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EFFECTS OF SAND BURIAL AND SEED SIZE ON SEED GERMINATION, SEEDLING EMERGENCE AND SEEDLING BIOMASS OF *ANABASIS APHYLLA*

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Abstract

Two greenhouse experiments were conducted to test the effectsof sand burial (0–2cm) and seed size (small, medium and large) on seed germination and seedling growth of *Anabasis aphylla*, which is typically used as a windbreak and for the fixation of sand in the Gurbantünggüt desert of Xinjiang, region of northwest China. The results showed that sand burial significantly affected seed germination, seedling emergence, survival and biomass of *A. aphylla*. The seed germination rate, seedling emergence rate, seedling survival rate and biomass were highest at the 0.2 and 0.5cm sand burial depths. At different burial depths, different sizes of *A. aphylla* seed showed a significant difference in the germination and emergence rate. At the same sand burial depth, the seedling emergence rate of the large seeds was significantly higher than that of medium and small seeds. At sand burial depth of 0.2–2cm, germination of large seeds and seedling survival rates were significantly higher than those at the same sand burial depth for medium seed germination, and the latter was significantly higher than for small seed. We speculate that tolerance to sand burial and diversity of seed size increased the adaption of *A. aphylla* to this environment, contributing to its dominance in the windy and sandy area of Gurbantünggüt desert.

Key words: *Anabasis aphylla*; Sand burial; Seed germination; Seed size; Seedling emergence; Seedling biomass.

Introduction

In dune systems, wind sand movement occurs frequently, and plants growing on the sand dunes are continually subjected to different degrees of sand burial (Zhu *et al*., 2005). Sand burial can change the microenvironment of plants including factors such as illumination, temperature, humidity, oxygen content of the roots, soil organic matter and soil microbe activity (Huang *et al*., 1997; Baskin *et al*., 2014). Therefore, there isconsiderable variation in psammophyte seed size, seed germination, seedling emergence evolution mechanism and seedling and adult plant survival. Sand burial is an important driving force controlling the distribution and communities of psammophyte (Huang *et al*., 2005). Sand burial depth directly affects seed germination, seedling emergence and survival. A sand burial depth that is appropriate for the plant could change the biotic and abiotic conditions of emergence, which can improve the viability of seedlings (Brown, 1997; Ren *et al*., 2002). As an environmental screen, sand burial has become an important factor in controlling vegetation distribution and composition in dune ecosystems (Zhu *et al*., 2005; Li & Zhao, 2006; An *et al*., 2011). In general, there was an optimal range of burial depth has been identified to maximize the seedling emergence and subsequent seedling growth (An *et al*., 2011).

Several factors such as inheritance, seed competition between the limited resources, seed size and seed number cause plants to produce different sizes of seed (Peng, 2001; An *et al*., 2011; Baskin *et al*., 2014). Different seed sizes may be related to different transmission ability, germination rate and germination time, and may also be associated with different size dynamics and survival ability in seedlings (Peng, 2001). Some studies have shown that the germination rate, seedling emergence and growth rate of the seeds were different as a result of their different sizes, and this phenomenon occurs in both intraspecific and interspecific crossing (Peng, 2001; Wang *et al*., 2012). The optimal burial depth of seedling emergence could be strongly influenced by seed size (Chen & Manu, 1999). Seed size plays a key role in the establishment of the juvenile phase of a plant's life cycle, and also represents a parameter that profoundly influences both germination characteristics and seedling traits (Benard & Toft, 2008). Larger seeds are generally superior to smaller seeds as they have a higher probability of emergence and trend to develop into seedlings with better competitive ability, higher survival rates, and better performance in later life stages (Stanton, 1984; Winn, 1988; Simons & Johnston 2000; Vaughton & Ramsey 2001; Benard & Toft, 2008). Theoretically, different seed sizes reflect the amount of material stored in the embryo, which has important ecological significance (Baskin *et al*., 2014).

Anabasis aphylla is a chenopodiaceous subshrub mainly distributed in the Xinjiang, region of northwest China, and has high capability to endure alkali salts, resist drought and limit sand drift, which is important to exploit and utilize land that has become salinized and alkali under drought, and thus to improve these environments by introducing plant cover (Chu *et al*., 2014; Wang *et al*., 2015). The *A. aphylla* population is mainly distributed in diluvial fan areas, lowland areas between dunes, Gobi and hillsides. The decoction of the leaves contains high percentages of antimicrobial components such as alkaloids and steroids (Yang *et al*., 2010). Therefore, it is also a medicinal plant that is used against plant diseases and insect pests. In recent years, there have been some studies on the responses of *A. aphylla* seeds germination to drought stress and salt (Chu *et al*., 2014).

