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Full Length Research Paper

Induced response in *Schima superba*: Effects of early-season herbivory on leaf traits and subsequent insect attack

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Physical, nutritional and allelochemical reactions generally occur in plant leaves in response to herbivory, and such responses can reduce to a certain extent, the performance and/or preference of subsequent herbivores. This study gave an additional evidence to the induced defense theory through the simulated herbivory in *Schima superba*, one of the common dominant trees in subtropical evergreen broadleaf forests, southern China. The results showed that the leaves that were damaged in the beginning of the leaf expansion had higher toughness and concentration of tannins, but lower water content and nitrogen content when compared with the control leaves. As a result, the damaged shoots had lower rates of herbivory but a higher shoot growth rate than control shoots. The results may imply that early-season herbivory on the leaves of *S. superba* reduced the nutritional quality of leaves and increased the amount of secondary compounds, therefore influencing later-season herbivory through the induction of plant responses that may act to reduce plant quality as food for herbivores.

Key words: Induced response, herbivory, early-season damage, nitrogen content, *Schima superba*.

INTRODUCTION

Young leaves of plants generally experience remarkably higher herbivore attacks than the mature leaves in trees (Brenes-Arguedas et al., 2006). In tropical regions, tree leaves suffer over 50% of herbivory during a relatively short period of leaf expansion. In particular, this is impressive when considering the fact that the average lifespan for leaves is 2 to 4 years (Coley and Aide, 1991). Young leaves are preferred by herbivores because they are tender and nutritious in comparison with the mature leaves on a tree (Feeny, 1970; Coley, 1983; Brenes-Arguedas et al., 2006). For defending herbivores, young leaves have evolved a variety of defenses including developmental, phenological, physical and chemical strategies, which are exclusive and critical in the expansion stage of leaves (Coley and Kursar, 1996; Kursar and Coley, 2003).

Plant defenses against herbivores had been generally assumed to be constitutive, that is, always expressed in plants. However, within the last 30 years, increasing evidences have indicated that many traits and processes protect plants against herbivores change following attacks and compensation for the lost tissue (Cornelissen and Fernandes, 2001). Defensive responses include chemical defenses such as synthesis of qualitative substances (production of small amounts of strongly poisonous

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Abbreviations: FW, Fresh leaves; **DW,** dry weight; **PBC,** protein-binding capacity.

compounds such as alkaloids, phenolic glycosides and cyanogenic glycosides) or quantitative substances (production of large amounts of indigestible compounds such as tannin, lignin and fiber), and physical defenses such as an increase in leaf toughness (Karban and Baldwin, 1997; Hattori et al., 2004; Keeling and Bohlmann, 2006). If the response of plant leaves occurs immediately after an attack by herbivores, it would be effective to reduce the current attacks. If it occurs slowly or in long time, it can be adaptive when current herbivory is a good predictor of future herbivory (Karban and Baldwin, 1997; Agrawal, 1998). Beside the defense mechanisms, plants can also compensate for the lost tissue by replacing it through growing leaves rapidly or increasing the photosynthetic capacity (Cornelissen, 1993; Honkanen et al., 1994; Dangerfield and Modukanele, 1996; Kudo, 1996; Strauss and Agrawal, 1999; Bergstom et al., 2000). For the last 20 years, many researchers have tried to demonstrate that induced defenses benefit plants in field experiments (Wold and Marquis, 1997; Cornelissen and Fernandes, 2001; Hattori et al., 2004), however, the dynamics of the induced responses after herbivore attacks are still rarely investigated, especially, the speed and lasting time of the induced responses which may suggest different defense strategies in plant (Haukioja and Neuvonen, 1985; Faeth, 1986; Kudo, 1996; Wold and Marquis, 1997).

