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AUTHOR(S):

Mizumachi, Eri; Mori, Akira S.; Akiyama, Reiko; Tokuchi, Naoko; Osawa, Naoya

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Title: Variation in herbivory-induced responses within successively flushing
***Quercus serrata* seedlings under different nutrient conditions**

Authors: Eri Mizumachi ^{1,2}, Akira S. Mori ^{1,3}, Reiko Akiyama ^{1,4}, Naoko Tokuchi ⁵, Naoya Osawa ¹

¹ Division of Environmental Science and Technology, Graduate School of Agriculture, Kyoto University

² (present address) Institute for Integrated Cell-Material Sciences (iCeMS), Kyoto University

³ (present address) Graduate School of Environment and Information Sciences, Yokohama National University

⁴ (present address) Department of Plant Ecology and Evolution, Evolutionary Biology Centre (EBC), Uppsala University

⁵ Field Science Education and Research Center, Kyoto University

Corresponding Author: Eri Mizumachi

Science Communication Group, Institute for Integrated Cell-Material Sciences (iCeMS), Kyoto University, Yoshida Ushinomiya-cho, Sakyo-ku, Kyoto 606-8501, Japan

Tel: +81-75-753-978 / Fax: +81-75-753-9785 / Email: emizumachi@icems.kyoto-u.ac.jp

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Abstract Herbivore damage can induce the host plant to alter the chemical and physical quality of its leaves, which is thought to be a plant strategy for avoiding further herbivory, termed induced response. In woody plants, many studies have considered variation in induced responses with resource availability, but few studies have examined this variation in relation to growth patterns of woody plants. We studied phenotypic variability of induced response within successively flushing *Quercus serrata* seedlings. *Q. serrata* seedlings were grown under controlled conditions. The controlled factors were herbivore damage (herbivore-damaged and -undamaged) and soil fertility (low and high). At each flush stage, concentrations of condensed tannin (CT), total phenolics (TP), and nitrogen (N) in leaves were analysed and leaf mass per area (LMA) was measured. CT and TP concentration of leaves and LMA were higher in herbivore-damaged seedlings. Leaves of the first flushes showed greater sensitivity to herbivore damage and had a higher CT concentration than leaves of the later flushes. Furthermore, seedlings growing in low-fertility soil showed greater induced response. The results suggest that *Q. serrata* seedlings showed induced response related to contributions of the tissue to current productivity. Leaves of the first flush showed greater induced response possibly because they play an important role in subsequent growth. The potential of *Q. serrata* seedlings to adjust the properties of leaves depending on herbivory and soil fertility in relation to growth patterns may be advantageous in forest floor where seedlings grow in heterogeneous soil fertility and are constantly exposed to herbivory.

Keywords: Herbivorous insect, Induced response, Oak, Phenolics, Leaf flushing, Soil fertility

42 **Introduction**

43

44 Leaf quality of host plants is an important property for herbivores in choosing their food
45 (e.g., Mattson 1980; Nykänen and Koricheva 2004). Leaves with high nitrogen (N)
46 concentration have been positively related to insect feeding and performance (Wait et al. 1998;
47 Lower et al. 2003). Concentrations of nitrogen and phenolic compounds within leaves are major
48 determinants of distribution and abundance of herbivores (e.g., Kytö et al. 1996; Lill and
49 Marquis 2001; Murakami et al. 2005). For example, condensed tannin (CT) and total phenolics
50 (TP) have negative effects on the growth and survival of insects (e.g., Mutikainen et al. 2000;
51 Lill and Marquis 2001; Nomura and Itioka 2002). Leaf mass per area (LMA) and leaf toughness
52 are often used as indices of leaf physical defences (e.g., Feeny 1970; Kudo 1996; Nabeshima et
53 al. 2001) and may vary in relation to leaf chemical properties (Poorter et al. 2009). Furthermore,
54 LMA and leaf toughness are recognised as important deterrents to herbivory (Coley 1983; Reich
55 et al. 1991; Choong 1996, Hanley et al. 2007).

