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\textbf{CORRELATIONS BETWEEN LEAFING PHENOLOGY AND TRAITS: WOODY SPECIES OF EVERGREEN BROAD-LEAVED FORESTS IN SUBTROPICAL CHINA}

\textbf{ABSTRACT:} Leaf phenology has been considered to evolve to maximize plant carbon gains, and it reflects plant life history strategies. To determine the effect of leaf traits on leaf phenology, leaf emergence (such as timing of leaf emergence, leaf expansion rate, durations of leaf expansion), leaf traits (leaf mass per area – LMA and leaf size), and their relationships were investigated for 40 woody species from 13 families in an evergreen broad-leaved forest, southeast China. Compared with understorey shrubs (23 species), trees species (17 species) were significantly later in timing of leaf emergence, greater in leaf area and leaf expansion rate. This is assumed to be a strategy for large-leaved tree species to decrease damage during leaf expansion. In terms of leaf size, the small-leaved species leafed out earlier than the species with large leaves, but the large-leaved species were greater in leaf expansion rate than their counterparts. Leaf expansion rate was positively correlated with leaf area and timing of leaf emergence. Leaf herbivore rate was positively correlated with leaf area and leaf expansion period, but negatively with LMA. These results suggest that large- and small-leaved species possibly employed different strategies to minimize herbivore damage. Small-leaved species avoid defoliator damage by early leafing, while large-leaved species have shorter expansion times and thereby shorter vulnerable time to herbivores. In general, dynamics of emergence and expansion of the woody species in the study forest indicate that the leaf phenology is of significance for species’ carbon gain and survival.

\textbf{KEY WORDS:} leaf area, leaf expansion, leaf emergence, herbivory, evergreen broad-leaved forest

\section{1. INTRODUCTION}

Leaf traits (\textit{e.g.} leaf size, leaf mass per area \textit{etc.}) and leaf phenology are adaptations of plants to environmental conditions, in particular, to climate factors in the sites, in a long term evolution, and that, in turn, has substantial implications for plant functioning at a leaf, whole-plant, and ecosystem level (Chabot and Hicks 1982, Coley 1988, Reich \textit{et al.} 1992, 1997). Empirical investigations have shown that the one of important aspects in leaf phenology evolution is to maximize leaf photosynthetic assimilation and minimize the loss of herbivory. It has been shown that both of physical and biotic factors can affect plant phenology to certain extent (Rathcke and Lacey 1985, Van Volkenburgh 1999; Kikuzawa 1983, Aide 1988, Moles and Westoby 2000). However, it is not very clear how the leaf traits may influence leaf phenology among plant species.

Although several studies have described the variation in leaf traits or leafing patterns...
of a broad spectrum of species of different life forms growing in a wide range of environments (Broadhead et al. 2003, Wright et al. 2004, Bertiller et al. 2005, Sekhwela and Yates 2007), only few studies analyzed simultaneously leafing patterns and leaf traits (Williams-Linera 2000, Sun et al. 2006) providing some evidence of relationships among leaf traits and leafing patterns in tree species. Addressing this topic could be important in providing possible evidence of ecological differentiation among coexisting species in subtropical zones.

In deciduous forests, plant growth was thought to be largely limited by the length of growing season. In order to increase photosynthetic capacity at the single leaf level and decreasing leaf damage by herbivores, early spring leafing can be an alternative choice, especially for understorey species (Chabot and Hicks 1982, Kikuzawa 1991). Similarly, for compensating their lower photosynthetic capacity per unit leaf mass, species with higher leaf mass per area (LMA) tend to extend more their leaf photosynthetic time by early leaf emergence and/or late leaf shedding compared to species with low LMA (Reich et al. 1991, Reich 1993, Ackerly and Reich 1999, Reich et al. 1999, Westoby et al. 2002, Wright et al. 2004). In evergreen broad-leaves forest, however, plant species have each specific leaf spans ranging from less than one year to more than five, and must have leaves which can endure winter coldness (Nitta and Ohsawa 1997). Therefore, understorey species can not get growth advantage by earlier leaf development. Farnsworth and Ellison (1996) found under dim light, that red-mangrove seedlings showed lower light compensation and saturation points than saplings or adults, suggesting compensation for lack of available light in a forest understorey. To increase interception of light and decrease self-shading, canopy architecture and leaf display of evergreen species also change with tree growth from shaded forest understorey to a higher canopy layer as light conditions improve (Rich et al. 1995, Yamada and Suzuki 1996). In consequence, a different leaf emergence pattern can be expected in evergreen broad-leaved forest than in deciduous forests.

