when the radicle was visible.

Data analysis

In this study, we used four germination parameters: Germination percentage (GP, %), first germination time (FGT), mean germination time (MGT), germination index (GI), and weighted germination index (WGI). Final GP for each treatment was calculated after 60 days. The GI is based on number of seeds that germinated and the germination rate. These parameters were also calculated from the following formulas (Figueroa and Armesto, 2001; Wu and Du, 2008; Bu et al., 2007a,b, 2008, 2009):

$$GP = 100 \times GN / SN$$

Where, GN is the total number of germinated seed and SN is the total number of seeds tested.
FGT is the number of days from seed sowing to first germination.

$$MGT = \sum_i G_i \times i \Big/ \sum_i G_i$$

Where, *i* is the number of days since the day of sowing (day 0) and G*i* is the number of seeds that germinated on day *i*. Only seeds that germinated were included in the calculation.

$$GI = \left(\sum_i (60 - i) \times G_i \right) \times 100 \Big/ (60 \times GN)$$

GI is a synthetic measure designed to reflect the synthetical germination ability which include germination rate and germination numbers. Where, *i* is the number of days since the day of sowing and *Gi* is the number of seeds that germinated on day *i*.

A weighted germination index (WGI) as described by Bu et al. (2009) was calculated with maximum weight given to the seeds that

Table 1. ANOVA results of effects of species and rumen juices treatments on seed germination properties for *Saussurea iodostegia* and *Saussurea japonica* (Significance was determined at P< 0.05).

Source	Dependent variable	d.f.	*F*	*P*-Value
Species	Germination percentage	1	5.11	0.00
	First germination time	1	3.96	0.07
	Mean germination time	1	0.65	0.43
	Germination index	1	2.45	0.00
	Weighted germination index	1	6.15	0.00
Treatment	Germination percentage	2	7.05	0.00
	First germination time	2	4.54	0.00
	Mean germination time	2	4.44	0.00
	Germination index	2	3.67	0.00
	Weighted germination index	2	6.72	0.00

Table 2. Mean (±S.D.) of germination percentage (GP), first germination time (FGT), mean germination time (MGT), germination index (GI) and weighted germination index (WGI) for seeds of *S. iodostegia* and *S. japonica* under different treatment.

Parameter	Species	Treatment		
		Distilled water	Yak rumen juice	Tibet sheep rumen juice
GP	*S. iodostegia*	2.71±0.39	0.00±0.00	0.00±0.00
	S. japonica	82.50±7.26	0.00±0.00	25.52±3.11
FGT	*S. iodostegia*	18.33±5.51	–	–
	S. japonica	5.00±0.00	–	7.00±0.00
MGT	*S. iodostegia*	19.33±6.43	–	–
	S. japonica	8.06±0.69	–	8.21±1.06
GI	*S. iodostegia*	0.07±0.02	–	0.00±0.00
	S. japonica	6.11±0.65	–	1.13±0.86
WGI	*S. iodostegia*	0.02±0.01	–	0.00±0.00
	S. japonica	0.73±0.07	–	0.16±0.12

–, Seeds had not germinated until 60th day.

germinated early and less to those that germinated late.

$$WGI = [60 \times n_1 + 59 \times n_2 + 58 \times n_3 + \ldots\ldots + 1 \times n_{60}] / (60 \times N)$$

Where, n_1, n_2, …, n_{60} are the number of seeds that germinated on the 1st, 2nd and subsequent days until the 60th day, respectively; 60, 59, ……, 1 are the weights given to the seeds that germinated on the 1st, 2nd and subsequent days until the 60th days. N is the total number of seeds placed in incubation.

Significant differences for all statistical tests were evaluated at the level of $P \leq 0.05$ with ANOVA. All data analyses were conducted using the Statistical Package for the Social Sciences (SPSS) for Windows, Version 13.0 (Chicago, IL, USA).

RESULTS AND DISCUSSION

There were significant effects of species on GP, GI and WGI (Table 1). *S. japonica* showed a significantly higher GP, GI and WGI, but a significantly shorter FGT and MGT than *S. iodostegia*. Seeds of *S. iodostegia* germinated less than 3%, and *S. japonica* germinated about 83% in distilled water treatment (Table 2).

ANOVA result showed that rumen juices treatments had significant effects on seed GP, FGT, MGT, GI and WGT (Table 1). Seeds of two species both showed no germination in Yak rumen juices treatment. Seeds of *S. iodostegia* also showed no germination in Tibet sheep rumen juices treatment. Tibet sheep rumen juices treatment significantly decreased seed GP, GI and WGI, and postponed FGT and MGT for *S. japonica* than distilled water treatment when compared (Table 2).

Our results showed that seeds germination of these two *Saussurea* species were significantly restrained by rumen juices of yak and Tibet sheep. Especially for yak rumen juices, it absolutely resulted in no germination of seeds of two species in this study. Livestock is considered as dispersal agents for seeds of plant species in plant

community, especially in degraded grasslands (Archer and Pyke, 1991; Gardener, 1993). Ocumpaugh (1996) had reported that fecal seeding of animals was better than broadcast seeding for seedling emergence and establishment of switchgrass in grassland. So, animals grazing potentially affected the cost and feasibility of this alternative re-vegetation strategy of seedling recruitment by affecting seed germination through seed grazing ingestion. Subsequently, animals grazing affected population dynamic and community structure.

Some researches had been done on the recovery and germinability of seeds that have passed through ruminant digestive tracts (Ozer, 1979; Thomson et al., 1990; Peinetti et al., 1993; Gökbulak, 2002; Gökbulak and Call, 2004; Cosyns et al., 2005). The germinability of grass seed generally decreases with length of time in the ruminant digestive tract (Gardener et al., 1993; Gökbulak, 2002). The seeds had lower germinability/viability when they passed through rumen of animals for about 1 - 2 days. It may be attributed to the fact that some seeds may be damaged by animal mastication; and some seeds were influenced by the rumen juices and change their physiological, biochemical matters or internal secretion within seeds, especially for seeds with soft seed coats. But Jones and Simao Neto (1987) had reported that seed intake by animals did not have a significant influence on seed germination percentage for *Paspalum notatum* and *Axonopus affinis*.

Our study suggested that yak and Tibet sheep grazing ingestion may affect species population dynamic by significantly restraining seed germination in alpine area of the Qinghai-Tibetan Plateau. However, more researches are needed to determine the appropriate physiological, biochemical or internal secretion mechanism which control seed germination when seed pass through animal body for more forbs species in plant community which had undergone heavy grazing pressure.

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