SEEDLING RECRUITMENT OF FORB SPECIES UNDER EXPERIMENTAL MICROHABITATS IN ALPINE GRASSLAND

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Abstract

Which factors limit plant seedling recruitment in alpine meadow of the Qinghai-Tibetan Plateau (QTP), China? This study examined the relative influence of seed mass and microsites (resulted from grazing disturbance) on field seedling emergence and survival of nineteen alpine herbaceous species with a range of traits in QTP. Seed mass had significant effects on seedling emergence and survival eliminating influence of light and nutrient variances among these species. The larger-seed species had more advantageous than the smaller-seed species in seedling survival, but it was disadvantage for seedling emergence, especially under high nutrient availability and low light intensity conditions. Light had obvious effects on seedling survival, but less effects on seedling emergence for these species. Moreover, nutrient and light treatments altered the regression relationships of seed mass and seedling emergence and survival and the order of significances was L25>L50>L100>L10>L4. These results suggested that seed mass may restrict seedling recruitment processes, however, light and nutrient availability all have significant effects on seedling emergence and survival for these alpine species. Moderate light intensity was propitious to seedling emergence and survival in alpine grassland. This suggests that ecological factors in alpine grassland provide a stochastic influence on different seed-mass species. These trends may help to explain why many small-seeded species of Asteraceae and Gramineae tend to be more abundant in disturbed habitats.

Key words: Alpine meadow, Light, Nutrient, Seed mass, Seedling emergence.

Introduction

Seed mass and resources availability are the main factors for controlling seedling establishment in plants (Turnbull et al., 2000; Moles & Westoby, 2004a, b; Eriksson, 2005). Seed mass has been regarded as an important plant property since the pioneering studies of Salisbury (1942), but a number of explicit studies on the ecological significance of seed mass has increased greatly in recent years (Harper, 1977; Westoby et al., 1996; Leishman et al., 2000; Fenner & Thompson, 2005; Wu et al., 2013). It is well established that seed mass has important effects on seed germination (Wu et al., 2013), seedling growth rate (Fenner, 1983; Shipley & Peter, 1990), competitive ability (Geritz et al., 1999; Coomes & Grubb, 2003), establishment success, species fecundity (Rees et al., 2001; Muller-Landau, 2003) and persistence in the seed bank (Moles et al., 2000). Large-seeded species have long been known to have higher survivorship during establishment than small-seeded species (Paz et al., 1999; Moles & Westoby, 2004a). But many plant species increased fecundity by producing more seeds and hence improved colonization ability— a selective advantage (Harper, 1977; Leishman, 2000). Given its links with so many demographic parameters, it is not surprising that seed mass has also been linked with patterns of relative abundance in several communities (Murray et al., 2005). The relations are typically triangular in form, with large-seeded species having low abundance while small-seeded species showing a wide range of abundances. The inverse relation between fecundity and seed mass undoubtedly plays an important role in determining the maintenance of plant diversity within communities (Rees et al., 2001; Coomes & Grubb, 2003).

Seedling recruitment not only depends on seed mass, but also depends on the quantity and quality of microsites available for seed to germinate and seedling to survive (Garcia-Fayos & Gasque, 2006). Microsite requirements can differ throughout the life of long-lived species (Schupp, 1995) and across the distribution range (Suding et al., 2003). Plants in alpine ecosystems must take advantage of the scarce spatial and periodical recruitment opportunities opened to them to colonize with sufficient seedlings for allowing population succession. Seedling recruitment in high-cold grassland habitats are dependent on environmental and biotic factors that are spatially and temporally variable, such as light and nutrient availability resulted from grazing disturbance (Shang et al., 2006; Wu et al., 2009). In most species, recruitment is concentrated during periods of favorable weather conditions and resource availability (Watson et al., 1997), and it also concentrates spatially in some favorable microsites (Mawster et al., 2003; Wu et al., 2013). Seedlings establishment of understory vegetation were determined light intensity through the canopy (Walters & Reich, 1996). Interactions of seeds with their environments determine seedling establishment patterns and influence the structure of both plant populations and communities (Chamber, 1995; Dalling & Hubbell, 2002). These studies have been complemented by studies on naturally developing seedlings in forest system (Caspersen & Kobe, 2001), or seedlings planted into natural forest vegetation (Baraloto et al., 2005). And Wu et al. (2011) found that grazing had significant effects on seedling recruitment
and offspring diversity and heavy gazing may alter community succession by affecting recruitment patterns among different plant functional groups.

