Nitrogen homeostasis in a willow leaf beetle, *Plagiodera versicolora*, is independent of host plant quality

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Abstract

While foliar nitrogen (N) content of host plants depends on environmental conditions, N content of herbivorous insects may remain relatively constant due to homeostasis. However, it is unknown to what extent insects can maintain their body elemental composition against natural variation in host plant quality. The present study examined the performance and N content of a willow leaf beetle, *Plagiodera versicolora* Laicharting (Coleoptera: Chrysomelidae), when fed leaves of host willow, *Salix eriocarpa* Franchet et Savatier (Salicaceae), with varying nutritional status.

Water content, toughness, and N content of willow leaves varied seasonally, and they affected performance of the leaf beetle. The leaf beetle achieved high performance when fed young leaves. On the other hand, the N content of the leaf beetle changed little, and it was independent of that of willow leaves, indicating strong N homeostasis of the leaf beetle. We discussed the function of N homeostasis in herbivorous insects in tritrophic level interactions.

Introduction

Foliar nitrogen (N) of plants varies with seasonal or ontogenetic development (Feeny, 1970; Mattson & Scriber, 1987), environmental conditions such as soil nutrients (Cotrufo et al., 1998; Lower & Orians, 2003), and herbivory (Faeth, 1986; Martinsen et al., 1998). Previous studies had frequently focused on the effects of foliar N on host-use patterns, survival, growth, and reproduction of herbivorous insects (e.g., Joern & Behmer, 1997; Fischer & Fiedler, 2000; Jiang & Cheng, 2004). In general, foliar N content is an appropriate indicator to measure plant quality for herbivorous insects, because many herbivorous insects prefer and perform best on plants with high N content (Mattson, 1980, but see Scriber, 1984).

On the other hand, insect N content may not be affected by environmental conditions (Fagan et al., 2002). For example, Slansky & Feeny (1977) demonstrated that while leaf N content of a crucifer plant was increased 4.1 times by fertilization, the N content of cabbage butterfly larvae that fed on the fertilized plant increased only 1.1 times. This is because the larvae enhanced their N accumulation rate on

poor-quality plants in order to maintain N homeostasis. Such a homeostasis in body-element composition has been widely recognized in several taxa, including bacteria, fungi, and zooplankton (Anderson & Hessen, 1991; Sterner & Elser, 2002), although its degree was variable. In herbivorous insects, many studies have examined N utilization efficiency to maintain body N content with varying food qualities, which were artificially manipulated by fertilization or by using artificial diets (Slansky & Feeny, 1977; Simpson & Raubenheimer, 2001; Lee et al., 2002, 2004). However, it is still unknown to what extent herbivorous insects can maintain their body-element composition when there is natural variation in host plant quality.

The present study examined the performance and body N content of a willow leaf beetle, *Plagiodera versicolora* Laicharting (Coleoptera: Chrysomelidae), when fed leaves of host willow, *Salix eriocarpa* Franchet et Savatier (Salicaceae), with varying nutritional status, observed under natural conditions. The N content of willow leaves can vary seasonally (Kudo, 2003), and the willow leaf beetle is multivoltine and occurs throughout the willow-growing season (Kimoto & Takizawa, 1994; Ishihara et al., 1999). Hence, the extent of N homeostasis in the leaf beetle is determined by comparing the N content of leaf beetles that

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fed on willow leaves of different ages. In addition, we discussed the function of N homeostasis of herbivorous insects in tritrophic level interactions.

Materials and methods

Sampling

Sampling of willow leaves and leaf beetles was conducted on a flood plain of the Yasu River in Shiga Prefecture, central Japan, where willow trees are the predominant woody plants and seven willow species occur sympatrically. *Salix eriocarpa* is a common riparian willow species in western and central Japan (Kimura, 1989), and leaves flush in early April in this area. *Plagiodera versicolora* feeds on several willow species (Kimoto & Takizawa, 1994) and is commonly found on *S. eriocarpa* throughout the willow-growing season.

