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Impacts of burial and insect infection on germination and seedling growth of acorns of *Quercus variabilis*

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ABSTRACT

Rodents usually exert important role, through their scatter seed hoarding behavior, on plant regeneration in the field. To investigate the effects of burial and insects infection on germination and seedling growth of acorns of *Quercus variabilis*, perfect and infected acorns were buried in the soil among four depths, 0 cm, 4 cm, 8 cm and 12 cm, to simulate the seed hoarding behavior by rodents in the field. The results showed that (1) the germination rate were both high, under 4 cm burial depth, for perfect and infected acorns (92% and 53% separately), and decreased significantly with increased burial depth; (2) perfect acorns germinated better in 4 cm burial depth group by autumn of first year; (3) there 32% (perfect) and 26% (infected) acorns with 0 cm burial depth, on the soil surface, germinated successfully; for infected acorns, the 4 cm depth group had the best germination or seedling recruitment in both the first year and the second year; (4) acorns of *Q. variabilis* exhibited dormancy period ca 7 months; (5) burial, infection, and the interaction between these two factors influenced several aspects including stem height, leaf weight, Tannic acid, and biomass within seedling growth; (6) the results from this study suggest that proper burial would be helpful for the germination and seedling growth, and seedlings of shallow buried acorns had an advantage in their early development; and (7) infection by insects will not inevitably influence seedling early development.

1. Introduction

Recruitment is a long and complex process in woody plant species, involving several sequentially connected life-history stages (Herrera et al., 1994). In plants, regeneration can be limited by the amount of seeds produced, by the effectiveness of dispersers, by the availability of microsites for seed germination and seedling establishment and by the activity of herbivores feeding on seeds, seedlings and juveniles (Schupp, 1995; Schupp and Fuentes, 1995; Zhang, 2001; Zhang and Wang, 2001; Lu and Zhang, 2004; Zhang et al., 2005). In addition, the effect of all these factors is in many cases non-exclusive.

Many previous studies indicated that seed germination and seedling establishment is an important stage in plant regeneration, while the germination of many oak species, under natural conditions, is very poor on soil surface without acorn burial and dispersal by rodents (Zhang and Wang, 2001). Burial is found to be a potential measure in reducing acorn predation by rodents (as well as other vertebrates) and an effective way for increasing seedling recruitment (Shaw, 1968; Borchert et al., 1989; Zhang, 2001; Zhang and Wang, 2001; Xiao et al., 2003, 2004). Yu et al. (2001, 2003) urged that insect infection influences strongly on acorn germination. Furthermore, time order of seedling emergence might exert important role for the subsequent survival and fitness in competitive capacity (Ross and Harper, 1972; Bush and Van Auken, 1991). Several days delay in emergence can be magnified into significant differences in final biomass and reproduction, especially under competitive situations (Ross and Harper, 1972). Despite much achievements being made in past decades, there were still some problems hindering our understanding on seedling establishment from acorns, for example, what is the suitable planting type (dense or scattered)? What is the proper burial depth for seed germination in the field?

Acorns (seeds of genus *Quercus*) have been believed to be an important food for wildlife, particular for rodents. Seed-eating rodents have a close relationship to acorns as seed predators, they especially play an important role as seed dispersers by scatter-hoarding, in which one or more acorns are separately buried in shallow depths in the soil (several centimeters under the litter) and are dispersed across a wide range (Harper, 1977; Vander Wall, 1990). Some of the scatter-hoarded acorns escaped from predation by hoarders and other animals, although most of them were ultimately preyed. Those escaped acorns, under suitable micro-environment and buried depth, will probably germinate and establish seedlings successfully.

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Cork oak, *Quercus variabilis* Blume, is distributed widely from Liaoning province, north China, to Guangdong province, south China, and was the dominant species in forest community in central area of China. To promote the forest regeneration among forest-degenerated area including our acorns collecting site, acorns of Cork oak were planted directly in soil for germination and seedling establishment. In this research, we intend to investigate: (1) the proper burial depth for acorns germination; (2) if acorns of *Q. variables* have a dormancy period in temperate area, and (3) the effect of insect infection on acorns germination and seedling growth.

