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锐齿槲栎橡子埋藏深度对发芽、幼苗出土和发育的影响

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摘要：锐齿槲栎林内活动的许多小型哺乳动物和鸟类不仅采食橡子，而且还常窖贮或在土壤中埋藏大量的橡子以便季后利用。这些埋藏的橡子构成了发育有效更新苗的主要种子来源。橡子被埋藏的深度和橡子的大小对幼苗的发育有潜在的影响。把橡子埋藏在6、12和18cm三个土层深度中的实验结果显示：随着埋藏深度的增加，橡子的发芽率和发芽橡子的幼苗出土率都有明显下降；由深埋橡子发育的幼苗出土比较困难，所需时间较长，也更易腐烂；橡子的大小对发芽和幼苗出土没有明显的影响；早出土幼苗在第一个生长季内由于有较长的生长发育期，季末发育的较好，所以，由浅埋橡子发育的幼苗在更新成功方面有优势。
关键词：橡子埋藏深度，橡子大小, 贮藏囤积, 动物觅食, 树种立苗
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Effect of acorn burying depth on germination, seedling emergence and development of *Quercus aliena* var. *acuteserrata*

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Abstract: Acorns of *Quercus aliena* var. *acuteserrata* are often predated by small mammals and birds in natural forests. These animals not only eat the acorns during the acorn ripening season, but also cache and hoard most of the remaining acorns on the forest floor in the soil for their future use. These buried acorns form the main seed resource for regeneration. Burying depth is potentially important for germination and for seedling development. The effects of burying depth on germination and seedling development in relation to acorn size were studied in an experiment, in which acorns were planted at 6cm-, 12cm- and 18cm-depth. The experimental results show: Fewer acorns germinated as burying depth increased. From the deeply buried acorns fewer seedlings emerged at a later time than of acorns buried less deeply. They appeared to have more difficulties to emerge aboveground than the seedlings from shallowly buried acorns. The deeply buried acorns and their seedlings also appeared to be more susceptible to rot. Acorn size did not significantly affect germination and emergence of the seedlings. As early emerged seedlings had longer developmental periods in their first growing season, and therefore grew better than late emerged seedlings, seedlings from shallowly buried acorns had an advantage.
Key words: Acorn burying depth, Acorn size, Cache and hoard, Animal predation, Seedling establishment

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In natural forest ecosystems plant seeds, especially large seeds with abundant reserves, are often predated by animals[1-4]. The main consumers of the seeds are small mammals and birds. In the temperate zone, many of these predators, such as squirrels and jays, not only eat the seeds during the seed ripening seasons, but also remove many seeds from trees and from the soil surface. They cache and hoard the seeds underground for their future use[5-10]. As some of these buried seeds may not be found again by the animals[8, 9], such caching and hoarding by animals is considered to be an important means of dispersal[2, 11, 12].

The burial is also thought to be very important for the regeneration of many species[13, 14]. It protects the seeds from predation by other animals[1, 6], and it promotes germination and establishment of seedlings by reducing the difficulty of radicle penetration into the soil[13]. Many big and water-rich seeds are short-lived and vulnerable to water loss[15]. Those seeds, exposed on the soil surface or on forest litter, are susceptible to rapidly losing moisture and their viability[13, 14].

Seed burying depth is an important factor for the fate of a seed[4, 16, 17]. The shallower a seed is buried, the easier the seed may be found again by predators. Furthermore, seeds buried at different depths may experience different environmental conditions affecting seed germination and seedling development, such as the concentration of oxygen and carbon dioxide, temperature and its fluctuation, water availability, nutrient availability, and others[4]. Deeply buried seeds have to invest more reserves into stems for their elongation before shoot apices can reach the ground surface and emerge.

