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Effects of Litter and Seed Position on Seedling Establishment of *Gentiana dahirica* Fischer

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Abstract

In recent three decades the medicinal plant *Gentiana dahurica* Fischer has been threatened by large scale exploitation for trade and land-use practices, such as over-grazing and reclamation. However, research into the population ecology and sustainable use of this highly-threatened medicinal plant is lacking, and so we analyzed the effects of different litter applications and seed positioning (surface-sown or buried) as part of a program to rehabilitate it. The results indicated that although litter and seed position per se had no significant effects on the seedling emergence of *G. dahurica*, interestingly, litter application did show positive effects on seedling survival, e.g. 100 g m^{-2} and 200 g m^{-2} litter increased it by 9.7% and 16.4%, respectively, compared to control. Moreover, the seedling leaf area increased with the quantity of applied litter when seeds were surface-sown, with the highest leaf area occurring in the 200 g m^{-2} treatment (9.7-fold of the control), and litter-covered treatments also significantly improved seedling root length and root diameter of *G. dahurica* compared to controls. Taken together, these data indicated that seedling establishment of G. *dahurica* benefited from accumulated litter, and it is thus suggested that fencing to exclude grazing for a period is the best method to protect and rehabilitate the threatened wild *G. dahurica* populations.

Keywords: grasslands, regeneration, rehabilitation, threatened plant species, litter

1. Introduction

Plants belonging to the *Gentiana* species are broadly used as traditional Chinese herbal medicines. For example, root of *Gentiana macropyhlla* and *G. dahurica* Fischer are used for the treatment of inflammatory diseases (ie. osteoarthritis and rheumatoid arthritis) (Yang, Ma, Zhou, & He, 2006; Wang et al., 2007).

Gentiana dahurica is distributed mainly in steppe grassland (Wang et al., 2007). In recent three decades, however, land use changing (reclamation etc.), heavy-grazing and over-exploitation of natural populations of medicinal plants have resulted in degradation of these native grasslands (Akiyama & Kawamura, 2007), and the micro-habitats of these medicinal plants, including *G. dahurica*, were largely destroyed (Lin, Chen, & Yang, 2007). Populations of *G. dahurica* are thus declining rapidly, and it is now recorded as the third levels protected Chinese medicinal plants (Lin et al., 2007).

The natural habitat restoration of *G. dahurica* was mainly depended on fencing. Fencing to exclude grazers or to reduce grazing intensity is one of the important management measures used to protect grasslands and to rehabilitate native vegetation (Carr & Turner, 1959). It has been observed that litter accumulates during fencing off (Rotundo & Aguiar, 2005; Liu, Huang, Han, & Sun, 2009), and this accumulation of plant litter can both have positive (Eckstein & Donath, 2005; Rotundo & Aguiar, 2005) and negative (Xiong & Nilsson, 1999; Jensen & Meyer, 2001) effects on seedling recruitment. These effects varied significantly among species in the same community and across microhabitats in the landscape, but it is known that these effects are related to the amounts of litter (Hovstad & Ohlson, 2008; Ruprecht, Donath, Otte, & Eckstein, 2008). Thus, the most important limits for seedling recruitment in many study sites appears to be the lack of safe germination sites due to overly thick litter (Ruprecht, Józsa, Ölvedi, & Simon, 2010). Seeds within the litter layer (but without contact with the soil) showed reducing seedling emergence and growth (Rotundo & Aguiar, 2005), as did those that were simply not buried in the soil (Huang & Gutterman, 1998).

Seed germination is a critical stage in the life cycle of plants, and it often controls population dynamics and biodiversity in grasslands (Tilman, 1993; Zobel, Otsus, Liira, Moora, & Mols, 2000), with major practical implications. The seed germination process plays an important role, since the dispersal of *Gentiana* plants and/or their reproduction occurs exclusively via seeds (Zhang, Wang, & Liu, 2004). Some previous studies have been conducted to investigate reproduction of *G. dahurica*, these concerned temperature, light and seed presoaking treatments (Lin, 2005; Lin et al., 2007), and the information about the population ecology of *G. dahurica* seeds is scarce, such as germination and seedling establishment patterns under natural conditions (eg. the effects of litter level and seed position). Therefore, in order to provide information for the conservation and future ecological study of *G. dahurica*, in this study we analyzed the effects of different litter applications and whether seeds were surface-sown or buried on the population ecology of *G. dahurica* in northern China.