Although the impact of induced responses on herbivore feeding, preference and performance has been evaluated in many systems (Karban and Baldwin, 1997), the impact of early-season damage on subsequent herbivory, as affected by induced traits, has been evaluated in very few natural systems (Rausher et al., 1993; Ernest, 1994; Wold and Marquis, 1997; Agrawal, 1998; Boege, 2004), and practically unexplored in subtropical evergreen broad-leaved forests. In order to assess the effects of early-season damage which promotes plant responses that translate into decreased herbivory, leaves of *Schima superba* were experimentally subjected to herbivory in the early growing season, and the induced responses and its subsequent effect on insect herbivores was examined. This tree may serve as a good model for the study of induced responses by insect herbivory, the early study indicated that the concentrated herbivory by *Neospastis simaona* Wang occurs in the period of leaf expansion (Wang et al., 2006). A common criticism of many studies investigating plant wound-induced responses to herbivory is that conclusions are often drawn from experiments that apply mechanical rather than real damage (Haukioja and Neuvonen, 1985; Van Alstyne, 1988); mechanical damage often fails to adequately mimic actual herbivory (Baldwin, 1990). This study therefore shows real damage treatment by insects. We examined the induction of response and its subsequent effect on insect herbivores in *N. simaona* by addressing the following questions: (1) Are there early-season damage induced changes in leaf quality? (2) Does early-season damage induced changes increase shoot performance? (3) Does the damage-induced changes affect herbivore foraging and (4) Do plants suffer less, subsequent to herbivory as a consequence of the damage-induced changes in their leaves?

MATERIALS AND METHODS

The study site is located in Meihuashan National Nature Reserve (25° 25´ N, 116° 50´ E, 1200 m a.s.l), Fujian Province, South China. The zonal vegetation is subtropical evergreen broad-leaved forests with *S. superb* as one of dominant tree species. The mean annual precipitation is about 1700 to 2200 mm, approximately 70% of which occurs from March to June. The mean annual temperature is 13 to 18℃, with an extreme monthly means of 7.5℃ in the coldest January and 22.9℃ in the warmest July. The soil type in the site is the forest brown soil.

Fifteen young trees of 2.0 to 3.0 m in height, were selected randomly in the mid-March 2007 before bud-break. Ten leaf buds were randomly selected, and they developed as shoots, randomly from the crown of each tree and were marked with plastic tags. Leaves were numbered sequentially from the base upward. Five of the ten shoots in each tree were used as control treatment, in which damage by insect herbivores was monitored during the growing season. The treatments were performed on the remaining 5 new shoots of each sampling tree. Early-season damage was produced by *N. simaona* larva. Larvae of *N. simaona* had been previously collected from non-experimental *S. superba* plants 1 to 3 days after egg hatching. Larvae were reared in the laboratory until they were 3rd-instar (approximately 2 to 3 cm long). Six days after bud-break (23 March 2007 for *S. superba*), 3 to 5 3rd-instar larvae, from laboratory reared on *S. superba* were placed on each shoot every morning and removed each evening until they reached the 25% leaf damage. Additional herbivory damages were performed on 4 unmarked sun shoots of each sample tree as a sample for analyzing leaf traits. To test whether induced response acts as a whole plant defense, herbivory pattern were also compared between treated trees and intact trees. Shoots collected from other 15 unmarked untreated young trees (overall control) were also sampled.

To evaluate the possible mechanisms of induced responses to early damage, 3 leaves per plant having the least herbivore damage (<5% leaf loss) were collected from all plants on day 12, 24, 48 and 72. For each leaf sample, leaf dry weight was measured using an electronic balance (Metter AE100, Germany). Water content was estimated as the difference between the weights of fresh leaves (FW) and dry ones (DW) per treatment (after dehydration at 70℃ for 48 h). Water content (%) was calculated with the formula, 100 × (FW - DW) / FW. Leaf N concentrations were determined using flow-injection autoanalyser (Skalar, Netherland).

Toughness was measured using a device that was constructed according to Feeny (1970). The leaf was clamped between two wood plates that were joined by two hinges. Thickness of one plate is 1 cm and the other one is 2 cm, each drilled with a 5 mm diameter hole. The number of grams of weight necessary to punch a 5 mm diameter rod through the leaf blades outside major veins gives an index of toughness.

The protein-binding capacity (PBC) of tannins in leaf extracts was measured using the radial diffusion assay due to its simplicity and widespread use (Hagerman, 1987). Tannin in the extract binds with the protein to form an opaque precipitate whose squared diameter is proportional to the tannin concentration of the extract. Plant tissue was extracted for an hour at room temperature with 50% v/v aqueous methanol, using a solvent to tissue ratio of 0.5 ml solvent

