Responses of *Artemisia frigida* Willd. (Compositae) and *Leymus chinensis* (Trin.) Tzvel. (Poaceae) to sheep saliva

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Abstract

Although studies show that grazing and browsing by herbivores have marked effects on host plants, the mechanisms remain unclear. The objective of this study is to determine the effects of sheep saliva on host plant growth. Sheep saliva was manually applied to clipped plants of two different life forms, a semi-shrub, *Artemisia frigida* Willd., and a herbaceous species, *Leymus chinensis* (Trin.) Tzvel. The results showed that sheep saliva significantly enhanced aboveground net primary productivity (ANPP) and the ratio of ANPP to belowground net primary productivity (BNPP) for both species. This indicated that sheep saliva promotes aboveground compensatory growth and allocation of photosynthate to aboveground for both plant species. Sheep saliva stimulated only tillering of *L. chinensis*. Regardless of saliva application, clipping significantly decreased BNPP and plant height, but significantly increased the number of branches or tillers for both plant species. The relative growth rates (RGRs) on both species were significantly greater after clipping with saliva compared with control and clipping without saliva treatments. In addition, RGR of the herbaceous species *L. chinensis* was faster than that of the semi-shrub *A. frigida* after application of saliva.

Keywords: Clipping; Inner Mongolia steppe; Net primary productivity; Relative growth rates

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1. Introduction

Research on plant–herbivore interactions has mostly used experiments that apply mechanical damage to simulate herbivore grazing (Agrawal et al., 1999; Bergman, 2002; Hjalten, 2004; Pilson and Decker, 2002). However, the mechanical damage is abiotic action to the host plant (Davis and Boyd, 2000; Krupnick et al., 2000; Tiffin and Inouye, 2000). The effects of applying herbivore saliva to the host plant result in controversial opinions because it is biotic action.

For many years, studies on the role of herbivore saliva in plant–herbivore interactions have focused mostly on graminoid species (Rooke, 2003), and there are conflicting viewpoints (Detling et al., 1980). Much research has inferred that ungulate saliva may increase regrowth of plants (Matches, 1992), and that there are substances in herbivore saliva, such as thiamine, that promote plant growth (Dyer et al., 1993; McNaughton, 1979, 1985; McNaughton et al., 1983; Reardon et al., 1972; Rhoades, 1985). However, other researchers have found that saliva had no significant effect on plant growth (Detling et al., 1980). Even the same researcher found different results with the same sample (Reardon et al., 1972, 1974).

There are two probable reasons for the differences among studies on the effects of herbivore saliva. First, the tested plant species are different. Herbaceous plants and woody species have different morphological and physiological characteristics (den Herder et al., 2004; Macfadyen, 1981; Rooke, 2003). Second, the response to herbivory or clipping may depend on the timing, type, extent of damage, availability of resources, and grazing history of the host plant (Gadd et al., 2001).

Recently, research on the effects of grazing on plants and plant communities in the Inner Mongolia steppe has been conducted (Chen and Wang, 2000; Wang et al., 2003). It is helpful for us to understand the mechanisms of grazing and the adaptive strategies of host plants to grazing animals. In the present study, two species were chosen for the experiment: *Artemisia frigida* Willd., a semi-shrub, which is a dominant species in the heavily grazed grassland community; and *Leymus chinensis* (Trin.) Tzvel., a herbaceous, which is a dominant species in the potential climax grassland community. These species were chosen because they are two major forage plants for grazing animals and they are different life-form species in the Inner Mongolia steppe. In this study, we addressed the following questions: (1) How does sheep saliva affect the growth of clipped plants? (2) Are there different responses between the two life forms to sheep saliva?