2. Materials and methods

2.1. Acorns collection

During the period of acorn maturation, late August, 2007, we collect Cork acorns from State-owned Huanglianshu Forest Farm (115°26′E, 39°58′N) of Jiyuan, Henan Province, China. Acorns of *Q. variabilis* are oval in shape, and with a size of 2.09 mm × 1.75 mm (long diameter × wide diameter) ($n = 400$), and weighs 3.65 ± 1.26 g ($n = 400$) in fresh seed.

2.2. Survey on infection rate of acorns

Once an acorn being infected by insect, finally a small hole will be left there, in the acorn shell, because of gnawing and crawling by larva. We can identify perfect acorns and infected ones via the hole above-mentioned. We randomly assigned a batch of acorns for infection rate survey, and did not stop inspecting the infected acorns, for about 60 days, till new hole-acorns were found, since those larvae within acorns do not fall in the same developing phase. We then can calculate the infection rate of current-year acorns.

2.3. Acorn planting

We got soil from agricultural land, and mixed them with fine sand (soil:sand = 2:1) to prevent hardening of soil surface after watering. The treated soil was loaded into plastic flower pot of 30 cm in height and 20 cm in diameter, with a small hole at the bottom. Total 200 perfect acorns, similar in size and mass, were selected for experiments, and were divided into four groups (i.e., 50 acorns in each group). We then planted the first 50 acorns, one in each, at 50 flower pots in a depth of 0 cm. Similarly, the other three groups of acorns were planted in depth of 4 cm, 8 cm, and 12 cm, respectively. With the same procedures, total 200 infected acorns were selected and planted. All the flower pots with acorn in it were placed in a semi-closed enclosure in the Agriculture Science and Technique Park of Zhengzhou, Henan Province, China, and watered with an interval of 3–5 days. Once the first seedling emerged, we investigated and record, every 2 days, the emerging time, seedling growth status including leaf number and seedling height.

The seedling growth was divided into four stages as follows: before germination, rooting, with under-ground stem, and emerging. Based on these categories, we counted number of emergence, seedlings, and rotten acorns, and measured, for emerging seedlings, the stem height, and length of axial root were checked and recorded. At the end of investigation, we excavated all the seedlings from flower-pot, and cleaned them with fresh water, and then dried each seedling in a oven to constant weight 120 ± 5 °C. Finally, the dried weight of leaves, stem above ground, stem below ground and root were scaled for the seedlings derived from perfect and infected acorns, respectively.

The research was carried out from September, 2007 to June, 2008.

2.4. Data analysis

SPSS for Windows (version 13.0) was employed in data statistics and analysis. Based on normal distribution of data, two-way ANOVA was used in determining the effects of burial depth, infection and interaction between burial and infection on acorn germination, and seedling growth. LSD was employed in checking the differences of seedling biomass between every two burial depth groups. Chi-square test was used for analyzing the differences of germination rates between perfect and infected acorns in each of four burial depths, and the germination rate among burial depth groups in perfect and infected acorns.

3. Results

3.1. Infection rate of acorns

Total 3625 acorns were checked randomly, and there were 1117 acorns were found infected by insect, the infection rate in current-year was 30.04% for Cork acorns collected from Taihangshan Mountains area, Jiyuan, Henan, Province China.

3.2. Impact of burial and infection on acorn germination

3.2.1. Perfect acorns

In late autumn of the first experimental year, seedlings derived from perfect acorns, among the burial depth of 0 cm, 4 cm, 8 and 12 cm, were 2, 33, 11 and 3, respectively, while there some new seedlings established in the second experimental year (Fig. 1). Combined those seedlings established in 2 years, the germination rate was higher in 4 cm burial depth group (92%) than that in other burial depths ($\chi^2 = 16.667$, df = 3, $P = 0.001$), the germination rate is the lowest in 0 cm burial depth (30%), that is to say, placing directly on soil surface was disadvantageous to acorn germination and seedling establishment. There were 10 acorns rotten in 12 cm burial depth group.