There are different resources heterogeneity and microhabitats because of different grazing disturbances in alpine meadows of the Qinghai-Tibetan plateau (Shang et al., 2006; Wu et al., 2009). Especially for different-degree degraded grasslands, as plant cover increases, light available to juvenile plants decreases and may become a limiting factor (Tilman, 1988). There were relatively few experimental studies on the seedling emergence and survival of plants from alpine plant communities (Koerner, 2003).

This paper presents an study on the relative role of light and nutrient availability and seed mass on the seedling recruitment of some central species for community construction of alpine meadow. The regimes of irradiance and temperature are very complex, and therefore we set up this experiment in field with some plants in the open and others under neutral shade screens, which were resulted from grazing disturbances (Wu et al., 2009). Many studies have been done for seed mass and seedling establishment under heterogeneous microsites (Leishman et al., 2000; Walters and Reich, 2000; Dalling & Hubbell, 2002), which mainly focus on forest saplings and have little information on herbaceous seedlings in grassland. Our study has been made on plants from alpine meadows on the Qinghai-Tibetan Plateau, where is marked heterogeneity in the physical conditions considered at the scale of an individual seedling. Thus, the main objectives of this study were to provide an additional comparative test of the relationships between seed mass and seedling emergence and survival. In summary, these questions addressed in this study were: 1) Do larger-seeded species have higher emergence and survival for these species in alpine meadow? 2) Do light and nutrient availability have effects on seedling emergence and survival in alpine grassland? and 3) What relationships between seed mass and early seedling survival under variational light and nutrient availability?

Material and Methods

Study area: The experiment was conducted in alpine meadow at an altitude of 3500 m in the eastern Qinghai-Tibetan Plateau at the Maqu Wetland Protection Area (33°06′–34°33′ N, 100°46′–102°29′ E), which was located in the eastern region of the Qinghai-Tibetan plateau in northwestern China. The main soil type is sub-alpine meadow soil. The soil horizon is about 80 cm. The vegetation consists mainly of arctic alpine and Chinese Himalayan plants. The vegetation of the area is typical alpine meadow (Wu, 1995) and is dominated by Sedges (e.g. Kobresia spp.), Graminoïds (e.g. Festuca spp., Poa spp., Agrostis spp.), Asteraceae (e.g. Saussurea spp.), and Buttercup (e.g. Anemone spp.). Species of the first two families are the main pioneer species, and species of the latter two families are the main dominant species in alpine meadow of this region. The climate of study area is alpine humid, mean annual temperature is 2.0°C, with the monthly average ranging from below -10°C in January to 11.7°C in July. Mean annual rainfall is 550 mm, and mean annual growing degree days are less than 150 days (Wu et al., 2013).

Study species: The 19 studied species (Table 1) were chosen to represent a wide range of seed size, which are the same as the coexisted and main species in the natural alpine meadow community of study area. Seeds of 19 native herbaceous plant species collected from the alpine meadow community were used in this study. Seeds were collected from more than fifty individuals in the same meadow community (N33°48', E102°08') for each species were pooled. Four species are very common, ten species are common, and other five species are rare in alpine meadow plant communities in eastern Qinghai-Tibetan Plateau. These seeds were collected from the Maqu county of Gannan County of Gansu Province of China in August-September. The seeds were stored at room temperature (15°C) until they were sown. Mean seed dry mass of 500 seeds were weighed.