2. Materials and Methods

2.1 Site Description

The experiment was conducted in greenhouse at National Field Station of Grassland Ecosystem (SaiBei) (NFSGE) (41º45′~41º57′N, 115º39′~115º48′E, alt: 1400 m), Saibei administrative region, Hebei province, China. It is located at the south of Xilingol steppe grassland. During the experiment period from July to September, mean temperature is 23.9 ºC and mean humidity is 59.0% in the greenhouse. The data was collected by automatic HOBO temperature and humidity apparatus. The humidity of the greenhouse is controlled by wet shade. PAR was 1790 µmol m^{-2} s^{-1} at 13:00 on July 10 (cloudless day).

2.2 Materials

Seeds of *G. dahurica* were obtained from its natural habitat in the Saibei administrative region in the autumn of 2007. Seeds were air dried at room temperature, cleaned and stored in paper bags. Mean seed mass (for 1000 seeds) was 0.19 ± 0.005 mg. Germination trials was conducted using 400 filleld seeds to determine seed viability (75.3%) (Association of Official Seed Analysts, 1988).

Litter was collected from the natural habitat of *G. dahurica* in a *Leymus chinensis* dominated grassland in Saibei administrative region. Litter was dead plant material (principally grass leaves eg. *L. chinensis, Agropyron cristatum*, and *Cleistogenes squarrosa*, and other were some forbs which only acount for less than 10% of the litter, such as *Artemisia eroipoda* Bunge, *A. tanaectifolia*, *Sausserea amara*, *Potentilla tanacetifolia*) which lying loose on the ground, and then was air dried. The amount of litter in the grassland after 4-8 years fencing ranged from 100 to 200 g m^{-2} in the middle of the growing season.

2.3 Experimental Design

A greenhouse experiment was conducted with a two-way factorial design with litter quantity and soil burial (seed position) as factors. Litter quantity had 4 levels: 0 g m^{-2} (no litter), 50 g m^{-2}, 100 g m^{-2}, and 200 g m^{-2}; Seeds were placed on the soil and were covered by litter (surface-sown treatment) or were buried up to 1 cm under the soil and then covered by litter (buried). Each treatment has 4 replicates. In total, 64 pots (20 cm in diameter and 16cm in height) were used. Pots were filled with soil collected from the field 0-20 cm depth in natural *L. chinensis* grassland (the soil type is Chestnut, and pH was 7.7, soil organic matter was 3.5%, total nitrogen was 0.25%, extractable phosphorous was 3.8 mg kg^{-1} and extractable potassium was 199.6 mg kg^{-1}). We sowed 20 seeds in each pot, and all seeds were sowed on 11 June 2008. After sowing, we water the pots every three days (water 10 times per month), as the 20-year average mean month (75 mm per month) precipitation in local, calculated from the date collected by weather bureau.

The seedlings per pot which emerged above the litter or soil were counted every three days until the end of September. The seedlings were marked with non-toxic colorized toothpicks to be able to differentiate newly emerged seedlings from those already present. On 28 September the final surviving seedlings were counted and the leaves of per seedling were tested, leaf area, and root diameter and root length of the seedlings was tested by choosing 10 seedlings randomly in each treatment.

2.4 Data Analysis

All statistical analyses were performed by fitting generalized linear models with the procedure of SPSS 12.0 (SPSS, Chicago, IL, USA). Type III sums of squares were used for the calculation of *F* statistics, and Duncan tests for *post-hoc* comparisons.

3. Results

3.1 Seedling Emergence and Survivorship

Plant litter quantity, soil burial and their interaction showed no significant effects on seedling emergence (Table

1). The effect of litter quantity on seedling emergence depended on seed position, with a positive response only when seeds were placed on the soil surface, and litter showed no significant effects under the seed burial treatment (Figure 1a). However, litter increased seedling survival significantly (Table 1), and 100 g m^{-2} and 200 g m^{-2} litter increased it by 9.7 and 16.4 %, respectively compared to the controls (Figure 1c, 1d).