Herbivory is also one of the major selective pressures in plant evolution, especially in shaping the leaf expansion process (Coley and Barone 1996, Moles and Westoby 2000, Westoby et al. 2002). During expansion, young leaves of plants generally experience remarkably higher herbivore attacks than their mature leaves (Brenes-Arguedas et al. 2006, Kursar and Coley 2003). Young leaves are preferred by herbivores, because they are tender, nutritious and contain lower levels of secondary metabolites than mature leaves (Coley 1983, Brenes-Arguedas et al. 2006). Moles and Westoby (2000) observed that small-leaved species expanded their leaves within a shorter duration than species with large leaves, which was associated with reduced damage incurred from herbivores, and they hypothesized that shorter expansion times were more likely to be evolutionarily selected for small-leaved species to reduce leaf damage by herbivores than for large-leaved species. But there is no further evidence showing whether or not this hypothesis by Moles and Westoby (2000) is a universal rule.

In this study we investigated leaf emergence patterns and leaf traits of woody species in an evergreen broad-leaved forest in southeast China. Timing of leaf emergence, duration of leaf expansion, and leaf expansion rate were measured and analyzed in relation of leaf traits and herbivory rate. This study intends (1) to clarify the pattern of leaf emergence and the interrelationships of leaf emergence with other leaf traits in an evergreen broad-leaved forest, and (2) to examine whether species with small leaves take less time to expand a leaf for reducing damage from insect herbivores.

2. 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, southeast China, with subtropical evergreen broad-leaved forest as zonal vegetation. In the study site, mean annual precipitation is about 1700–2200 mm, of which approximate 70% occurs from March to June. Mean annual temperature is 13–18°C, with extremes of monthly means being 7.5–8.3°C in the coldest January and 22.9–23.8°C in the warmest July. As a result of this seasonality, the vegetation consists
primarily of a subtropical evergreen broad-leaved forest. The soil type in the site is the forest brown soil according to the soil classification system of China.

3. METHODS

In this study the woody plants were divided into two functional groups, trees and shrubs, due to difference in ecological niches. Totally 40 woody plant species of 13 families were selected as sample plants in the plot constituting 75% of total woody plant species. The most highly represented plant families include Fagaceae, Lauraceae and Theaceae. Among these species, tree species is dominated by *Schima superba* Gardn. et Champ. (Theaceae), *Castanopsis fabric* Franch. (Fagaceae), *C. eyrei* (Champ.) Tutch. (Fagaceae), *Cyclobalanopsis glauca* (Thunb.) Oerst. (Fagaceae) and some others, with an average canopy height of 16.2 m. Understorey shrub layer include *Camellia fraternal* Hance (Theaceae), *Eurya muricata* Dunn (Theaceae), *Ilex purpurea* Hassk. (Aquifoliaceae), *Symlocos sumuntia* Bunch.-Ham. (Symplcaceae) and so on. Average plant height of the understorey shrub species varies between about 0.5 and 1.2 m. All the woody plant species cited above are indigenous broad-leaved evergreen species in the study area.

For the sampled tree species, their crowns were in the upper canopy and were exposed directly to sunshine with little shading by other trees. For the sampled shrub species, they were mature individuals, regardless of the light attenuation level above the plant crown. For each species at least 8 indviduals were located and five random branches with tips on the outer edge of the plant crown were selected. Young leaves were sequentially numbered upward from the base part of the branch.

