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Leaves damaged by insect herbivory have consistently different biomechanical traits compared to undamaged leaves within individual plants

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ABSTRACT

Leaf herbivory by insects is a widespread phenomenon across ecosystems, significantly impacting plant growth, survival, and community structure and biodiversity maintenance. While past research has mainly focused on interspecific and intraspecific differences in insect herbivory and its correlation with leaf traits, intra-individual variation in the susceptibility of leaves to insect herbivory remains under-investigated. Here, we investigated 5583 leaves from 584 seedlings of 40 woody species from a subtropical forest in southwestern China, focusing on five key leaf traits: size, specific leaf area (SLA), water content, thickness, and toughness. Our results revealed significant differences in these trait properties between insect-damaged and undamaged leaves from the same individual plant, with damaged leaves being larger, thicker, and harder, but having lower SLA and water content. These patterns were consistent across species, suggesting a conserved mechanism influencing the evolution of traits resisting insect herbivory. Furthermore, our findings offer valuable insights for future research on herbivory, particularly in terms of experimental design. Relying solely on traits from intact leaves may compromise the reliability of the leaf trait effects on herbivory. Traits of damaged leaves should also be considered to ensure the robustness of herbivory research.

1. Introduction

Leaf herbivory by insects is ubiquitous in various ecosystems (Salazar and Marquis, 2012; Wang et al., 2024; Zhang et al., 2016; Herbivory Variability Network, 2023) and can directly influence the growth and survival of individual plants, particularly in the early life stages, such as seedlings (Moles and Westoby, 2004; Kulikowski et al., 2022). Furthermore, the way in which insect herbivory alters or impairs plant vigor can exert a substantial influence on plant community diversity and structure through antagonistic effects on plant competitiveness (Cardenas et al., 2014; Burkepile and Parker, 2017; Cope et al., 2021; Allbee et al., 2023).

Notable variation in the extent of leaf herbivory by insects has been observed both among and within plant species (Zhang et al., 2016; Martini et al., 2021; Herbivory Variability Network, 2023), arising from interspecific and intraspecific heterogeneity in multiple leaf traits, particularly defensive traits such as biomechanical and chemical resistance profiles (Agrawal, 2011; Caldwell et al., 2016). Plant chemical defenses include a variety of secondary metabolites, the core structures of which are predominantly terpenoids, alkaloids, benzenoids, phenylpropanoids, flavonoids, or N-containing compounds, providing plants with a variety of toxic or repellent compounds to deter insect herbivores (War et al., 2012; Wang et al., 2019; Yactayo-Chang et al., 2020). Chemical defenses may be direct, involving compounds that affect leaf tissue palatability or that inhibit the insect's growth, development or reproduction, or indirect, involving the release of volatile allelochemical compounds (i.e., Herbivore Induced Plant Volatiles, HIPVs; War et al., 2011) that can suppress herbivorous insects by attracting their natural enemies (Dicke et al., 1990; Turlings and Wäckers, 2004; Belete, 2018).