56 Conversely, herbivores can affect the quality of damaged leaves and leaves produced after
57 implementation of the damage (e.g., Karban and Myers 1989; Karban and Baldwin 1997).

Consequently, in a seasonal environment, leaf damage early in the growth season affects the distribution and abundance of herbivores, which, in turn, causes changes in leaf quality later in the growth season (Hunter 1987; Wold and Marquis 1997; Boege 2004). These changes in leaf quality caused by herbivory thus seem to be one of important plant strategies for avoiding additional herbivore damage; this strategy is termed “induced response” (e.g., Karban and Myers 1989; Karban and Baldwin 1997).

Induced response varies with environmental factors such as the availability of nutrients, light and water (e.g., Hunter and Schultz 1995; Mutikainen et al. 2000; Nabeshima et al. 2001; Baraza et al. 2004). The pattern of carbon allocation toward induced response depends not only on the external availability of resources, but also on the internal characteristics of the plant (Nykänen and Koricheva 2004). Nykänen and Koricheva (2004) conducted a meta-analysis of 68 studies, and showed that the plant responses induced by herbivory depend on plant type (evergreen or deciduous) and inherent growth rate; phenolic compounds of leaves increase following damage in deciduous and fast-growing species, but not in evergreen or slow-growing species. Therefore, in clarifying induced response in woody species, it is important to take into consideration the variations in leaf quality within plant.

Several studies on *Quercus* species have shown a close connection between leaf quality and the abundance and performance of herbivorous insects (e.g., Feeny 1970; Rossiter et al. 1988; Hunter and Schultz 1995; Forkner et al. 2004). In Japan, *Quercus* species, including *Q. serrata*, are important components of deciduous temperate forests (Ozawa et al. 2000; Ohsawa et al. 2008) and have been the subject of understanding interactions between plants and herbivores. Large numbers of herbivorous insects, such as Lepidopterans use *Q. serrata* as a host plant (Teramoto 1993; Teramoto 1996). The extent of leaf damage by herbivorous insects in *Quercus* species changes temporally among years (Furuno and Saito 1981) and spatially among branches, even within the same individual (Yamasaki and Kikuzawa 2003; Nakamura et al. 2008). Like other woody plants, *Quercus* species could respond to the temporal and spatial fluctuations in herbivore damage because they have semi-autonomously modular units (Watson 1986; Sprugel et al. 1991). *Quercus* seedlings growing in forest floor may respond especially well to severe herbivore damage by adjusting the pattern of leaf flushing because they are constantly exposed to insect attacks during most of growing season.

In this study, we focused on induced response in relation to growth characteristic of seedlings of *Q. serrata*. *Quercus* seedlings/saplings can show successively flushing, which can

show several growth flushes within a growing season (e.g., Borchert 1975; Charr et al. 1997a, b).

We previously demonstrated that herbivore damage increased both the total number of flushes

and the probability of producing a later flush in *Q. serrata* seedlings (Mizumachi et al. 2004,

2006). Our previous studies also showed that the probability of flushing was prominent in

seedlings grown in high-fertility soil than ones grown in low-fertility soil (Mizumachi et al. 2004,

2006). While these studies demonstrated the nutrient level dependent effect of herbivory on the

pattern of flushing, induced response to herbivory at different nutrient levels in *Q. serrata*

remains to be known. Here, we investigated phenotypic variability of induced response within *Q.*

serrata seedlings. We examined whether herbivory induces change in chemical and physical

properties of leaves in different flushes, and whether the response varies depending on the soil

fertility. The results will be discussed in relation to the growth pattern under different soil

nutrient availability.

Materials and Methods

105 Plant materials

106

107 We used a group of 120 *Quercus serrata* Thunb. Ex Murray seedlings purchased from
108 the Kutsuki Village Forest Association (Shiga Prefecture, Japan) in December 2001. These
109 seedlings had been grown in uniform environment prior to the experiment. The roots of each
110 seedling were washed to remove any remnants of soil, and then all seedlings were transplanted
111 into plastic pots (44 cm in diameter, 24 cm in depth) with 500 ml of kanuma soil (pumice) at the
112 bottom and filled with sand. The mean seedling height at the start of the experiment was $43.4 \pm$
113 0.4 cm (mean $\pm$ SE). Seedling height did not differ significantly among the treatments (Scheffé's
114 range test, $P > 0.05$). All seedlings were watered to saturation for 10 min daily by an automatic
115 sprinkler (Sprinkler Thinker DC-1, Irrigation Control Equipment, Galcon[®]).