3.2.2. Infected acorns

For infected acorns, the germination among four burial depths was similar than that for perfect acorns, and the differences of total germination rates was significant ($\chi^2 = 13.234$, df = 3, $P = 0.004$).

The infection by insect larva was harmful to acorn germination and acorn germination. Compared to perfect acorns, there some rotten infected acorns were found in all burial depths but for 0 cm, while the germination rate was lower in infected acorns for all burial depth than that in perfect acorns. At 4 cm burial depth, for example, the germination rate was significantly lower than perfect ones ($\chi^2 = 6.761$, df = 1, $P = 0.011$). We thus claimed that the infected acorns might have a decreased viability.

To see the influence of infection on germination rate, we compared the percentages of established seedlings derived from perfect and infected acorns among four burial depth groups (Fig. 1). The difference of germination rate between perfect and infected acorns were both insignificant both in 0 cm ($\chi^2 = 0.143$, df = 1, $P = 0.705$) and 4 cm ($\chi^2 = 2.513$, df = 1, $P = 0.113$) burial depth groups, while infection heavily affected germination rates in 8 cm ($\chi^2 = 5.070$, df = 1, $P = 0.024$) and 12 cm ($\chi^2 = 7.364$, df = 1, $P = 0.007$) burial depth groups. Furthermore, there were significant differences in germination rate among four burial depth group both in perfect ($\chi^2 = 16.667$, df = 3, $P = 0.001$) and infected acorns ($\chi^2 = 13.234$, df = 3, $P = 0.004$). The percentage of perfect acorn-derived seedlings was higher than that of infected acorns (Fig. 1). The result from this research indicated that infection would decrease the possibility of acorns to germinate with the increasing in burial depths.