Nevertheless, these chemical defenses can be costly for plants to

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synthesize (Neilson et al., 2013; Bixenmann et al., 2016) and so plants also utilize leaf biomechanical traits (Salgado-Luarte et al., 2023), the properties of which can impose a significant obstacle to insect herbivores and often influence patterns of herbivory more strongly than leaf chemistry (Clissold et al., 2009; Salgado-Luarte et al., 2023; Zhu et al., 2024). While plant structural traits such as thorns, cuticles, and trichomes play a significant role in protecting plants from attack by larger vertebrate herbivores (Hanley et al., 2007; Balaji and Jambagi, 2024), simpler leaf properties can substantially deter insect herbivores. For example, smaller leaves are often less vulnerable to insect herbivory than larger ones, and this pattern may be explained by two complementary mechanisms: (1) larger leaves provide greater feeding efficiency and better shelter for herbivores (Brown and Lawton, 1991; Harrison et al., 2018; Zhu et al., 2024); and (2) according to the plant apparency theory (Feeny, 1976), larger leaves are more conspicuous and thus more easily detected by herbivores, resulting in higher feeding pressure. Plant species with a larger specific leaf area (SLA) exhibit faster growth rates and are often more palatable due to a trade-off between investment in rapid growth (associated with larger SLA) and investment in chemical defenses (toxins or repellents), which can lead to more severe herbivory damage (Coley et al., 1985; Onoda et al., 2017; Agrawal, 2020). Phytophagous insects are limited by their size and feeding apparatus in their ability to penetrate, chew, and digest plant tissue (Hochuli, 1996; Clissold, 2007) and therefore generally prefer feeding on easier to access resources (Poorter et al., 2004; Zhu et al., 2024). Consequently, plants can enhance physical resistance to chewing insects by building tougher leaves (Martini et al., 2022; Gianoli et al., 2024). Tougher leaves not only slow down insect feeding rate but also hinder nutrient absorption (Clissold et al., 2009). These effects of leaf toughness on herbivory are also related to various other leaf traits. For instance, high water content generally indicates lower investment in cell wall construction (Ryser, 1996), while thinner leaves tend to be soft, making them more susceptible to insect phytophagy (Zhu et al., 2024). In response, insects have co-evolved stronger mandibular anatomy (Krenn, 2010; Silva-Sanzana et al., 2020) and adaptations to resist plant defenses (Hochuli, 1996; Endara et al., 2017), and so this plant-insect ‘arms race’ exemplifies a ‘Red Queen’ game (Van Valen, 1973; Brockhurst et al., 2014; War et al., 2018; Beran and Petschenka, 2022) driving adaptation in both taxa over millions of years.

Most previous research examining plant biomechanical resistance to insect herbivory has explored the correlation between leaf traits and herbivory intensity among plant species or among individuals of the same species (Peeters, 2007; Carmona et al., 2011; Schuldt et al., 2012; Burkepile and Parker, 2017; Zhang et al., 2023a). Moreover, most studies opt to measure the traits of intact leaves (i.e., leaves undamaged by insects) to explain the differences of herbivory (Muiruri et al., 2019; Li et al., 2021; Martini et al., 2022). At the individual plant level, regardless of whether a plant experiences high or low overall herbivory pressure, there is typically considerable variation in damage among its leaves—some leaves may sustain severe damage while others exhibit minimal or even no herbivory (Caldwell et al., 2016; Wetzel and Meek, 2019; Cosmo et al., 2021), though entire plants may be completely defoliated in some extreme cases. This raises the question of whether this is attributable to specific differences in leaf trait properties between insect-damaged leaves and undamaged leaves growing on the same plant. If so, relying solely on traits from intact leaves may compromise the reliability of the leaf trait effects on herbivory. Therefore, elucidating the trait differences among various leaves within the same plant and their impact on intraindividual herbivory intensity variations is crucial for comprehending the aforementioned issues.

In this study, we investigated a total of 5583 leaves from 584 seedlings, which are the life-stage most vulnerable to insect damage, belonging to 40 woody species in a subtropical forest in southwestern China. Focusing on five key leaf biomechanical traits (size, SLA, water content, thickness, and toughness) that are commonly used as indicators of leaf herbivory, we addressed two fundamental questions: 1) Do these selected traits explain intra-individual variation in leaf herbivory, i.e., do they systematically differ between damaged and undamaged leaves within the same plant? 2) Are the evaluated trait-herbivory relationships consistent across a broad diversity of plant species?

2. Materials and methods

2.1. Study site

This study was conducted in a subtropical forest in the Ailao Mountains in southwestern China (24°32′N, 101°01′E, altitude of 2045 m). Annual temperature averaged 11.3 °C with 1778 mm of precipitation per year. The canopy layer is dominated by the tree species *Lithocarpus hancei, Lithocarpus xylocarpus, Castanopsis wattii*, and *Schima noronhae*. Vertebrate herbivores are few in this forested region, and chewing insects are the primary herbivores consuming woody plants (Zhang et al., 2023b; Yuan and Wang, 2025); therefore, we focused exclusively on chewing insect herbivory in this study.

