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# Effects of Simulated Partial Cotyledon Herbivory on Seedling Growth in *Quercus crispula* Acorns

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**Abstract** - We examined the effects of partial cotyledon herbivory on developmental growth of seedlings from *Quercus crispula* acorns by simulated cotyledon removal. Although the size was smaller, seedlings developed from partially cotyledon-removed acorns showed higher RGRs with lower cotyledon reserves than those from intact ones. This suggests the existence of compensatory responses to herbivory involving enhanced utilization of cotyledon reserves in acorns.

## I. Introduction

*Quercus* acorns contain a large amount of resources in their cotyledons [1, 2], and as a result they are highly attractive to many kinds of predators [3-6]. Although acorns are frequently attacked by seed predators and suffer from cotyledon herbivory, it has been reported that consumed acorns can germinate and develop into seedlings if the embryo is not damaged [7, 8], suggesting one of the adaptive function of oak acorns to partial cotyledon herbivory. However, there are few studies to clarify quantitatively how strongly relative growth rates (RGRs) of oak seedlings are influenced by partial cotyledon herbivory before acorns germinate.

The initial growth of oak seedlings appears to depend largely on cotyledon reserves in acorns [9-11]. Branco et al. [8] found that germination (i.e., root elongation) speeds tended to increase with greater degrees of cotyledon herbivory in *Quercus suber* acorns. This suggests the occurrence of rapid resource translocation from cotyledon reserves into roots and/or hypocotyl, resulting in a higher RGR of seedlings developed from partially cotyledon-consumed acorns as compared with those from intact ones. The aim of this study tests the above prediction in *Quercus crispula* acorns.

## II. Plant materials

*Quercus crispula* Blume (syn. *Q. mongolica* var. *grosseserrata* (Blume) Rehd. et Wils.) (Fagaceae) is, a shade-tolerant climax species [12], which is predominantly deciduous and is found throughout the cool-temperate forests of Japan. Acorns of this species at the pre-dispersal stage were infested and eaten by insect larvae, *Curculio* spp. (Curculionidae), *Cydia glandicolana* and *C. danilevskyi* (Tortricidae)[6]. At the post-dispersal stage, the main seed

predators are small rodents, mice and voles [13-16]. Although these small rodents can eat acorns completely, partial consumption (cotyledons) was also frequently observed in laboratory experiments [17, 18], suggesting that the occurrence of acorns that are partially consumed by predators but still have sound embryos may not be rare in nature, as reported in oaks in north America [7].

We randomly collected acorns that had recently fallen to the forest floor (having been deposited on the fresh leaf litters) under the canopy of a mature tree (ca. 50 cm in diameter at the breast height), in a cool-temperate deciduous forest of Arimine (ca. 1000 m a.s.l.), which is located in Toyama Prefecture, central Japan.

## III. Methods

### A. Root developmental stage

We selected sixty sound acorns collected in early October of 1999; 50% of the cotyledons were immediately cut off of twenty acorns, on another twenty acorns 75% of the cotyledons were cut off using a sharp knife, and the remaining twenty acorns were untreated as controls. Thus, we simulated herbivory as 50% or 75% of the cotyledon but embryos were left intact. All samples were weighed (fresh weight).

We filled a plastic box (35 (L) x 25 (W) x 15 (D) cm) with soil (garden soil, S. K. Agri. Co. Ltd., Gunma, Japan), moistened it thoroughly and sowed the samples 1 cm below the surface in a 5 x 6 grid ( $n = 30$ : 10, 50% cotyledon-removed acorns; 10, 75% cotyledon-removed acorns; 10, controls). The surface of the soil was covered with moistened vermiculite. The plastic box was covered with a lid that had small perforations to retain the moisture and prevent drying out. The remaining 30 samples were treated in the same manner. We held the two plastic boxes with samples in an incubator at 5°C during two months. At the end of this period, each sample was harvested, washed thoroughly, and divided into root, hypocotyl, cotyledon and shell. Then they were dried at 80°C for 48 hrs, and weighed. The RGR was calculated using the following formula:

$$\text{RGR} = (\log W_2 - \log W_1) / (t_2 - t_1),$$

where  $W_1$  (mg) and  $W_2$  (mg) were dry weight at the time  $t_1$  (wk) and  $t_2$  (wk), respectively. Values for  $W_2$  were total dry

weight with the exception of the shell at time  $t_2$ , i.e., root, hypocotyl, and cotyledon. Values for  $W_1$  were the initial dry weight of cotyledon and hypocotyl including embryo, estimated using the relationship between fresh and dry weights of acorns ( $n = 10$  samples for each treatment) using the following equations;

$$Y = 0.46 X + 0.08 \quad (r^2 = 0.89, P < 0.01, \text{ for intact acorns}),$$

$$Y = 0.45 X + 0.04 \quad (r^2 = 0.89, P < 0.01, \text{ for 50\% removed}),$$

$$Y = 0.52 X - 0.05 \quad (r^2 = 0.90, P < 0.01, \text{ for 75\% removed}),$$

where  $X$  (g) was the fresh weight of acorns and  $Y$  (g) was the dry weight of cotyledons and hypocotyls excluding shells. The decreased rate of cotyledon dry mass from  $t_1$  to  $t_2$  was also calculated for each sample.

### B. Seedling emergence stage

We selected sixty sound acorns collected in early October of 2001, and simulated herbivory as the same manner as mentioned above under section A. We incubated samples at 5°C over 6.5 months. After that, each sample was individually transplanted into a pot (12 cm in diameter and 12 cm in depth), and was grown in a sunny location in Toyama University over a 1.5 month period. Then, height and basal diameter were measured for each emerged seedling. Each sample was harvested, washed thoroughly, and divided into leaf, stem, root, cotyledon and shell. They were then dried at 80°C for 48 hrs, and were weighed. The RGR was calculated as the formula described in section A. In the formula,  $W_2$  was the actual total dry weight with the exception of the shell at time  $t_2$ . Values of  $W_1$  represented the initial dry weight of cotyledons and hypocotyls including embryo, and were calculated using the relationship between fresh and dry weight of acorns ( $n = 25$  samples for partially cotyledon-removed acorns, and  $n = 50$  for intact acorns) using the following equations;

$$Y = 0.47 X - 0.13 \quad (r^2 = 0.96, P < 0.01, \text{ for intact acorns}),$$

$$Y = 0.43 X - 0.03 \quad (r^2 = 0.98, P < 0.01, \text{ for 50\% removed}),$$

$$Y = 0.44 X - 0.05 \quad (r^2 = 0.91, P < 0.01, \text{ for 75\% removed}),$$

where  $X$  (g) was the fresh weight of the acorn and  $Y$  (g) was dry weight of the seedling excluding the shell. The decrease in cotyledon dry mass from  $t_1$  to  $t_2$  was also calculated for each sample.

## IV. Results

Acorns germinate and develop the root from fall to spring in nature. At this stage, the root grows using resources only in cotyledon. Therefore, lower (negative) RGR and greater rates of cotyledon mass decrease leads to more rapid growth of roots. Acorns with more heavily damaged cotyledons, 75% removed, exhibited lower RGRs and higher rates of cotyledon mass decrease compared with acorns with 50% removal and controls (Fig. 1, left figures). This suggests rapid translocation of resources stored in cotyledons to roots

in heavily cotyledon-damaged acorns. Half of cotyledon-removed acorns showed no significant difference in RGRs and rates of cotyledon mass decrease compared with controls at the root development stage.

