Annals of Botany **94**: 377–384, 2004
doi:10.1093/aob/mch153, available online at www.aob.oupjournals.org

Interspecific Variation of Plant Traits Associated with Resistance to Herbivory Among Four Species of *Ficus* (Moraceae)

HUI XIANG and JIN CHEN*

Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Mengla, Yunnan Province, P. R. China 666303

Received: 5 December 2003 Returned for revision: 2 March 2004 Accepted: 13 May 2004 Published electronically: 26 July 2004

- *Background and aims* To understand the defensive characteristics of interspecies varieties and their responses to herbivory damage, four species of *Ficus* plants (*Ficus altissima*, *F. auriculata*, *F. racemosa* and *F. hispida*) were studied. They were similar in life form, but differed in successional stages. Of these, *Ficus altissima* is a late successional species, *F. hispida* is a typical pioneer and *F. auriculata* and *F. racemosa* are intermediate successional species. We addressed the following questions: (1) What is the difference in plant traits among the four species and are these traits associated with differences in herbivory damage levels? (2) What is the difference in the damage-induced changes among the four species?
- *Methods* Herbivory damage was measured in the field on randomly planted seedlings of the four species of the same age. Defences to herbivory were also tested by feeding leaves of the four species to larvae of *Asota caricae* in the laboratory. A total of 14 characters such as water content, thickness, toughness, pubescence density on both sides, leaf expansion time, lifetime and the contents of total carbon (C), nitrogen (N), phosphorous (P), potassium (K), magnesium (Mg) and calcium (Ca) were measured. Leaf calcium oxalate crystal (COC) density, total Ca and N content, leaf toughness and height were measured to investigate induced responses to artificial herbivory among the four species.
- *Key results and conclusions* Herbivory damage in the four studied species varied greatly. The pioneer species, *F. hispida*, suffered the most severe herbivory damage, while the late successional species, *F. altissima*, showed the least damage. A combination of several characteristics such as high in content of N, Ca and P and low in leaf toughness, lifetime and C : N ratio were associated with increased herbivore damage. The late successional species, *F. altissima*, might also incorporate induced defence strategies by means of an increase in leaf COC and toughness.

Key words: Calcium oxalate crystals, defensive characteristics, *Ficus*; herbivory, induced defence.

INTRODUCTION

Almost all plant species are consumed by herbivorous animals, among which insects are especially conspicuous in most terrestrial communities (Futuyma, 2000). Insect herbivory is often detrimental to plants and causes a significant loss of plant growth, survival and fecundity and, therefore, a reduction in plant fitness (Belsky, 1986; McNaughton, 1986; Crawley, 1997; Bigger and Marvier, 1998; Lehtila and Strauss, 1999). Evolutionary interactions between herbivores and plants have resulted in an impressive variety of adaptations, and herbivory pressure has led to the evolution of chemical, mechanical and phenological defences in plants (Rosenthal and Janzen, 1979; Coley, 1983). Empirical studies have demonstrated that there is a wide range of characters involved in plant defence. These include the timing of leaf flush (Aide, 1992, 1993), leaf water and nutritional components such as N content (Coley, 1983; Filip *et al*., 1995) and mineral composition (Alonso and Herrear, 2000), fibre content, toughness, phenolic content, secondary chemical substances and proteinase inhibitors (PIs) (Coley, 1983; Dudt and Shure, 1994; Marquis *et al*., 2001; Underwood *et al*., 2002). Furthermore, plants' resistance to herbivory may also be involved with different defensive strategies such as constitutive resistance and induced resistance (Herms and Mattson, 1992; Zangerl *et al*., 1997).

There are large differences among plant species in both the amount and type of anti-herbivore defences in leaves. Plants of different successional status, or those adapted to different habitats, might have different defensive characteristics (Coley, 1983, 1988; Dudt and Shure, 1994; Marquis *et al*., 2001). However, most of these studies only conducted research at the community level; data on different successional species of one genus are relatively few.

There are many defensive chemicals in plants, amongst which are calcium oxalate crystals (COC) (Sunnell and Healey, 1985; Perera *et al*., 1990; Ward *et al*., 1997; Molano-Flores, 2001; Ruiz *et al*., 2002). Although COC is supposed to deter herbivores, a direct link between herbivory and COC production has rarely been found (Ward *et al*., 1997; Molano-Flores, 2001; Ruiz *et al*., 2002) and the mechanism remains uncertain.

With about 800 species distributed worldwide in tropical and subtropical zones, *Ficus* (Moraceae) is one of the largest genera of woody plants. Furthermore, *Ficus* is the most diverse genus of woody plants with regard to habit, growth forms and life forms (Berg, 1990). In this study, we selected four species of *Ficus* that were the same in life form, but different in successional status. We addressed the following

---

* For correspondence. E-mail biotrans@bn.yn.cninfo.net

two questions. (1) What is the difference in plant traits among the four species and are these traits associated with differences in herbivory damage levels? (2) What is the difference in the damage-induced changes among the four species?

MATERIALS AND METHODS

Study site and species

The study was carried out in the Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, China. The climate in this area is tropical to subtropical humid monsoonal. The annual average temperature is 21·7 °C, the annual rainfall is about 1500 mm and the annual relative humidity is 83 % (Xu and Tao, 1988). The climate is characterized by a distinct rainy season (May–October) followed by a cool dry season (November–February) and a hot dry season (March–April).