2.2. Experimental design

A total of 108 randomly distributed plots (1 $m \times 1$ m) were sampled in the forest, with a minimum 5-meter buffer between adjacent plots. Sampling was conducted across three periods to capture seasonal contrasts: 34 plots in June 2023 (early wet season), 36 in October 2023 (late wet season), and 38 in May 2024 (early wet season). In each plot, all woody seedlings (basal diameter <1 cm with fully expanded leaves) were identified to the species level by an expert botanist (Mr. Jinhua Qi) through comprehensive morphological examination of leaves (shape, venation, margin) and stems, supported by our research group’s decade-long seedling monitoring experience in this forest (Yuan and Wang, 2025). For each individual seedling plant, the number of undamaged leaves and leaves damaged by insects were counted. For each plant, we randomly collected six undamaged leaves and six leaves damaged by chewing insects (or all available leaves if fewer than six were present per damage category). The leaves were immediately placed in sealed plastic bags and transported to the laboratory for trait measurements and herbivory estimation. Upon arrival, we added moist towels to the sealed bags containing leaves awaiting measurement to minimize dehydration during the processing period.

For each leaf, we measured leaf size, specific leaf area (SLA), thickness, toughness, and water content. All measurements were completed within three hours of collection to minimize trait changes caused by leaf detachment, particularly to prevent water loss. Importantly, paired damaged and undamaged leaves from the same individual were measured consecutively under identical conditions, ensuring any temporal changes would affect both leaf types equally. Leaf size was measured by using a digital scanner. For damaged leaves, missing tissue areas were reconstructed by interpolating intact portions from the scanned images using ImageJ version 1.53c (National Institutes of Health, USA). Leaf damage was quantified as the proportion of leaf area consumed by chewing insects. Both the thickness and toughness of each leaf were measured six times at different sites on the leaf: along both sides of the main vein (midrib), 3 sample sites (i.e., the middle of the leaf, and at one quarter and three quarters along the midrib) were selected. Each site was located between the midrib and the leaf margin while avoiding primary and secondary veins. Thickness was measured using a caliper with an accuracy of 0.01 mm. Toughness was estimated as the force required to pierce the leaf, using a digital force gauge, with an accuracy of 0.001 N (1.00 mm in diameter, Model: HP-20, HANDPI Instrument Co., LTD., Zhejiang, China). The mean value of these six measurements was then calculated to represent the properties of the entire leaf. The fresh and dry weight (drying in an oven at 65 °C to constant weight, about 72 h) for each leaf were measured using an electronic balance with an accuracy of 0.001 g. From this, the water content and SLA for each leaf were calculated, where SLA is the area of a

Table 1
Comparative statistics of leaf traits of damaged ($n = 2901$) versus undamaged ($n = 2682$) leaves from 40 species (584 individuals). For each trait, the table shows mean ± standard deviation (SD), observed range (in parentheses), and distribution overlap coefficient between damaged and undamaged leaves.

Trait	Damaged leaves Mean ± SD (Min, Max)	Undamaged leaves Mean ± SD (Min, Max)	Overlap coefficient
SLA (cm^2/g)	222.87 ± 121.09 (58.43, 1694.00)	253.84 ± 139.04 (64.67, 1711.00)	0.96
Water (%)	58.76 ± 12.31 (7.14, 93.79)	61.88 ± 12.45 (10.81, 95.83)	0.89
Size (cm^2)	15.50 ± 9.77 (0.58, 74.59)	13.87 ± 9.45 (0.52, 81.19)	0.97
Thickness (mm)	0.112 ± 0.040 (0.020, 0.285)	0.110 ± 0.040 (0.013, 0.287)	0.96
Toughness (N)	0.717 ± 0.263 (0.129, 2.451)	0.675 ± 0.271 (0.077, 1.973)	0.93

fresh leaf divided by its dry mass.

2.3. Statistical analysis

To assess differences in leaf traits between insect-damaged and undamaged leaves, we fitted Bayesian phylogenetic mixed models with Gaussian distributions using the “brm” function in the “*brms*” package (Bürkner, 2018). Prior to analysis, we evaluated pairwise correlations among all leaf traits (leaf size, thickness, toughness, water content, and SLA) using Pearson’s correlation analysis. All traits exhibited significant correlations ($P < 0.05$), except for toughness and water content ($P > 0.05$). To avoid multicollinearity, we analysed each variable separately rather than employing multivariate approaches. All leaf trait variables were $\log_{10}$-transformed to improve the normality of residuals, and then standardized (mean = 0, SD = 1). We incorporated phylogenetic structure by constructing a species-level tree using “V.PhyloMaker” function in the “*V.PhyloMaker*” package (Qian and Jin, 2015) and converting it into a variance-covariance matrix via “vcv.phylo” function. This matrix, along with species name and individual ID, was included as random effects in the models. For each trait, we ran four Markov chain Monte Carlo (MCMC) chains with 10,000 iterations per chain, discarding the first 5000 iterations as warm-up (total: 20,000 posterior samples per model). Model convergence was verified using the Gelman–Rubin diagnostic (all R-hat < 1.01), with default priors applied.