Table 1. Study species, family, species code, seed dry mass (mg/100seeds), and relative abundance in alpine meadow. Nomenclature and assignment to families follows Wu (1995).

<table>
<thead>
<tr>
<th>Species</th>
<th>Family</th>
<th>Seed dry mass (mg/per seed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ligularia przewalskii</td>
<td>Asteraceae</td>
<td>1.0120 ± 0.004</td>
</tr>
<tr>
<td>Tripolium vulgare Ness.</td>
<td>Asteraceae</td>
<td>0.1784 ± 0.002</td>
</tr>
<tr>
<td>Senecio.diversipinnus Ling</td>
<td>Asteraceae</td>
<td>0.6203 ± 0.003</td>
</tr>
<tr>
<td>Saussurea morfolia Chen</td>
<td>Asteraceae</td>
<td>1.3921 ± 0.005</td>
</tr>
<tr>
<td>Saussurea japonica (Thunb)DC</td>
<td>Asteraceae</td>
<td>1.3256 ± 0.005</td>
</tr>
<tr>
<td>Saussurea iodostegia Hance</td>
<td>Asteraceae</td>
<td>3.3068 ± 0.270</td>
</tr>
<tr>
<td>Saussurea mongolica(Franch.)</td>
<td>Asteraceae</td>
<td>1.0806 ± 0.002</td>
</tr>
<tr>
<td>C pleurocaule (Franch) R Good.</td>
<td>Asteraceae</td>
<td>1.9222 ± 0.007</td>
</tr>
<tr>
<td>Anemone rivularis Buch-Ham.</td>
<td>Ranunculaceae</td>
<td>6.9599 ± 0.210</td>
</tr>
<tr>
<td>Aconitum gymnastrum Maxim.</td>
<td>Ranunculaceae</td>
<td>0.9026 ± 0.035</td>
</tr>
<tr>
<td>Truolius farrei Stapf</td>
<td>Ranunculaceae</td>
<td>0.5092 ± 0.024</td>
</tr>
<tr>
<td>Ranunculus brotherrsii var. tanguticus</td>
<td>Ranunculaceae</td>
<td>0.4005 ± 0.027</td>
</tr>
<tr>
<td>Salvia.prezvasklai Maxim.</td>
<td>Labiateae</td>
<td>5.4034 ± 0.310</td>
</tr>
<tr>
<td>Draacocehalum ruyshchiana</td>
<td>Labiateae</td>
<td>2.3568 ± 0.140</td>
</tr>
<tr>
<td>Medicago minima Bart</td>
<td>Fabaceae</td>
<td>1.8733 ± 0.008</td>
</tr>
<tr>
<td>Astragalus mahoschamicus -Hand.-Mazz.</td>
<td>Fabaceae</td>
<td>1.1910 ± 0.007</td>
</tr>
<tr>
<td>Hedysarum tanguticum Fedtsch</td>
<td>Fabaceae</td>
<td>3.5938 ± 0.420</td>
</tr>
<tr>
<td>Descurainia sophia</td>
<td>Brassicaceae</td>
<td>0.1640 ± 0.025</td>
</tr>
</tbody>
</table>
Methods: Effects of seed mass, light and nutrient availability on seedling emergence and survival were determined through the establishment of pots across the ten combinations (L100N10, L100N100, L50N10, L50N100, L25N10, L25N100, L10N10, L10N100, L4N10, L4N100) of five light treatments and two nutrient treatments at the start of the spring. Others were in the nature field environments. For the 100% daylight treatment (referred to as L100) no screen was used. For other four treatments different densities of black plastic screening were used. We used two types of screenings with different density. The four compages of these two types of screenings form the four kinds of shadings. The screens allowed the penetration of about 50% (L50), 25% (L25), 10% (L10) and 4% (L4) of full daylight 100% (L100) respectively. L100 and L50 presented rock-bottom light availability of seriously degraded meadow community, L25 presented rock-bottom light availability of lightly degraded meadow community, L10 presented rock-bottom light availability of normal meadow community, and L4 presented rock-bottom light availability of fertilized meadow community. Light intensity within each shade screen was measured using a Decagon Model SF80 Sunfleck Ceptometer (Decagon Devices, Inc. Pullman, Washington, USA) on a cloudless day. Thirty-time repeats light measurements of three periods of time of a day (photon flux density, µmol m⁻² s⁻¹) using the single sensor setting were taken from each light regime. We used the full-strength Hoagland solution as one nutrient treatment because it was easy to control (Wang et al., 2014). The two nutrient treatments were N10 (addition of 10% of full-strength Hoagland’s solution) and N100 (full-strength Hoagland’s solution). N100 presented soil nutrient availability of degraded meadow community and N100 presented soil nutrient availability of normal meadow community. The nutrient solutions were added every 5 days and 200ml were sprinkled uniformly over each pot. Apart from the normal rain, the seedlings were well watered during the all growth seasons and all treatments were given the same amount of water.