116 In early April 2002, the winter buds of *Q. serrata* seedlings were beginning to unfold
117 (defined as 'first flush'). These shoots that elongated from the winter buds were termed 'the first
118 shoots'. During the growth season, most of the seedlings had more than one flush. The shoots
119 formed after the first flush were considered 'the second shoots', and the following shoots were
120 'the third shoots' and 'the fourth shoots'. These flushes and shoots emerging after the first flush

were defined as ‘later flush’ and ‘later shoots’, respectively. Bud-break of the last flush occurred in early October 2002. Because a two-dimensional diagram was drawn to illustrate the branching structure of each seedling when new shoots elongated during the growing season, we could identify when each shoot elongated. Maximum number of flushes was five among all seedlings and four among seedlings sampled.

Experimental design

The study was carried out at Kitashirakawa Experimental Station of Kyoto University in Kyoto, Japan (35.02°N, 135.47°E). The average annual temperature at the station is 15.9°C (Field Science Education and Research Center, Kyoto University). *Quercus serrata* is found naturally distributed around the experimental station. Seedlings were grown in two greenhouses (H1, 10 × 7.5 m, 4 m in height; H2, 9.5 × 4.4 m, 3.5 m in height). These greenhouses were located on the same site with no obvious difference in environmental conditions and the distance between two greenhouses was within 25m. Therefore, we have treated the data of the greenhouses equally for analyses. The roofs were made of transparent plastic. The sides were

constructed using nylon mesh to allow free air circulation. In this experiment, we manipulated herbivore damage and soil fertility, but we did not manipulate temperature and light conditions in the greenhouses.

We controlled the herbivore damage by size of nylon mesh. The sides of the herbivory-undamaged blocks were made of 1 × 1 mm nylon mesh. This mesh size effectively reduced herbivore damage (the leaf area loss in herbivore-undamaged blocks were less than 3%). Because a few insect invasions did occur, we checked all seedlings and removed invasive insects from the herbivory-undamaged blocks every 2 days. On the other hand, those in the herbivory-damaged blocks were made of 20 × 20 -mm mesh to allow insect herbivores free access. *Quercus serrata* and other *Quercus* species (e.g. *Q. glauca* and *Q. acutissima*) are growing also outside the greenhouses. Therefore we can regard that the seedlings in these blocks were damaged naturally by insect herbivores.

Leaf damage within this experimental system was caused mainly by the following generalist herbivorous insects: larvae of Lepidoptera belonging to the families Oecophoridae, Lecithoceridae, Noctuidae, Geometridae, Lymantriidae, and Arctiidae; larvae of Hymenoptera belonging to the family Tenthredinidae; and adults of Coleoptera belonging to the families

Attelabidae and Scarabaeidae (Ishii H and Osawa N, unpublished data).

Seedlings in each block were randomly assigned to fertilisation treatments (low- and high-soil fertility) with the application of 25:5:20 NPK fertiliser (Peters Professional, HYPONeX JAPAN[®]) every 2 weeks from April to November 2002. The concentration of fertiliser was adjusted to obtain two levels of soil fertility. Half of the seedlings in each block were grown under low soil fertility (20 kg N ha⁻¹ year⁻¹); the other half grew under high soil fertility (200 kg N ha⁻¹ year⁻¹).

Plant measurements

A two-dimensional diagram was drawn to illustrate the branching structure of each sapling when new shoots elongated during the growing season. For each shoot, all leaves were roughly categorized into one of seven classes based on leaf damage, which was determined visually by estimating the percentage of leaf area loss: 0%, damage class 0; 1-5 %, damage class 1; 6-25 %, damage class 2; 26-50 %, damage class 3; 51-75 %, damage class 4; 76-99 %, damage class 5; 100 %, damage class 6. The assessment of leaf damage class was done when

each shoot has stopped elongating and the leaves have just fully unfolded. Leaf damage for each shoot was calculated as an average value of these damage classes.