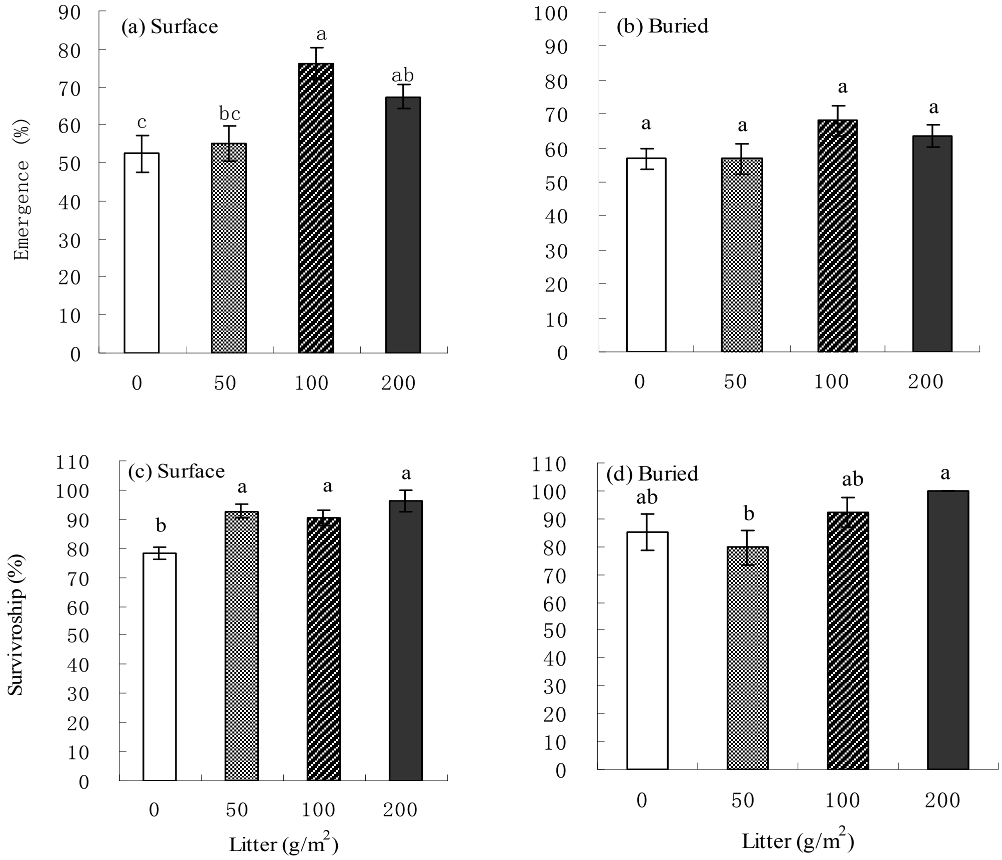

Figure 1. Seedling emergence of *G. dahurica* from surface (a) and buried (b) seeds, seedling survivorship from surface (c) and buried (d) seeds. Bars represent treatment means±1SE (n=4). Different small letters show significant differences (Duncan test at *P*<0.05) under the same soil burial treatment (surface or buried)

3.2 Seedling Growth Performance

The litter treatment had significant effects on all measured growth performance indicators, such as the number of leaves per seedling, leaf area, root length and root diameter, whereas soil burial or seed position significantly affected only the number of leaves and root length. The interaction between litter application and soil burial also influenced growth performance parameters significantly (with the exception of root diameter) (Table 1).

The number of leaves per seedling and the leaf area of *G. dahurica* increased significantly with the quantity of applied litter when seeds were sown onto the soil surface (Figure 2a, 2c), but there were no significant effects on these parameters when seeds were covered with soil (Figure 2b, 2d). In the case of the surface-sown seeds, the highest increase in leaf area occurred under the 200 g m^{-2} litter application, which resulted in an increase of 9.7-fold that of control plants. On average, and when the soil burial and surface-sown treatments were combined, the highest leaf area also occurred under the 200 g m^{-2} litter application, resulting in a leaf area that was 2.5-fold that of seedlings from the non-litter treatments. The litter covering treatments all significantly improved seedling root length and root diameter (Figure 2e-h), but root lengths under the 200 g m^{-2} applied litter treatment when seeds were covered with soil (Figure 2f), and root length and root diameter under this level of litter application with both seed burial treatments, were lower than those under the 50 and the 100 g m^{-2} litter applications (Figure 2g, 2h).