The investigation of leaf emergence started from early March 2006. The number of leaves present was recorded at 3-day interval. In this study, for a plant individual, timing of leaf emergence was identified when 10% of the buds on the branches leafed out; leaf emergence was considered to stop when the number of leaves was not changed during the three successive observation days. Leaf appearance was defined as the date when the lamina separated from the shoot axis at a length of about 0.5 cm, observed using field glasses. The leaves mostly were sampled at the upper crown of the plants. For the plant individuals taller than we could reach, 6-m high aluminium ladders were used so that leaf number could be conveniently recorded. We only sampled leaves at mid-crown positions for those individuals of canopy species for which the upper canopy was unreachable by using the ladder.

In order to quantify average area per leaf and leaf expansion rates throughout the growing season in 2006, we randomly chose six leaves per branch and measured their length and width to an accuracy of 0.1 m using vernier calipers. These measurements were taken during recording leaf number. Leaves were considered to have ceased to expand when their length and width had remained unchanged for three successive observation days. Leaf area was calculated subsequently from non-destructive leaf length and width measures, from regressions of leaf area on leaf length times width obtained for a sample of 100 randomly collected leaves from each species ($R^2$ between 0.942–0.986). The mean leaf area was considered to represent the leaf area at maturity for each species.

In addition, at the end of leaf expansion, between 2–8 weeks depending on the species, 200 mature leaves of each species were sampled from the same branches used for the leaf emergence investigation and brought to the laboratory. Leaf area was measured using a digital area meter (Li-3000, USA). The area of leaf eaten by insects on these leaves was also measured with a plastic grid to determine the percent of leaf area eaten while young. Missing leaves were scored as 100% damage. Plants or leaves that were obviously damaged by falling debris were not included. Subsequently, these leaves were dried to constant (after drying at 70°C for 48 hours). LMA was calculated by dividing leaf dry mass by leaf area for each leaf, and mean LMA was used to indicate the species’ characteristics.

Timing of leaf emergence of a species was calculated as the period from the March 1 to the date of leaf emergence of the species. Duration of leaf emergence for a species was calculated from the date the species leafed out to the date the species ceased leaf emergence.
Duration of leaf expansion was defined as the duration from the date the species leafed out to the date the species ceased expanding. Leaf expansion rate was calculated by dividing the mean leaf area by duration of leaf expansion. Before analysis, all data were tested for normal distribution. Student’s t-test for independent variables was employed to test the difference in leaf area, LMA, and leaf expansion rate between canopy trees and understory shrubs. The non-parametric Mann-Whitney U-test was used to compare timing of leaf emergence, duration of leaf emergence, and duration of leaf expansion, because those data were not normally distributed.

We chose 40 woody species with a view to forming a phylogenetic tree having only dichotomous branching (Fig. 1). The correlations between different functional traits and cross-correlated evolutionary divergences were determined with Phylogenetically Independent Comparative Methods (PIC) of Compare, version 4.6b (http://compare.bio.indiana.edu/). The calculation method followed Martins (2004). A phylogenetic tree was constructed following ECCAS (1974–1999). Regression of evolutionary divergence data used standard model I techniques.

4. RESULTS

Mean value of leaf area (2143 mm²) in the canopy trees was significantly larger than that in the understory shrubs species (about 1200 mm²) (Table 1). Similarly, the canopy trees had significantly higher leaf expansion rate (57.82 mm² day⁻¹) compared with the understory shrubs (30.80 mm² day⁻¹; Table 1). The understory shrubs emerged earlier about 10 days than the canopy trees.