Furthermore, we examined the differences in leaf traits between insect-damaged and undamaged leaves for each species represented by more than 30 individuals. For each leaf trait, we conducted separate analyses using linear mixed models through the “lmer” function in

Table 2
Summary of Bayesian phylogenetic mixed models testing trait differences between damaged and undamaged leaves. Models were fit separately for each trait across 40 species (584 seedlings). Values represent posterior means with 95 % credible intervals (L95CI, U95CI) and effective sample sizes (ESS).

Parameter	Estimate	SE	L95CI	U95CI	Rhat	Bulk ESS	Tail ESS
Size	0.16	0.03	0.11	0.22	1.00	29987	15530
SLA	−0.28	0.03	−0.33	−0.22	1.00	26771	15251
Water	−0.24	0.03	−0.29	−0.19	1.00	44034	14749
Thickness	0.05	0.02	0.02	0.08	1.00	27971	15237
Toughness	0.15	0.02	0.11	0.20	1.00	33205	14158

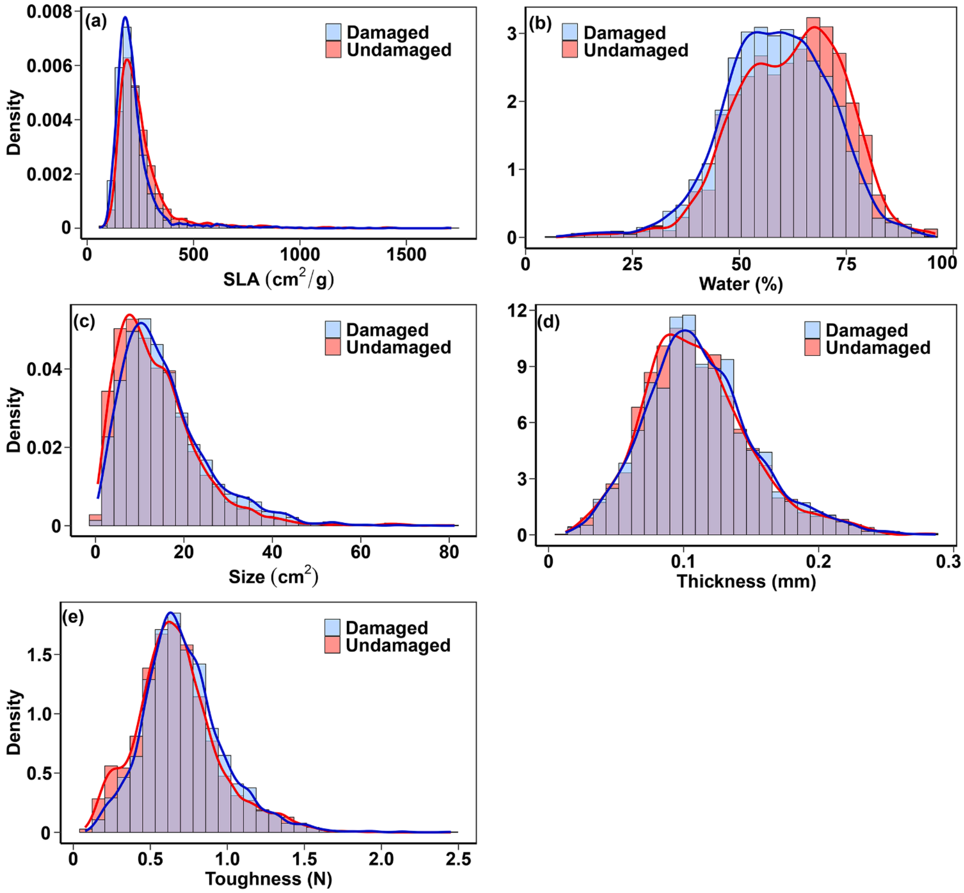

Fig. 1. Frequency distributions of (a) specific leaf area (SLA), (b) water content, (c) leaf size, (d) thickness, and (e) toughness comparing damaged versus undamaged leaves.