In early October 2002, we randomly selected three seedlings per treatment (12 seedlings in total) for analysing chemical and physical properties of leaves. The experimental system was planned for continuous research; therefore, we sampled a minimum number of seedlings. The leaves of each flush within sampled seedlings were photocopied separately. The images were scanned and then processed with an image analysis program (NIH image ver. 1.63, National Institutes of Health, MD, USA) to obtain leaf area of each shoot. After photocopying, the leaves were oven-dried at 70°C for 2d. Dry mass of leaves was measured to determine the leaf mass per area (LMA). Dried leaves were ground into fine powder with a mill (TI-100, CMT CO. LTD., Tokyo, Japan). The nitrogen (N) concentration was determined by gas chromatography with an NC analyser (SUMIGRAPH, NC-900, SUMIKA Chemical Analysis Service, LTD., Tokyo, Japan). The shoots analysed were: (1) the first shoots (120 for LMA, and 100 for N), (2) the second shoots (50 for LMA, 36 for N), and (3) the third and fourth shoots (43 for LMA, 33 for N). The nitrogen analysis required at least 20 mg of dried leaf, and therefore we could not analyse several small shoots.

For each seedling, we used five first shoots and the subsequent shoots produced from the five first shoots for analyses of condensed tannin (hereafter CT) and total phenolics (hereafter TP). Because several shoots yielded small amount of leaves for chemical analyses, we could not analyse all selected shoots. The categories of shoots analysed included (1) the first shoots (53 for CT and 56 for TP), (2) the second shoots (27 for CT, 27 for TP), and (3) the third and fourth shoots (22 for CT, 23 for TP). The sample leaf powders were extracted with 50% methanol for 24 h. The concentration of CT was quantified with a spectrophotometer (UV-1200, SHIMADZU, Kyoto, Japan), using cyaniding chloride as a standard (Porter et al. 1986). The concentration of TP was quantified with a spectrophotometer (U-1000, HITACHI, Tokyo, Japan), using tannic acid as a standard (Waterman and Mole 1994). Phenolic compounds, such as CT and TP, in leaves have been commonly used as indicators of chemical defence against herbivores (e.g., Feeny 1970; Forkner and Hunter 2000; Mutikainen et al. 2000; Nabeshima et al. 2001; Forkner and Marquis 2004; Matsuki et al. 2004; Murakami et al. 2005; Koike et al. 2006).

As a preliminary analysis of initial properties of selected seedlings, we compared the lengths of the first shoots among sampled seedlings within each treatment. A one-way analysis

of variance (ANOVA) revealed no noticeable differences among individuals within the same treatment (one-way ANOVA, herbivory-damaged under low soil fertility, $F_{2,12} = 0.500$, $P = 0.619$; herbivory-undamaged in low-fertility soil, $F_{2,12} = 1.619$, $P = 0.239$; herbivory-undamaged under high soil fertility, $F_{2,10} = 2.071$, $P = 0.177$), except for one treatment (herbivory-damaged in high-fertility soil, $F_{2,12} = 9.724$, $P = 0.003$). In this treatment, several first shoots were eaten by insect herbivores before elongation finished, and the difference among individuals is thus likely to reflect the damage by herbivory rather than intrinsic variation among the seedlings. Accordingly, we analysed the data without distinction among individuals within each treatment.

Statistical analysis

For each flush stage, differences in leaf qualities were tested using a two-way ANOVA with two between-subject factors (herbivore damage and soil fertility). However, the third and the fourth shoots were tested together, because the harvested seedlings had few flush-stage shoots. When a significant interaction was detected between herbivore damage and soil nutrient fertility, Tukey's HSD test was performed for comparisons among the four treatments.

For each treatment, differences in CT concentration and LMA between the periods of shoot production (the first shoots and the later shoots) were tested using Student's *t*-test. CT concentration and LMA scarcely decreased as the season progressed, while N concentration and TP concentration were unstable over the growing season (Salminen et al. 2004; Migita et al. 2007). For this reason, we did not make comparisons of N concentration and TP concentration in leaves between periods of shoot production.

