Biomass Allocation and Chemical Defense in Defoliated Seedling of Quercus serrata with Respect to Carbon-Nitrogen Balance

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Biomass Allocation and Chemical Defense in Defoliated Seedlings of *Quercus serrata* with Respect to Carbon-Nitrogen Balance

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Abstract - We studied the effect of nutrient availability and defoliation on biomass allocation and chemical defense. Current-year seedlings of *Quercus serrata* were grown in an experimental garden under three levels of nutrient availability and all leaves were manually defoliated. In the present study, defoliation did not induce chemical defense. Plant nitrogen concentration (PNC), which was used as a measure of the carbon-nutrient balance in the plant, significantly decreased just after defoliation but recovered to similar or higher levels than that of undefoliated plants at 3 or 6 weeks following the defoliation. Thus defoliation did not decreased nitrogen status in the plants. Leaf nitrogen concentration (LNC) was significantly higher in defoliated plants. Concentrations of total phenolics and condensed tannins were significantly lower in defoliated plants and tannin concentration in leaves was negatively correlated with LNC, suggesting that the amount of defensive compounds was controlled by the carbon-nutrient balance at a leaf level. Thus the leaf-level carbon-nutrient balance is important for chemical defense but the effect of carbon-nutrient balance may change depending on situation or species.

I. Introduction

Plants are subject to herbivory, which sometimes reduces productivity and reproduction of plants significantly. Responding to damage by herbivores, plants often alter chemical composition of newly flushing or remaining leaves (induced defense). Accumulation of secondary compounds such as tannins and decrease of nitrogen concentration may be effective to avoid further herbivory. It has been established that the carbon-nutrient balance within plants is responsible to induce chemical defense [1, 2, 3]. Leaf nitrogen concentration (LNC) is generally higher than that of roots and stems. Thus loss of leaves decreases nitrogen concentration of a whole-plant. When new organs are produced, nitrogen may be limitted while carbon is relatively abundant. New leaves may have lower LNC than those that had been defoliated by herbivoers, and contain a greater amount of secondary compounds, which are produced from excess carbon. This hypothesis, known as the carbon-nutrient balance hypothesis [1], has been tested by many experiments.

On the other hand, plant ecophysiologists have indicated that the carbon-nutrient balance surrounding plants affects various plant traits. One of the most known traits is biomass allocation between root and shoot [4, 5]. Plants need both carbon and nutrient to grow. Given that the role of root and shoot are absorption of nutrient and carbon, respectively, optimal allocation of biomass to root and shoot changes with balance between carbon and nutrient availability surrounding the plant. More allocation to roots is effective to compensate for lower nutrient uptake rates under low nutrient availabilities, while more allocation to shoot ameliorates growth under low light availabilities.

Previously, many authors have investigated responses of biomass allocation to defoliation. Defoliation suppresses root growth and accelerates shoot growth [5]. Consequently, the shoot/root ratio after defoliation recovers similar level before the defoliation [4]. However, these studies have not paid attention to a possibility that defoliation may also alter the carbon-nutrient balance within the plants. Given that both nutrient deficiency and defoliation will reduce PNC, we can expect that defoliation alters biomass allocation to root and shoot as well as nutrient deficiency. Otherwise, is biomass allocation independent of alteration in carbon-nutrient balance by defoliation?

The question addressed in the present study is whether empirical responses of plants under nutrient deficiency are applicable to those after defoliation. If the responses to defoliation are solely explained by the alteration in PNC, we can apply the mathematical models of optimal biomass allocation under various nutrient availabilities to prediction of that after defoliation. Modification of such models may contribute to modelling of induced defense after defoliation. On the other hand, since the induced defense is not solely explained by the carbon-nutrient balance hypothesis [3], biomass allocation after defoliation may also be affected by factors other than the carbon-nutrient balance. The first hypothesis to be tested was that changes in PNC due to defoliation alter biomass allocation between organs as well as those due to nutrient deficiency. Furthermore, we also determined concentrations of nitrogen and tannins in leaves to test the second hypothesis that changes in PNC due to defoliation induce chemical defense as well as those due to nutrient deficiency.