Willow leaf quality

In mid-May 2004, 10 *S. eriocarpa* trees were chosen randomly, and the seasonal changes in leaf quality were monitored from late May to late September, which corresponds with the oviposition period of *P. versicolora*. However, because four study trees were heavily damaged by flooding in July, they were replaced with less damaged trees. Six mature leaves per tree were randomly collected monthly to determine their quality. Because most trees of *S. eriocarpa* produced secondary leaves in July, we also collected six mature secondary leaves three times between late July and late September. The leaves collected were immediately brought to the laboratory in a cooler box. Three leaves were used to measure toughness using a penetrometer (Model 9500, Aiko Inc., Yokohama, Japan) that recorded the force necessary for a rod (2 mm in diameter) to penetrate the leaf tissue. The remaining three leaves were weighed, dried in an oven at 60 °C for 72 h, and then weighed again. The dried leaves were then ground, and the N content was measured using an elemental analyzer (JM 1000 CN, J-Science Co. Ltd, Kyoto, Japan).

Performance and N content of leaf beetle

Egg masses of the leaf beetle were collected from *S. eriocarpa* trees monthly from late May to late September 2004. This period corresponded with the leaf sampling. The leaf beetle larvae were reared on willow leaves during each sampling period. Therefore, there were eight treatments in the experiment: larvae were reared on the primary leaves in May, June, July, August, and September, and on the secondary leaves in July, August, and September. Egg masses collected were placed separately in plastic cases (70 × 120 × 30 mm) in the laboratory. Larvae were used for the experiment within 24 h after hatching. Ten larvae were

put together in a plastic case lined on the bottom with wet paper and reared until adult eclosion in an environmental chamber at 23 °C, 70% r.h., and L16:D8. The larvae were provided with one or two nearly intact (<5% damage in leaf area) mature leaves taken from 10 *S. eriocarpa* trees during each sampling period. The leaves from the 10 willow trees were mixed and were provided randomly to each larval group to decrease the effects of individual trees. Leaves were replaced with new ones that were collected from field trees every other day and were removed after the larvae had pupated. Survival rate and developmental time until adult eclosion were recorded. Within 24 h after eclosion, adults were dried in an oven at 60 °C for 72 h and weighed. Then, the dried adults were ground, and N content was measured using the elemental analyzer. Twelve replicates were conducted for each treatment.

Statistical analysis

Seasonal changes in leaf quality were tested by one-way ANOVA with Fisher's protected least-significant difference (PLSD) as post hoc test. Although our monitoring design had been set up for the repeated-measure ANOVA, we performed one-way ANOVA because of the unexpected replacement of several study trees due to the flooding mentioned previously. This was not fully adequate in the strict sense, but the statistical results from one-way ANOVA would not be overestimated because the one-way ANOVA is less powerful than the repeated-measure ANOVA for consequential data (Zar, 1999). Survival rate, developmental time, adult weight, and N content of the leaf beetles were also analyzed by ANOVA with Fisher's PLSD as post hoc test. Data of survival rate were arcsine square root-transformed prior to the analysis. Tree means and experimental group means were used in each analysis for the willow and the leaf beetle, respectively. The difference between coefficients of variation (CV) of willow N and leaf beetle N content was determined by Z-test (Zar, 1999). The degree of N homeostasis of the leaf beetle was determined by linear regression analysis between willow N and leaf beetle N content. Nitrogen content was standardized as mean value = 1 prior to the regression analysis. Therefore, when the leaf beetle has no N homeostasis, the slope of the regression line = 1. The difference of the slope from 1 was determined using 95% confidence limits (CL).

Results

Seasonal changes in leaf quality

The leaf quality of *S. eriocarpa* varied seasonally (Table 1). Water content decreased continuously throughout the season in both primary and secondary leaves (Figure 1A). Primary leaves contained less water than secondary leaves

Table 1 ANOVA table for seasonal changes in leaf quality of *Salix eriocarpa*

	Primary leaves			Secondary leaves		
	d.f.	F	P	d.f.	F	P
Water content	4	12.93	<0.0001	2	38.30	<0.0001
Toughness	4	7.81	<0.0001	2	78.73	<0.0001
N content	4	17.77	<0.0001	2	8.23	0.0016

Table 2 ANOVA table for the performance and N content of *Plagiodera versicolora* reared on willow leaves collected at different periods