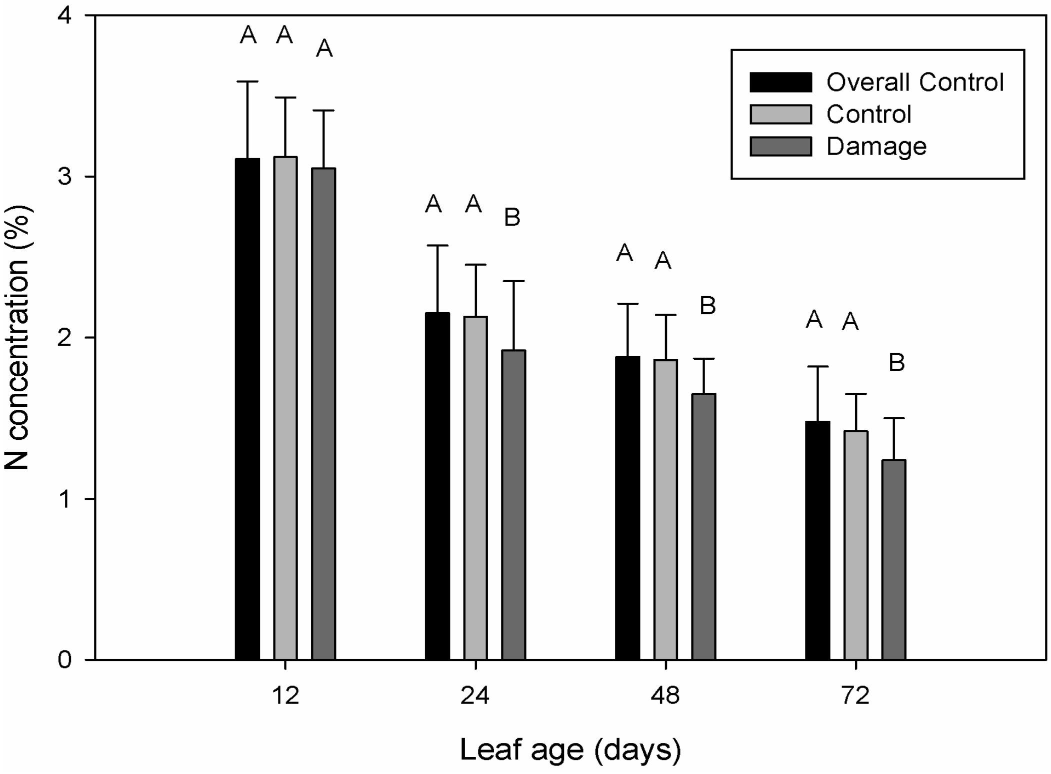

Figure 1. Nitrogen concentration (mean ± 1 SE) of leaves of *S. superb*. Error bars represent SE. Means with different letters are significantly different from each other at $P < 0.05$.

per 100 mg tissue. To estimate the tannin concentration, 60 µl of plant extract was placed in a Petri dish containing a mixture of agar and protein (bovine serum albumin; Sigma). Tannin in the extracts binds with the protein to form an opaque precipitate whose squared diameter is proportional to the tannin concentration of the extract (Hagerman, 1987). A regression equation relating the square of the precipitate-ring diameter to the tannic acid concentration was calculated using a series of tannic acid standard. The average of three replicates per leaf was used for statistical analysis.

In March 2007, 3rd-instar larvae of *N. simaona* were taken from laboratory maintain, and were reared on *S. superba*. They were starved for 24 h and then placed on leaf rectangles (1.5 × 3 cm) in a cone-shaped bottle. Experiments were divided into two groups as follows. (1) 3rd-instar larvae were fed separately with the leaves of different treatments at three leaf rectangles per larva. (2) Mixed feeding: 3rd-instar larvae were fed with mixed leaf rectangles of three different treatments at two rectangles per treatment. Twenty-four hours later, the larvae were removed and the herbivory damaged square was calculated using grid cards. Leaf palatability was directly determined from the herbivory damaged square and eight replications were used for each case.

To determine if the early-season damage reduces subsequent insect attack, the damaged area after treatment was compared between artificially damaged and control leaves. The herbivory rate every 6 days for 48 days was recorded, excluding the former attacked ones. Actual leaf area and leaf loss areas were measured on the marked shoots every time with a plastic grid (10 grids /cm^2) to determine herbivory rate of leaf area eaten while leaf is expanding.

Herbivory rate (%) = 100 × leaf loss area / (actual leaf area + leaf loss area)

Missing leaves were scored as 100% damage. Shoots or leaves that were obviously damaged by falling debris were not included. The rate on a whole shoot was expressed as the average herbivory of every leaf.

