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BRIEF COMMUNICATION

# Influence of insect-infested cotyledons on early seedling growth of Mongolian oak, *Quercus mongolica*

X.F. YI*,** and Z.B. ZHANG*,***

*State Key Laboratory of Integrated Management of Pest Insects and Rodents in Agriculture, Institute of Zoology,*
*The Chinese Academy of Sciences, Datun Road,Chaoyang District, Beijing 100101, China**
*College of Agriculture, Henan University of Science and Technology,*
*Tianjin Road 70, Jianxi District, Luoyang 471003, China***

## Abstract

Insect-infested (II) acorns germinated 3 d earlier than the healthy (H) ones. However, germination ratio of II-acorns was strongly decreased compared with H-acorns and there were great differences in activities of amylase. We found an apparently lower net photosynthetic rate and total chlorophyll contents of the first true leaf of II-acorns than of the H-ones. Maximal photochemical efficiency of photosystem 2 (PS2, $F_v/F_m$) decreased in seedlings germinated from II-acorns than from the H-ones. Infestation of insects, especially for weevil (*Curculio* spp.) had significantly negative effects on length of taproots, height of plants, dry mass (DM) of roots and the first fully expanded true leaf. Leaf area and total N content of the first true leaf declined due to limitation of resource reserves in cotyledons. Damage of cotyledons caused by weevil accounted much for poor development of seedlings germinated from II-acorns. A mutual relationship between seedling establishment and seed-infesting insects may exist due to high predation on H-acorns by small rodents.

*Additional key words*: *Curculio*; dry mass; leaf; leaf area; net photosynthetic rate; nitrogen content; photochemical efficiency; photosystem 2; plant height; root; weevil.

Species in the family of Fagaceae produce large-sized seeds. Mongolian oaks belong to typically large-seeded tree species of the northern hemisphere (Harper *et al.* 1970). Higher plants with this reproductive strategy put much effort in generating each single seed, *e.g.* large individual mass (Farmer 1997). Large seeds may have a higher probability to survive because scatter-hoarding animals store larger seeds in lower densities to protect them from competitors (Stapanian and Smith 1978, Clarkson *et al.* 1986) and they provide large metabolic reserves for seed germination and seedling growth (Westoby *et al.* 1992). This is favourable for the early development of seedlings (Jurado and Westoby 1992) and consequent opportunities to survive. The larger amounts of reserve compounds in big seeds favour the better development of vigorous root systems enabling to occupy large volumes of soil (Venable 1992, Allsopp and Stock 1995) or to reach deep soil layers with larger moisture availability (Bacilieri *et al.* 1993, Mosandl and Kleinert 1998). Furthermore, large amount of reserves always leads to earlier successful establishment in severe environments (Merouani *et al.* 2001).

Large seed reserves help the seedling overcome temporal deficits in saccharide synthesis during the early stage of germination when seeds are buried in a deep soil layer inaccessible to irradiation (Tripathi and Khan 1990, Leishman and Westoby 1994). This positive effect of seed size on seedling growth affects not only final seedling size, but also its fast growth rate as well as final successful establishment of plants. Xiao *et al.* (2007) reported that large mass is a mechanism to the joint evolution of resistance and tolerance to insect infestation.

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***Corresponding author; fax: 86+10-64807099, e-mail: zhangzb@ioz.ac.cn

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Mongolian oak (*Quercus mongolica*) possesses a large seed of 2.52×1.84 cm and a large fresh mass of 4.40±0.51 g. Despite high proportion of acorns suffering from infestation by insects, *e.g*. *Curculio* spp., large seed mass in acorns could tolerate partial consumption by seed predators, because damaged acorns can germinate and even establish viable seedlings. In nature, most insect-infested (II) acorns can still germinate to form a seedling despite one or both cotyledons are destroyed by *Curculio* or other insect larva (see Fig. 1). However, information is still rare for better understanding the difference in growth biology and ecological adaptability of II- and healthy (H) acorns of *Q. mongolica*. We intended to define how much the acorn is affected by II and evaluate the role of acorns of *Q. mongolica* in regeneration of forests.

Fig. 1. Germination of healthy (*A*) and insect-infested (*B*) acorns.