Four *Ficus* tree species were selected for the study. *Ficus altissima* is a typical rainforest plant or late successional species and seedlings are shade-tolerant (Xu *et al*., 1996). *Ficus hispida* is a typical pioneer species in this area. *Ficus auriculata* and *F. racemosa* are intermediate in terms of successional status and both occur on the edge of rainforests or beside roads.

In June 2002, seeds of the four species were collected from ripe fruit of several individual trees of the four species in a nearby forest and germinated in washed sand. After 6 weeks, 60 seedlings of approximately the same height and biomass of each species were selected and transplanted into pots (diameter 15 cm) containing manured soil at one seedling per pot. Pots were then placed in a mothproof glasshouse. After 10 weeks, at the beginning of October 2002, potted seedlings were moved to an experimental field (20 m × 15 m) for further study. Twenty seedlings of each species were used for an investigation on herbivory rates and a plant characteristic census.

Herbivory level

Herbivory rates were monitored at the end of the wet season (October 2002), dry and cool season (February 2003) and dry and hot season (April 2003). Total leaf area and the damaged area were quantified by placing a clear grid card under the leaf and counting the squares. The herbivory rate of one leaf was expressed as the percentage of leaf area damaged. The rate on a whole seedling was expressed as the average herbivory of every leaf.

Larvae feeding experiment

In November 2002, woolly bear (larvae of *Asota caricae* Boisduval, a common lepidopter on *Ficus* plants) were collected from the field. They were starved for 24 h and then placed on leaf rectangles (1·5 cm × 3 cm) in a cone-shaped bottle. Experiments were divided into two groups as follows. (1) Separate feeding: young (body length 1·35 ± 0·18 cm) and old larvae (body length 2·51 ± 0·27 cm) were used. Larvae were fed separately with the first mature leaves of the seedlings of the four species at five leaf rectangles per larva. (2) Mixed feeding: old larvae (body length 2·51 ± 0·27 cm) were fed with mixed leaf rectangles of the four species of seedlings at two rectangles per species. 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 five replications were used for each case.

Census of plant characteristics

The water content, thickness, toughness, pubescence density on both sides, leaf expansion time, lifetime and contents of total C, N, P, K, Mg and Ca were measured in this study. The first and/or second mature leaves were selected and morphological and mechanical characteristics of 20 duplicated samples (20 seedlings of each species) were measured in the field. Thickness was estimated using a vernier calliper (precision 0·02 cm) and toughness measured with a punchameter modelled after a design by Feeny (1970). Upper and lower pubescence density was determined as the number of pubescences per view (×20) under a microscope. The number of days required to expand from bud burst to a fully matured size and then to senescence were measured on tagged leaves and based on daily records. From these values, leaf expansion and lifetimes were determined. Three duplicates were measured for each species on chemical analysis. Leaf material was collected and oven dried for 48 h (70 °C) in the laboratory. Water content was estimated as the difference between the wet and dry leaf mass. Dry samples were then sent to the Biogeochemical Laboratory of the Kunming Division of the Xishuangbanna Tropical Botanical Garden for the measurement of chemicals. The C and total N were determined using a wet digestion procedure (Kalra and Maynard, 1991). Phosphorous, K, Mg and Ca contents were determined from atomic absorption spectrum-photometry (AAS, Type 932GBC; Scientific Equipment Pty Ltd, Australia).

Artificial herbivory treatment

Seedlings of the four species were placed in field cages, the roofs of which were rainproof and shaded. The cages were enclosed with nylon net that prevented insects from entering. For each species, artificial herbivory was produced by clipping rectangular sections from the leaves. A different percentage of the area from the right-hand side of the blade of each leaf was removed without severing the central vein. The area of the leaf removed was similar to that lost under natural conditions (personal observation). This equated to: *F. altissima* (10·0 %), *F. auriculata* (16·7 %), *F. racemosa* and *F. hispida* (33·3 %). This treatment was applied to every other leaf, excluding the cotyledons. Young leaves were periodically cut until they reached maturity, with the set areas maintained. Cages that enclosed the four species with unclipped leaves were used as controls. For each treatment and control, four replications of five individual seedlings from five pots per species were set, giving a total of eight cages.

At the end of the experimental period (February 2003), a chemical analysis was run. Total N and Ca contents and toughness were measured as described in the previous section. COC densities were estimated following the method of Finley (1999). Twenty leaves were selected and the crystal density of each leaf was determined on the left-hand side of the leaf blade at the tip, middle and base. In each of these regions, crystals densities were expressed as the number of crystals per view (×10 magnification) under a microscope. Young and mature leaves were measured separately.

The height of the seedlings at the beginning and end of the experimental period were measured and plant growth determined as the height increment.