The leaf damage of each flush-stage was analysed with Wilcoxon rank sum test under low and high soil fertility. All statistical analyses were performed with JMP ver. 6.0 software (SAS Institute 2005).

Results

Condensed tannin concentration

For leaves of the first shoots, the effects of herbivore damage and soil fertility on the condensed tannin (CT) concentration were significant (two-way ANOVA, herbivore damage, *F*

235 $F_{1,49} = 35.712$, $P < 0.0001$; soil fertility $F_{1,49} = 15.276$, $P = 0.0003$). The CT concentration was
236 significantly higher in herbivore-damaged seedlings than in undamaged seedlings, and
237 was significantly higher in seedlings in low-fertility soil than in high-fertility soil (Fig.
238 1). There was no significant interaction between herbivore damage and soil fertility
239 (two-way ANOVA, $F_{1,49} = 0.314$, $P = 0.578$, Fig. 1). For leaves of the second shoots,
240 the effects of herbivore damage on CT concentration were significant (two-way
241 ANOVA, $F_{1,23} = 10.272$, $P = 0.004$). The CT concentration was significantly higher in
242 herbivore-damaged seedlings than in undamaged seedlings (Fig. 1). The effects of soil
243 fertility on CT concentration were not significant (two-way ANOVA, $F_{1,23} = 0.024$, $P =$
244 0.878 , Fig. 1). There was no significant interaction between herbivore damage and soil
245 fertility (two-way ANOVA, $F_{1,23} = 0.155$, $P = 0.697$, Fig. 1). For leaves of the third
246 and fourth shoots, CT concentration did not differ among the four treatments (two-way
247 ANOVA, $F_{1,18} = 2.862$, $P = 0.066$, Fig. 1).

248 In herbivore-undamaged seedlings in high-fertility soil, the CT concentration of leaves
249 was not significantly different between flush periods (Table 1). In the other treatments, CT
250 concentration of leaves in the first shoots was higher than in the later shoots (Table 1).

Total phenolics concentration

For leaves of the first shoots, the effects of herbivore damage and soil fertility on the total phenolics (TP) concentration were significant (two-way ANOVA, herbivore damage, $F_{1,52} = 4.800$, $P = 0.033$; soil fertility, $F_{1,52} = 24.909$, $P < 0.0001$). The TP concentration was significantly higher in herbivore-damaged seedlings than in undamaged seedlings, and was significantly higher in seedlings in low-fertility soil than in high-fertility soil (Fig. 2). There was no significant interaction between herbivore damage and soil fertility (two-way ANOVA, $F_{1,52} = 0.188$, $P = 0.667$, Fig. 2). For leaves of the second shoots, TP concentration was not significantly different among treatments (two-way ANOVA, $F_{1,23} = 1.850$, $P = 0.166$, Fig. 2). For leaves of the third and fourth shoots, effects of soil fertility on TP concentration were significant (two-way ANOVA, $F_{1,19} = 14.415$, $P = 0.001$); the TP concentration in seedlings in low-fertility soil was significantly higher than in high-fertility soil (Fig. 2). The effects of herbivore damage on TP concentration were not significant (two-way ANOVA, $F_{1,19} = 3.129$, $P = 0.093$, Fig. 2). There was

no significant interaction between herbivore damage and soil fertility (two-way

ANOVA, $F_{1,19} = 0.265$, $P = 0.613$, Fig. 2).