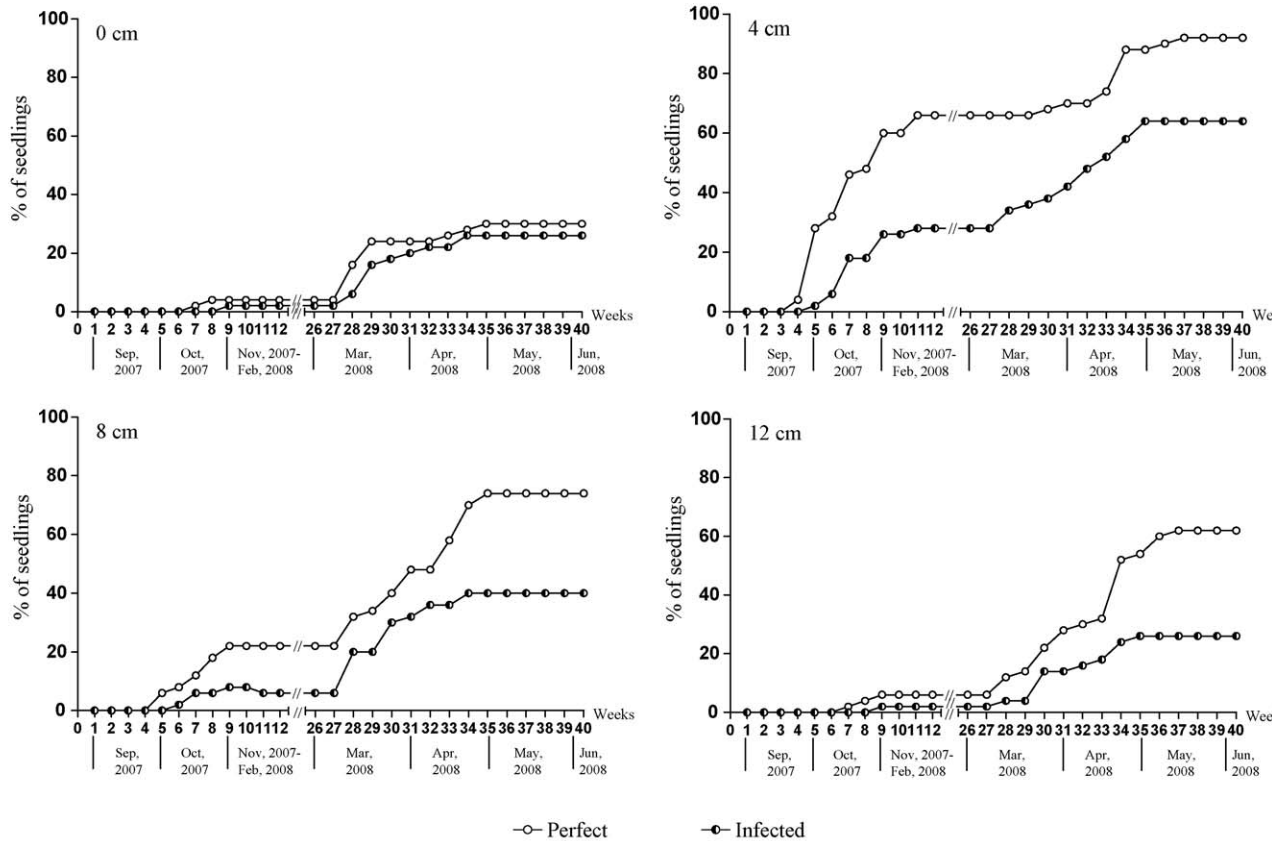

Fig. 1. Effects of infection on germinating rate of acorns of *Quercus variabilis* among various burial depth groups.

3.3. Impacts of burial and infection on time of seedling emergence

3.3.1. Perfect acorns

In the first experimental year, all the established seedlings experienced several development phases including emergence, growth, leaves withering, and growth pause. Except for 0 cm burial depth, the emerging time was delayed with the increasing of burial depth (Fig. 2), and the first seedling emerged from 4 cm burial depth 24 days after acorns planting, whereas the first seedling derived from 8 cm to 12 cm burial depth emerged 34 days and 48 days, respectively. Generally, the peak emergence time fell in 30–60 days after acorns planting, and the total emerging time lasted for about 50 days (Fig. 2).

In spring of the second experimental year, new leaves sprout from those pre-year-emerged seedlings. Furthermore, some new seedlings emerged from all burial depths (Fig. 2). The first seedling was found in 0 cm burial depth. The peak emergence time fell in 203–245 days after acorns planting, and the emergence time in this year lasted for 55 days (Fig. 2).

3.3.2. Infected acorns

In the current-year of acorns planting, process of seedling establishment for infected acorns was similar to perfect acorns. The emerging time, however, was later infected acorns, and lasted relative short time (Fig. 3). The first seedling, in 4 cm burial depth, derived from infected acorn emerged 27 days after acorns planting, was found while in burial depths of 0 cm, 8 cm, and 12 cm, the first seedling were found after planting in 51 days, 39 days, and 48 days, respectively. The total emergence time of infected acorns lasted for 45 days (Fig. 3).

There were some new seedlings emerged in the second experimental year, and the emergence process of these seedlings was showed in Fig. 3. The first seedling was found in 0 cm group

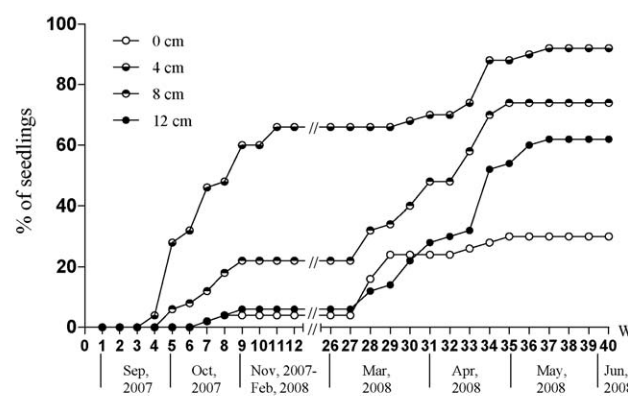

Fig. 2. Germinating rate of perfect acorns of *Quercus variabilis* during September 2007 and June 2008.