Timing of leaf emergence varied substantially between species, ranging from late-March to mid-June of 2006, spanning over 50 days. Regression analyses showed that timing of leaf emergence was significantly related to both leaf area and LMA (Figs. 2 and 3). Leaf area was positively correlated to timing of leaf emergence in both cross-species regression analysis (Fig. 2 and Table 3) and phylogenetic regression (Table 2). The smaller the leaf area, the earlier it leafed out. Species with leaf areas less than 500 mm² leafed out in the first 5 days, while large leaved species such as *Michelia maudiae* Dunn (Magnoliaceae) leafed out when some small-leaved species finished the process of leaf emergence. In contrast, LMA was negatively correlated to timing of leaf emergence in both cross-species analysis (Fig. 3 and Table 3) and phylogenetic regression (Table 2). The larger in LMA the species was, the earlier it leafed out. Most of the species that leafed out in the first 5 days were larger than 50g m⁻² in LMA, but there are also some exceptions. Multiple regression analyses showed that timing of leaf emergence significantly depended on leaf size and LMA ($R^2 = 0.721$, $F (2, 37) = 37.752$, $P < 0.001$). No significant difference in the timing of leaf emergence was found between the canopy and understory species (Table 1). The canopy species leafed out over a relatively long period, ranging between the 4th and 20th days. But the earliest leafed species mostly is the shortest understory species such as *Adinaandra millettii* (Hook. et Arn.) Benth. et Hook. f. ex Hance (Theaceae).

Leaf expansion rate fluctuated strongly among the woody species in the evergreen broad-leaved forest. The fastest reached 92.33 mm² day⁻¹ in *Daphniphyllum macropadum*
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Franch (Ericaceae), more than 17 times that of the slowest in *Ilex purpurea* Hassk. (Aquifoliales).

Leaf area positively correlated with expansion rate, as shown by both cross-species analysis (Fig. 4 and Table 3) and phylogenetic regression analysis (Table 2). Most species had a leaf expansion rate lower than 50 mm² day⁻¹, and only a few of the species with leaf area larger than 2000 mm² expanded at a faster rate.

Timing of leaf emergence was found to have a strong positive correlation with leaf expansion rate at the cross-species level (Table 3), and also in the phylogenetic regression analysis (Table 2). The earlier the species leafed out, the slower it expanded. This is consistent with the correlation between timing of leaf emergence and leaf area, and the correlation between leaf area and leaf expansion rate. There was also a significant difference in mean leaf expansion rate between canopy trees and understorey shrubs (Table 1). The mean leaf expansion rate of canopy trees was more than 57.82 mm² day⁻¹ significantly larger than that of understorey species (Table 1).

The amount of leaf tissue lost to insect herbivores during leaf expansion was generally high, but showed substantial inter-specific variation, for instance, some species lose more than 50% of their area during the few weeks of expansion, while the others lose less than 20%. Damage rates are positively correlated with leaf expansion (Fig. 5; r² = 0.29, P < 0.01). Although species with rapid leaf expansion have a shorter window of vulnerability, they suffered significantly higher leaf loss.

Most species leafed out within a short time period of less than 10 days, and some of them even took only 4–5 days to reach their maximum leaf number. Few species had a duration of leaf emergence longer than 15 days. Duration of leaf expansion is relatively long and more than 90% of the species took more than 20 days to expand their leaves to final size. However, there was no significant difference in the durations of either leaf emergence or expansion between canopy and understorey species (Table 1). Duration of leaf emergence and expansion were not related to leaf traits and timing of leaf emergence (Table 3).