Leaf mass per area

For leaves of the first shoots, there was significant interaction between herbivore damage

and soil fertility (two-way ANOVA, $F_{1,116} = 15.751$, $P = 0.0001$, Fig. 3). The LMA in

herbivore-damaged seedlings was significantly higher than in undamaged seedlings,

especially in low-fertility soil (Fig. 3). For leaves of the second shoots, effects of

herbivore damage on LMA were significant (two-way ANOVA, $F_{1,46} = 34.245$, $P <$

0.0001); the LMA in herbivore-damaged seedlings was significantly higher than in

undamaged seedlings (Fig. 3). However, the effects of soil fertility on LMA were not

significant (two-way ANOVA, $F_{1,46} = 0.014$, $P = 0.908$, Fig. 3). There was no

significant interaction between herbivore damage and soil fertility (two-way ANOVA,

$F_{1,46} = 3.691$, $P = 0.061$, Fig. 3). For leaves of the third and fourth shoots, there was

significant interaction between herbivore damage and soil fertility (two-way ANOVA,

$F_{1, 39} = 9.047$, $P = 0.005$, Fig. 3); the LMA in herbivore-damaged seedlings was significantly higher than in undamaged seedlings, especially under low-fertility soil conditions (Fig. 3).

In herbivore-damaged seedlings in low-fertility soil, LMA in the later shoots was greater than in the first shoots (Table 1). In the other treatments, LMA was not significantly different between flush periods (Table 1).

Nitrogen concentration

For leaves of the first shoots, the effects of soil fertility on the nitrogen (N) concentration were significant (two-way ANOVA, $F_{1, 96} = 61.974$, $P < 0.0001$). The N concentration of seedlings in high-fertility soil was significantly higher than in low-fertility soil (Fig. 4), but the effects of herbivore damage on the N concentration were not significant (two-way ANOVA, $F_{1, 96} = 1.931$, $P = 0.168$, Fig. 4). There was no significant interaction between herbivore damage and soil fertility (two-way ANOVA, $F_{1, 96} = 2.747$, $P = 0.101$, Fig. 4). For leaves of the second shoots, the N concentration was not

significantly different among treatments (two-way ANOVA, $F_{1,32} = 2.121$, $P = 0.117$,
Fig. 4). For the leaves of the third and fourth shoots, the effects of soil fertility on N
concentration were significant (two-way ANOVA, $F_{1,29} = 18.001$, $P = 0.0002$). The N
concentration in high-fertility soil seedlings was significantly greater than in
low-fertility soil seedlings (Fig. 4). The effects of herbivore damage on the N
concentration were not significant (two-way ANOVA, $F_{1,29} = 0.239$, $P = 0.629$, Fig. 4).
There was no significant interaction between herbivore damage and soil fertility
(two-way ANOVA, $F_{1,29} = 0.524$, $P = 0.475$, Fig. 4).

Leaf damage

The leaf damage of the second shoots in high-fertility soil was significantly higher than in
low-fertility soil (Wilcoxon rank sum test, $P = 0.1566$, Fig. 5). While, soil fertility did not
affect the leaf damage of the first, third, forth or fifth shoots (Wilcoxon rank sum test, first shoot,
 $P = 0.0099$; third shoot, $P = 0.0577$; forth shoot, $P = 0.1445$, fifth shoot, $P = 0.7582$, Fig. 5).

Discussion

The effect of insect damage on leaf quality at each flush stage

The increase in phenolic compounds in leaves of the herbivore-damaged seedlings and the increase in LMA in every flush stage of *Quercus serrata* seedlings in this study can be regarded as induced resistance against insect damage. Leaf damage by herbivorous insects increased the condensed tannin (CT) concentration of leaves of the first and the second shoots, and also increased the total phenolics (TP) concentrations of leaves of the first shoots (Figs. 1 and 2). Similar to our findings, the increases in CT and TP concentrations induced by herbivores have been previously described (Haukioja 1990; Karban and Baldwin 1997; Nykänen and Koricheva 2004). Increases in these phenolic compounds in leaves of a certain flush stage can be interpreted as defensive reactions against further herbivory. Moreover, in our study, leaf damage by herbivorous insects significantly increased the LMA in every flush stage, especially when seedlings were grown in soil with low fertility (Fig. 3). This suggests that the increase in LMA is a response to the herbivore attacks. Our result is related to findings of a previous study that showed a negative correlation

between LMA and defoliation by herbivores within a crown of *Fagus crenata* (Yamasaki and Kikuzawa 2003).