Ripe seeds were cleaned and stored at room temperature (about 15°C) in darkness and wet over winter and keep the natural moisture and temperature regimes on their locality, until the experiment was being. The 900 seeds/ species from the seed collections used in this study were weighed to determine average seed mass and being in this experiment. The seeds of 19 species were sown on 3rd May. All petri dishes and pots were set up by complete randomized design under each light treatment. The seeds were sown into pots and these pots were standing on the soil surface underneath the shade cloth. In each plastic pot (40 cm × 40 cm × 40 cm) which were buried into the ground, 30 seeds were sown uniformly, which avoided the competition among seedlings, and every species in ten treatments were repeated 3 plots. There are 570 pots in this experiment. Circular pots were placed to avoid competition for resources and light for seedlings. Seedling emergence was evaluated after sowing, and the exact location of the seedlings on each circular surface was monitored. From emergence of first seedling, new seedling emergence and establishment were monitored daily; all seedlings of emergence and survival were recorded. The establishment numbers of seedlings were accounted at the 100 days after emergence. Successful establishment was accounted at the September.

We monitored the all seedlings emergence and survival numbers daily during the growth-season. They were calculated by the following formula:

- Seedling emergence percentage = \( \frac{\text{Emerged seedlings}}{\text{Total seedlings sown}} \times 100 \)
- Seedling survival percentage = \( \frac{\text{Survival seedlings in the end}}{\text{Total seedlings sown}} \times 100 \)

We performed ANCOVA to detect effects of seed mass under different resources availabilities (with covariant variables of light and nutrient) on seedling emergence and survival. In addition, the interaction effects of seed mass, light and nutrient availability on seedling emergence and survival were detected. Effects of seed mass and family on seedling emergence and survival were analyzed by ANOVA. The relationships between seed mass and survival and the response variables of seedling emergence and survival percentage were showed by linear regression under different nutrient and light availability. To observe the trend of seedling emergence and survival responding to light intensity, we performed a linear regression to examine the relationship between seedling emergence and seedling survival under 5 light intensities. To approach normality and reduce the heterogeneity of variances, seedling emergence and survival percentage values were log-transformed. All statistical analyses were conducted with the 11.5 version of the SPSS package (SPSS Inc. Chicago).

Results

Seedling emergence and survival to seed mass: Seed mass had significant effects on seedling emergence percentage (p<0.0001) and survival percentage (p<0.0001) eliminating influence of light and nutrient variances among the 19 investigated species (Table 2). In addition, family had a significant effect on seedling survival percentage (p<0.0001) and non-significant effect on seedling emergence percentage (p=0.15), as an inherent factor (Table 3).

There is a weak negative relationship between seed mass and seedling emergence percentage under N100 (r²=0.08) and N10 (r²=0.034) treatments. However, for seed mass and seedling survival percentage, there is a positive relationship under N100 (r²=0.0393), but no relationship under N10 (r²=0) (Fig. 1).