	Primary leaves			Secondary leaves		
	d.f.	F	P	d.f.	F	P
Survival rate	4	9.80	<0.0001	2	13.63	<0.0001
Developmental time	4	49.23	<0.0001	2	14.88	<0.0001
Adult dry weight	4	15.17	<0.0001	2	13.62	<0.0001
N content	4	0.99	0.42	2	0.21	0.81

in July and September. Toughness of primary leaves increased in June and decreased in August (Figure 1B). Secondary leaves were very soft in July and hardened rapidly in August compared to primary leaves. The N content of primary leaves was high in May and June and decreased rapidly in July (Figure 1C). There were no significant differences between the N content of primary and secondary leaves in July, August, and September. Throughout the season, foliar N content ranged from 1.91 to 2.56% in dry weight.

Performance and N content of leaf beetle

The performance of the leaf beetle varied among the treatments (Table 2). Survival rate was high, developmental

time was short, and body size was larger when the larvae were fed young leaves, i.e., primary leaves in May and secondary leaves in July (Figure 2A–C). Leaf beetle performance decreased with leaf aging. In addition, the larvae
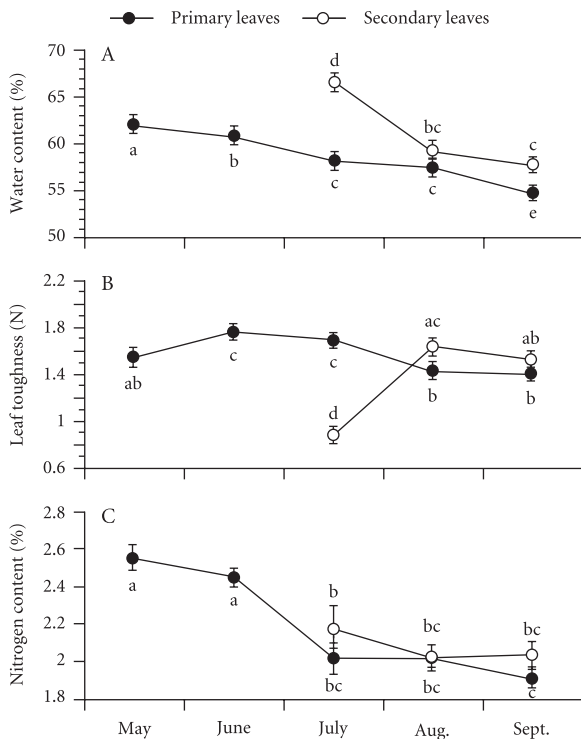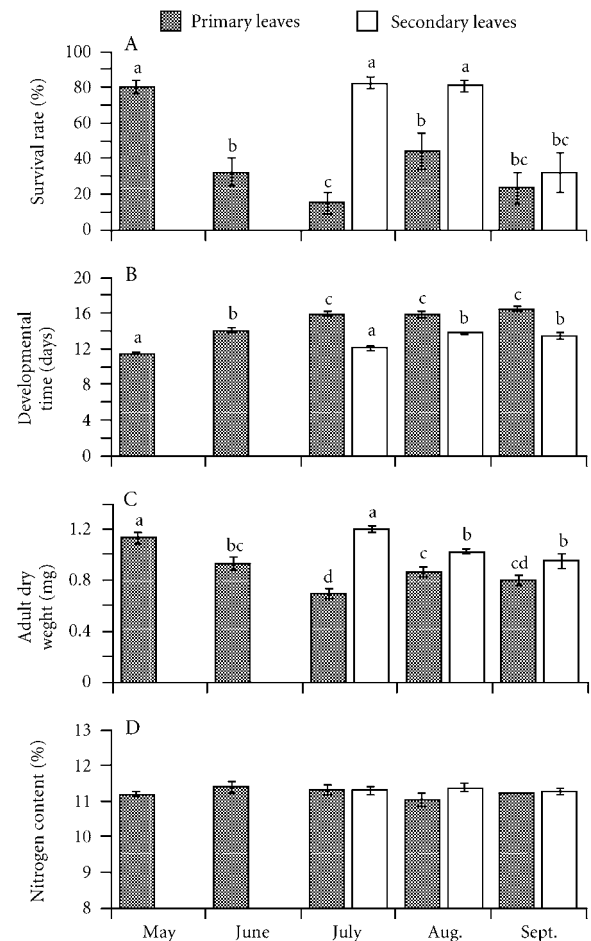

Figure 1 Seasonal changes in (A) water content, (B) leaf toughness, and (C) N content of *Salix eriocarpa* leaves. Means ± SE are presented. Different letters indicate significant differences (Fisher's PLSD, P<0.05).