II. Materials and methods

Current-year seedlings of *Quercus serrata*, a deciduous broad-leaved tree, were used in the experiment. On 18 May 1999, seeds were sawn in a pot (1.5 L volume) filled with washed river sand. Seedlings were grown in the experimental garden of Tohoku University. We supplied

commercial nutrient solution (N, P, K and micronutrients are included; Hyponex, Murakami bussan, Tokyo) every week with three nutrient levels: 0, 0.5, and 5 mg N per week (low, middle, and high nutrient treatment, respectively).

On 25 July, we applied manual defoliation where all leaves of target individuals were removed. From this day, plants were harvested 4 times every 3 weeks. Leaves, stems and roots were separated and dried for more than 3 days at 70°C after determining leaf area. Nitrogen concentration of organs were determined with an NC-analyzer (NC-80, Shimadzu, Kyoto). Phenolic compounds were extracted from the milled samples with 50% methanol at 90°C for 5 h. Concentrations of condensed tannins were determined according to [6].

III. Results

Plant biomass was significantly affected by day, nutrient treatment, and defoliation (Fig. 1). Undefoliated plants showed positive growth rates through the experiment and their final biomass tended to be higher under higher nutrient availabilities. In defoliated plants negative or small growth was found for 3 weeks following defoliation and the subsequent growth rates tended to be higher in higher nutrient availabilities. Consequently defoliated plants had smaller biomass than undefoliated plants when compared at the same nutrient treatment.

Here PNC on a dry mass basis is regarded as a measure of nitrogen level relative to carbon. Defoliation significantly decreased PNC (Fig. 2), because leaf nitrogen concentration (LNC) was higher than PNC (Figs. 2 and 3). However, while PNC of undefoliated plants decreased with time, that of defoliated plants increased. At the end of experiment, there was no significant difference in PNC between defoliated and undefoliated plants under the low and middle nutrient treatments and defoliated plants had significantly higher PNC than undefoliated plants under the high nutrient treatment

Defoliated plants produced leaves immediately after defoliation and their expansion almost finished until 3 weeks after defoliation. LNC was significantly affected by day, nutrient treatment, and defoliation (Fig. 3). New leaves of defoliated plants had significantly higher LNC than leaves of undefoliated plants.

Leaf mass ratio (LMR, leaf mass per total plant mass) was significantly affected by day, nutrient treatment and defoliation. Fig. 4 shows LMR plotted against PNC. In undefoliated plants, LMR was positively correlated with PNC irrespective of day and N treatments. Similar trend was found in defoliated plants but the regression line of LMR on PNC was significantly different in the Y-intercept between defoliated and undefoliated plants. It should be noted that defoliated plants grown at the high nutrient treatment had LMR comparable to undefoliated plants but when compared at the same PNC defoliated plants had lower LMR.

Fig. 5 shows tannin concentration plotted against LNC.

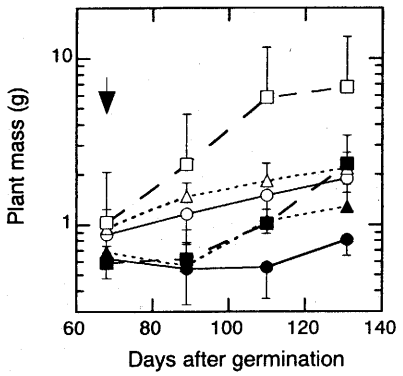

Fig. 1. Changes in plant biomass after defoliation. Open and closed symbol indicates undefoliated and defoliated plants, respectively. Circle, triangle, and square symbol indicates the low, middle, and high nutrient treatment, respectively. Mean and SD are shown (n = 6). Arrow in the figure indicates the day of defoliation.

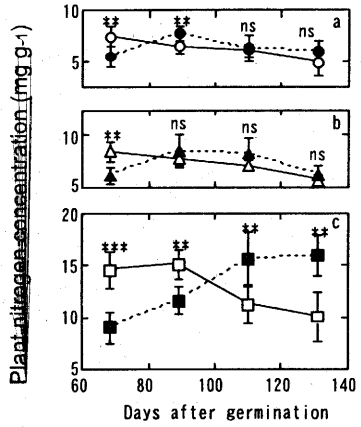

Fig. 2. Changes in plant nitrogen concentration after defoliation. a, b, and c shows the low, middle, and high nutrient treatment, respectively. Significance was assessed for each date with student t-test (ns, not significant; **, P<0.01; ***, P<0.001). Symbols are as in Fig. 1.