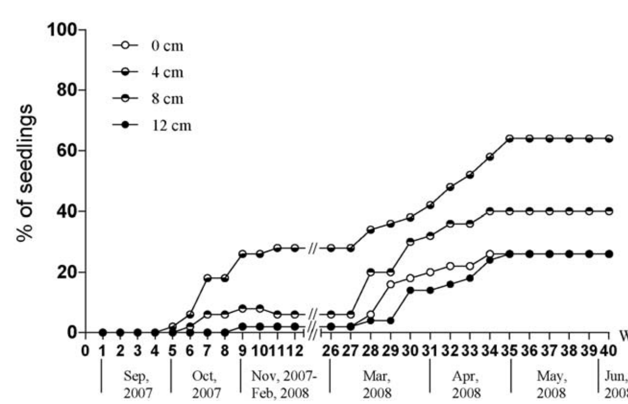

Fig. 3. Germinating rate of infected acorns of *Quercus variabilis* during September 2007 and June 2008.

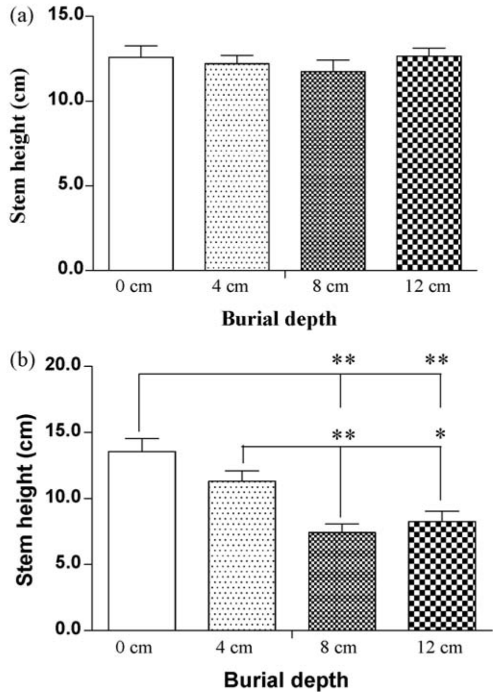

Fig. 4. Stem heights of seedlings derived from perfect (a) and infected acorns (b) under different burial depths.

and 200 days after acorns planting, and seedlings from the other burial depths emerged soon after, and lasted for 35 days (Fig. 3).

3.4. Impact of burial and infection on seedling growth

3.4.1. Stem height of seedlings

We investigated growth status of seedlings derived from perfect and infected acrons (Figs. 4 and 5). Burial depth

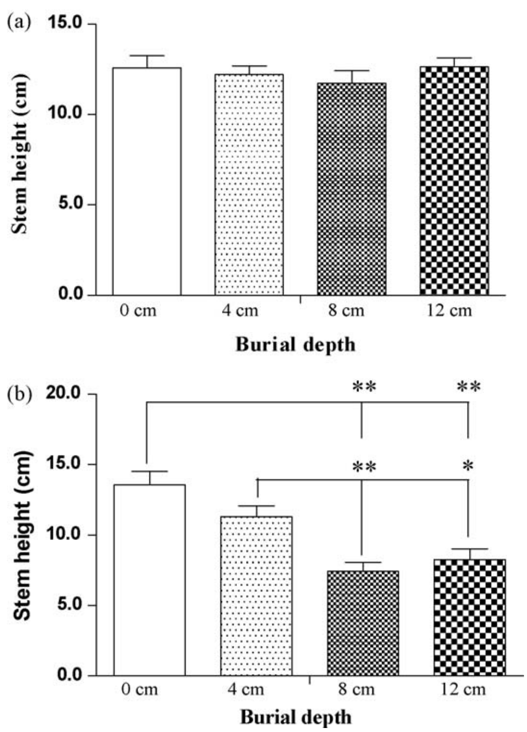

Fig. 5. Leaf weight of seedlings among four burial depth groups in perfect (a) and infected acorns (b).