2. Materials and methods

2.1. Study site

The Inner Mongolia Grassland Ecosystem Research Station established an experimental grazing field in 1989. It is located at 43°37’N, 116°43’E, mostly at an elevation of 1000 m above sea level or more. The regional climate is continental, with a 30-year average annual rainfall of approximately 350 mm (200–500 mm), 60–70% of the total occurring from June to August. Annual mean temperature is −0.4 °C and average monthly temperature is −23 °C in January and 17.9 °C in July. There are 150–180 favorable days for plant growth per year. The predominant plant species are *A. frigida* and *Cleistogenes squarrosa* (Trin.) Keng (Wang et al., 2001).
2.2. Plant materials

*A. frigida* is a widely distributed deciduous semi-shrub with small hairs on the leaves and stems. The plant varies in height from 10 to 40 cm, depending on grazing intensity. It is an important species in the grassland of Inner Mongolia due to its relatively high herbage quality. Many large ungulate livestock, particularly horses and sheep, preferentially graze it during spring, early summer, and autumn (Li et al., 2002; Wang, 2000, 2001). It has strong tolerance to drought and is one of the dominant species on degraded grasslands (Wang et al., 2001; Zhou et al., 2004).

*L. chinensis*, a perennial herbaceous species, is distributed widely in the eastern region of the Eurasian steppe zone (Chen and Wang, 2000). It is highly palatable for grazing animals and is used for grazing grassland and haymaking. The plant has long, strong rhizomes which distribute horizontally about 10 cm under the ground surface with vigorous vegetative propagation (Wang et al., 2004).

2.3. Experimental design

In late May 2004, the experiment was conducted in a fenced plot that had not been grazed since 1989. Twenty-eight quadrates of 0.0625 m² (0.25 × 0.25 m) were established in each species-dominated community. Quadrates were chosen so as to be as similar as possible in plant size (including plant height and number of branches) and at least 1 m apart. Eighteen of 28 quadrates were selected and randomly divided into three groups and labeled. A monoculture of *A. frigida* or *L. chinensis* was maintained through clipping other plants over the experiment within the 18 quadrates. Meanwhile, the *L. chinensis* population within 0.5 m of the peripheries of the quadrates was also clipped throughout the experiment to prevent rhizomes from growing into the plots. Treatments included: (1) no treatment (abbreviated “T₀”); (2) clipping without saliva (abbreviated “T₁”); and (3) clipping with saliva (abbreviated “T₂”). For *T₁* and *T₂* treatments, plants were manually clipped with scissors on June 5, 2004, to approximately 20% of plant height. This was above the majority of the apical meristems.

Sheep saliva was collected by inserting a sponge into the mouth of the sheep, which was a 2-year-old wether, at the experimental site. The sponge was sterilized with 70% alcohol and then dried before it was used. When enough saliva was collected in a tube, the chosen plants were clipped and saliva was applied immediately to the damaged parts. About 2.5 ml of saliva was applied to each treated quadrat. All treatments were performed within 3.5 h, alternating between the two treatments (clipping alone vs. clipping with applying saliva) to prevent any temporal bias.

The clipped parts of each quadrat were collected. The treatments were applied only once at the beginning of the experiment.

2.4. Plant measurements

Preceding treatment application on 5 June 2004, we accurately counted branch numbers and measured the height of *A. frigida* and *L. chinensis* on all 28 quadrates. The branch number of *A. frigida* was based on its tertiary branches. Living shoots were cut at ground level. Root samples were then taken using a soil corer (0.25 × 0.25 × 0.3 m) directly beneath the crown of the host plants, and washed. Other plant roots were carefully removed by
hand. All samples were oven-dried at 65°C to a constant weight. On 10 August 2004, preceding the final harvest, re-measurements were taken on the \( T_0, T_1, \) and \( T_2 \) treatments for both species, and plant heights were also measured.

### 2.5. Data analysis

We used non-destructive estimation based on the data from the 10 quadrates for each species to get initial aboveground biomass for each plot on 5 June 2004. There were significant positive correlations between aboveground and belowground biomass and the number of branches for both species (\( A.\ frigida, \) aboveground biomass: \( R^2 = 0.914, \) \( n = 10, \) \( p < 0.001; \) \( L.\ chinensis, \) aboveground biomass: \( R^2 = 0.945, \) \( n = 10, \) \( p < 0.001; \) \( L.\ chinensis, \) belowground biomass: \( R^2 = 0.872, \) \( n = 10, \) \( p < 0.001 \)). The initial biomass of the 18 quadrates for each species was estimated using the linear model. There were no significant differences for either species between the 18 quadrates and the 10 quadrates (\( p > 0.05 \)).