Statistical analysis

A principal components analysis was applied to extract the principal leaf characteristics. An ANOVA and multi-comparison (LSD) were applied to compare interspecific differences of leaf herbivory level and leaf palatability to *A. caricae* larvae when fed each species separately, and non-parametric analyses were used when fed with mixed diet. Correlations were used to analyse relationships between leaf characteristics and damage levels and between leaf characteristics and leaf palatability to *A. caricae* larvae. An ANOVA and multi-comparison (LSD) were performed to compare species characteristics and a *t*-test was used to compare COC density, toughness, Ca and N content, height increment between the artificial herbivory treatment and control. All statistical analyses were performed using Nosa 3·2 (Nosa Workgroup, The Fourth Military Medical University, Xi'an, China).

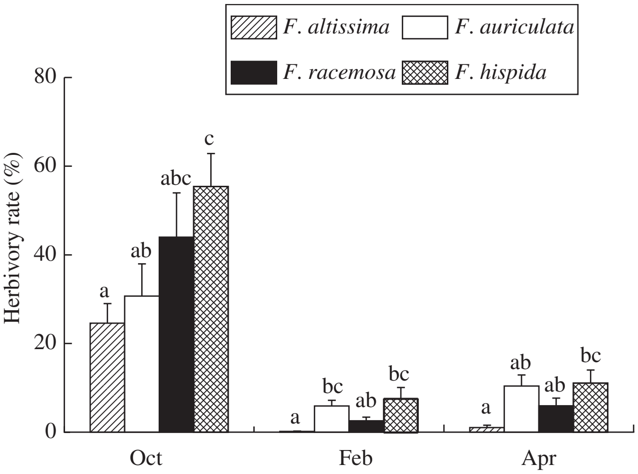

FIG. 1. Herbivory damage of leaves in the seedlings of four *Ficus* species (mean ± 1 s.e., $n = 20$). Means showing the same letter are not significantly different.

RESULTS

Herbivory damage to the seedlings

All four species of seedlings were subjected to considerable herbivory attack when leaf damage was measured at the end of the wet season (October 2002). There was also a significant interspecific variation in the damage level ($P < 0·05$), with *F. hispida* suffering the highest damage and *F. altissima* the lowest (Fig. 1).

The damage level at the end of dry and cool season (February 2003) and dry and hot season (April 2003) was low in all four species (Fig. 1).

Preferences of A. caricae *larvae to leaves of the four* Ficus *species*

Both young and old larvae showed certain preferences to the four *Ficus* species when fed the leaves separately. The consumed area of *F. altissima* was significantly smaller than that of *F. auriculata*, *F. racemosa* and *F. hispida*. Although for the latter three species the old larvae did not show any significant preference, the young larvae favoured *F. hispida* (Fig. 2A and B).

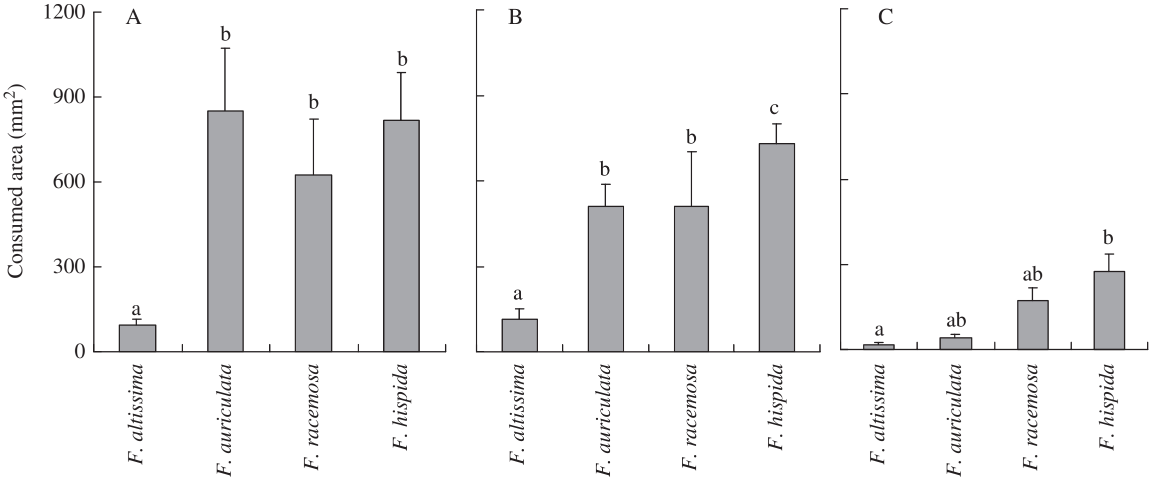

FIG. 2. Comparison of leaf area damage to four species of fig seedlings (A and B, larvae fed with leaves from all four species; C, larvae fed a mixture of leaves) (mean ± 1 s.e., $n = 5$). Means showing the same letter are not significantly different.