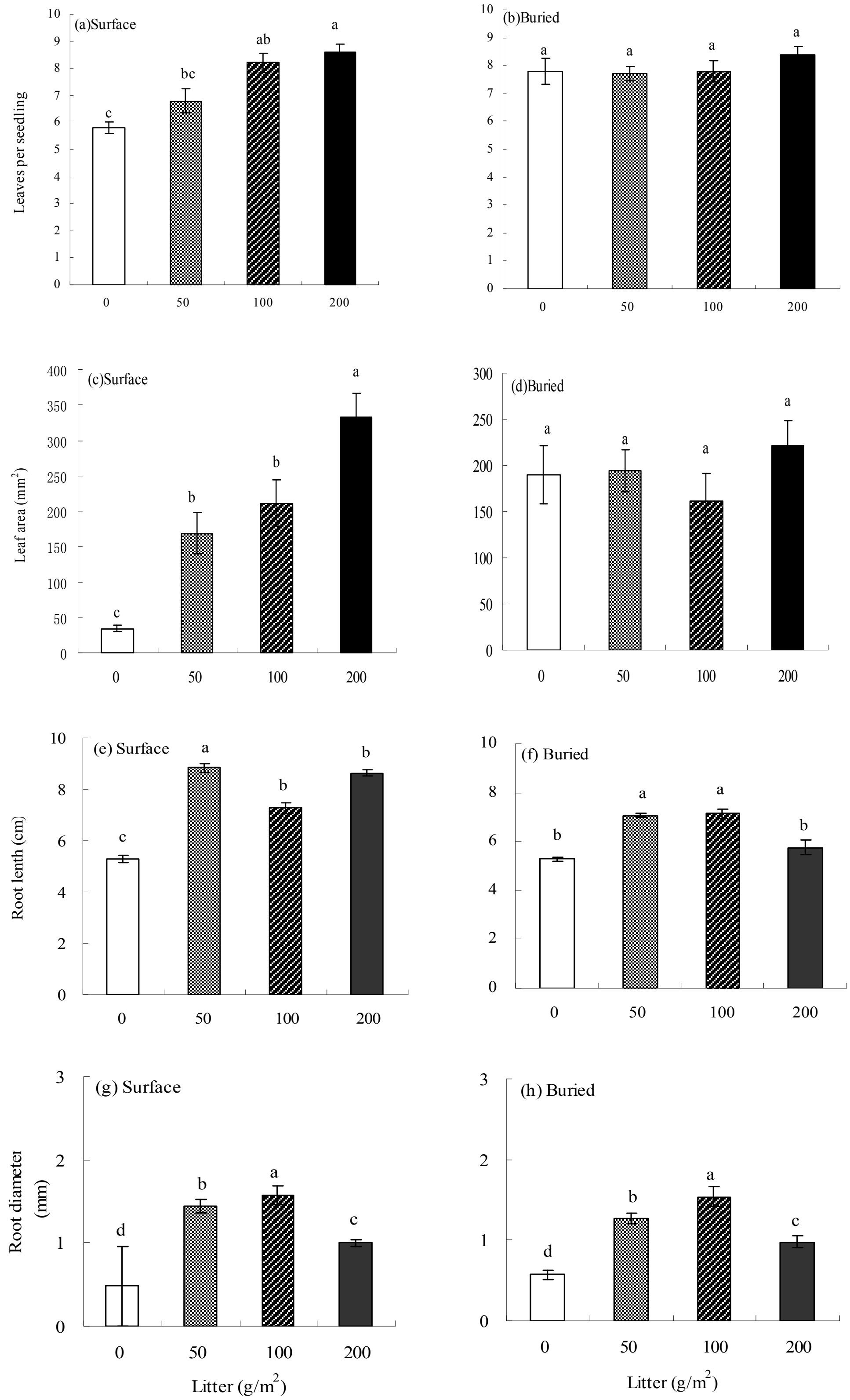

Figure 2. Seedling number of leaves for *G. dahurica* from surface (a) and buried seeds, leaf area of per seedling from surface (c) and buried (d) seeds, root length from surface (e) and buried (f), and root diameter from surface (g) and buried (h) seeds at the end of growing season. Bars represent treatment means±1SE (n=10). Different small letters show significant differences (Duncan test at P<0.05) under the same soil burial treatment (surface or buried)