Table 1

Two-way ANOVA of burial depths on stem heights of seedlings derived from perfect and infected acorns.

Source	Sum of squares	df	Mean square	*F*	*P* value
Perfect acorns	16.095	3	5.365	0.478	0.698
Error	1358.779	121	11.230		
Total	1374.874	124			
Infected acorns	365.214	3	121.738	9.950	<0.000
Error	831.998	68	12.235		
Total	1197.213	71			

insignificantly affects stem height (above ground) perfect acorn-origined seedlings (Fig. 4, Table 1) ($F = 0.478$, df = 3,121, $P = 0.698$). For infected-acorn-derived seedlings, however, stem heights decreased significantly with the increasing of burial depths ($F = 9.950$, df = 3,68, $P < 0.001$) (Fig. 5, Table 2).

3.4.2. Leaf weights of seedlings

Effects of burial depths and infection on seedling leaf weights were showed in Fig. 5 and Table 2. For those successfully established seedlings, leaf weight was significantly influenced by acorn type ($F = 59.800$, df = 1, $P < 0.001$) and burial depth ($F = 94.663$, df = 3, $P < 0.001$). Furthermore, there was significant interaction between burial depth and acorn type on leaf weights and the interaction between the two above-mentioned factors ($F = 8.163$, df = 3, $P < 0.001$), leaf weights decreased with the increase of burial depths (Fig. 5).

3.4.3. Variance of Tannic acid of seedlings

We sampled endosperm, foliar bud, leaves, and fallen leaves, with the developing stages of acorns, to understand the changes of concentration of Tannic acid in germination and seedling establishment, and the changes of leaves derived from perfect and infected acorns (Fig. 6). The differences of Tannic acid concentration in the four above-mentioned parts were significant in between-groups ($P < 0.05$) (Fig. 6). The quantity of Tannic acid was the most in endosperm, and then in foliar bud, leaves, and fallen leaves, respectively. The difference of Tannic acid in perfect acorns-origin leaves was significantly lower than that in infected ones ($t = 4.437$, df = 6, $P = 0.004$) (Fig. 6).

3.4.4. Variance of biomass of seedlings derived from perfect and infected acorns

Biomass of seedlings were heavily influenced by burial depths ($F = 3111.190$, df = 3,200, $P < 0.001$) and acorn type ($F = 63.645$, df = 1,200, $P < 0.001$), and the interaction between burial depth and acorn type ($F = 47.993$, df = 3,200, $P = 0.000$) (Table 3). For the seedlings derived from perfect acorns, the seedling biomass in 4 cm burial depth group were significantly larger than that in 8 cm and 12 cm burial depth groups (LSD, both $P < 0.05$) (Fig. 7), while the difference of biomass was insignificant between 4 cm and 0 cm burial depth groups (LSD, $P = 0.464$), though the biomass in 4 cm group was larger than that in 0 cm group. The biomass of seedlings derived from infected acorns decreased sharply with the increasing of burial depth (LSD, all $P < 0.05$) (Fig. 7).

4. Discussion

Under natural conditions, the infection rate of acorns varied heavily, from 30% to 70%, even to 100%, according to tree species, forest stands, and spat-temporal differences (Sork et al., 1993; Koenig et al., 1994; Crawley and Long, 1995; Yu et al., 2001, 2003; Xiao et al., 2003; Wang et al., 2008). The insects found in acorns of *Q. agrifolia* were *Curculio occidentis* and *Cydia latiferreana*, and the infection rate was 38% for this oak (Lewis, 1992); For Liaodong oak,

Table 2
Two-way ANOVA of acorn type and burial depth on leaf weights of seedlings of *Quercus variables*.

Source	Sum of squares	df	Mean Square	*F*	*P* value
Intercept	63.970	1	63.970	5664.543	<0.001
Acorn type	0.675	1	0.675	59.800	<0.001
Burial depth	3.207	3	1.069	94.663	<0.001
Acorn type × burial depth	0.277	3	0.092	8.168	<0.001
Error	2.112	187	0.011		
Total	92.247	195			

Table 3
Variances of Biomass of seedlings derived from perfect and infected acorns.