The aboveground net primary productivity (ANPP) of both plant species for the \( T_1 \) and \( T_2 \) treatments was the sum of present biomass (including standing dead and litter) in August and biomass removed in June (Wang and Wang, 2001). The belowground net primary productivity (BNPP) of both plant species was the difference between the belowground biomass on August 10 and the belowground biomass on June 5 (Chen and Huang, 1988). The relative growth rate (RGR) of both species was determined according to the following formula (Ishikawa and Kachi, 2000):

\[
RGR = \frac{\ln(W_2) - \ln(W_1))}{T_m},
\]

where \( W_i \) is the plant dry weight at the time \( T_i (i = 1 \) and 2) and \( T_m \) the treatment period in days (67 days in the present study).

Data were statistically analyzed using the two-way ANOVA procedure of the SPSS 10.0 software (Chicago, IL, USA) to test the effects of both plant species (\( A.\ frigida \) and \( L.\ chinensis \)) and the three treatments (\( T_0, T_1, \) and \( T_2 \)) and their interaction (species × treatments) on the growth-related variables (i.e., plant height, the number of branches, ANPP, BNPP, the ratios of ANPP to BNPP, and RGR). Type III sums of squares were used. Because of differences in the magnitudes of the variables between both plant species, a log10 transformation was applied so that they could be compared. Additionally, the correlations between tested parameters were analyzed using Pearson’s correlation matrix.

### 3. Results

Plant species had significant influence on plant height, number of branches, ANPP, ratio of ANPP to BNPP, and RGR, whereas no significant effect was found on BNPP (Table 1).

Treatments had a significant effect on all of the test variables (Table 1). Clipping significantly decreased plant height and increased branch numbers of both plant species (\( P < 0.001 \)), whereas saliva did not affect the heights of either plant species (\( P = 0.313 \)) and only increased branch numbers of \( L.\ chinensis \) as compared to the clipping only treatment (Fig. 1). Furthermore, clipping alone (without saliva) significantly increased ANPP of \( A.\ frigida \) (\( P = 0.045 < 0.05 \)), and clipping with saliva significantly increased ANPP of both species (\( P = 0.003 \)). This indicated that saliva promoted compensatory growth of
aboveground biomass of the plants. However, regardless of saliva application, clipping significantly decreased BNPP of both plants ($P < 0.001$) (Fig. 1). Clipping alone significantly increased the ratios of ANPP to BNPP only in *A. frigida*. (Fig. 2), but

<table>
<thead>
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<th>Factors</th>
<th>d.f.</th>
<th>Plant height</th>
<th>Number of branches</th>
<th>ANPP</th>
<th>BNPP</th>
<th>Ratios of ANPP to BNPP</th>
<th>RGR</th>
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<tr>
<td>S</td>
<td>1</td>
<td>59.66**</td>
<td>67.52**</td>
<td>72.95**</td>
<td>5.37 ns</td>
<td>73.75**</td>
<td>19.71**</td>
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<td>T</td>
<td>2</td>
<td>13.99**</td>
<td>80.63**</td>
<td>20.57*</td>
<td>5.51*</td>
<td>23.36**</td>
<td>4.83*</td>
</tr>
<tr>
<td>S x T</td>
<td>2</td>
<td>0.87 ns</td>
<td>1.89 ns</td>
<td>22.41**</td>
<td>0.15 ns</td>
<td>0.06 ns</td>
<td>1.93 ns</td>
</tr>
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ns, no significant; *$P < 0.05$; **$P < 0.01$.

Values are $F$ ratios and their significances for effects.