In this study, we buried different seed sizes of *A. aphylla* at various sand depths in a greenhouse to examine the effects of sand burial depth and seed size on seed germination, seedling emergence and survival, we also tested the biomass allocation at various sand burial depths, and assessed the optimal depth for *A. aphylla* establishment in response to sand burial and seed size. We mainly aimed to understand the ecological adaptation strategies of *A. aphylla* under sand burial conditions. This study not only enriches the plant strategic theory, but also provides a scientific basis for the wind screening, sand fixation and vegetation restoration in dune ecosystems.

Materials and Methods

Seed collection and study site: The Gurbantünggüt Desert on the southern edge of the Junggar Basin in Xinjiang (45°22′43.4″N, 84°50′32.5″E; elevation is 843 m) was selected to gather the study materials. The annual mean temperature varies from 5°C to 9°C in this area. Minimum winter temperatures vary from −30°C to −41°C, while maximum summer temperatures are between 30°C and 40°C. Snowmelt at the end of winter, together with the rainfall that occurs in summer, amounts to an annual precipitation of 100 to 150 mm and annual potential evaporation is >2000 mm (Wang *et al*., 2014). Freshly matured *A. aphylla* seeds were collected in November 2013 from plants growing in the desert on the southern edge of the Junggar Basin The cleaned seeds then were stored at −18°C. Before the experiments, we selected only full seeds for use in the experiment.

Experimental design

Seed size and mass: Randomly selected 1000 seeds of *A. aphylla* and weighed with an electronic balance. Four replications were used in the experiment. Then, randomly selected 30 seeds and weighed with 1/10000 scales electronic balance one by one, analyzed the proportion of different mass of *A. aphylla* seeds.

Effects of sand burial and seed size on seed germination and seedling emergence: We randomly selected 1000 full seeds of *A. aphylla* and weighed these seeds together using an electronic balance. Four replications were used in the experiment. We then randomly selected 30 seeds and weighed them individually with a 1/10000 electronic balance and subsequently analyzed the proportion of different masses of *A. aphylla* seeds.

Before the experiment, seeds were divided based on the weight standard: large, medium and small, each group containing 720 seedsin total. The hundred-grain weight of large seed was 0.2361–0.2513 g. The hundred-grain weight of medium seed was 0.0914–0.1152 g. The hundred-grain weight of small seed was 0.0623–0.0846 g.

For each group, selected seeds (30 each) were planted at 0, 0.2, 0.5, 1, 1.5 and 2 cm depths (labeled 1, 2, 3, 4, 5 and 6 respectively) in plastic pots (15 cm in diameter, 20 cm in depth) and filled with sterilized and sifted sand. There were four replications per treatment. The drainage outlet at the bottom of the pots was covered with thick paperboard to prevent the loss of sand while allowing drainage of excess water. During the experiment, pots were watered daily using tap water and to keep the sand moist. This watering process was halted after one week.

The laboratory experiment was commenced on 4 December 2013 in the light incubator. Temperature was maintained at 20°C in the laboratory. After 15 days of germination and seedling emergence, the experiment was terminated. During the experiment, emerged seedlings were counted daily. Seedling emergence was defined as the first appearance of a seedling at the seed surface. Then we calculated the number of seeds that germinated and the seedling emergence.

Effects of sand burial and seed size on biomass: At the end of the experiment, the surviving seedlings were removed from the treatment pots. The seedlings were oven dried, weighed with 1/10000 scales electronic balance to determine the seedling biomass.

Statistical analysis

The effects of sand burial depth and seed size on the germination rate, seedling emergence rate and seedling biomass were analyzed using one-way and two-way analysis of variance at the 0.05 significance levels.. Different capital letters indicate the same size of seed in different sand burial depth significant at 0.05 levels. Different lowercase letters indicate different sizes of seeds under the same sand burial depth significant at 0.05 levels. The data were analyzed using SPSS 11.5 software, and the figures were created with Origin 8.0.