Before analysis, all data were tested for normal distribution, and when necessary, the data were log$_{10}$-transformed. One-way ANOVA was used to compare leaf area, toughness, water content, tannin concentration and leaf herbivory at different times of harvest. Tukey's honestly significant difference post hoc test for significant differences among means was used. All statistical analyses were performed with SPSS software (SPSS Inc.).

RESULTS

Leaf traits

Most of the *S. superba* trees began to produce leaves from 21 to 25 Mach, 2007 in the study area. Each leaf bud produced an average of 10 leaves ranging from 8 to 14. The leaf area abruptly increased to the maximum from the 24th day after bud-break and then remained unchanged.

At first collection date, there was no difference in N concentration of damaged and the control leaves (Figure 1). However, from day 24 after bud-break, there were significant differences in leaf nitrogen concentration between the damaged leaves and the control leaves, which was lower in damaged leaves than that in control

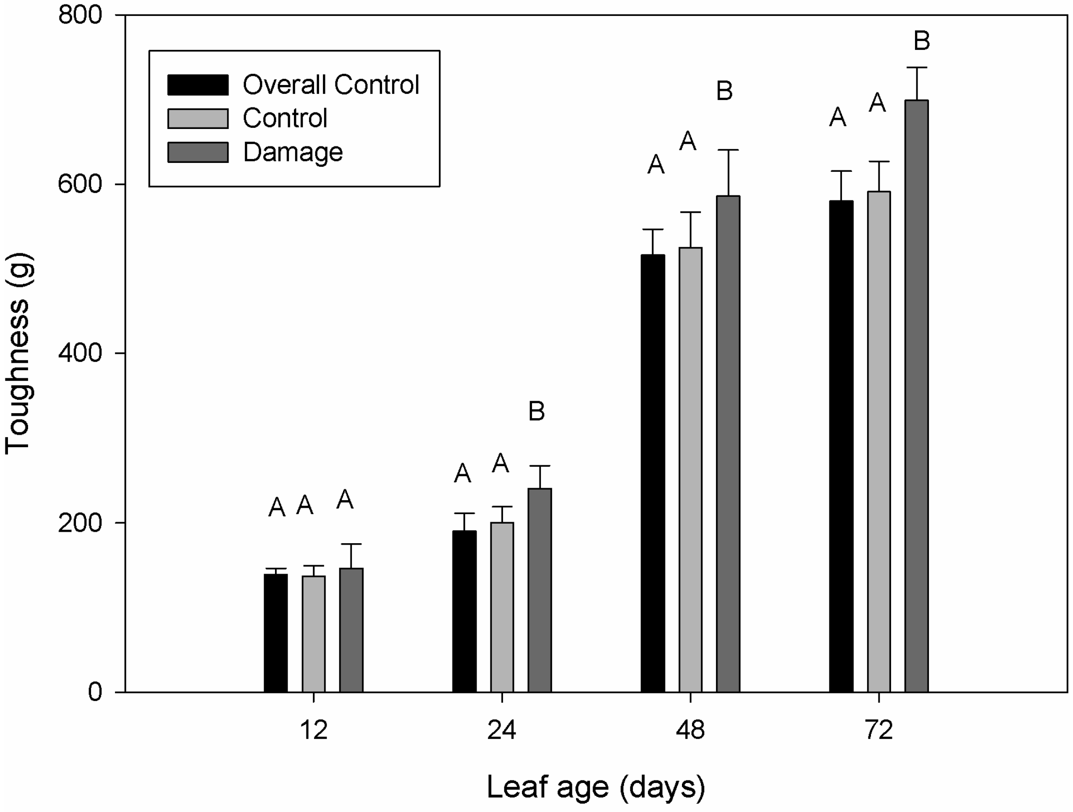

Figure 2. Toughness (mean ± 1 SE) of leaves of *S. superb*. Error bars represent SE. Means with different letters are significantly different from each other at $P < 0.05$.

leaves (df = 2.0, F = 7.84, $P < 0.05$) (Figure 1). The leaves from damaged shoots showed a higher toughness than leaves from control shoots (df = 2.0, F = 9.87, $P < 0.01$) (Figure 2). A trend for increased toughness from the first to the last sample was also noted. Leaf toughness increased very slowly during leaf expansion, and then rapidly rose until the leaf reached the full size. In the control shoots, this decrease was of approximately four-fold from the first to the last sample, while on damage shoots an increase of approximately five times was found.