5. DISCUSSION

The present study showed that the timing of leaf emergence of woody species in an evergreen broad-leaved forest varied greatly, with the earliest occurring about 57 days in advance of the latest. The understorey species emerged leaves earlier than canopy species. Comparisons among species indicated

| Table 2. Phylogenetic regression analysis for the relationship between leaf traits and timing of emergence for the woody species in the study stand in Meihuashan, southeastern China (n = 40). |
|----------------|----------------|----------------|----------------|----------------|
| Leaf area vs. timing of leaf emergence | 1,26 | 212.43 | <0.001 |
| LMA vs. timing of leaf emergence | 1,26 | 5.87 | 0.026 |
| Leaf area vs. leaf expansion rate | 1,26 | 1017.89 | <0.001 |
| Leaf expansion rate vs. leaf herbivory rate | 1,26 | 237.36 | <0.001 |
| Timing of leaf expansion vs. leaf expansion rate | 1,26 | 12.66 | 0.002 |

| Table 3. Cross-species correlations among leaf traits for the woody species in the study stand in Meihuashan, southeastern China (n = 40). |
|----------------|----------------|----------------|----------------|----------------|
| LA | LMA | TLE | DLEm | DLEe | LER |
| LMA | -0.231 | -0.375 | | | |
| TLE | 0.555 | -0.375 | -0.375 | -0.094 | -0.055 |
| DLEm | -0.094 | 0.322 | -0.094 | -0.077 | 0.041 |
| DLEe | 0.253 | -0.106 | -0.077 | 0.412 | |
| LER | 0.253 | -0.106 | -0.077 | 0.412 | |
| LHR | 0.852 | 0.549 | -0.070 | -0.205 | 0.379 |

LA: leaf area, LMA: leaf mass per unit area, TLE: timing of leaf emergence, DLEm: Duration of leaf emergence, DLEe: duration of leaf expansion, LER: leaf expansion rate, and LHR: leaf herbivory rate (n = 40). Significance level: *** P < 0.001; ** P < 0.01; * P < 0.05; and ns: P > 0.05.
that timing of leaf emergence was related to leaf traits. Cross-species correlation analysis showed that the timing of leaf emergence was negative correlative with leaf size and LMA. Moreover, the results of phylogenetic regressions were very similar to those of cross-species regressions, indicating the relationship between leaf traits and timing of leaf emergence results from ecological adaptation of the species, but not just from the phylogenetic divergence of the species. However, there was no significant relationship found between leaf size and the length of leaf expansion period, inconsistent with the hypothesis that small-leaved species should have a shorter leaf expansion duration than species with large leaves.

Early leaf emergency has been suggested to be of great significance to understorey species (Sun et al. 2006). In the present research, we found the understorey species leafed out earlier than the tree species. Some factors

Fig. 1. Phylogenetic relations of 40 woody plants in the evergreen broad-leaved forests in south-eastern China.
may contribute to this result. First, some shade-tolerant plants may have the photosynthetic light response that are adapted to low irradiance, understorey plants can operate under elevated photo flux density by leaf out earlier. Second, early emergence for understorey species will increase the time period for photosynthesis. It can compensate for low rates of photosynthesis understorey or even allow plant to initiate photosynthesis early in the spring before conditions are suitable for leaf growth (Chabot and Hicks 1982). Moreover, the leaf value of photosynthetic capacity decreases with time (Gill et al. 1998). A plant would leaf out early in spring to extend leaf out early in spring rather than retain old leaves for a longer time.

It is widely recognized that leaf phenology has evolved to maximized leaf carbon gain (Kikuzawa 1991, Ackerly 1999). Some models on leaf phenology at the single leaf level (Chabot and Hicks 1982), at the shoot

Fig. 2. The relationship between timing of leaf emergence (day) and leaf area (mm²) for the woody plants in the study stand, south-eastern China (n = 40).

Fig. 3. The relationship between timing of leaf emergence (day) and LMA (g m⁻²) of the woody species of evergreen broad-leaved forest (n = 40).
level (Ackerly 1999), and at the whole plant level (Kikuzawa 1991, 1995) have predicted that species with higher leaf construction cost and/or low photosynthetic capacity have a longer leaf photosynthetic time. Such relationships between LMA, leaf photosynthetic capacity, and leaf photosynthetic time, have been well established among a variety of species and ecosystems (Reich et al. 1991, 1992, Reich 1993, Reich et al. 1999, Wright et al. 2004). In the present study, we found that woody species with higher LMA leafed out earlier than those with lower LMA, indicating the importance of early leaf emergence to the species with higher leaf construction cost. Cost-benefit analysis for the timing of leaf emergence has been applied to several temperate woody species (Seiwa 1999). It is suggested that early leaf emergence has both advantages (such as more carbon gain and less herbivore damage) and disadvantages including loss by frost damage. In the present study, possibly due to the low temperature or unpredictable frost in early spring, species