Results

Effects of sand burial depth and seed size on seed germination of *A. aphylla*: The germination rate was significantly affected by both sand burial depth and seed size, but there was no significant interaction effect of sand burial depth and seed size. At all studied sand burial depths, different sizes of seed showed similar germination trends. At the sand surface (0 cm sand burial depth), only about 70% of seeds were able to germinate. The germination rate was highest at 0.2 cm. From 0.5 to 2 cm, the germination rate of the medium and small seed was relatively lower, while there was no significant difference in the large seed. At each sand burial depth, different sizes of seed showed significant differences under all the treatments (except there was no significant difference between the large and medium seeds at 0.2 cm burial depth) (Fig. 1).

Effects of sand burial depth and seed size on seedling emergence of *A. aphylla*: The emergence rate was significantly affected by both burial depth and seed size, but there was no significant interaction effect of sand burial depth and seed size had no significant effect. At all studied sand burial depths, different sizes of seed showed a similar emergence trend. About 50% of seeds were able to emerge at the sand surface. The emergence rate was highest at 0.2 cm burial depth. From 0.5 to 2 cm, the emergence rate of the seedlings decreased. Only a limited number of seedlings reached the sand surface for seeds buried under 1–2 cm of sand and no seedling emergence of small seeds was observed at 1.5–2 cm sand burial depth (Fig. 2).

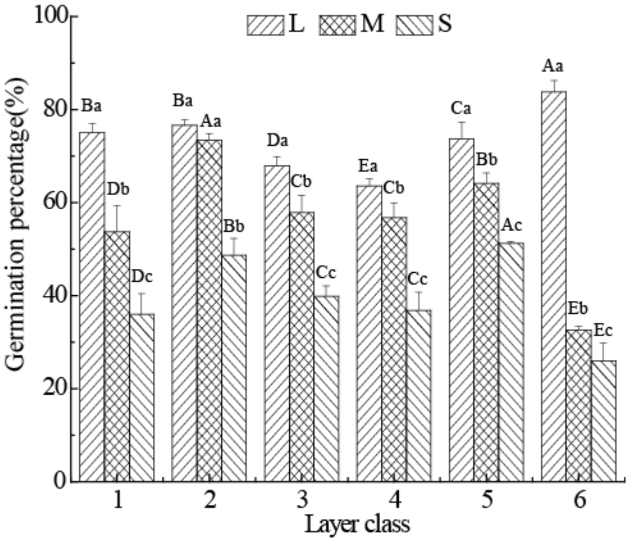

Fig. 1. Germination percentage of *A. aphylla* seed at different seed sizes.

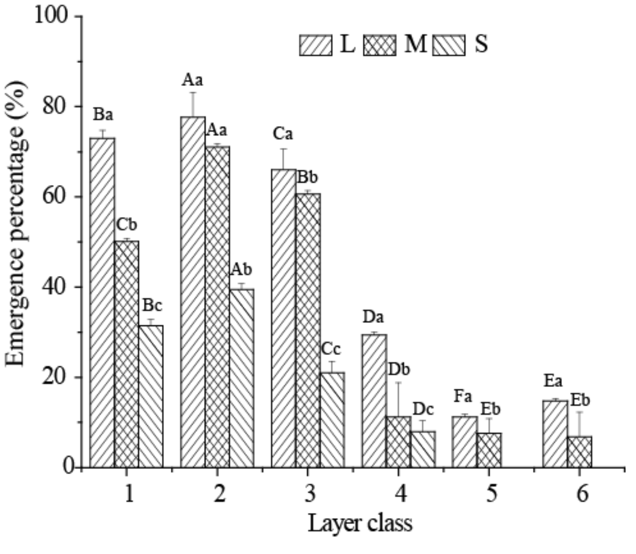

Fig. 2. Emergence percentage of *A. aphylla* seed at different seed sizes.