Acorns of *Q. mongolica* were collected during autumn 2005 from a mixed stand of conifers and broad-leaved species in Chenqing Forest Centre of north-eastern China. Elevation of the study area is 320–507 m and averages at 360 m. This area belongs to Xiaoxing'an Mountain Region, with a warm temperate continent monsoon climate. The mean annual temperature averages 0.4 °C, precipitation is 540 mm per year. 100 seeds of H- and 100 seeds of II-acorns were analysed for length, diameter, fresh mass, and amylase activity prior to sowing. 20 plastic pots (250 mm in height and 200 mm in diameter) were divided into two groups and filled with nutrient-free sand. Each 5 H- and II-acorns were sown in 20 plastic containers at a depth of 2 cm. All plastic pots were put into a *FPG3-300AY-12* irradiated feeding box (temperature of 10.0–50.0±0.5–1.0 °C) under "visible light" of 800 μmol $m^{-2}$ $s^{-1}$, 14-h photoperiod, day-night temperature of 25/15 °C, and relative humidity of 60±10 %. Balanced nutrient solution (Hoagland) was supplied once 7 d after germination and plants were watered three times per week.

When the first true leaf was fully expanded in H-acorns (H-acorn germinates later than the II-one), height of plant was measured. Photosynthetic parameters were recorded using portable photosynthesis system (*CIRAS-1*, *PP Systems*, UK) under 800 μmol $m^{-2}$ $s^{-1}$ (leaf temperature 28 °C, ambient $CO_2$ concentrations). Chlorophyll (Chl) *a* fluorescence indicators were measured using a pulse amplitude-modulated fluorometer (*FMS-2*, *Hansatech*, Norfolk, UK) after 20-min dark adaptation, to investigate saccharide accumulation and capability of photosynthetic apparatus in true leaves. Then the whole plant was removed from the container and the roots and shoots were washed with water until sand particles were completely eliminated. Length of taproot, leaf area, and fresh mass (FM)/area ratio were measured prior to desiccation. Clean plants were separated into root, stem, and leaves. These organs were dried at 80 ºC for 48 h to constant mass and weighed. Total nitrogen content was determined by using the Kjeldahl method. All data were analyzed using *SPSS for Windows 11.0*. Traits related to H- and II-acorns were tested for significance by Independent-Samples T Test (Levene's test).

Acorns selected for germination experiment did not differ in seed length, seed diameter, and FM between H- and II-acorns and thus enabled uniform treatment condition except for infestation. Time to germination was significantly different for H- and II-acorns (Table 1). II-acorns germinated 3 d earlier than the H-ones. However, germination ratio of the II-acorns was significantly decreased compared with the H-acorns. Activities of amylase of H- and II-acorns were analyzed with an attempt to link it to germination duration; the activity of II-acorns was higher than that of H-ones (Table 1).

Net photosynthetic rate ($P_N$) of the first true leaf of II-acorns was lower than that of the H-one under an irradiance of 800 μmol $m^{-2}$ $s^{-1}$ (Table 1). Also total contents of Chl *a* and *b* were lower in the first true leaf from II-acorns. Maximal photochemical efficiency of PS2 ($F_v/F_m$) decreased in seedlings germinated from II-acorns than that in the H-ones, while actual photochemical efficiency of PS2 in the light ($\Phi_{PS2}$) showed only a little change.

The final evaluation of seedling successful establishment depends on the length of taproots, height of plants, dry mass (DM) of root system, and DM and area of the first fully expanded true leaf. Infestation by insects, mainly by *Curculio* spp., had significant effects on the above mentioned parameters. Furthermore, total N content of the first true leaf declined due to limitation of resource reserves. Damage of cotyledons accounted for poor development of seedlings germinated from II-acorns (Table 1), the impact of cotyledon reserve was nevertheless of a key role in the early stage of seedling establishment.