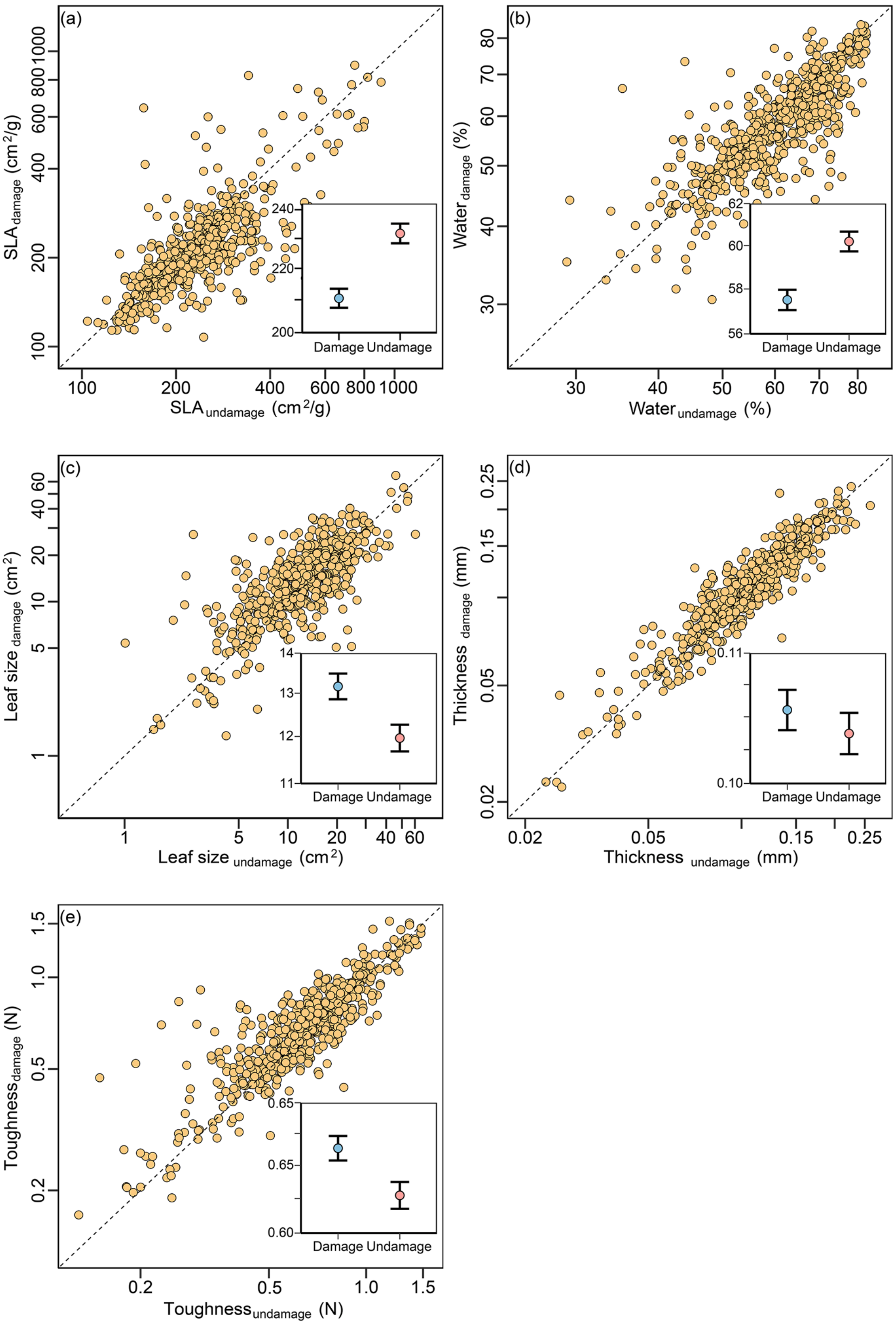

Fig. 2. Within-individual trait differences between damaged and undamaged leaves: (a) leaf size, (b) leaf thickness, (c) leaf toughness, (d) leaf water content, and (e) specific leaf area (SLA). All values are presented on $\log_{10}$-transformed scales. Dotted lines represent $y = x$. Inset plots in the bottom right of each panel display the mean ± SE for damaged and undamaged leaves.

package "*lme4*" (Bates et al., 2015), with individual ID as a random effect. All statistical analyses were performed using R version 4.2.2 (R Core Team, 2022).