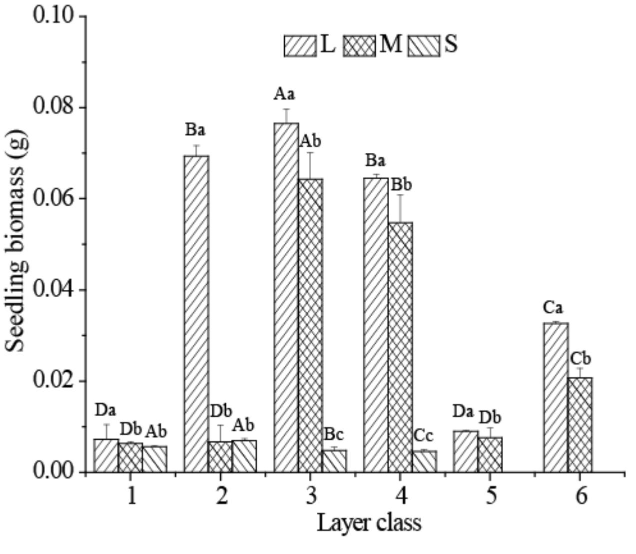

Fig. 3. Biomass of *A. aphylla* seedlings at different seed sizes.

Effects of sand burial depth and seed size on seedling survival and biomass of *A. aphylla*: After 5 days of the experiment, several *A. aphylla* seedlings died in succession, and seedling mortality was most serious at 0 cm sand burial. At 0.2 cm sand burial, there was a large number of deaths after 7 days without water. At 0.5 cm sand burial, there was a higher survival rate for *A. aphylla* seedlings; the survival rate of large seed reached 93%, whereas that of the small seed was only 56%. For 1–2 cm sand burial, there was a higher survival rate, especially for the large seed, which reached 100% (Table 1).

Seedling biomass of *A. aphylla* was significantly affected by both sand burial depth and seed size. The large seed had a higher biomass at 0.2 and 0.5 cm sand burial depth, and biomass at 0.5 cm sand burial was significantly higher than that at other burial depths. However, seedlings from all three sizes of seeds had low biomass at 0 cm sand burial. Moreover, the biomass of small seeds was lower at all studied burial depths (Fig. 3).

Overall trend of seedling emergence: This experiment showed that the seedling emergence rate was fastest and most consistent at 0.2 cm sand burial depth, and the number of seedlings was highest on the fifth day. However, the seedling emergence rate of seeds at the surface was highest on the ninth day. Germination from different seed sizes showed similar trends from the surface to 1 cm sand burial depth (Fig. 4).

Discussions

Effects of sand burial depth on the emergence of *A. aphylla*: In dune habitats, the plant seeds will be buried in the sand at different depths because of many factors (Yang *et al*., 2012). Like other dune plants, there are four potential fates after the *A. aphylla* seed is buried in the sand (Manu, 1994): (1) seed germinates and seedling emerges; (2) seed germinates but no seedling emerges; (3) seed becomes dormant and a part of the soil seed bank; (4) seed dies due to multiple factors. The results of this study indicate that the seed of *A. aphylla* was consistently able to germinate at sand burial ranges from 0–2cm. The germination rate significantly decreased as sand burial depth increased when sand burial depth was above 0.5 cm. We also found that seedling emergence was highest at 0.2 cm burial depth, but decreased with increased burial depth from 0.5 to 2 cm.. This indicates that *A. aphylla* seeds are able to germinate but the seedling is unable to emerge at more than a certain burial depth, The seedling emergence at shallow burial depth was greater than at the surface and at deep burial depths, with 0.5 cm of sand providing optimal seedling emergence. These results support the findings of some previous studies (Zhang & Maun, 1990; Yang *et al*., 2012; He *et al*., 2013). At 0–2 cm burial depth, the germination rate of large *A. aphylla* seeds was greater than 75%, but the emergence rate significantly decreased as sand burial depth increased.