For five light treatments different-degree negative relationships between seed mass and seedling emergence percentage is present. But, seedling survival percentage is positively related to seed mass for the 19 investigated species, especially under low light treatments (Fig. 2).
Table 2. ANCOVA analysis (Seed mass effects with covariant variables of light and nutrient) and interaction effects on seedling emergence and survival (Significance was determined at p<0.05, Tukey-Kramer HSD).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Emergence</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>F</td>
</tr>
<tr>
<td>Seed mass</td>
<td>18</td>
<td>4.970</td>
</tr>
<tr>
<td>Light</td>
<td>4</td>
<td>40.720</td>
</tr>
<tr>
<td>Nutrient</td>
<td>1</td>
<td>5.626</td>
</tr>
<tr>
<td>Seed mass × Light</td>
<td>72</td>
<td>1.922</td>
</tr>
<tr>
<td>Seed mass × Nutrient</td>
<td>18</td>
<td>1.075</td>
</tr>
<tr>
<td>Light × Nutrient</td>
<td>4</td>
<td>1.694</td>
</tr>
<tr>
<td>Seed mass × Light × Nutrient</td>
<td>72</td>
<td>0.785</td>
</tr>
<tr>
<td>Error</td>
<td>380</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>570</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Linear relationships between mean seed mass and (left figure) seedling emergence percentage and (right figure) seedling survival percentage (logarithmic scale for both axes) under nutrient treatments, n=19 species. Two nutrient availability treatments were separated. The regression lines were drawn and R-squared were given.

Fig. 2. Linear relationships between mean seed mass and (left figure) seedling emergence percentage and (right figure) seedling survival percentage (logarithmic scale for both axes) under light treatments, n=19 species. Five light availability treatments were separated. The regression lines were drawn and R-squared were given. The continuous lines are significant (p<0.01).
Seedling emergence and survival to light and nutrient availability: Light had significant effects on seedling emergence (p<0.0001) and survival (p<0.0001) percentage for the 19 investigated species. Nutrient treatments also had significant effects on seedling emergence (p=0.018) and survival (p<0.0001) percentage. Meanwhile, light and nutrient all had more significant interaction effects on seedling survival percentage (light × seed mass: p<0.001; nutrient × seed mass: p<0.05) than seedling emergence percentage (light × seed mass: p=0.015; nutrient × seed mass: p=0.375). In addition, light and nutrient treatments presented a significant interaction effects on seedling emergence percentage (p<0.001), but non-significant effects on seedling survival percentage (p=0.194). The three factors had no interaction effect on seedling emergence and survival percentage (Table 2).

In addition, nutrient and light treatments altered the regression relationships of seed mass and seedling emergence and survival percentage (Figs. 1 & 2). Meanwhile, seedling emergence and survival percentage presented significant positive relationships. Light intensity variance changed their regression relationships, the order of significances was L25>L50>L100>L10>L4 (Fig. 3).

For these species, seedling emergence and survival percentage usually reached maximum under moderate light treatments and different species had different response to light and nutrient variances (Figs. 4 & 5). The high light intensity was propitious to seedling emergence and survival in alpine grassland of the Qinghai-Tibetan Plateau.

Table 3. Effects of seed mass and family by ANOVA analysis on seedling emergence and survival (Significance was determined at p<0.05, Tukey-Kramer HSD).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Emergence F</th>
<th>P</th>
<th>Survival F</th>
<th>( R^2 )</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed mass</td>
<td>45.798</td>
<td>&lt;0.0001</td>
<td>16.635</td>
<td>0.5027</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Family</td>
<td>1.903</td>
<td>0.150</td>
<td>12.751</td>
<td>0.3568</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Note: Four families were included except for Brassicaceae

Discussion

Our results allow us to consider the regeneration potential by seedling recruitment of native species, which showed different establishment ability in different light conditions. Patterns of seedling emergence and survival demonstrate that light and nutrient conditions can have strong effects on relationships between seed mass and seedling emergence and survival. Seed mass may restrict seedling emergence and survival processes (Wang et al., 2014). High light regimes and nutrient addition have a more positive effect on the seedling survival of these species in alpine meadow.