Table 1. Two-way ANOVA analysis of effects of plant litter, soil burial and plant litter × soil burial interaction on the seed emergence, seedling survivorship and growth performance of *Gentiana dahurica*

Date	Source of Effects		
	litter	Soil burial	Interaction
Emergence	ns	ns	ns
Survivorship	**	ns	ns
Number of leaves	***	*	**
Leaf area	***	ns	***
Root length	***	***	***
Root diameter	***	ns	ns

* $p<0.05$; ** $p<0.01$; *** $p<0.001$; ns=not significant.

4. Discussion

Gibberellin promoted the seed germination of *G. dahurica* under dark incubation conditions (Lin, 2005) and gibberellin can substitute for red light requirments for germination of the seeds (Kallio & Piiroinen, 1959). Higher germination percentage of *G. dahurica* was obtained under light incubation conditions with presoaking in H_2SO_4 (Lin et al., 2007). Those previous studies indicated that *G. dahurica* was a light requirement species for germination (Lin, 2005; Lin et al., 2007). In the present study we found that light quantity was significantly lower beneath litter (reduction of 20%, 61% and 70% beneath 50 g, 100 g and 200 g m^{-2} litter, respectively, data was not shown) and the R/FR-ratio decreased from 1.3 without litter to 1.1, 1.0 and 0.9 in presence of 50 g, 100 g and 200 g m^{-2} litter (data was not shown). While germination of *G. dahurica* was not hampered by a litter layer perhaps the light quantity and quality was enough though it decreased. Jensen and Gutekunst (2003) found that small-seeded species had a higher light requirement for germination and their germination was decreased by litter layer due to reduction of light quantity and the R/FR-ratio. The germination requirements value for light quantity and the R/FR-ratio maybe different among species. Hence the lowest light quantity and the R/FR-ratio for *G. dahurica* germination need to be studied in future.

Litter application showed a positive effect on seedling emergence and survival of *G. dahurica*, but only when seeds were surface-sown i.e. it did not increase seedling emergence overall or under the soil burial treatment. This is inconsistent with previous studies which have shown that seed burial increased seed emergence and survival. For example, Huang and Gutterman (1998) and Liu and Han (2008) showed that seed germination percentage was higher for grass seeds which were buried at a depth of 1-2 cm under soil than those placed on the soil surface, probably because soil burial maintains the environment around the seeds moist and thus prevents seeds and seedlings from. desiccation. The difference between our study on *G. dahurica* and these other studies might be due to seed size and areas of contact between the seeds and the surrounding soil. In addition, small seeds may be more easily buried or trapped in the soil.

Litter application had positive effects on growth performance parameters, such as leaf area and root length, especially for unburied seeds. This is supported by many other studies (Zobel et al., 2000; Eckstein & Donath, 2005), which have shown that most of sown species (70-80%) had established their permanent populations. These positive effects may be due to the protection of seeds from drying out, as even little quantities of litter are able to prevent seedling desiccation, and whether these effects are positive or inhibitory depends upon species-specific characters such as seed size (Eckstein & Donath, 2005). While these positive effects are generally positively correlated with amount of applied litter, very high litter applications (200 g m^{-2}) inhibited the below-ground growth of *G. dahurica* seedlings compared to low and intermediate applications of litter. Positive effects occurred at low and intermediate quantities of litter, while high litter quantities led to inhibitory effects (Hovstad & Ohlson, 2008).

In addition to physical effects on seed germination, litter may have chemical effects on some species seedling establsihment (Facelli & Picket, 1991). While there was no clear evidence for chemical effects of *L. chinensis* dominated grassland litter on seedling establsihment of *L. chinensis, B. inermis* and *A. cristatum* in our another studies(data was not published). Whether the litter leachates from *L. chinensis* dominated grassland have chemical effects on *G. dahurica* or not need to be further studied.

Our results indicate that the seedling establishment of *G. dahurica* benefitted from accumulated litter, and we suggest that fencing to exclude grazing for a period (perhaps no more than 4-5 years) is the best method to protect the threatened wild *G. dahurica* resources.

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