TABLE 1. *Interspecific differences in leaf characteristics of seedlings of four* Ficus *species (mean ± s.d.)*

Species	C (% d. wt)	N (% d. wt)	C/N	P (% d. wt)	Ca (% d. wt)	K (% d. wt)	Mg (% d. wt)	Toughness (mN)	Pubescence upper (no./view)	Pubescence lower (no./view)	Thickness (cm)	Water content (%d. wt)	Expansion time (day)	Lifetime (day)
	n = 3	*n* = 3	*n* = 3	*n* = 3	*n* = 3	*n* = 3	*n* = 3	*n* = 20	*n* = 20	*n* = 20	*n* = 20	*n* = 3	*n* = 20	*n* = 20
F. altissima	49·16 ± 0·35^{c}	1·71 ± 0·11^{b}	36·10 ± 1·84^{b}	0·23 ± 0·02^{a}	2·01 ± 0·35^{a}	1·58 ± 0·08^{a}	0·40 ± 0·09^{a}	6871·4 ± 1103·7^{c}	6·5 ± 4·2^{a}	32·7 ± 10·9^{a}	0·048 ± 0·007^{c}	81·63 ± 4·55^{c}	17·53 ± 5·62^{b}	159·21 ± 21·20^{c}
F. auriculata	44·28 ± 2·00^{b}	1·26 ± 0·06^{a}	35·24 ± 3·21^{b}	0·21 ± 0·03^{a}	2·27 ± 0·22^{ab}	1·47 ± 0·13^{a}	0·67 ± 0·05^{a}	3616·5 ± 756·0^{b}	2·3 ± 2·0^{a}	25·1 ± 12·7^{a}	0·038 ± 0·004^{b}	68·54 ± 4·18^{ab}	15·25 ± 3·04^{b}	120·11 ± 23·91^{b}
F. racemosa	45·69 ± 0·68^{b}	2·70 ± 0·04^{c}	16·92 ± 0·49^{a}	0·27 ± 0·01^{a}	2·95 ± 0·10^{c}	1·61 ± 0·05^{a}	0·52 ± 0·11^{a}	1433·3 ± 427·9^{a}	4·6 ± 1·6^{a}	25·0 ± 11·7^{a}	0·020 ± 0·001^{a}	71·31 ± 1·04^{b}	12·21 ± 3·03^{a}	52·16 ± 20·13^{a}
F. hispida	41·08 ± 0·85^{a}	2·35 ± 0·20^{c}	17·48 ± 1·22^{a}	0·28 ± 0·02^{a}	2·77 ± 0·20^{bc}	1·51 ± 0·31^{a}	0·45 ± 0·06^{a}	3183·7 ± 931·9^{b}	43·9 ± 36·5^{b}	115·0 ± 22·0^{b}	0·046 ± 0·003^{c}	64·92 ± 0·19^{a}	22·07 ± 4·51^{c}	101·64 ± 26·95^{b}

Means showing the same letter are not significantly different.

When fed mixed leaves of the four species, the interspecific difference was very significant. This indicated considerable feeding preferences. Species in order of damage level from highest to lowest were: *F. hispida*, *F. racemosa*, *F. auriculata* and *F. altissima* (Fig. 2C).

Interspecific differences in leaf characteristics

Other than the contents of total K, P and Mg, all 14 leaf characteristics investigated showed significant interspecific differences (Table 1). *Ficus altissima* had relatively high levels in terms of leaf C, C : N ratios, toughness, thickness, water content and lifetime, but low Ca content. For *F. hispida*, leaf C content and C : N ratios were low, but N contents were high, and leaf expansion times were significantly long. It also had dense pubescence and a high Ca content. *Ficus auriculata* had a lower leaf N content and a higher C : N ratio, while *F. racemosa* had a higher leaf N content, which was associated with a short leaf expansion time and lifetime. It also had a lower leaf thickness and toughness.

Two principal components, PC 1 and PC 2 (percentage variance is 79·21 %, which could be accepted), were extracted from the set of leaf traits (Table 2). The contents of total N, Ca, P, toughness, lifetimes and C : N ratios had higher factor scores on PC 1. Pubescence, thickness and expansion time had higher factor scores on PC 2.

The first principal component explained a significant portion of the interspecific variation in herbivory damage at the end of the wet season and the leaf palatability determined by the mixed diet feeding experiment. However, the relationship between the second principal component and herbivory damage was not significant (Table 3). A partial correlation analysis also indicated that no single trait from the traits scored high on PC 1 was significantly related to the interspecific variation in herbivory damage (data not presented).

TABLE 2. *Eigenvalues, percentage of the variance explained by the first two principal components (79·21 %) and factor scores of leaf characters listed in Table 1*

	PC 1	PC 2
Eigenvalue	6·77	4·32
Percentage variance (%)	48·33	30·88
Characters	Factor scores	
C	0·72	−0·47
N	−0·83	−0·20
C/N	0·98	0·05
P	−0·89	0·14
Ca	−0·98	−0·22
K	−0·12	−0·54
Mg	0·05	−0·36
Toughness	0·84	0·40
Pubescence – upper	−0·58	0·80
Pubescences – lower	−0·55	0·83
Thickness	0·47	0·88
Water content	0·73	−0·25
Expansion time	−0·10	0·99
Lifetime	0·85	0·51

Induced responses to artificial herbivory damage

Density of COC in the leaflets. Considerable quantities of calcium oxalate druses were found in the leaves of *F. altissima*, *F. auriculata* and *F. hispida*, but not in the leaves of *F. racemosa*. The shape or number of crystals varied among the species. Responses to the artificial herbivory damage treatment varied among the former three species. For *F. altissima*, artificial herbivory damage induced the production of COC in both mature and young leaves. The artificial herbivory treatment did not cause a significant difference in the amount of COC in either the mature or young leaves of *F. auriculata* (Fig. 3). In *F. hispida*, herbivory decreased the production of COC in both the mature and young leaves (Fig. 3).