Fig. 4. The relationship between leaf expansion rate (mm² day⁻¹) and leaf area (mm²) of the woody species of evergreen broad-leaved forest (n = 40).

Fig. 5. The relationship between leaf expansion rate (mm² day⁻¹) and leaf herbivory rate (%) of the woody species in evergreen broad-leaved forest (n = 40).
with large leaves emerged late, while most small-leaved species emergence early. Apparently, there is a trade-off between timing of leaf emergence and expansion rate. Many species have been reported to be seriously attacked by herbivores during leaf expansion (Coley and Barone 1996). Plants are assumed to develop several strategies to cope with herbivores and one of them is to shorten leaf expansion duration by reducing final leaf size and increasing leaf expansion rate, and thus minimize the vulnerable period of exposure to herbivores (Kursar and Coley 2003, Moles and Westoby 2000). The research of Moles and Westoby (2000) has shown that the species with small leaves have a shorter expansion duration than the large-leaved species in Australia. However, in this study, we found no significant relationship between leaf expansion duration and leaf traits, similar to the result of Sun et al. (2006). In the present research, we found herbivory was positively correlated with leaf area. Plant may decrease their leaf loss by herbivores through some ways. One is through reducing their final leaf size. At the same expansion rate, small-leaved species would have shorter expansion periods than species with large leaves (Moles and Westoby 2000). Insect searching behaviour might lead them to large leaves easily than to small leaves (Feeny 1976). And small-leaved species would be exposed to a narrower spectrum of species that complete their larval development on an individual than would large-leaved species. The other way is to increase leaf expansion rate. The species that leafed out late perhaps employed this approach. Because young leaves are vulnerable to herbivores until they finish expanding and begin to toughen, rapid expansion should shorten this window and reduce herbivory (Aide and Londoño 1989). However, species with more rapid leaf expansion suffer significantly higher herbivory (Table 3, Fig. 5). This counter-intuitive result is because other traits are correlated with rapid expansion. For example, nitrogen is positively correlated with expansion rate, presumably because of increased metabolic demands within the rapidly growing leaf. And the species with fast expansion have less effective secondary metabolites.

In general, the species with higher LMA and smaller leaf size leafed out earlier than their counterparts in the evergreen broad-leaved forest. Such a combination may have an additional advantage in that it permits less energy loss when these early emerging species are under the influence of frost. This indicates that leaf size and LMA are two important factors affecting timing of leaf emergence, as suggested by the result of multi-regression analyses. The species with larger LMA need to leaf out earlier to obtain positive carbon balance. Under the conditions of frequent frost and herbivore damage, the combination of higher LMA with smaller leaf area allows less leaf loss during leaf emergence and expansion than the combination of higher LMA with larger leaf area. The phenomenon that the species with larger LMA but smaller leaves leafed out earlier may result from such an evolutionary balance between carbon gain and carbon loss.

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6. REFERENCES

ondary metabolite accumulation during leaf development in two tropical tree species with different leaf expansion strategies – Oecologia, 149: 91–100.


Martins E.P. 2004 – COMPARE, version 4.6b. Computer programs for the statistical analysis of comparative data. Distributed by the author at http://compare.bio.indiana.edu/. Department of Biology, Indiana University, Bloomington IN, USA.


Williams-Linera G. 2000 – Leaf demography and leaf traits of temperate-deciduous and tropical evergreen-broadleaved trees in a Mexican mountain cloud forest – Plant Ecol. 149: 233–244.


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