We showed for *Q. serrata* seedlings that N concentration of damaged leaves did not differ from that in intact leaves (Fig. 4). In contrast, Kudo (1996) reported that nitrogen (N) concentration of leaves of *Q. crispula* decreased following artificial damage. The differences in N concentration between damaged leaves and intact leaves were explained by differences in N allocation to leaves in mid-summer; N was not allocated to damaged leaves in mid-summer, although it was allocated to intact leaves in the same season (Kudo 1996). On the other hand, the discrepancy between the results for *Q. serrata* and *Q. crispula* might be caused by species-specific differences in regrowth processes between the two plants. *Quercus serrata* seedlings produced later shoots in mid-summer (Mizumachi et al. 2004, 2006); as a result, the N, which was expected to be allocated to intact leaves in mid-summer, was distributed to the later shoots.

Variability of induced responses

348 The CT concentration varied among different flush stages, while no such
349 difference was detected for TP concentration. The CT concentration of leaves in the first
350 flush was higher than that in leaves in the later flush, except for herbivore-undamaged
351 seedlings in high-fertility soil, which showed no significant differences between flush
352 periods (Table 1). This indicates that the difference in CT concentration between flush
353 periods did not merely arise from leaf age. The difference in CT concentration between
354 flush periods could reflect contribution of different flushes to subsequent growth. For
355 example, Matsuki et al. (2004) showed that *Betula platyphylla* and *B. ermanii* invest highly in
356 defence in their early-season leaves, which make a major contribution to subsequent growth. In
357 contrast, *B. maximowicziana* invests its defences in late-season leaves, which are more
358 important to its growth (Matsuki et al. 2004). In case of successively flushing *Quercus* species,
359 leaves of the first and second shoots act as a carbon source during the production of
360 subsequent-flush shoot (Dickson et al. 2000). Therefore, leaves of the first shoots play a highly
361 important role in growth of subsequent-flush shoots (Alaoui-Sossé et al. 1996; Mizumachi et al.
362 2006). Our findings support this idea by showing that leaves of the first shoots, which would
363 contribute greatly to subsequent growth, had higher CT concentration (Table 1) and the effects

of herbivore damage on leaf qualities were more clearly in leaves of the later shoots (Figs. 1–3).

In contrast to CT concentration, TP concentration of leaves in the first shoots was not higher than that of leaves in the second shoots, and the third and fourth shoots (Fig. 2). The results are related to the seasonal variation in TP; concentrations of TP and hydrolyzable tannins, which comprise the dominant group of phenolic compounds, are higher in younger oak leaves (Rossiter et al. 1988; Mauffette and Oechel 1989; Salminen et al. 2004).

Our data showed that, for leaves of first flush and leaves of third and forth flush, increase in CT and TP concentration in *Q. serrata* seedlings in low-fertility soil was greater than those in high-fertility soil (Figs. 1-3) in accordance with several previous studies finding higher induced response in low-fertility soil (Koricheva et al. 1998, Hunter and Schultz 1995; Ruohomäki et al. 1996; Hikosaka et al. 2005; Cornelissen and Stiling 2006; Koike et al. 2006). However leaf damage observed in these flushes did not differ between low- and high-fertility soil (Fig. 5). In the second flush, in contrast, leaf damage was higher in high-fertility soil than in low-fertility soil (Fig. 5), while induced response did not differ between the different soil fertility levels (Figs. 1-3). Thus, the effect of soil fertility on leaf quality and leaf damage varied among flush stages. These intriguing patterns may be understood in terms of that the timing of herbivory could

influence induced responses. For example, during the elongation of the first flush, leaf damage was caused mainly by the larvae of Lepidoptera, which feed on leaves before the leaves have finished unfolding. While, during the elongation of the second flush, leaf damage was caused by the adults of Coleoptera, which feed on leaves after the leaves have finished unfolding. The leaf properties, such as CT and TP concentration and LMA, might be affected by at what stage of leaf expansion did herbivory occur. Our experimental set-up in this study was such that the sampling of all leaves was conducted at one time at the end of the growing season. Sequential sampling in accordance with leaf expansion may allow us to examine whether the higher induced defence observed in some treatments has the potential to protect the plants better against subsequent insect damage.