Quercus aliena var. *acuteserrata* is one of the most important deciduous tree species in the montane deciduous broad-leaved forests in mid- and south-China (south of the Yellow river). Its acorns (seeds) are rich in reserves, which confers an advantage in supporting seedling survival and growth of the species. However, this trait also leads to a higher risk of being predated. Seeds of this species can hardly be found on the forest floor after the seed-ripening season, but they can be found at various depths in the soil where predators have buried them (Guo Ke, personal observation). As seed germination and seedling emergence are critical processes of seedling establishment and population regeneration, we were interested to know how burying depth affects seed germination and seedling emergence of this species. In an experiment with three burying depths, the following hypotheses were examined: 1. deeply buried seeds germinate worse (slower and fewer) than shallowly buried seeds; 2. fewer seedlings from deeply buried seeds will emerge from the soil than from shallowly buried seeds; 3. as larger seeds contain larger amounts of reserves enhancing emergence of seedlings[18, 19], more seedlings of larger seeds are expected to emerge from deep soil than of smaller seeds.

1 Materials and Methods

Seeds of *Q. aliena* var. *acuteserrata* were collected in early October 1996 from the forests in Daba Forest Farm, Nanjiang County, Sichuan, China, and taken to Utrecht, The Netherlands. They were stored in a refrigerator at about 3^{o}C.

On January 15, 1997, 150 seeds were randomly selected and divided into three groups, 50 seeds each group. They were subjected to three burying depth treatments, i.e. 6cm, 12cm and 18cm, respectively. After being weighed, they were planted into plastic pots, one acorn per pot. The pots were 15cm in diameter and 30cm in depth, with some small holes in the bottom allowing excess water to drain off. The substrate in the pots was a mixture of compost and river sand, at a volumetric proportion of about 1: 2.

The pots were placed on a mesh in a greenhouse at Utrecht University, The Netherlands. They were watered twice a week. The temperature in the greenhouse was about 17℃ during the night and 20℃ during the day time. At the end of May all pots were moved outdoors.

Seedling emergence was monitored. Seedling height, number of leaves, and leaf size were measured twice per week before June 2 and one time per week until July 29, when they were harvested.

On July 29, all individuals were harvested. Seedling height (length of the aboveground part of the stem, StemA), number of leaves and leaf area of each emerged seedling were measured. Lengths of the tap-root and the belowground part of the stem (StemB) were measured after they had been washed. Each seedling was separated into roots, belowground part of the stem, aboveground part of the stem (StemA), leaves and the remains of the acorn. Biomass of these parts was weighed after drying.

Four plant developmental stages were recognized: pre-germinated seed (non-germinated seed), radicle seedling (with the root but without stem), non-emerged seedling (with only belowground part of the stem) and emerged seedling. Based on this classification of developmental stages and survival states (rotted or not) a census was conducted on the harvest data (see Table 1).

A one-way analysis of variance (ANOVA) was performed to compare the differences in acorn fresh weight with seedlings of different developmental stages and survival states. An analysis of covariance (ANCOVA), with original acorn fresh weight and duration (days) from planting to seedling emergence as two covariates and with seed-burying depth as the main factor, was performed for seedling height (length of aboveground part of the stem), total stem length, tap-root length, number of leaves, leaf area, and biomass of the emerged seedlings. Data of seedling biomass were log-transformed prior to the performance of the ANCOVA, so that they could meet the general assumptions of ANCOVA. An ANCOVA, with original acorn fresh weight as a covariate and with burying depth and seedling developmental stage as two main factors, was performed for the dry weight of acorn remains at harvest.

2 Results

Seedlings developed best in the 6cm-depth treatment and worst in the 18cm-depth treatment (Table 1). About one third of the seeds buried at the 12cm- and 18cm-depth treatments did not germinate, compared to one seventh at the 6cm-depth treatment. 36 and 21 seedlings successfully emerged from the seeds buried at the 6cm- and 12cm-depth treatments, respectively, but only 6 seedlings emerged from the seeds buried at the 18cm-depth treatment.