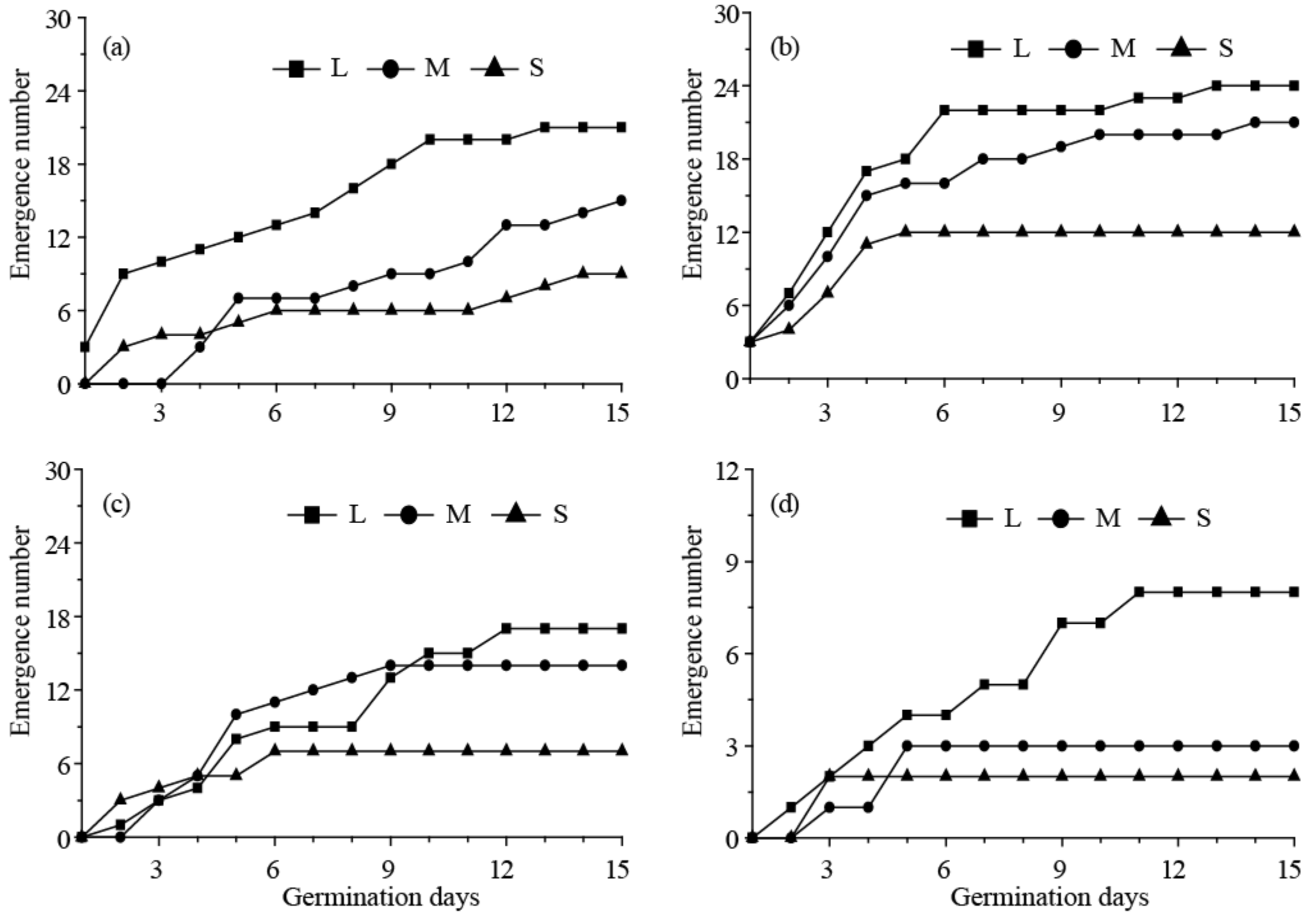

Fig. 4. Surface(0 cm) layer (a), 0.2 cm layer (b), 0.5cm layer (c), 1cm layer (d) of *A. aphylla* seed emergence over time.

Table 1. Effect of burial depth and seed size on survival percentage of *A. aphylla* seedling.

Sand burial depth (cm)	Survival rate of large seed (%)	Survival rate of medium seed (%)	Survival rate of small seed (%)
0	24Da	16Eb	13Dc
0.2	42Ca	34Db	19Cc
0.5	93Ba	78Cb	56Bc
1	100Aa	75Cb	80Ab
1.5	100Aa	100Aa	0Eb
2	100Aa	98Bb	0Ec

In summary, as sand burial depth increased, *A. Aphylla* seedling emergence rate decreased and emergence time was extended. Moreover, there was no seedling emergence of small seeds at 1.5 cm and 2 cmsand burial, whian may be because the small seeds store less energy and could not maintain the seedlings growth through the sand layer. We further confirmed that seeds could germinate but seedling could not always pass through the sand layer. In addition, there was no significant difference in seed germination rate under the surface layer and other sand burial treatments, but the emergence rate significantly decreased as sand burial depth increased, while the survival rate and biomass were very low. In the field, *A. aphylla* seed on the surface has been shown to germinate in the transient snowmelt period (about 15 days) (Liu *et al*., 2010). However, in the current study, the seed radicle may have found it difficult to implant into the soil, which is likely to lead to low seed survival rate at the surface. This also indicates that sand burial has a major impact on *A. aphylla* seedling regeneration.