Leaf Ca and N contents. No significant differences were found in the total Ca content between the control and artificial herbivory treatments. For Ca content, the *P*-values of *F. altissima*, *F. auriculata*, *F. racemosa* and *F. hispida* were 0·45, 0·85, 0·22 and 0·72, respectively (*t*-test). A similar case was found for the N content; *P*-values were 0·33, 0·56, 0·18 and 0·49, respectively (*t*-test).

Leaf toughness. Artificial herbivory damage increased the toughness of *F. altissima*, both in the mature and young leaves. It also increased the toughness of *F. auriculata* in the mature leaves, but had no influence on the young leaves. Toughness of the other two species was not significantly affected (Fig. 4).

Plant growth. Artificial herbivory damage had no influence on plant growth in all four species (Fig. 5). The *P*-values of *F. altissima*, *F. auriculata*, *F. racemosa* and *F. hispida* were 0·23, 0·84, 0·12 and 0·37, respectively (*t*-test).

DISCUSSION

Insect herbivory of the four *Ficus* species varied significantly. The pioneer species, *F. hispida*, suffered a significantly high herbivory attack, while the late successional

TABLE 3. *Results of correlation analysis as significance (*P*-value) and proportion variance (*r*) explained by between the principal components and as to leave damage and leave consumed area*

	PC 1 *P*, *r*	PC 2 *P*, *r*
Leaf damage at the end of the wet season	*0·038, −0·96	ns, 0·27
Leaf damage at the end of dry and cool season	ns, −0·52	ns, 0·50
Leaf damage at the end of dry and hot season	ns, −0·33	ns, 0·33
Consumed leaf area of separate species (old larvae)	ns, −0·61	ns, 0·09
Consumed leaf area of separate species (young larvae)	ns, −0·82	ns, 0·25
Consumed leaf area of larvae fed a mixed diet	*0·050, −0·97	ns, 0·20

ns, Not significant; *, significant.

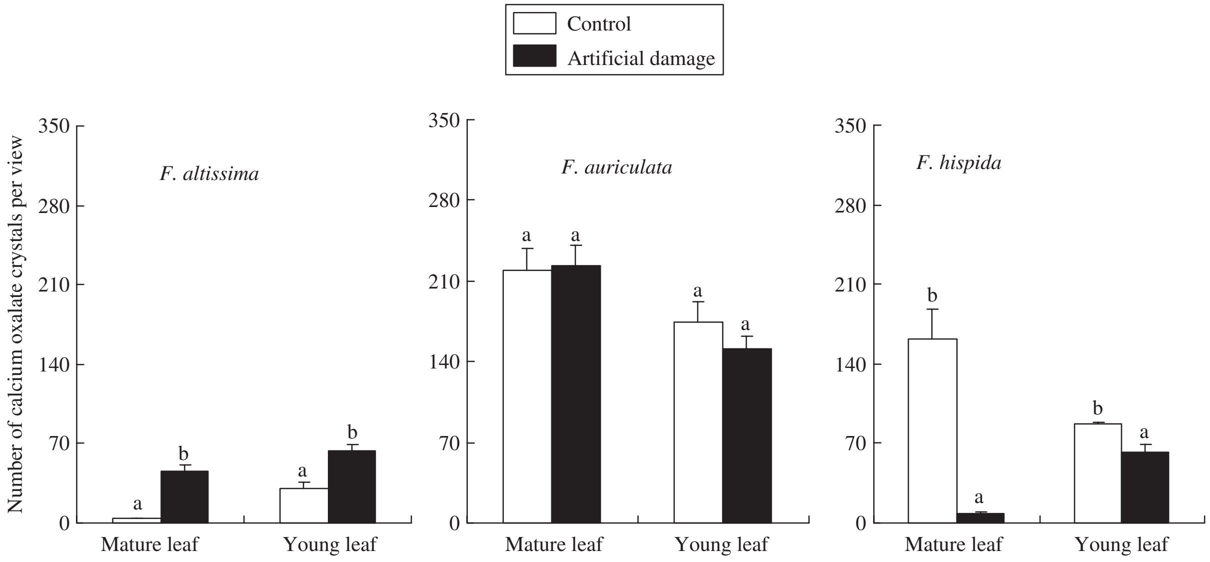

FIG. 3. Calcium oxalate crystals in leaflets (viewed under ×10 magnification) (mean ± s.e., *n* = 60). Means showing the same letter are not significantly different.