In contrast, many seedlings from the seeds buried at the 18cm- and 12cm-depth treatments remained at the developmental stage of non-emerged seedling. Most of these seedlings lost their primary stem tips and proceeded height growth with lateral buds or became multi-branched in the soil. In addition, all rotted individuals remained at two developmental stages, pre-germinated seeds and non-emerged seedlings. Increases in burying depth tended to increase the rotting probability of seeds and non-emerged seedlings (Table 1).

Acorn fresh weight did not appear to affect seed germination and seedling emergence. The original acorn fresh weight did not differ significantly, between the seedlings at different developmental stages and survival states (Table 1).

Table 1 Results on survival and developmental stage of seedlings in the three depth treatments, and the mean original acorn fresh weights. (one-way ANOVA results of the mean weight: n=150, F-ratio=0.29, p=0.920).

Treatments (burying depths)	6cm	12cm	18cm	Mean±SE of acorn fresh weight (mg)
Rotted seeds (not germinated)	1	--	3	1873 ± 83
Rotted seedlings (non-emerged)	2	5	7	1974 ± 70
Pre-germinated seeds	7	14	14	1975 ± 52
Radicle seedlings	2	2	4	1948 ± 67
Non-emerged seedlings	2	8	16	2024 ± 44
Emerged seedlings	36	21	6	1981 ± 35

Dry weight of acorn remains at the harvest date tended to be lower for seedlings that were more developed (Table 2). The weight was significantly affected by the original acorn fresh weight, the developmental stage and the interaction between stage and burying depth, but not by depth alone (Table 2).

Table 2 Dry weight (mean±SE, mg) of acorn remains. The effects of original acorn fresh weight, the developmental stage and the interaction were significant (p=0.000, 0.000, 0.007, respectively), but the effect of burying depth alone was not (p=0.092).

	Pre-germinated seed	Radicle seedling	Non-emerged seedling	Emerged seedling
6cm	611±37	521±110	447±80	317±17
12cm	698±49	495±156	450±30	321±21
18cm	538±36	490±86	447±25	281±34

Seedlings tended to emerge earlier with decrease in burying depth (Fig. 1). For the six earliest emerged seedlings at each of the 6cm-, 12cm- and 18cm-depth treatments, on average it took 49, 71 and 130 days, respectively, to emerge. The duration from planting the acorns to seedling emergence were 77.2±4.1, 111.6±8.3 and 130.0±15.6 days (mean±SE) for all emerged seedlings at the 6cm-, 12cm- and 18cm-depth treatments, respectively.

Fig. 1 Cumulative number of emerged seedlings after planting during the experiment

For the 63 emerged seedlings, the duration from planting to seedling emergence appeared to be not related to the seed fresh weight (Fig. 2).

Fig. 2 Relationship between acorn fresh weight and duration from planting acorns to seedling emergence (for all points, r=0.105, t=0.826, p=0.412)

Burying depth did not significantly affect seedling height (StemA length), i.e. the length of the aboveground part of the stem (Table 3). This made the total stem length (Stem length, including the belowground part) increase with the burying depth. The mass of the belowground part of the stem was significantly affected by the burying depth, which determined the length of this part. Root length, number of leaves, leaf area and other components of biomass were not significantly affected by burying depth (Table 3).

Table 3 Mean size (mean±SE) of emerged seedlings at harvest date and the ANCOVA results (p-value) with acorn fresh weight (Fresh-W) and duration since planting to emergence (Days) as the covariates and seed-burying depth (Depth) as the main factor.