Effects of seed size on seedling emergence of *A. aphylla*: Seedling emergence of large *A. aphylla* seeds under sand burial was significantly higher than that of medium seed and small seeds. In addition, at 0.5 cm sand burial depth, medium seed emergence was significantly higher than small seed, which supports the finding of Nie and Zheng (2005). The greater the seed mass, the greater the chance that a seedling could occur from a deeper burial depth, which has been shown in previous studies using seeds from the same species and different species (Zhu *et al*., 2005; Li *et al*., 2006). Although all three size categories of *A. aphylla* seed could germinate at the sand burial depths covered by this experimental design, but from different seedling emergence of small seed in each burial treatment was lower. In addition, the seedling emergence of large and medium seed was significantly lower overdue 0.5 cm sand burial. Large seeds also a showed higher germination and survival rate in deep sand burial (2 cm), indicating that large *A. aphylla* seeds were more adapted to deeper sand burial. In summary, it is clear that seed germination and seedling emergence were consistently affected by seed mass.

Some *A. aphylla* seedlings did not reach the surface, which may be due to the limited energy stored in the seed; the energy could not produce sufficient length hypocotyls to extend to the sand surface. Seed size was closely related to seed energy content, seed energy content has been shown to influence the seed germination, seedling emergence and also the seed setting ability of mature plants (Zhang & Maun, 1990；Dawn, 2013). Previous studies have shown that the germination rate of large seed is higher than that of small seed for some plants (Greipsson & Davy, 1995; Yanful & Maun, 1996a), however, other plants showed the opposite response (Stanton, 1984). Moreover, some plants had different size seeds but the germination rate was similar (Chen & Maun, 1999；Huang *et al*., 2005). The results of this study showed that the seedling emergence rate of large *A. aphylla* seeds was significantly higher than that of medium and small seed at the same sand burial depth. We found that after seed germination whether the seedling reached the surface was dependent on seed size and sand burial depth, the greater the seed size, the more energy stored, and seedlings were more likely to reach the surface (Yanful & Maun, 1996b; Yang *et al*., 2007; Li *et al*., 2011). This has been verified by the seedling emergence and biomass in our study, the larger the seed, the higher the seedling survival rate. The biomass of seedlings from large seeds was significantly higher than that of medium seeds, whereas the biomass of seedlings from medium seeds was significantly higher than that of small seeds.

In the dune ecosystems, the frequent occurrence of sand burial has a significant impact on the survival of plants and has a selective effect of the plant suitability (Maun, 1994; Wang *et al*., 2006). Remarkably, *A. aphylla* is not a typical desert plant, and it is mainly distributed in the proluvial fan and interdune lowland (Huang *et al*., 1997), however, observed seed germination and seedling emergence in the field is dependent on the sand burial environmental conditions, such as autumn sandstorm and micro terrain and other factors. In early spring snowmelt conditions, *A. aphylla* is subject to adequate moisture conditions and the seeds on the exposed surface can germinate, however, the root may find it difficult to implant the soil, which may cause difficulties in seedling emergence. In contrast, *A.aphylla* seeds can germinate even under deep sand burial, but the emergence is difficult and it is also very difficult to achieve the purpose of settlement. Therefore, *A. aphylla* seeds had a higher survival rate at 0.2–0.5 cm sand burial depth, which could reduce the drought stress from the rapid temperature increase after the snowmelt period.

Conclusions

The seed of *A. aphylla* had a high germination rate; the seeds on the exposed surface could germinate, but the root was unable to consistently implant the soil, leading to limited seedling emergence. In addition, the seed emergence was limited under deeper sand burial (2 cm), resulting in a low survival rate. However, in moderate sand burial (0.2–0.5 cm) there was a higher germination rate and seedling survival rate. Moreover, large seed of *A. aphylla* contains more energy-generating material and the resistance of its seedlings may also be stronger. As a result, large seeds show a higher seedling survival rate, and also a greater advantage in seedling establishment.

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