Source	Sum of squares	df	Mean Square	*F*	*P* value
Intercept	829.968	1	829.968	3111.190	<0.001
Acorn type	16.979	1	16.979	63.645	<0.001
Burial depth	38.409	3	12.803	47.993	<0.001
Acorn type × burial depth	8.251	3	2.750	10.310	<0.001
Error	53.354	200	.267		<0.001
Total	1208.556	208			

Q. liaotungensis, the infection rate was 45.4% (Yu et al., 2001, 2003), while the infection rate of Monggu oak, *Q. mongolica*, reached 58.2% in Mt. Xiaoxing'an area (Wang et al., 2008). It was believed that the infection rate will be low in mast-seeding year and vice versa (Bonal et al., 2007; Maeto and Ozaki, 2003; Wang et al., 2008). In this research, infection rate of Cork oak, in current-year, was relative lower (30.04%) to other oak species above-mentioned, which might because of the mast-seeding of Cork oak in 2007 (personal observation, unpublished data).

In our study, a few acorns in 0 cm burial depth emerged and established seedling successfully, this was possibly because the harden surface of soil makes it difficult for seedling to root below soil, and withered to die eventually under heavy sunlight. The seedlings from deeper burial depths seemed to have more difficulties in emerging above ground than those seedlings from shallow buried acorns. The deeply buried acorns and their seedlings also appeared to be more susceptible to rot. Our conclusion supports the experiment conducted by several authors. Su et al. (2007) had planted six plant species in nine burial depths (i.e., 0 cm, 0.5 cm, 1 cm, 2 cm, 3 cm, 4 cm, 6 cm, 8 cm, and 12 cm) to investigate the seed emergence, and found that emergency rate decreased sharply with the burial depths increasing. Yang et al. (2007) found that emergence rate of seeds of *Bromus inermis* Leyss decreased with the burial depth increased from 1 cm to 14 cm. Guo et al. (2001) buried acorns of *Quercus aliena* var. *acuteserrata* in depths of 6 cm, 12 cm, and 18 cm, respectively; the results showed that fewer acorns germinated as burying depth increased. The results from our research indicated that suitable shallow burial will promote acorns emergence and seedling growth, while it was disadvantageous to recruitment for acorns either uncovered or deeper buried.

Seed germination and seedling establishment will complete under favourable conditions including temperature and wetness (Michael and Paul, 2000; Kos and Poschlod, 2007, 2008). The variance of germination and seedling recruitment, both in perfect acorns and infected ones, might reflect the influence of temperature and wetness among different burial depths. In this research, we provided the four burial depth groups with same water management, however, the wetness in upper layer of soil would be weaker than that in the deeper parts because of evaporation, while the soil temperature in upper parts would be higher than deeper parts owning to much sunlight. Under the optimal configuration between sunlight and temperature, acorns thus had better germination and seedling growth. The result from this study showed that the 4 cm depth group, for both the perfect and infected acorns, had better germination by autumn in the first year, which indicated that the favorite condition for germinating of acorns of Cork oak was possibly the 4 cm depth group, which was coincidently with burial depths for those scatter-hoarding rodents in the field (Vander Wall, 1990, 1993; Zhang et al., 2005). And all the seedlings from 4 cm depth group grew well in the next year.