Water content only differed among treatments on the first collection date, with leaves control shoots showing higher water than leaves from damaged shoots (df = 2.0, F = 8.64, $P < 0.01$). Nevertheless, on day 12, differences in water content between the overall control and damage treatment was found, while in the control, damage leaves did not show difference statistically ($P > 0.05$). Form day 48, water content did not differ among treatments (df = 2.0, F = 0.363, P = 0.569) (Figure 3).

We also found a significant difference in tannin concentration (Figure 4). Tannin concentration in experimentally damaged leaves was higher than control and overall control, on each of the four sampling dates (df = 2.0, F = 18.92, $P < 0.001$). On control shoots, this decrease was approximately 32.4% from the first to the last sample, while on damaged shoots, a decrease of approximately 25.6% was only noted.

Preferences of *N. simaona* larvae to leaves

Larvae showed certain preferences to leaves of different treatments when fed the leaves separately. The consumed area of damaged leaves was significantly smaller than that of overall control and control leaves (Figure 5a). When fed mixed leaves of the three treatments, the difference was also very significant, indicating considerable feeding preferences of larvae (Figure 5b).

Effects of early-season damage on levels of subsequent herbivory

The cumulative percentage of leaf loss area by herbivory increased abruptly from day 12 to 24 after bud-break and gradually rose in the following days (Figure 6). The cumulative herbivory of early-season damaged shoots increased gradually in comparison with control leaves, and the extent of herbivory was significantly lower in early-season damaged shoots from day 18. The final leaf area loss in the damaged leaves (average 7.11%) was

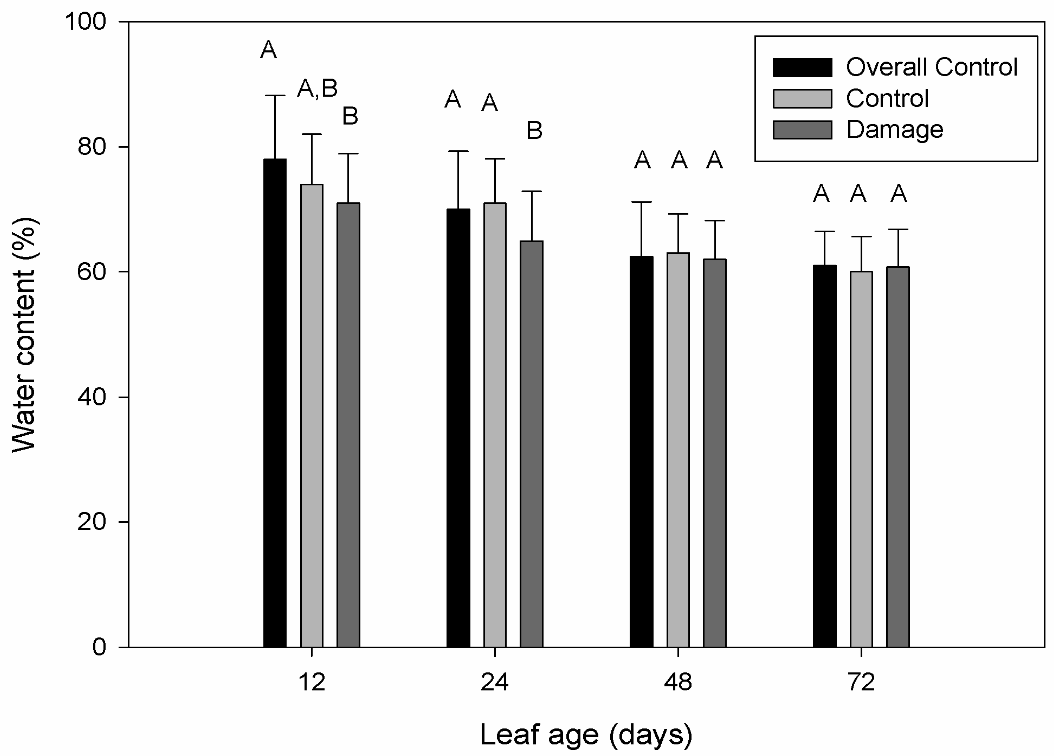

Figure 3. Water content (mean ± 1 SE) of leaves of *S. superb*. Error bars represent SE. Means with different letters are significantly different from each other at P < 0.05.