In natural conditions, II-acorns account for 30–40 % (even much more) of total acorns produced by an

Table 1. Traits related to germination and seedling growth, characteristics of photosynthesis, and chlorophyll fluorescence parameters for healthy (H) and insect-infested (II) acorns. Different letters in the same line indicate significant difference at $p<0.05$.

| Characteristic | H | II |
|---|---|---|
| Length [cm] | 2.50±0.09 a | 2.48±0.07 a |
| Diameter [cm] | 1.84±0.09 a | 1.77±0.08 a |
| Fresh mass [g] | 4.40±0.51 a | 4.13±0.15 a |
| Time to germination [d] | 20.30±1.62 a | 16.80±1.18 b |
| Amylase activity [mg $kg^{-1}$(maltose) $s^{-1}$] | 6.00±0.67 a | 7.83±0.67 b |
| Germination [%] | 78.00±10.80 a | 46.00±12.40 b |
| Chlorophyll *a* [g $kg^{-1}$] | 1.34±0.04 a | 1.08±0.07 b |
| Mass/area [g $m^{-2}$] | 51.2±1.3 a | 49.8±1.4 b |
| Net photosynthetic rate [μmol($CO_2$) $m^{-2}$ $s^{-1}$] | 7.28±0.11 a | 5.78±0.27 b |
| $F_v/F_m$ | 0.76±0.03 a | 0.71±0.03 b |
| $\Phi_{PS2}$ | 0.34±0.03 a | 0.32±0.02 a |
| Taproot length [cm] | 21.58±1.27 a | 16.57±1.03 b |
| Plant height [cm] | 8.25±0.92 a | 5.94±1.17 b |
| Dry mass of roots [g] | 2.67±0.25 a | 1.64±0.17 b |
| Dry mass of first true leaf [mg] | 91.15±3.01 a | 63.92±3.09 b |
| Dry mass of stem [g] | 0.47±0.03 a | 0.46±0.03 a |
| N content [%] | 0.86±0.05 a | 0.74±0.06 b |
| Leaf area [$cm^2$] | 18.47±1.42 a | 14.25±1.30 b |

individual mother tree. The II-acorns showed lower rate of germination despite time to germination was shorter than in the H-ones.

Fast germination of II-acorns can be attributed to fast start-up of reserved nutrients, *e.g.* starch and protein. Higher activity of amylase caused by II is responsible for the acceleration of substrate decomposition, re-synthesis, and transfer. There might be an evolutionary trait that acorns compete for nutrition with insects. Reduction in seedling growth, indicated by decreases in length of taproots, height of plants, DM of roots, and DM and average area of the first fully expanded true leaf, as a consequence of cotyledon infestation by *Curculio* spp. were also observed. The results were consistent with the effect of cotyledon excision carried out by Frost and Rydin (1997), Milberg and Lamont (1997), and Bonfil (1998), but not with results of Sonesson (1994) and Andersson and Frost (1996), who reported that cotyledon extirpation had no effect on growth in oaks. Furthermore, these results were strengthened by similar studies (Fukumoto and Kajimura 2000, Branco *et al*. 2002), which showed that early growth of young seedlings was greatly reduced with increasing acorn damage. The significant reduction in plant growth observed in seedlings germinated from II-acorns corroborates this idea.

High $P_N$ and $\Phi_{PS2}$ are positively related to successful establishment and later survival. Sharp reduction in $P_N$ and $F_v/F_m$ indicated lower ability to occupy positions for seedlings from II-acorns than from the H-ones. Although leaf area of a single plant germinated from II-acorn was lower than those from the H-one, survival rates were not affected, *i.e.* mortality rate was zero.

In nature, rodents significantly remove more H-acorns than II-acorns, and the disappearance of infested acorns is slower than that of H-acorns (Xiao and Zhang 2003). Also seed-eating mammals and birds reject more infested seeds (Johnson and Adkisson 1985). In such way, II-acorns have a great possibility of establishment than the H-ones with high rate of predation. Advantages of H-acorns would be counteracted by high rate of predation in the earlier stage of germination and seedling establishment. II-acorns can prevent birds from damaging seedlings because most jays (*e.g.* blue jay *Cyanositta stelleri*) discriminate infested seeds more accurately (Johnson and Adkisson 1985). Differential consumption and removal of H- and II-acorns by small rodents and birds may be important for natural regeneration of *Q. mongolica*. Insects, as pre-dispersal seed predators, often reduce seed quality to attract seed-eating vertebrates, and consequently affect seed-eating vertebrates' decisions to forage and cache (Smith and Reichman 1984, Vander Wall 1990). There may be a mutual relationship between seedling establishment and seed-infesting insects.

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