Induced responses in the subtropical evergreen, broad-leaf tree *Schima superba*: Effects of simulated herbivory on leaf quality and subsequent insect attack during leaf expansion

Resuestas inducidas en el árbol siempreverde subtropical de hoja ancha *Schima superba*: Efectos de herbivoría simulada en la calidad foliar y ataque de insectos subsiguiente durante la expansión de la hoja

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Abstract. Induced responses to herbivory are physical, nutritional, and allelochemical traits that change in plants following disturbances, and reduce the performance and/or preference of leaf tissues on herbivores. This study gave evidence to the induced defense theory through the simulated herbivory in *Schima superba*, one of common dominant trees in subtropical evergreen, broadleaf forests in southern China. Results showed that leaves damaged at the beginning of leaf expansion would develop into having a larger area, higher toughness and higher tannin concentrations, but a lower water content compared with control leaves. As a result, they experienced lower herbivory rates than controls. These results indicate that simulated herbivory on leaves of *S. superba* (1) reduced leaf nutrition, and (2) increased the leaf physical and biochemical defense as a result of a localized induction to herbivory, therefore altering insect herbivore attacks.

Key words: Induced response; Herbivory; Simulated herbivory; Nitrogen content; *Schima superba*.

Resumen. Las respuestas inducidas ante el ataque de herbívoros incluyen características físicas, nutricionales y aleloquímicas que cambian en las plantas luego de disturbios, y que reducen la performance y/o preferencia de los tejidos vegetales a los herbívoros. Este estudio proveyó evidencia a la teoría de defensas inducidas a través de la simulación de herbivoría en *Schima superba* Este es uno de los árboles dominantes comunes en los bosques subtropicales siempreverdes, de hoja ancha, en el Sur de China. Los resultados mostraron que las hojas dañadas al comienzo de la expansión foliar podrían desarrollar una mayor área foliar, hojas más duras y de mayor concentración de taninos, pero un menor contenido de agua, comparado a las hojas control. Como resultado, estas hojas tuvieron una menor tasa de herbivoría que los controles. Estos resultados indican que la simulación de herbivoría en hojas de *Schima superba* (1) redujeron el estado nutricional de las hojas, y (2) incrementaron las defensas físicas y bioquímicas de las hojas como resultado de una inducción localizada a la herbivoría, alterando por lo tanto los ataques de herbívoros insectos.

Palabras clave: Respuestas inducidas; Herbivoría; Herbivoría simulada; Contenido de N; *Schima superba*.

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INTRODUCTION

Almost all plant species are consumed by herbivores, among which insects are especially conspicuous in most terrestrial communities (Futuyma, 2000). Evolutionary interactions between herbivores and plants have resulted in varieties of adaptations, and herbivory pressure has led to the evolution of chemical, mechanical and phenological defenses in plants (Coley, 1983). Plant defenses against herbivores had been generally assumed to be constitutive, that is, always expressed in plants. However, evidence is accumulating that many of the traits and processes that defend plants against herbivores change following attacks, and compensating responses for the lost tissue arise (Cornelissen & Fernandes, 2001).

Induced defensive responses usually include chemical defenses such as synthesis of qualitative substances (e.g., production of small amounts of strongly poisonous compounds such as alkaloids, phenolic glycosides, and cyanogenic glycosides). Quantitative substances (e.g., production of large amounts of indigestible compounds such as tannins, lignin and fiber), and physical defenses (e.g., increases in leaf toughness are also produced) (Karban & Baldwin, 1997; Hattori et al., 2004; Keeling & Bohlmann, 2006). If the response of plant leaves occurs immediately after a herbivore attack, it would be effective to reduce current attacks. If it occurs slowly, it can be adaptive when current herbivory is a good predictor of future herbivory (Karban & Baldwin, 1997; Agrawal, 1998). Besides defense mechanisms, plants can compensate for the lost tissue by a rapid replacement of this tissue (e.g., rapid leaf growth, increased photosynthetic capacity) (Cornelissen, 1993; Honkanen et al., 1994; Dangerfield & Modukanele, 1996; Kudo, 1996; Strauss & Agrawal, 1999; Bergstrom et al., 2000).