Our study demonstrated that seed mass had significantly negative effects on seedling emergence and survival percentage. But the former researches advanced that the species were larger-seed had more advantage in seedling establishment (Moles & Westoby, 2004a). The larger-seed species have advantage in seedling survival, while the smaller-seed species had advantage in seedling emergence. It may be a fundamental trade-off during seedling emergence and survival course for different seed-mass species. Moreover, the survival advantages of larger-seed species are bigger than the emergence advantage of smaller-seed species. Positive associations between seed mass and seedling survival were restricted to small-seeded species, while negative associations were restricted to medium and large-seeded species (Gross, 1984). So, the larger-seed species have larger advantage for seedling establishment, especially under high nutrient availability and low light intensity conditions.

Light and nutrient availability all have significant effects on seedling emergence and survival for these alpine species, which are consistent with other studies. All have been shown the importance of environmental stress on the seedling establishment of species (Alpert et al., 2000) and for many species, establishment is sensitive to stress (Turnbull et al., 2000). Our results confirm that the seedling recruitment of these species in alpine meadow was affected by the light and nutrient availability. In addition, light and nutrient treatments can change the relationships between seed mass and seedling emergence and survival for these alpine species. This suggests that ecological factors in alpine grassland provide a stochastic influence on different seed-mass species. These trends may help to explain why many small-seeded species of Asteraceae and Graminoids tend to be more abundant in different micorsites.
Light is a key factor determining seedling mortality in the shade (Saverimuttu & Westoby, 1996, Moles & Westoby, 2004b). The importance of microsites differences in maintaining diversity has been implicated in many studies (Dalling & Hubbell, 2002, Coomes & Grubb, 2003). Survival of seedlings in shade will depend on the total amount of reserves in the seed and the rate of carbon fixation and respiration of the seedlings (Leishman et al., 2000). Our study demonstrate that seedling survival should be positively related to seed mass in low light with this relation becoming obscure as irradiance increases. In addition, we also demonstrate that high light intensities are most suitable for seedling emergence and survival in alpine meadow of the Qinghai-Tibetan Plateau.

For the species included here, there are no fixed relationships between seed mass and seedling emergence and survival percentage, because their relationships are changing under different microsites. This suggests that successful establishment was not only dependent of seed mass, but also the light and nutrient availability. These features may make regeneration by seeds is more important than generally assumed. This suggests that local biotic and abiotic interactions dynamics jointly determined the composition recruitment of alpine meadow communities. Focusing on patterns of seedling recruitment variation naturally arised through environment constraints or through species evolutionary considerations lead to a predictive theory of vegetation dynamics. However, seeds from different plant families are used for the experiment, additionally, it is a pity that no stratification measures are applied. These all can highly influence the outcome of such experiment.
Fig. 5. Changes in cumulative seedling survival percentage (%) under two nutrient treatments and five light treatments over first years for the nineteen species planted in the field experiment. Data are the means (± standard error) and the corresponding proportion of emergence seedlings planted into each of 570 plots. Species are identified by Wu (1995) (see Table 1).

A firm conclusion, however, would require the further study of more species and the observation of the effects of seed mass on the demographic fate of individuals throughout the whole life cycle in field community. From an evolutionary perspective, the direction of selection on seed mass depends on the combined effects of seed mass on different fitness components for different growth stages. Large-seeded species must have experienced direct (or correlated) selection favoring the larger seeds relative to their close relatives in the past (Leishman et al., 2000; Walters & Reich, 2000; Moles & Westoby, 2004a), but our data suggest that the larger seeds are not necessarily advantageous during other phases of the life cycle, e.g. seed germination, seedling performance and adult reproduction stages (Paz et al., 1999; Wu et al., 2013). Further studies that compare survival patterns and the subsequent reproductive stages leading to recruitment at different sites, as well as over longer time periods, are especially needed.

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