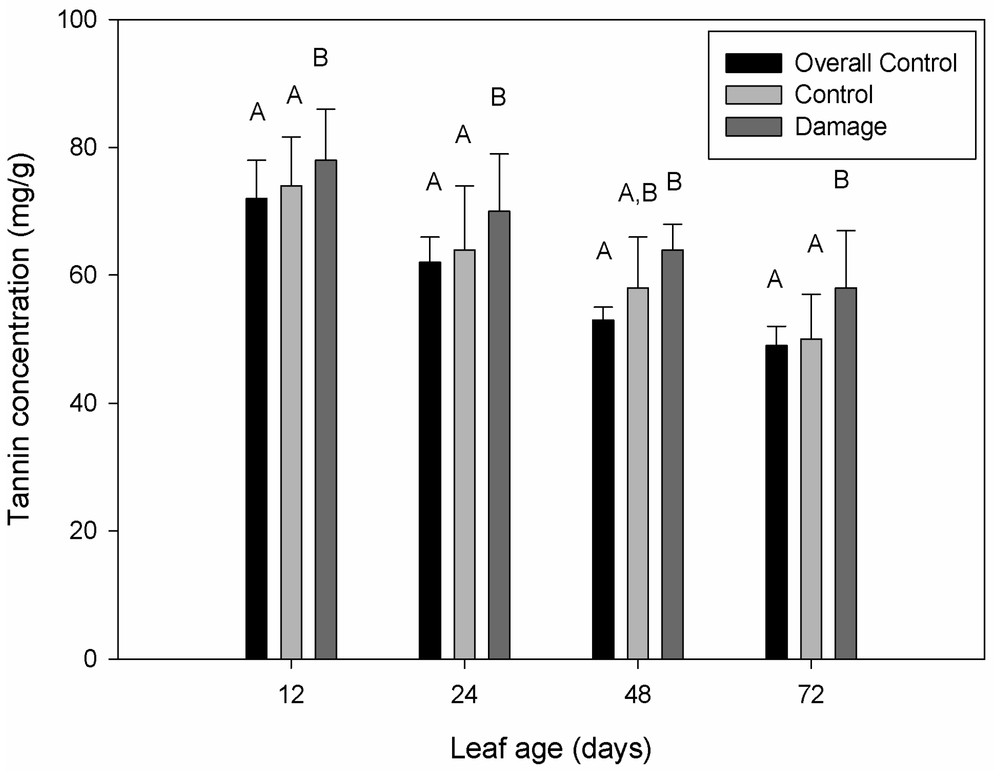

Figure 4. Tannin concentration (mean ± 1 SE) of leaves of *S. superb*. Error bars represent SE. Means with different letters are significantly different from each other at P < 0.05.