![Figure 1](image_url)

Fig. 1. The effect of clipping and sheep saliva on four characteristics (mean ± 1 SE) of *Artemisia frigida* and *Leymus chinensis*: (a) plant height; (b) the number of branches; (c) the aboveground net primary production (ANPP) within 67 days; and (d) the belowground net primary production (BNPP) within 67 days. White bars, $T_0$ (no treatment); gray bars, $T_1$ (clipping without saliva); black bars, $T_2$ (clipping with saliva). The data are untransformed values and are presented on a per square meter basis. Bars with the same letter indicate no significant difference at the $P \leq 0.05$ level.
clipping with saliva significantly increased the ratio in both species. This indicated that saliva stimulated plants to invest relatively more resources to aboveground parts compared to belowground parts. According to the RGR, the two life-form plants showed different response to clipping and saliva treatments (Fig. 3). After clipping with saliva, the RGR of both plants was significantly greater than on the control or clipping without saliva treatments \((P<0.001)\). The RGR of the herbaceous species \textit{L. chinensis} was faster than that of the semi-shrub \textit{A. frigida} after application of saliva \((F_{1,10} = 11.304, P = 0.007<0.01)\). Significant interaction between plant species and treatments was observed only for ANPP (Table 1).

The number of branches was significantly positively correlated with the ANPP and negatively correlated with the BNPP. Negative correlations between plant height and
branch number and ANPP were observed (Table 2). These results indicated that trade-off occurred between plant height and branch production (i.e., clipping decreases plant height but increases branching).

4. Discussion

In this study, the different responses of both plant species on branch or tillering to sheep saliva could be mainly due to their different life forms (Fig. 1). A few researches proved that application of herbivore saliva led to significantly more branches than without saliva treatment on woody plants (Bergman, 2002; Rooke, 2003), because woody plants undergo secondary growth originating from secondary or lateral meristems and have obviously apical dominance, whereas non-woody plants undergo primary growth (Bergman, 2002). However, no significant effect of saliva on branching in the semi-shrub was shown in this study. The reason could be an effect of the differences in growth stage (sapling versus mature level) and ecosystems adaptation. Nevertheless, sheep saliva stimulated tillering or branches of the grass *L. chinensis* (Fig. 1) which has clonal propagation through its tillering and rhizomes (Wang et al., 2004).

Consistent with the findings of Dyer et al. (1993) and Paige and Whitham (1987), our study showed that saliva resulted in aboveground compensatory growth of both plants. However, Rooke (2003) did not find significant effects of saliva on the aboveground biomass of the woody species *Combretum apiculatum* (Combretaceae). Apart from methodological differences between the trials (measurements on sapling versus mature level), these different results could be explained by the fact that the species are adapted to different ecosystems (tropical climate versus continental climate).

Our results showed increase of the ratios of ANPP to BNPP after clipping regardless of saliva application probably because the photosynthetic rate of the residual plant parts increased (Painter and Detling, 1981; Wallace et al., 1984) and more current photosynthate was allocated to new shoots (Hjalten, 2004) even moving from roots to shoots (Belsky, 1986; Bjorkman et al., 2000).
Compared with herbaceous species, the woody species had much lower RGR after clipping with saliva, which was partially due to the low photosynthetic rates (Reich et al., 2003). The maximal photosynthetic rate was 6.2 μmol m⁻² s⁻¹ for the semi-shrub *A. frigida* (Zhou et al., 2004), whereas it was 17.8 μmol m⁻² s⁻¹ for *L. chinensis* (Du and Yang, 1983). Therefore, the results showed that herbivore saliva does have a potential positive effect on plant growth which was agreement with other report (Bergman, 2002).

5. Summary

There were different responses of different life form plants to sheep saliva. After application of saliva, the tillering of only *L. chinensis* significantly increased, and its RGR was faster than that of the semi-shrub *A. frigida*. Sheep saliva promoted aboveground growth and increased allocation of photosynthate to aboveground for both plant species. In addition, the RGRs on both species were significantly greater after clipping with saliva compared with control and only clipping.

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References