	StemA length (cm)	Stem length (cm)	Root length (cm)	Number of leaves	Leaf area (cm^2)	Root mass (mg)	StemB mass (mg)	StemA mass (mg)	Leaf mass (mg)	Bio-mass (mg)
6cm	11.1 ±0.8	16.9 ±0.8	28.5 ±1.0	6.3 ±0.6	96.6 ±9.7	2148 ±235	562 ±54	290 ±39	500 ±50	3501 ±362
12cm	9.5 ±1.0	20.4 ±0.9	23.4 ±1.4	5.8 ±0.9	75.0 ±12.7	944 ±181	524 ±70	156 ±31	328 ±63	1952 ±337
18cm	7.8 ±2.1	24.3 ±2.1	16.3 ±2.0	5.0 ±1.6	52.5 ±21.2	560 ±251	583 ±198	104 ±50	251 ±120	1497 ±612
Fresh-W	0.072	0.092	0.081	0.690	0.575	0.014	0.033	0.011	0.186	0.015
Duration	0.000	0.002	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000
Depth	0.783	0.000	0.148	0.605	0.641	0.584	0.000	0.771	0.797	0.594

Acorn fresh weight significantly affected root mass and stem mass of both parts (below and above ground), but not leaf mass. Seedling size (biomass) was mainly determined by the time of emergence (Table 3).

3 Discussion

Seed-burying depth had significant effects on seed germination and seedling development of the species, but acorn fresh weight had not, except for seedling biomass. The hypotheses that deeply buried seeds germinate worse (slower and fewer), and that fewer seedlings from these seeds emerge from the soil, were confirmed. The hypothesis that more seedlings emerge from larger seeds, however, were not confirmed.

Four individuals rotted at the seed stage and 14 at the non-emerged seedling stage (with the stem in the soil), but no one at the developmental stage of radicle seedlings. This appeared to be related to the duration of the developmental stage. The duration from radicle protrusion to flushing of the plumular axis usually was about two weeks or shorter (Guo Ke, personal observation). It was much shorter than the duration of the pre-germinated seed stage (from planting to germination) and the duration of the non-emerged seedling stage (with the belowground part of the stem). As seedlings from deeply buried acorns took longer to emerge than seedling from shallowly buried acorns, it was not surprising that the number of rotted individuals increased with the burying depth of the acorns (Table 1).

Many seedlings, especially at the 18cm-depth treatment, remained at the non-emerged seedling stage. These seedlings appeared to have difficulties to emerge aboveground, because their main stem tips had been frequently replaced. Some stems of seedlings grew with more than one branch simultaneously (multi-stemmed) below ground.

Deeply buried seeds usually emerge later than shallowly buried seeds[18]. The shallowly buried acorns germinated better and their seedlings emerged earlier (Fig. 1). This suggests that these seedlings have a longer developmental period in the first growing season than the seedlings from deeply buried acorns, thereby allowing the tissues of those seedlings to be better lignified before the coming of harsh winter conditions. It will improve survival chances of seedlings during the extreme adverse conditions in winter. In addition, late emerged seedlings usually are more vulnerable to the attack of predators and pathogens in natural forests[20].

However, shallowly buried seeds can more easily be found again by predators[4]. Many animals, such as squirrels, not only predate ungerminated acorns of this species, but also are sufficiently intelligent to dig up the acorns of emerged seedlings (personal observation). Shallowly buried acorns of emerged seedlings can easily be dug up, but the predators often give up the effort of digging up the deeply buried acorns of emerged seedlings (personal observation).

Thus, seed burying depth is important for the seedling to successfully establish in nature. For this species, 18cm-depth often seems to be too deep for seed germination and for seedling development, however.

It was surprising that seedling height was not significantly affected by burying depth (Table 3). The initial height of the species is determined by the moment when the first whirl of leaves flushes. On the belowground part of the stems, many nodes can be seen. On those nodes the leaves should have flushed had the nodes been exposed aboveground. This suggests that flushing of leaves cannot start until the shoot emerges aboveground. Flushing of the first whirl of leaves usually starts about one week after emergence. When the leaves flush, most matter from the reserves (even some from the stem) is transferred to the developing leaves and at that time stem growth is transitorily limited (but does not completely stop). These constraints may explain that the initial height might not be affected by burying depth (at least as long as the cotyledons still carry sufficient reserves to maintain stem growth). As most seedlings did not further extend their stems before the harvesting date, the initial length of the aboveground part of the stem determined the seedling height in our experiment.