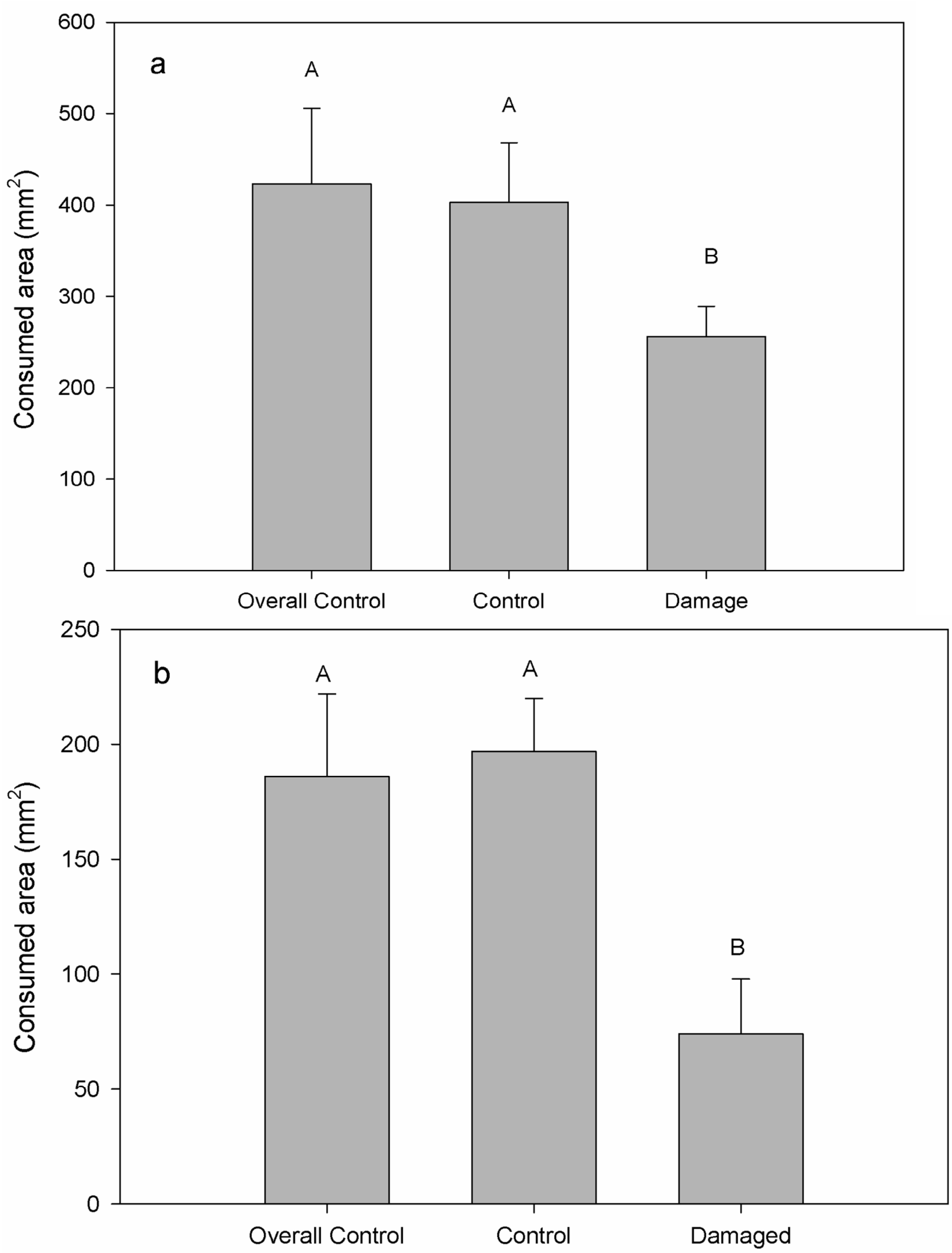

Figure 5. Comparison of leaf area damage between treatments (a, larvae fed with leaves separately; B, larvae fed with a mixture of leaves) (mean ± 1 SE, n = 5). Different letters are significantly different from each other at $P < 0.05$.

significantly lower than that of control shoots (overall control = 16.98%; control = 19.56%). And the herbivory of control shoots was higher than that of overall control shoots. There were no significant differences among trees in the same treatment ($P = 0.578$, one-way ANOVA after arcsine transformation).

DISCUSSION

The results showed that leaf quality of *S. superba* changed with leaf expansion, of which leaf nitrogen concentration and leaf water content decreased and leaf toughness increased during leaf expansion in both the