We previously demonstrated that in high-fertility soil the probabilities of producing subsequent shoots were higher (Mizumachi et al. 2006), and the number of shoots and total shoot length produced in one growing season were greater (Mizumachi et al. 2004). It means soil fertility affected not only the concentration of CT and TP in leaves, but also the growth patterns of *Q. serrata* seedlings (Mizumachi et al. 2004, 2006). In low-fertility soil, the contribution of the first shoots to the growth within one growing season is relatively greater than

in high-fertility soil. In the present study, seedlings in low-fertility soil were better defended against herbivore damage than those in high-fertility soil (Figs. 1–3). Taken together, these results suggest that *Q. serrata* seedlings adjust the balance between induced response and growth according to resource availability: seedlings in low-fertility soil have higher level of induced response while showing relatively low potential for production of new photosynthetic organs compared to those growing on more fertile soil.

In summary, this and our previous studies (Mizumachi et al. 2004, 2006) showed that *Q. serrata* seedlings have the ability to produce later shoots and the ability to strengthen defensive properties of leaves against herbivore damage. Furthermore, *Q. serrata* seedlings appear to adjust the balance between these abilities according to resource availability. Given that invertebrate herbivores prefer seedlings over older woody plants (Boege and Marquis 2005) and that *Q. serrata* is host to 346 lepidopterans species (Teramoto 1996), the potential of *Q. serrata* seedlings to adjust their physiological and morphological properties depending on herbivory and soil fertility can be advantageous in forest floor where seedlings constantly are exposed to herbivory.

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Fig. 1 Condensed tannin (CT) concentration (means $\pm$ s.e.) in leaves of shoots produced at each flush stage in *Quercus serrata* seedlings in treatments of herbivory and soil fertility.

Fig. 2 Total phenolics (TP) concentration (means $\pm$ s.e.) in leaves of shoots produced at each flush stage in *Quercus serrata* seedlings in treatments of herbivory and soil fertility.

Fig. 3 Leaf mass per area (LMA; means $\pm$ s.e.) in leaves of shoots produced at each flush stage in *Quercus serrata* seedlings in treatments of herbivory and soil fertility. Different letters are significantly different by Tukey's HSD test ($P < 0.05$).

Fig. 4 Nitrogen (N) concentration (means $\pm$ s.e.) in leaves of shoots produced at each flush stage in *Quercus serrata* seedlings in treatments of herbivory and soil fertility.

576 **Fig. 5** Leaf damage class (means $\pm$ s.e.) of shoots produced at each flush stage in

577 *Quercus serrata* seedlings in treatments of soil fertility.

Table 1 Leaf properties (means $\pm$ standard error) of shoots produced each flush period in *Quercus serrata* seedlings subjected to herbivore damage at various soil nutrient levels

Leaf property		Flush period		<i>P</i>
		First flush	Later flush	
CT	Damaged-Low	10.19 $\pm$ 0.82 (n = 13)	4.99 $\pm$ 1.05 (n = 8)	0.0010
	Damaged-High	7.31 $\pm$ 0.47 (n = 13)	4.19 $\pm$ 0.54 (n = 10)	0.0003
	Undamaged-Low	5.97 $\pm$ 0.36 (n = 14)	2.54 $\pm$ 0.47 (n = 8)	< 0.0001
	Undamaged-High	3.81 $\pm$ 0.48 (n = 13)	3.07 $\pm$ 0.36 (n = 23)	0.2279
LMA	Damaged-Low	6.19 $\pm$ 0.20 (n = 21)	6.83 $\pm$ 0.17 (n = 28)	0.0169
	Damaged-High	5.38 $\pm$ 0.17 (n = 29)	5.87 $\pm$ 0.22 (n = 18)	0.0871
	Undamaged-Low	4.91 $\pm$ 0.07 (n = 51)	4.94 $\pm$ 0.15 (n = 10)	0.8704
	Undamaged-High	5.15 $\pm$ 0.16 (n = 20)	5.47 $\pm$ 0.11 (n = 43)	0.1157

Differences were tested with Student's *t*-test.

CT, condensed tannin content in leaves; LMA, leaf mass per area.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5