Figure 2 (A) Survival rate, (B) developmental time, (C) adult dry weight, and (D) N content of *Plagiodera versicolora* reared on willow leaves collected at different periods. Means ± SE are presented. Different letters indicate significant differences (Fisher's PLSD, P<0.05).
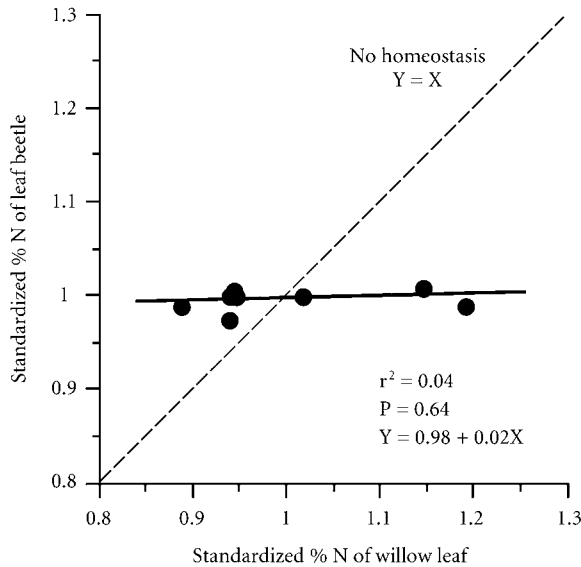
Figure 3 Relationship between standardized N contents of *Salix eriocarpa* leaves and *Plagiodera versicolora*. The dotted line indicates no homeostasis.

achieved higher performance when fed secondary leaves than primary leaves in the same period (Figure 2A–C).

The N content of the leaf beetle did not differ significantly among the treatments (Table 2 and Figure 2D). Throughout the experiment, the N content of the leaf beetle ranged from 11.05 to 11.40% in dry weight. This range was relatively smaller than that of the willow leaves (CV = 0.01 for the leaf beetle, CV = 0.11 for the willow leaves; Z = 4.39, P<0.001). There was no significant correlation between the standardized N content of the willow leaves and the leaf beetle (Figure 3). The slope of the regression line was 0.02 (95% upper CL = 0.11), which was smaller than 1. These results imply that the N content of the leaf beetle was independent of foliar N of the host willow, indicating strong N homeostasis of the leaf beetle.

Discussion

Leaf quality and insect performance

Leaf quality of *S. eriocarpa* for *P. versicolora* decreased throughout the season; water and N content decreased while toughness increased. This seasonal pattern was also reported in another willow species, *Salix miyabeana* (Kudo, 2003). Seasonal changes in leaf quality would result from the aging process of individual leaves (Feeny, 1970; Mattson, 1980; Raupp & Denno, 1983), because new leaf production of *S. eriocarpa* mostly occurred in April (Miyamoto & Nakamura, 2004). The performance of the leaf beetle varied in response to seasonal changes in the leaf quality of

S. eriocarpa. It was high when fed young leaves in May and decreased consistently thereafter. In addition, we found secondary leaf flush of *S. eriocarpa* in July, and these leaves were of high quality as food for the leaf beetle. Seasonal change in leaf N content can be one of the important factors resulting in the difference in performance of herbivorous insects (Mattson, 1980). However, not only N content but also water content, toughness, and defensive chemicals changed with leaf aging (Mattson, 1980), and these leaf characteristics also influence the performance of many herbivorous insects (Mattson & Scriber, 1987; Casher, 1996; Donaldson & Lindroth, 2004). In the present study, the leaf beetle performance differed between primary and secondary leaves, although no difference in N content was detected between these leaves. This is probably because defensive chemicals in leaves may have affected the beetle performance. For example, tannins are known to be contained in the leaves of many tree species (Kraus et al., 2003), including willows (Ayres et al., 1997; Hayashi et al., 2005), and increase with leaf aging (Feeny, 1970). These compounds can decrease the performance of several herbivorous insects by reducing N intake or N utilization efficiency (Simpson & Raubenheimer, 2001; Nomura & Itioka, 2002; but Ayres et al., 1997). Further phytochemical analysis is required to examine the cause of the different performance of the leaf beetle.