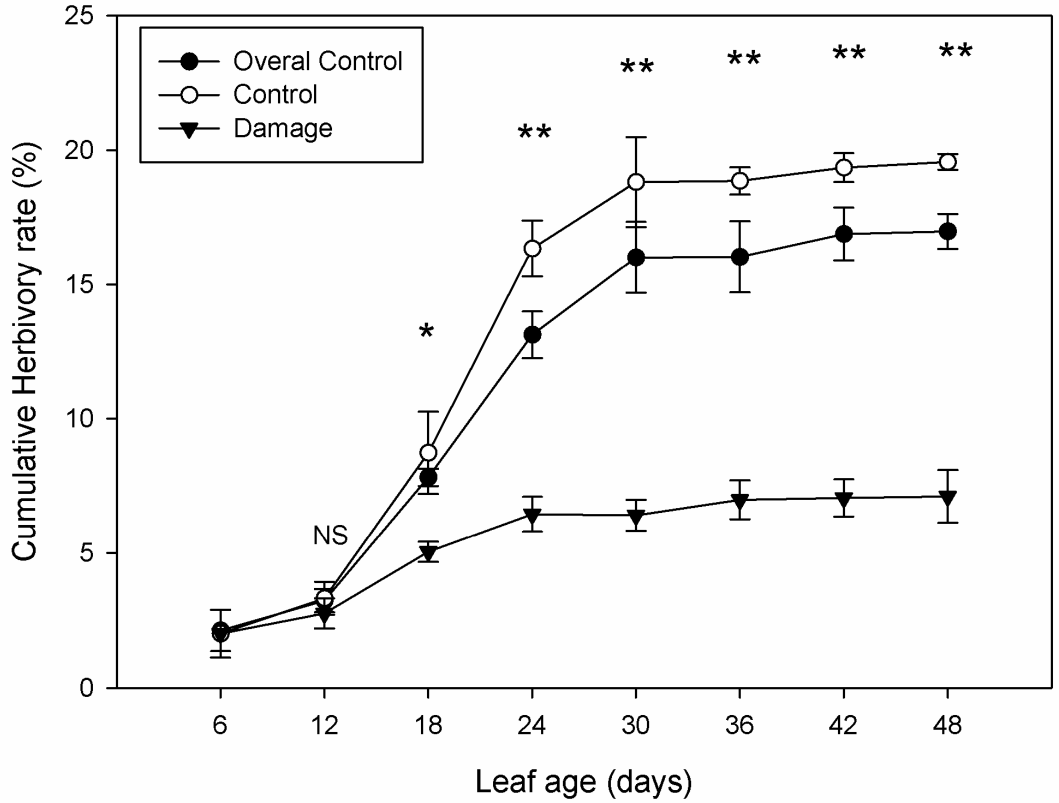

Figure 6. Cumulative leaf loss area (mean ± 1 SE) of leaves of *S. superb*. Error bars represent SE.

treatment and control leaves. Such a decline in leaf quality with development of leaves was considered to be a defense strategy against herbivory, especially for tropical trees with long lifespan leaves (Coley and Barone, 1996; Kursar and Coley, 2003). When compared with the mature leaves, the toughness decreased but water content was higher in young leaves of *S. superba*. So there were relatively greater losses in young leaves than in mature leaves, showing that insect attack occurred highly in young leaf stage (phenological window). While the leaves reached the full size, leaf toughness increased abruptly, and leaf water content decreased rapidly in leaves of *S. superba*. These changes in leaves might improve defense level of mature leaves against herbivory. However, leaf tannin concentration as a defense substance decreased during leaf expansion in *S. superba* and in other trees (Coley and Barone, 1996; Kursar and Coley, 2003). A possible reason is that higher tannin concentration in young leaves might be a make-up defense when leaf constituent of defense such as toughness is not enough (Coley and Barone, 1996; Kursar and Coley, 2003).

It has been assumed that herbivory might induce leaf defense and leaves would increase their defense substance concentration and decrease their nutrition or quality after damage (Wold and Marquis, 1997). Based on this assumption, it was expected that damaged leaves would have lower leaf quality than control leaves, and these include higher toughness, higher tannin concentration and lower water content. This report was consistent with this assumption. Although there is still debate concerning the adaptive significance of plant secondary metabolites (Smith et al., 1992; Berenbaum, 1995), in the present study, the lower nitrogen concentration and water content, and higher concentration of tannins in damaged leaves indicates their lower quality in herbivores, which is reflected in the lower rate of herbivore attack on these damaged shoots over control treatments.

Leaf toughness is widely recognized as a mechanical defense and is negative to herbivory damage (Coley, 1983; Dudt and Shure, 1994; Xiang and Chen, 2004). Rapid changes in leaf toughness of damaged *S. superba* leaves may be a mechanism to disperse herbivore damage. In this study, an induced response occurred before day 12 and these were sustained throughout the study period, at least for N concentration, toughness and tannin concentration. But the water content was just found to be different before day 24, and was not sustained throughout the experiment. Wold and Marquis (1997) also did not find differences between damaged and undamaged leaves in a three months study. This study demonstrated that induced changes in host plant leaf quality can significantly affect the tendency of *N. simaona* to affect leaves of the plant, that is, make the larvae eat more control leaves than induced leaves. Induced leaves with poor quality may represent a poor resource for herbivores, so it may stimulate the herbivores to consume other good quality leaves. Tang et al. (2006) indicated that dispersed damage by herbivores is less detrimental to the fitness of the plant than more concentrated damage. With this, the total negative effects of herbivory on the whole tree are reduced. But contradictory results indicate that insects might consume more leaves to compensate for the low-nutrient quality (Cornelissen and Fernandes, 2001). In the present study, lower herbivory in early-season damaged shoots and higher herbivory rate in overall control shoots was found, indicating that the poor resource did increase herbivore mobility and make greater dispersal of feeding damage by herbivores because of the energetic way of moving themselves.

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