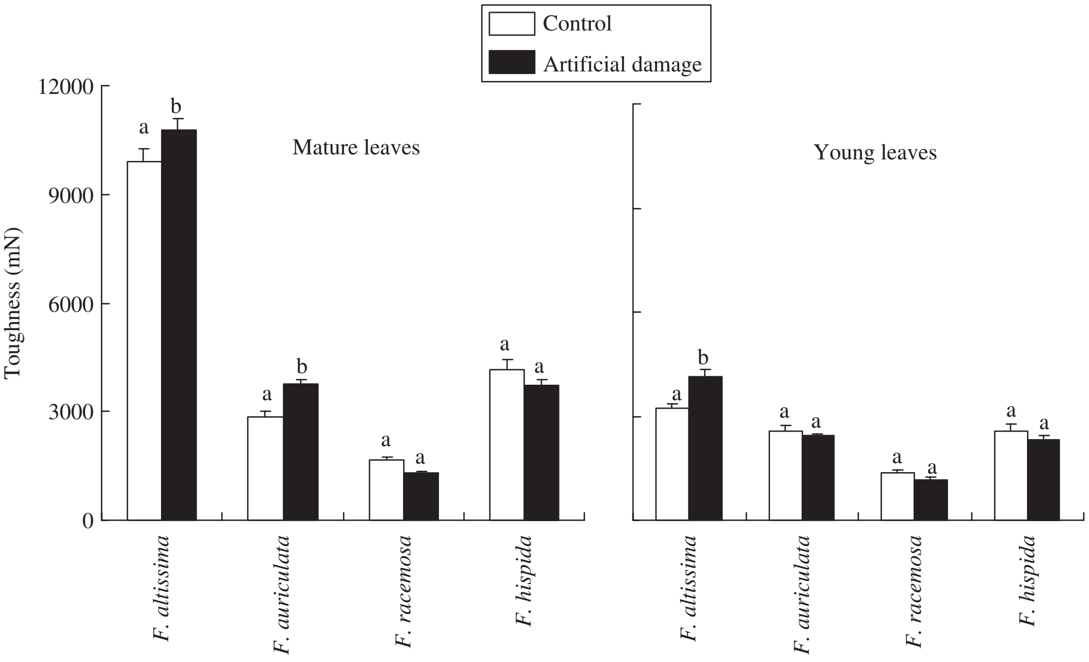

FIG. 4. Leaf toughness in different treatments (mean ± s.e., *n* = 20). Means showing the same letter are not significantly different.

species, *F. altissima*, received relatively low herbivory damage (Fig. 1). This pattern was also illustrated in the mixed leaf feeding experiments with *A. caricae* larvae (Fig. 2A and B). Plant species with different life histories or successional status may vary in their defence to herbivory damage. Pioneers tend more towards growth, and so are often poorly defended, while late successional species are usually well defended and often suffer less herbivory damage (Coley, 1983; Dudt and Shure, 1994). However, previous studies often used community comparisons and, thus, did not exclude phylogenetic effects (Leps *et al*., 2001; Marquis *et al*., 2001). In the study reported here, the differences in herbivory damage of plants from the same genus, *Ficus*, were compared, which means that phylogenetic effects could be excluded to some extent.

A total of seven characteristics was extracted on PC 1 with high factor socres by principal components analysis. Total leaf N, Ca and P were positively correlated, while toughness, lifetimes and C : N ratios were negatively correlated (Table 2). A combination effect of the above

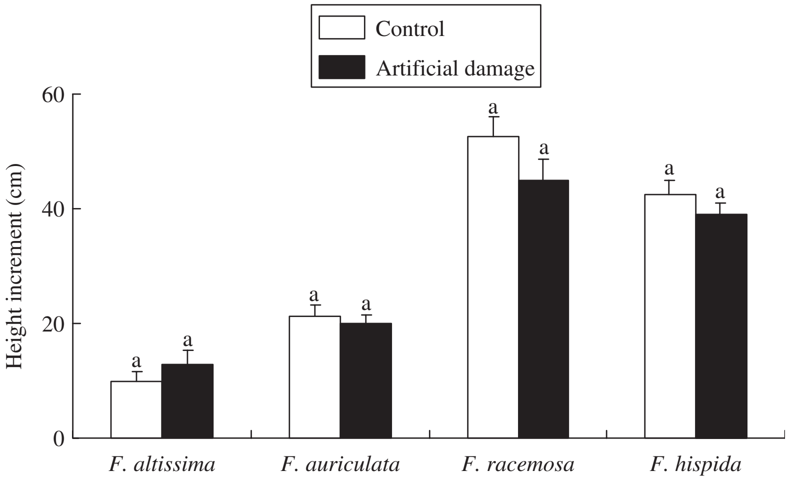

FIG. 5. Height increment of the seedlings in different treatments (mean ± s.e., $n = 20$). Means showing the same letter are not significantly different.

seven characteristics was significantly correlated with interspecific variations in insect damage. Leaf N has been reported to influence insect herbivory and Stamp and Casey (1993) found that low leaf N contents might negatively affect the preference and performance of insect herbivores. In some plant systems, a reallocation of nutrients occurs in the damaged foliage. This includes a decrease in N content (Wold and Marquis, 1997; Cornelissen and Fernandes, 2001), which implies the importance of leaf N to insects. Contradictory results indicate that insects might consume more biomass to compensate for the N deficiency (Price *et al*., 1980). Leaf toughness is widely recognized as a mechanical defence and is negatively related to herbivory damage (Coley, 1983; Dudt and Shure, 1994). Macromolecular substances such as lignin, cellulose and phenolic contents might contribute to leaf toughness (Coley *et al*., 1985), which would increase leaf construction costs. Long-lifetime leaves have a greater risk of exposure to herbivory and tend to synthesize these macromolecular substances as a defence (Coley *et al*., 1985; Southwood, 1986; Williams *et al*., 1989). The C : N ratio of leaves influences their quality. Bryant's 'carbon-nutrition balance theory' predicted that when insects fed on leaves with high C : N ratio, their development would slow down (Bryant, 1983). However, the influence of the leaf C : N ratio on defence is complicated and has strong environmental plasticity (Coviella *et al*., 2002). Although leaf total P content was also a principal component, there was no significant difference among the four species. Nonetheless, when relating a single trait of the above seven characteristics to the degree of herbivory damage, there were no significant relations detected. This suggested that *Ficus* plants such as *F. altissima* might develop a combination of characters for resistance to herbivory.