For the last years, researchers have tried to demonstrate that induced defense benefits plants in field experiments (Wold & Marquis, 1997; Cornelissen & Fernandes, 2001; Hattori et al., 2004). However, the dynamics of the induced response after defoliation are still under investigation. Major questions relate to the speed and lasting effect of the induced response, which may suggest different defense strategies among plants (Haukojoa & Neuvonen, 1985; Faeth, 1986; Kudo, 1996; Wold & Marquis, 1997).

Most efforts for understanding the mechanisms that might account for the resistance changes following herbivory have focused on changes in leaf quality and chemical constituents of plants. Following herbivore attack, the nutritional quality of the attacked plant tissue may decrease, and the amount of secondary compounds and physical defenses may increase. Thus, wound-induced responses are thought to have a defensive function due to their strong effect on herbivore performance (e.g. Agrawal, 1998, 1999; Boege, 2004).

In this study, we experimentally manipulated leaf damage in a subtropical evergreen, broad-leaf tree, Schima superba to assess the defensive function of induced responses. This plant species may serve as a good model for the study as it is attacked by different guilds of insect herbivores, and such attack is concentrated during the period of leaf expansion (Wang et al., 2006). Several studies have measured the relationship between herbivory pattern and seasonal changes in leaf chemical and physical traits in many systems (reviewed by Karban & Baldwin, 1997). Nevertheless nothing is known about this type of plant defense in subtropical evergreen, broad-leaf forests.

The effects of herbivory were assessed on leaf performance during the expansion of Schima superba leaves. They were experimentally subjected to simulate a herbivory early in the growing season, and induced responses and their subsequent effects on insect herbivores were examined. The following aspects were focused in this study: 1) the relationship between the patterns of herbivore's attack, and the changes in leaf traits during leaf expansion, 2) the responses of leaf traits to stimulated herbivory, and 3) the efficacy of induced responses to subsequent herbivory.

MATERIALS AND METHODS

Study site. 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-leaf forest, with Schima superba as one of the dominant tree species. The mean annual precipitation is about 1700-2200 mm, approximately 70% of which occurs from March to June. The mean annual temperature is 13-18°C, with extremes of monthly means of 7.5°C in the coldest month (January) and 22.9°C in the warmest month (July). The soil type in the site is the forest brown soil according to the soil classification system of China.

Sample trees and its insect herbivore. Schima superba is one of the dominant tree species in evergreen, broad-leaf forests in subtropical China. It is a common canopy tree in the area of Meihuashan Mountain, commonly about 20 m tall, that flowers and fruits from June to August. Leaf flush occurs at the beginning of early spring. In this study, leaf damages were primarily caused by Neospastis simaona wang, and an unidentified Liparidae (Lepidoptera).

Simulated damage on leaves. We chose at random 15 young S. superba trees at the understory (2.0 m - 3.0 m in height) in mid-March 2007 before bud-break in the sample stand. Ten leaf buds were randomly selected, which developed as shoots, from the crown of each tree. We marked them with plastic tags. Young leaves were marked as they emerged from the bud. Five of the ten shoots in each tree were used as controls. Artificial herbivory was produced by clipping rectangular sections from the leaves to find out how current year shoots responded to insect herbivory. Thirty percent of the area from the right-hand side of each leaf blade was removed, without severing the central vein, 6 days after bud-break (23 March 2007 for S. superba). The area of the leaf removed was...
similar to that lost under natural conditions (personal observation). Artificially damaged and control leaves were attacked by natural herbivores after the treatment.

**Leaf traits.** Additional cutting treatments were performed on leaves of 8 unmarked shoots on each sample tree. Dynamic changes on leaf traits were assessed on leaves from unmarked shoots. They were collected and examined at 6-day intervals for potentially inducible traits which might affect herbivory foraging and growth. Leaves were immediately placed in plastic bags, and stored on dry ice until analyses were carried out.