Nitrogen homeostasis of herbivorous insects

In contrast to changes in the N content of the willow leaves, the N content of the leaf beetle remained constant, and it was little affected by foliar N of the host willow. This suggests that *P. versicolora* has a strong N homeostasis against natural variation of host plant quality. Therefore, the leaf beetle should increase N accumulation by prolonging the feeding period, increasing N use efficiency, and/or decreasing body size to maintain N content in the body, when fed leaves having poor N content (Mattson, 1980). Conversely, herbivores should release N in excess of their requirements to maintain homeostasis, when fed foods with too much N (Anderson et al., 2005). In this context, the primary leaves in May and June could contain more N than required by the leaf beetle (two points at right side in Figure 3), and the beetles could excrete this surplus N. Such a compensatory feeding strategy on poor-quality diet or the excretion of excess nutrients to maintain N homeostasis is well known in other herbivorous insects (Slansky & Feeny, 1977; Obermaier & Zwölfer, 1999; Lee et al., 2002, 2003; Raubenheimer & Simpson, 2004). In addition, we examined the degree of N homeostasis in two other herbivorous insects from previous studies for which N data of both host plants and insects are available. Slansky & Feeny (1977) examined the growth efficiency

of the cabbage butterfly, *Pieris rapae*, when fed different host Cruciferae species or *Brassica oleracea* with varying fertilization levels. They demonstrated that while host plant N varied from 1.48 to 6.11%, insect N varied from 7.11 to 9.40%. Using the method of the present study, the slope of the regression line was 0.02 (95% upper CL = 0.12), indicating strong N homeostasis in the butterfly. Similarly, Fox & Macauley (1977) examined the growth of a leaf beetle, *Paropis atomaria*, on several *Eucalyptus* species. They demonstrated that while host plant N ranged from 0.49 to 1.85%, insect N varied only from 4.6 to 8.6%. The slope of the regression line was 0.23 (95% upper CL = 0.44). This indicates that *Pa. atomaria* also has N homeostasis, but not pronounced.

Thus, herbivorous insects generally have N homeostasis, although its degree is variable. Such N homeostasis has long been ignored in studies of tritrophic level interactions, because the nutritional quality of herbivorous insects as prey for predators was seldom measured (but see Francis et al., 2001). For example, several recent studies have demonstrated that high quality of host plants can indirectly increase insect performance at the third or fourth trophic level via herbivorous insects, i.e., bottom-up trophic cascades (Teder & Tammaru, 2002; Harvey et al., 2003; Kagata et al., 2005). However, the quality of herbivorous insects as prey was not measured in these studies. One hypothesis for the mechanism causing the bottom-up cascading effect is that higher plant quality may improve nutritional quality of herbivorous insects as prey, which may subsequently increase the performance of predatory and parasitic insects (Mayntz & Toft, 2001; Hunter, 2003; Raubenheimer & Simpson, 2004). This hypothesis assumed that the nutritional quality of herbivorous insects varies with host plant quality. However, the present study did not support this assumption, at least in case of N, which is an essential element for insect growth (Mattson, 1980; White, 1993; Denno & Fagan, 2003), as the N content of the leaf beetle was little affected by a change in host-leaf quality observed in the field. Herbivorous insects are the mediators transmitting the bottom-up effects from plants to predatory insects. Hence, quantitative and qualitative changes in herbivorous insects in response to variable plant traits may greatly influence higher trophic levels. In this context, discriminating homeostatic and non-homeostatic traits in herbivorous insects is important to understand how bottom-up effects from host plants transmit to higher trophic levels.

Acknowledgements

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