In the study reported here, traits that had high factor scores on PC 2 (i.e. pubescence density, thickness and expansion times) were not significantly correlated to herbivory damage (Table 3). Although pubescence is considered as a defensive trait, poorly defended pioneers are often pubescent (Coley, 1983). Young leaves with high levels of nutrition develop rapid expansion to escape herbivory. In this study, *F. racemosa*, which had a relatively high N content, had a short leaf expansion period (Table 1).

Among the characteristics investigated for the response to the artificial herbivory treatment, the COC density and leaf toughness showed induced responses (Figs 3 and 4), while the total N content and Ca content were not significantly affected. COC density and leaf toughness were significantly increased in the late successional species, *F. altissima*, as a response to the artificial herbivory treatment. To date, knowledge of the function of COC is limited. In plants, COC may have a role in defence against herbivores and/or in accumulating excess Ca. However, how plants regulate the production of COC under herbivory has not been resolved. The few relevant studies focus on whether COC production is constitutive or induced (Ward *et al*., 1997; Molano-Flores, 2001; Ruiz *et al*., 2002), but the results are rather contradictory. Although defensive mechanisms might vary among different plant systems (Sanchez-Alonso and Lachica, 1988), results in this study did not totally support the view that COC might just be a compensatory defence of young leaves (Finley, 1999) despite the fact that the COC density in young leaves was significantly higher than in mature leaves, which supported Finley's results. Under herbivory damage, the COC of *F. altissima* increased without an increase in total Ca content. This indicated that the Ca resource for COC synthesis might come from the internal environment and supported the view that COC synthesis was an active process (Webb, 1999). The COC production in *F. auriculata* was not increased by artificial herbivory damage and *F. hispida* was even reduced by it, which obscured the current hypothesis that COC might have a defensive function.

In conclusion, herbivory damage in the four species studied greatly varied. The pioneer species, *F. hispida*, suffered the most severe herbivory damage, while the late successional species, *F. altissima*, had the least damage. The combination of several characteristics, i.e. high contents of N, Ca and P and low leaf toughness, lifetime and C : N ratios, were ascribed to a high degree of herbivory resistance. The late successional species, *F. altissima*, might also incorporate induced defence strategies by means of an increase in leaf COC and toughness.

ACKNOWLEDGEMENTS

We thank Wei-Xiu Li, Qing Dao, Zhi-Lin Bai and Ling Zhang, for their help in field investigations and experiments, and Yun Fu for her help with leaf chemical analyses. Jin Chen also thanks C. C. Horvitz for her constructive discussions on part of the experiment design. This study was supported by a grant from the Chinese Academy of Sciences (KSCX2-SW-105).

LITERATURE CITED

Aide TM. 1992. Dry season leaf production and escape from herbivory. *Biotropica* **24**: 532–537.

Aide TM. 1993. Patterns of leaf development and herbivory in a tropical understory community. *Ecology* **74**: 455–466.

Alonso C, Herrear C. 2000. Seasonal variation in leaf characteristics and food selection by larval noctuids on an evergreen Mediterranean shrub. *Acta Oceologica* **21**: 257–265.

Belsky AJ. 1986. Does herbivory benefit plants? A review of the evidence. *American Naturalist* **127**: 870–892.

Berg CC. 1990. Reproduction and evolution in *Ficus* (Moraceae): traits connected with the adequate rearing of pollinators. *Memoirs of the New York Botanical Garden* **55**: 169–185.

Bigger DS, Marvier MA. 1998. How different would a world without herbivory be? A search for generality in ecology. *Integrative Biology* **1**: 60–67.

Bryant JP, Chapin FS, Klein DR. 1983. Carbon nutrient balance of boreal plants in relation to vertebrate herbivory. *Oikos* **40**: 357–368.

Coley PD. 1983. Herbivory and defensive characteristics of tree species in a lowland tropical forest. *Ecological Monographs* **53**: 209–233.

Coley PD. 1988. Effects of plant growth rate and leaf lifetime on the amount and type of antiherbivory defense. *Oecologia* **74**: 531–536.

Coley PD, Bryant JP, Chapin FS. 1985. Resource availability and plant antiherbivore defense. *Science* **230**: 895–899.

Cornelissen GT, Fernandes WG. 2001. Induced defences in the neiotropical tree *Bauhinia brevipes* (Vog.) to herbivory: effects of damage-induced changes on leaf quality. *Trees* **15**: 236–241.