Leaf area and dry weight were measured using a digital area meter (Li-3000, USA) and an electronic balance (Metter AE100, Germany), respectively. Leaf water content was estimated as the difference between fresh (FW) and dry (DW) weights per treatment (after dehydration at 70°C during 48 hours). Leaf water content (%) was calculated as: 100 × (FW - DW) / FW. Leaf N concentrations were determined using flow-injection autoanalyser (Skalar, Netherland).

Toughness was measured with a penetrometer which followed the design of Feeny (1970). The leaf was clamped between two wood plates, each drilled with a 5-mm diameter hole. The weight (grams) necessary to go through the leaf using a 4-mm diameter rod gave us an index of toughness. Measurements were taken using the penetrometer immediately upon leaf collection. Five sections of tissue were sampled in each leaf between main veins in the adaxial surface.

Tannins consist of multiple structural units containing phenolic groups, and are functionally defined by their capacity to bind proteins (Hagerman, 1987). We measured the protein-binding capacity (PBC) of tannins in leaf extracts using the radial diffusion assay due to its simplicity and widespread use. Tannins in the extract bind to proteins to form an opaque precipitate whose squared diameter is proportional to the tannin concentration in that extract (Hagerman, 1987). Extraction from plant tissues was made during an hour at room temperature with aqueous methanol (50% v/v). A solvent was used maintaining a ratio of 0.5 ml solvent per 100 mg leaf tissue. Sixty µl of plant extract were placed in a Petri dish containing a mixture of agar and protein (bovine serum albumin; Sigma) to estimate the tannin concentration. A regression equation relating the square of the diameter of the precipitate (ring form) to the tannin concentration was calculated using a series of tannic acid standards. The average of three replicates per leaf was used for statistical analysis.

**Leaf area loss.** Damaged areas after treatment were compared with artificially damaged and control leaves to determine if simulated damages reduce subsequent insect attacks. Actual leaf areas and leaf area losses were measured on marked shoots every time using plastic grids (10 grids/cm²) to determine herbivory rates while the leaf was expanding. Herbivory rate (%) = 100 × leaf area loss / (actual leaf area + leaf area loss). Missing leaves were scored as 100% damage. Shoots or leaves that were obviously damaged by falling debris were not included.

**Statistical analyses.** Before analysis, all data were tested for normal distribution, and homoscedasticity of variance when necessary; data were log10-transformed. One-way ANOVA was used to compare leaf area, toughness, water content, tannin concentration and leaf area loss at the different harvesting times. All statistical analyses were performed with SPSS software (SPSS Inc.).

**RESULTS**

**Leaf traits.** Most of *S. superba* trees began to produce leaves from 21 to 25 March in 2007. Each leaf bud produced about 10 leaves (range = 8-14). Leaf area increased abruptly during the first 24 days after bud-break; then, it remained stable. From 12 days after bud-break onward, treated leaves showed larger (p<0.05) leaf area than control leaves (Fig. 1).

Leaf nitrogen concentration was high during early leaf expansion, and it decreased thereafter. Leaf nitrogen concentration was not affected by simulated herbivory during early leaf expansion. However, from day 30 onwards, damaged leaves had a lower (p<0.05) nitrogen concentration than control leaves (Fig. 2).

Like nitrogen concentrations, leaf water content was highest on young leaves, and it decreased with leaf age. Artificially damaged leaves had significantly lower (p<0.05) water contents than control leaves. When leaves reached full size, water content decreased much faster on artificially damaged than on control leaves (Fig. 3).
Leaf toughness increased from day 30 onward after bud-break (Fig. 4). Thereafter, it increased rapidly until the leaf reached full size. Leaves from damaged shoots showed higher toughness than those on control shoots (Fig. 4).