There was no significant difference in initial acorn fresh weight between the developmental stage groups at harvest date in our experiment (Table 1). This suggests that the failure of germination was not related to seed size and that seed size did not significantly affect emergence of the seedlings. This result is consistent with the findings of Chen and Maun[17] that seed germination and seedling emergence of *Cirsium pitcheri* were not related to seed size, but is in contrast with the findings of Tripathi and Khan[19] that heavy seeds of *Quercus dealbata* and *Quercus griffithii* germinated earlier and showed better germination than light seeds and seedlings produced from the heavy seeds survived and grew better. The divergence appears to result from the different ranges of seed size, as extremely small seeds of a species usually show markedly lower viability and vigor than the normal and larger ones[5]. The acorn fresh weights of *Q. aliena* var. *acuteserrata* in this experiment ranged from 1164 to 2569mg: the largest was about 2.2-fold the smallest. The acorn dry weights of *Q. dealbata* and *Q. griffithii* ranged from 283 to 1283mg and from 245 to 1080mg, respectively: the largest was about 4.5-fold the smallest. Cornelissen, Zhong and Werger[21] found that smaller chestnuts of *Castanopsis fargesii* germinated significantly earlier than bigger ones. They ascribed this to the higher ratio of contact area of chestnut-substrate and chestnut size of the smaller seeds. We do not know the exact time of seed germination in this experiment, but we did not find a significant correlation between acorn fresh weight and emergence time (Fig. 2).

The depletion (consumption) of seed reserves depended on seedling developmental stage rather than on burying depth (Table 2). This demonstrated that at a certain developmental stage shallowly buried acorns had not exported less resources to developing seedlings than deeply buried acorns. Larger seeds generally produced more robust (thick) initial roots and stems, but contrary to the common supposition[4, 22] that more seedlings from larger seeds succeed in emerging from greater depths, our experiment did not confirm this. In fact, seedling failure to emerge from deeply buried acorns in this experiment resulted from the death of their main tips and lateral branching rather than from small reserves in the acorns.

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Fig. 1 Cumulative number of emerged seedlings after planting during the experiment

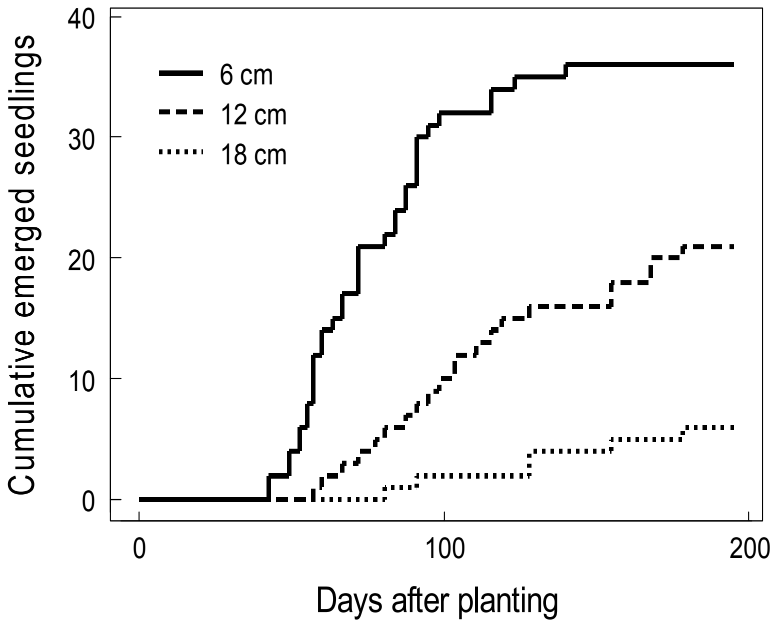

Fig. 2 Relationship between acorn fresh weight and duration from planting acorns to seedling emergence (for all points, r=0.105, t=0.826, p=0.412)