Coviella CE, Robert DS, Trumble JT. 2002. Plant allocation to defensive compounds: interactions between elevated CO_2 and nitrogen in transgenic cotton plants. *Journal of Experimental Botany* **53**: 323–331.

Crawley MJ. 1997. Plant-herbivore dynamics. In: Crawley MJ, eds. *Plant ecology, 2nd edn.* Oxford: Blackwell Science, 401–474.

Dudt JF, Shure DJ. 1994. The influence of light and nutrients on foliar phenolics and insect herbivory. *Ecology* **75**: 86–98.

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.

Filip V, Dirzo R, Maass JM, Sarukhán J. 1995. Within- and among-year variation in the levels of herbivory on the foliage of trees from a Mexican tropical deciduous forest. *Biotropica* **27**: 78–86.

Finley DS. 1999. Patterns of calcium oxalate crystals in young tropical leaves: a possible role as an anti-herbivory defense. *Revista de Biologia Tropical* **47**: 27–31.

Futuyma DJ. 2000. Some current approaches to the evolution of plant-herbivore interactions. *Plant Species Biology* **15**: 1–9.

Herms DA, Mattson WJ. 1992. The dilemma of plants: to grow or defend. *Quarterly Review of Biology* **67**: 283–335.

Kalra YP, Maynard DG. 1991. *Methods manual for forest soil and plant analysis.* Alerta Canada: Forestry Canada, Northwest Region, Northern Forestry Center.

Lehtila K, Strauss SY. 1999. Effects of foliar herbivory on male and female reproductive traits of wild radish, *Raphanus raphanistrum*. *Ecology* **80**: 116–124.

Lepš J, Novotný V, Basset Y. 2001. Habitat and successional status of plants in relation the communities of their leaf-chewing herbivores in Papua New Guinea. *Journal of Ecology* **89**: 186–199.

McNaughton SJ. 1986. On plants and herbivores. *American Naturalist* **128**: 765–770.

Marquis RJ, Diniz IR, Morais HC. 2001. Patterns and correlates of interspecific variation in foliar insect herbivory and pathogen attack in Brazilian cerrado. *Journal Tropical Ecology* **17**: 127–184.

Molano-Flores B. 2001. Herbivory and concentrations affect calcium oxalate crystal formation in leaves of Sida (Malavaceae). *Annals of Botany* **88**: 387–391.

Perera CO, Hallett IC, Nguyen TT, Charles JC. 1990. Calcium oxalate crystals: the irritant factor in kiwifruit. *Journal of Food Science* **55**: 1066–1069.

Price PW, Bouton CE, Gross P, McPheron BA, Thompson JN, Weis AE. 1980. Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. *Annual Review of Ecological Systems* **11**: 41–65.

Rosenthal GA, Janzen DH. 1979. *Herbivores: their interaction with secondary plant metabolites*. New York: Academic Press.

Ruiz N, Ward D, Saltz D. 2002. Calcium oxalate crystals in leaves of *Pancratium sickenbergeri*: constitutive or induced defence? *Functional Ecology* **16**: 99–105.

Sanchez-Alonso, Lachica M. 1988. Oxalate salts in the leaves of plum (*Prunus salicina* L.) and cherry (*P. avium* L.). *New Phytologist* **108**: 505–508.

Southwood TRE, Brown VK, Reader PM. 1986. Leaf palatability, life expectancy and herbivore damage. *Oecologia* **70**: 544–548.

Stamp NE, Casey TM. 1993. *Caterpillars: ecological and evolutionary constraints on foraging*. New York: Chapman & Hall.

Sunell LA, Heley PL. 1985. Distribution of calcium oxalate crystal idioblasts in leaves of taro (*Colocasia esculenta*). *American Journal of Botany* **72**: 1854–1860.

Underwood N, Rausher M, Cook W. 2002. Bioassay versus chemical assay: measuring the impact of induced and constitutive resistance on herbivores in the field. *Oecologia* **131**: 211–219.

Ward D, Spiegel M, Saltz D. 1997. Gazelle herbivory and interpopulation differences in calcium oxalate content of leaves of a desert lily. *Journal of Chemical Ecology* **23**: 333–346.

Webb AW. 1999. Cell-mediated crystallization of calcium oxalate in plants. *Plant Cell* **11**: 751–761.

Williams K, Field CB, Mooney HA. 1989. Relationships among leaf construction cost, leaf longevity, and light environment in the rain forest plants of the genus *Piper*. *American Naturalist* **133**: 198–211.

Wold EN, Marquis RJ. 1997. Induced defense in white oak, effects on herbivores and consequences for the plant. *Ecology* **78**: 1356–1369.

Xu ZF, Tao GD. 1988. *Tropical wild flowers and plants in Xishuangbanna*. Beijing: Agricultural Press.

Xu ZF, Zhu H, Yang DR, Ying SH, Wang H, Li BG. 1996. Species diversity and ecological significance of figs in tropical rainforests of Southern Yunnan. In: *Collected research papers on tropical botany, Vol. IV*. Kunming: Yunnan University Press, 1–15.

Zangerl AR, Arntz AM, Berenbaum MR. 1997. Physiological price of an induced chemical defense: photosynthesis, respiration, biosynthesis, and growth. *Oecologia* **109**: 433–441.