Tannin concentrations declined in artificially damaged and control leaves during leaf expansion. After 12 days from bud-breaking, tannin concentrations were significantly higher on artificially damaged than on control leaves (Fig. 5).
Effects of simulated herbivory on the frequency and levels of subsequent herbivores. The cumulative percentage of leaf area loss by herbivory increased abruptly from day 12 to day 24 after bud-break, and it gradually rose over the following days (Fig 6). Cumulative herbivory on artificially damaged leaves increased gradually in comparison with control leaves, and the extent of herbivory was significantly lower in damaged leaves from day 18 onward. The final leaf area loss on treatment leaves (average 6.1%) was significantly lower than that on control leaves (average 16.8%). There were no significant differences among trees in the same treatment (p=0.63, one-way ANOVA after arcsine transformation).

Fig. 6. Comparison of changes between artificially damaged leaves and control leaves with cumulative herbivory. Error bars represent SE. **p<0.01, *p<0.05, NS: p>0.05 in each leaf age, n= 45.

Our results showed that leaf quality of S. superba changed drastically with leaf expansion. Leaf nitrogen concentration and leaf water content decreased, and leaf toughness increased during leaf expansion in both the treatment and control leaves. The decline in leaf quality with leaf development can be considered a defense strategy against herbivory, especially for evergreen trees with long lifespan leaves (Coley & Barone, 1996; Kursar & Coley, 2003). Water content was higher in young than mature leaves of S. superba. However, leaf nitrogen concentrations as a defense substance decreased during leaf expansion in S. superba and in other trees (Coley & Barone, 1996; Kursar & Coley, 2003). Higher tannin concentrations in young leaves might be a make-up defense, when leaf constitutive defenses (such as toughness) are not enough (Coley & Barone, 1996; Kursar & Coley, 2003).

There were no significant differences among trees in the same treatment (p=0.63, one-way ANOVA after arcsine transformation).
fense, and different plant species may follow different strategies. Evergreen species differ from deciduous species in their lack of major storage reserves in stem or roots. Leaves comprise a larger proportion of the total source of stored nutrients than in deciduous trees, and reserves cannot be mobilized from other parts to compensate leaf loss (Bryant et al., 1983).

In summary, S. superba demonstrated defensive and compensatory responses as a result of early-season herbivory in this study. In addition, food quality (plant tissues) for herbivores was reduced, which decreases late-season damage.