White oak (WO, section *Quercus*) acorns do not exhibit seed dormancy and will start to germinate very soon after maturing and falling to the ground (Fox, 1982; Steele et al., 2001). In our research, however, acorns germination lasted 50 days in the first experimental year, and 250 days in the second experimental year after acorns planting. Furthermore, the established seedlings in second year occupied over 50% of total seedlings both in perfect and infected acorns. This result indicated that acorns of *Q. variabilis* exhibited a certain dormancy period, ca 7 months, in our study area. Under favorable conditions, the acorns may complete

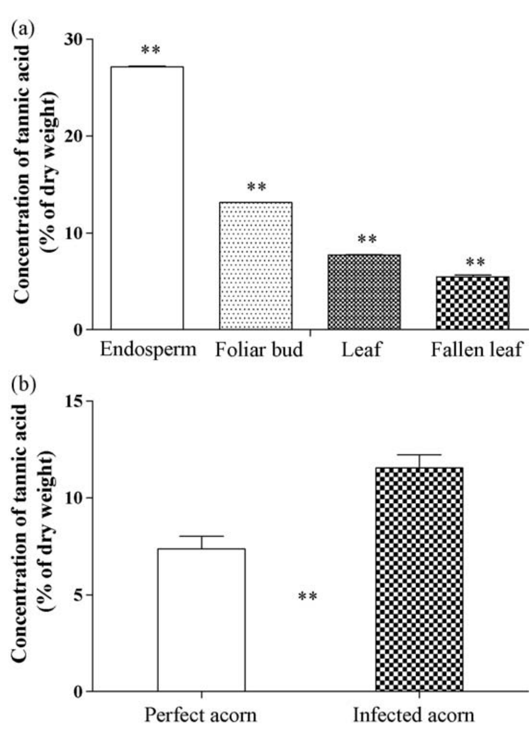

Fig. 6. Variance of concentration of Tannic acid in the course of germination and seedling establishment (a) among different developmental stages; (b) between perfect and infected acorns.

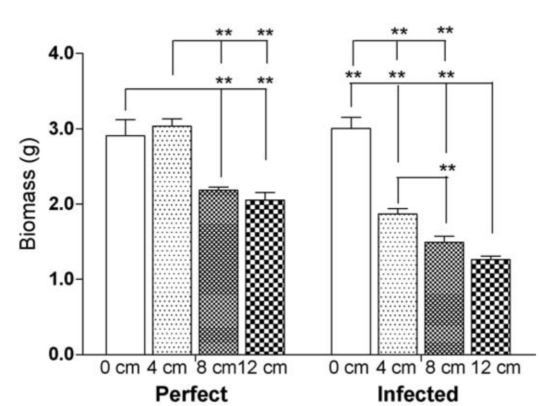

Fig. 7. Variance of biomass of seedlings derived from perfect and infected acorns.

germination and seedling establishment, or they will go to dormancy status until necessary microenvironment comes. Based on the observation on seed germination behavior of *Ceratocrpus arenarius*, Gao et al. (2008) found that seeds of this plant species kept viability and germinated successively 210 days after seeds planting, and the total germination rate reached over 80%.

Infection by insects on acorns exerted remarkable impacts on acorn germination, seedling survival, stem height, leaf weight, andÂseedling biomass. In 4 cm burial depth, germination rate of perfection acorns reached 92%, whereas was 62% in infected acorns, the reason may be that the nutrients within acorns were not enough for germination with loss of partial components. Once an acorn was infected by insect, it would be susceptible to attack of bacterium with the increasing of burial depths, which made acorn more easily rotten. For example, in 12 cm burial depth, there were 10 perfect acorns rotten, while the number reached 20 for infected acorns.

Before germination, Tannic acid level in infected acorns was significantly higher than that in perfect ones, which indicated that infected acorns were not preferred food item for rodents in the field, and these acorns, however, should have more chances to germinate. That is to say, slight infection will not hinder the germination of acorns of Cork oak. Tannic acid level decreased with the developmental stages of seedling (Fig. 6), which will reduce the damage on early seedlings by rodents.

Some rodent species usually selected perfect seeds and shallowly buried them scattered in soil, while the infected seeds were consumed *in situ* (Vander Wall, 1990; Xiao et al., 2004; Zhang et al., 2005). Scatter-hoarding on seeds by small rodents will exert positive role on seed germination and seedling establishment (Vander Wall, 1990; Lu and Zhang, 2004). We intend, in future, to investigate the relationship between infected acorns and rodents and seedling establishment in the field.

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