Defoliated plants tended to have lower tannin concentrations. The data points fell on one regression line when plotted against LNC.

IV. Discussion

A. Plant and leaf nitrogen concentration in defoliated plants

The carbon-nutrient balance hypothesis has insisted that, if fed organs have higher nitrogen concentration, herbivory causes nitrogen deficiency in the plant, leading to a decrease in LNC [3]. In the present study, as has been expected, PNC just after defoliation was lower than that of undefoliated plants (Fig. 3), because LNC was higher than PNC (Fig. 4). However, PNC recovered for 3 or 6 weeks. Under the low and middle nutrient treatments, PNC in defoliated plants increased and achieved similar levels of undefoliated plants, and under the high nutrient treatment PNC in defoliated plants became significantly higher than that in undefoliated

plants (Fig. 2). These results indicate that defoliation does not always causes nitrogen deficiency.

Under the high nutrient treatment, increase in PNC after defoliation (Fig. 2) is ascribed to high rates of nitrogen uptake (data not shown). Under the low and middle nutrient treatments, on the other hand, increase of PNC was found (Fig. 2) though nitrogen uptake was very small after defoliation. This increase may be ascribed to loss of biomass (Fig. 1) probably due to respiration. After leaf loss, plants cannot photosynthesise so that respiratory loss of carbon resulted in relative increase in nitrogen status in the defoliated plants. Therefore, to predict nitrogen status after defoliation, we may need to consider not only nitrogen concentration in lost organs but also respiratory loss in biomass.

LNC was found to increase after defoliation irrespective of nutrient availability in the present study (Fig. 3). This result is surprising because many studies have reported that woody species decrease their LNC after defoliation [1, 2, 7]. However, increased LNC after defoliation seems to be common in herbaceous species [8]. Increased LNC increases leaf photosynthesis, known as compensatory photosynthesis. There seems no consistent theory that satisfactory explains difference in responses to defoliation between woody and herbaceous species.

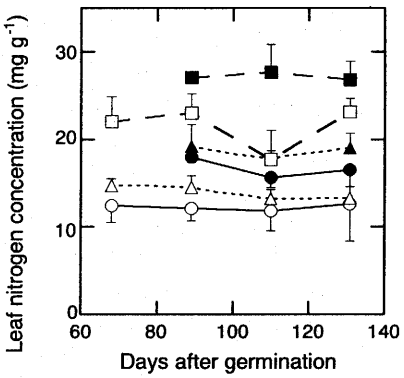

Fig. 3. Changes in leaf nitrogen concentration after defoliation. Symbols are as in Fig. 1.

B. Biomass allocation in defoliated plants

For undefoliated plants, a single, positive correlation was found between LMR and PNC irrespective of plant size and nutrient availabilities (Fig. 4), as has been shown in previous studies [9]. This is known as an adaptive adjustment of resource acquisition; when nitrogen is a limiting resource, plants allocate biomass more to roots to increase nitrogen uptake rates per plant [9]. For defoliated plants, a similar correlation was found but the regression was significantly different in the Y-intercept, i.e., defoliated plants had lower LMR when compared at the same PNC. The simplest interpretation of the difference may be that leaf growth in defoliated plants was insufficient to recover leaf mass ratio to the level in undefoliated plants. However, plant mass of defoliated plants increased from 0.6 to 2.3 g at the high nutrient treatment (Fig. 1), which seems to be enough for the recovery of the balance between leaf and other organs. Thus, there may be another meaning of alteration in the relationship between LMR and PNC.

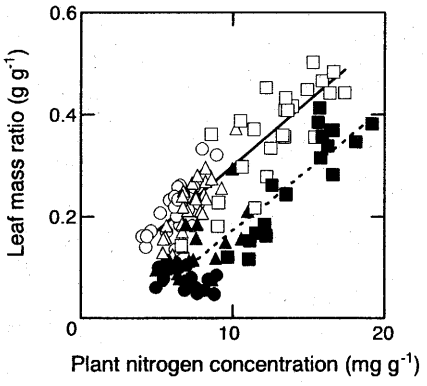

Fig. 4. Relationship between leaf mass ratio (leaf mass per total mass) and plant nitrogen concentration. Symbols are as in Fig. 1.