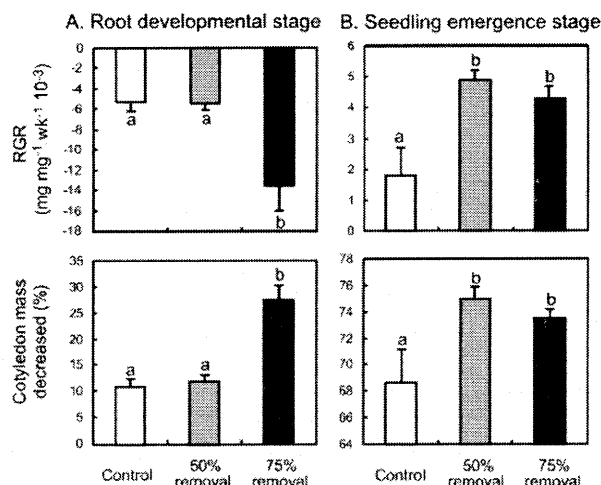


Fig. 1. The relative growth rate (RGR) and decrease rate of cotyledon mass (%) (mean + 1 SE) for individuals at root developmental stage (A) and at seedling emergence stage (B). Different letters show significant differences among treatments at  $P < 0.05$  level (Kruskal Wallis test and Mann-Whitney  $U$  test).

In contrast to individuals (acorns) at the root development stage, high (positive) RGR is expected for individuals at the seedling emergence stage if newly emerged leaves produce hydrocarbons by photosynthesis at rates that exceed seedling-constructive costs. Our results showed positive RGRs at the seedling emergence stage regardless of cotyledon-removal treatments (Fig. 1, right-top figure). Significantly higher RGRs and higher rates of cotyledon mass decrease (Fig. 1, right-bottom figure) were observed in individuals that developed from cotyledon-removed acorns than in controls. This suggests that acorns, with partially consumed cotyledons, can develop into seedlings more rapidly and with higher utilization of cotyledon-reserves compared with intact acorns. The height and basal diameter of seedlings were not significantly different between individuals with half of cotyledons removed and controls (Fig. 2). However, acorns with heavily -removed cotyledons (75% removal) grew into smaller seedlings.

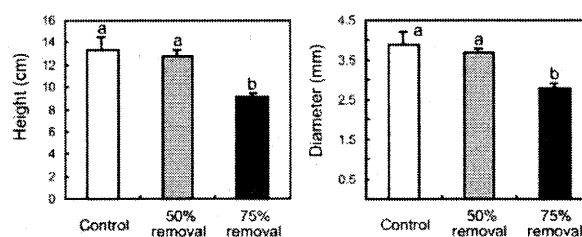


Fig. 2. The height and basal stem diameter (mean + 1 SE) of seedlings. Different letters indicate significant differences among treatments at  $P < 0.05$  level (Kruskal Wallis test and Mann-Whitney  $U$  test).

## V. Discussion

Juveniles developed from acorns with 75% cotyledon-removal grew more rapidly during the root development stage as shown in Fig. 1A. Furthermore, we observed no large difference in root elongation (ca. 10 mm on average) at this stage between juveniles of cotyledon-removed acorns and those from intact acorns. Several studies have reported higher germination speeds in insect-damaged acorns than in intact acorns of *Quercus* spp. [8, 19]. Our results demonstrated higher utilization of cotyledon-reserves in acorns with heavy cotyledon removal than in intact acorns (Fig. 1A, left-bottom figure). Thus rapid resource-translocation from cotyledon to root and/or hypocotyl during the root development stage seems to enable acorns with partial cotyledon-removal to develop into seedlings with high RGRs as shown in Figs. 1B and 2. We observed faster leaf emergence in juveniles developed from acorns with partial cotyledon removal than in those from intact acorns. More rapid germination and translocation of cotyledon reserves to root and shoot may lead seedlings to emerge faster above the ground, and to produce hydrocarbons by photosynthesis in earlier season, suggesting one of the compensatory responses to partial cotyledon herbivory.

In our study, however, we did not specifically determine the reason why acorns with partial removed cotyledons germinated and grew more quickly compared with intact acorns, because a likely cause for this relationship may be that acorns with partial cotyledon removal would be able to uptake water faster from a section made by cut-off treatments in our experiments. Kikuzawa and Koyama [20] demonstrated that smaller-sized seeds had higher efficiencies of water absorption required to germinate because of higher ratios of surface area relative to volume. Thus mechanism may act on acorns with partially removed cotyledons- and/or acorns naturally damaged by insects [8, 19]. In addition, acorns might have surplus resources required for seedling establishment. Further studies are necessary to clarify ecological and evolutionarily meanings of surplus cotyledon-reserves in *Quercus* seeds [7, 10, 21].

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