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REFERENCES

Agrawal, A.A. (1998). Induced responses to herbivory and increased plant performance. Science 279: 1201-1202.
Agrawal, A.A. (1999). Induced responses to herbivory in wild radish: effects on several herbivores and plant fitness. Ecology 80: 1713-1723.
Bergstrom R, C. Skarpe & K. Danell (2000). Plant responses and herbivory following simulated browsing and stem cutting of Combretum apiculatum. Journal of Vegetation Science 11: 409-414.
Boege, K. (2005). Influence of plant ontogeny on compensation to leaf damage. American Journal of Botany 92: 1632-1640.
Bryant, J.P., F.S. Chapin III & D.R. Klein (1983). Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. Oikos 40: 357-368.
Coley, P.D. (1983). Herbivory and defensive characteristics of tree species in a lowland tropical forest. Ecological Monographs 53: 209-233.
Coley, P.D. & J.A. Barone (1996). Herbivory and plant defenses in tropical forests. Annual Review of Ecology and Systematics 27: 305-335.
Coley, P.D. & T.A. Kursar (1996). Anti-herbivore defences of young tropical leaves: physiological constraints and ecological trade-offs. In: Mulkey S.S. and Chazdon R.L. (eds) Tropical forest plant ecophysiology. Chapman & Hall, New York, pp 305-336.
Cornelissen, J.H.C. (1993). Growth, morphology and leaf characteristics after simulated herbivory in Chinese subtropical evergreen saplings. Ecological Research 8: 143-150.
Cornelissen, T.G. & G.W. Fernandes (2001). Induced defenses in the neotropical tree Bauhinia brevipes (Vog.) to herbivory: effects of damage-induced changes on leaf quality and insect attack. Tree 15: 236-241.
Dangerfield, J.M. & B. Modukanele (1996). Overcompensation by Acacia erubescens in response to simulated browsing. Journal of Tropical Ecology 12: 905-908.
Ernest, K.A. (1994). Resistance of creosote bush to mammalian herbivory: temporal consistency and browsing-induced changes. Ecology 75: 1684-1692.
Faeth, S.H. (1986). Indirect interactions between temporally separated herbivores mediated by the host plant. Ecology 67: 479-494.
Feeny, P. (1970). Seasonal changes in oak leaf tannins and nutrients as a cause of spring feeding by winter moth caterpillars. Ecology 51: 565-581.
Futuyma, D.J. (2000). Some current approaches to the evolution of plant herbivore interactions. Plant Species Biology 15: 1-9.
Hagerman, A.E. (1987). Radial diffusion method for determining tannin in plant extracts. Journal of ChemicalEcology 13: 437-449.
Hattori, K., T.A. Ishida, K. Miki, M. Suzuki, & T. Kimura (2004). Differences in response to simulated herbivory between Quercus crispula and Quercus dentate. Ecological Research 19: 323-329.
Haukioja, E. & J. Koricheva (2000). Tolerance to herbivory in woody vs. herbaceous plants. Evolutionary Ecology 14: 551-562.
Haukioja, E. & S. Neuvonen (1985). Induced long-term resistance of birch foliage against defoliators: defensive or incidental. Ecology 66: 1303-1308.
Herms, D.A. & W.J. Mattson (1992). The dilemma of plants: to grow or defend. Quarterly Review of Biology 67: 283-335.
Honkanen, T., E. Haukioja & J. Suomela (1994). Effects of simulated defoliation and debudding on needle and shoot growth in Scots pine Pinus sylvestris - implications of plant source-sink relationships for plant-herbivore studies. Functional Ecology 8: 631-639.
Keeling, C.I. & J. Bohlmann (2006). Genes, enzymes and chemicals of plant defense against insects and pathogens. New Phytologist 170: 657-675.
Kudo, G. (1996). Herbivory pattern and induced responses to simulated herbivory in Quercus mongolica var. grosseserrata. Ecological Research 11: 283-289.
Kursar, T.A. & P.D. Coley (2003). Convergence in defense syndromes of young leaves in tropical rainforests. Biochemical Systematics and Ecology 31: 929-949.
Rosati, A., G. Esparza, T.M. De Jong, & R.W. Parry (1999). Influence of canopy light environment and N availability on leaf photosynthetic characteristics and photosynthetic nitrogen-use efficiency of field-grown nectarine trees. Tree Physiology 19: 173-180.
Straw, S.Y. & A.A. Agrawal (1999). The ecology and evolution of plant tolerance to herbivory. Trends in Ecology and Evolution 14: 179-185.
Stout, M.J., W.C. Rice & D.R. Ring (2002). The influence of plant age on tolerance of rice to injury by the rice water weevil Lissorhoptrus oryzophilus (Coleoptera: Curculionidae). Bulletin of Entomological Research 92: 177-184.
Wang Hong-Wei, Yong-Li Cai, Kai Li & Yu-Peng Tai (2006). Insect herbivory patterns on leaves of 11 plant species in the evergreen broad-leaved forests of Tianzhu National Park (in Chinese with English abstract), Zhejiang. Biodiversity Science 14:145-151.
Wold, E.N. & R.J. Marquis (1997). Induced defense in white oak: effects on herbivores and consequences for the plant. Ecology 78: 1356-1369.
Zangerl, A.R. & F.A. Bazzaz, (1992). Theory and pattern in plant defense allocation. In: Fritz RS and EL Simms (eds.), Plant Resistance to Herbivores and Pathogens. University of Chicago Press, Chicago. pp 363-391