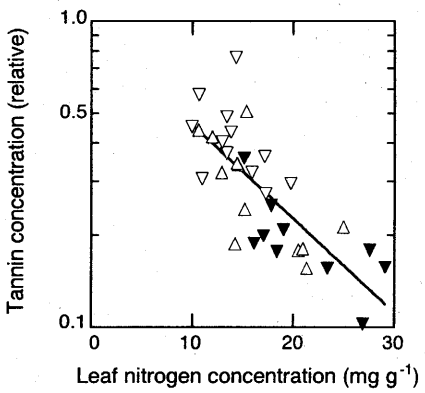

Fig. 5. Tannin concentrations as a function of leaf nitrogen concentration. Undefoliated plants harvested at 0 (△) and 6 (▽) weeks after defoliation and defoliated plants harvested at 6 weeks after defoliation (?) are shown.

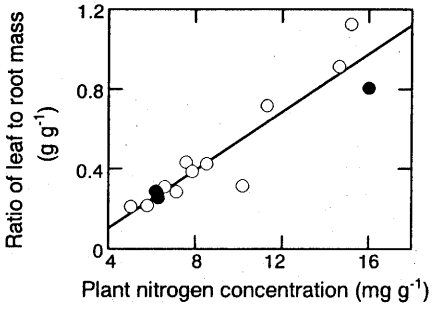

Fig. 6. The ratio of leaf to root newly produced after defoliation as a function of plant nitrogen concentration. Open circle denotes undefoliated plants, where mass of harvested root and leaf is used. Closed circle denotes defoliated plants. Leaf mass of defoliated plants means leaf mass at 9 weeks after defoliation. Root mass of defoliated plants means root mass at 9 weeks after defoliation minus minimum root mass observed though the experiment

Fig. 6 shows the ratio of leaf to root mass plotted against PNC. For undefoliated plants, the ratio was simply calculated from attached leaves and roots at the harvests. For defoliated plants, we calculated mass of leaves and roots that were newly produced after defoliation. The relationship between the leaf/root ratio and PNC was a positive function and there was little difference between defoliated and undefoliated plants. This result suggests that defoliated plants regulate biomass allocation so as to maintain the balance between newly produced tissues, rather than the balance between total tissues.

C. Chemical defense

Despite that defoliated plants have shown to accumulate defensive compounds such as tannins in leaves [1, 7], defoliated plants in the present study had significantly lower concentrations of tannins (Fig. 5). Together with the fact that LNC was higher in defoliated plants, we conclude that defoliation did not induce defensive response in the studied plants. This result suggests that accumulation of tannins is regulated in response not to defoliation. It is remarkable that there was a strong correlation between tannin concentration and LNC irrespective of growth conditions and of defoliation treatment (Fig. 5). Similar correlation was found in previous studies [2]. Thus the chemical defense is suggested to be induced not by changes in carbon-nutrient balance.

D. Conclusion

The present study showed that defoliation brings about alteration in biomass allocation. Defoliated plants had lower LMR at the low and middle nutrient treatments. Although defoliated plants at the high nutrient nutrient had LMR comparable to undefoliated plants grown under the same nutrient availability, the relationship between LMR and PNC was clearly different between defoliated and undefoliated plants. This suggests that the change in biomass allocation after defoliation is not solely explained by the carbon-nutrient balance. However, there was a similar relationship between the newly produced leaf to root ratio and PNC irrespective of defoliation treatment. This suggests that defoliated plants had lower LMR so as to compensate for decreased root activity for nutrient uptake, and thus implies the importance of the carbon-nutrient balance for biomass allocation after defoliation. Together with the fact that concentration of defensive compounds was negatively correlated with LNC, we conclude that the carbon-nutrient balance is an important factor for both biomass allocation and chemical defense. However, the chemical response to defoliation observed in the present study was not consistent with previous studies. In previous studies using woody species, defoliated plants had a higher level of phenolics and lower LNC while the inverse was the case in the present study. However, when we also see herbaceous species, inconsistent results are found between literatures. In many studies using herbaceous studies, defoliation increased LNC or photosynthetic capacity, known as compensatory photosynthesis. Part of the inconsistency may be explained by different effect of defoliation on the carbon-nutrient balance, namely, after defoliation the C/N ratio sometimes increases but decreases in other cases. Still it seems difficult to specify the condition where defoliation increases or decreases the C/N ratio. Further study is needed to understand plant responses to defoliation via the carbon-nutrient balance.

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