3. Results

Overall, we investigated a total of 5583 leaves (2901 damaged, 2682 undamaged) from 584 individuals across 40 woody species (Table S1). All five leaf traits exhibited substantial variation in both damaged and undamaged leaves (Table 1). Notably, frequency distribution overlap coefficients (ranging 0–1) between damaged and undamaged leaves were exceptionally high for all traits: SLA (0.96), water content (0.89), size (0.97), thickness (0.96), and toughness (0.93), indicating nearly identical trait distributions regardless of damage status (Fig. 1). Furthermore, we conducted parallel analyses focusing on each species represented by more than 30 individuals. These species-level examinations revealed patterns remarkably consistent with the overall trends: (1) all dominant species maintained substantial trait variation in both damaged and undamaged leaves (Table S2), and (2) exhibited similarly high overlap coefficients across all measured traits (Figure S1).

Table 3

Summary of linear mixed models (LMM) analyzing leaf trait differences between damaged and undamaged leaves across five dominant species (each with sample size > 30 individuals). Separate models were fitted for each trait.

Variables	Mean	SE	*t*-value	*P*-value
Daphne papyracea				
Leaf size	0.027	0.038	0.706	0.483
SLA	−0.057	0.013	−4.259	**<0.001**
Water	−0.006	0.004	−1.449	0.154
Thickness	0.019	0.007	2.656	**0.011**
Toughness	0.027	0.010	2.857	**0.007**
Litsea elongata				
Leaf size	0.044	0.015	3.029	**0.004**
SLA	−0.028	0.007	−3.818	**<0.001**
Water	−0.023	0.007	−3.172	**0.003**
Thickness	0.001	0.008	0.082	0.935
Toughness	0.016	0.006	2.664	**0.010**
Machilus amblei				
Leaf size	−0.003	0.018	−0.156	0.876
SLA	−0.025	0.011	−2.199	**0.031**
Water	−0.012	0.007	−1.642	0.104
Thickness	0.004	0.007	0.525	0.601
Toughness	0.036	0.012	3.123	**0.002**
Stewartia teropetiolata				
Leaf size	0.094	0.022	4.277	**<0.001**
SLA	−0.064	0.012	−5.229	**<0.001**
Water	−0.023	0.005	−4.914	**<0.001**
Thickness	0.017	0.008	2.056	**0.045**
Toughness	0.012	0.008	1.470	0.147
Symplocos ramosissima				
Leaf size	0.122	0.026	4.715	**<0.001**
SLA	−0.056	0.015	−3.632	**0.001**
Water	−0.015	0.007	−2.069	**0.043**
Thickness	0.011	0.010	1.052	0.297
Toughness	0.013	0.009	1.439	0.156

When all individuals were analyzed in combination, SLA and water content of damaged leaves were significantly lower than that of undamaged leaves (Table 2; Fig. 2a, b). In comparison, damaged leaf size, thickness, and toughness were all significantly greater than for undamaged leaves (Table 2; Fig. 2c-e).

When each of the five species represented by more than 30 individuals was analyzed separately, similar patterns were detected to the overarching pattern (Table 3). Damaged leaves had a smaller SLA than undamaged leaves in all five of these species (Fig. 3a). Damaged leaves had a lower water content but were of a larger size than undamaged leaves in *Litsea elongata, Stewartia pteropetiolata*, and *Symplocos ramosissima*, but not in *Daphne papyracea* and *Machilus gamblei* (Fig. 3b, c). Damaged leaves were thicker than undamaged leaves in *Stewartia pteropetiolata* and *Daphne papyracea*, but not in the other three species (Fig. 3d). Damaged leaves were harder than undamaged leaves in *Daphne papyracea, Litsea elongata*, and *Machilus gamblei*, but not in the other two species (Fig. 3e).

4. Discussion

Our results revealed substantial variation in all five leaf traits in both damaged and undamaged leaves. Addressing our first research question, we found significant differences in trait values between damaged and undamaged leaves. Compared to the undamaged leaves, leaves damaged by insect herbivory were larger, thicker, and harder, but with a smaller SLA and lower water content. These patterns remained robust when examining our second question - analyses combining all species as well as separate assessments of more abundant species yielded similar results, indicating that herbivory-resistant traits exhibit consistent patterns across species, although we note some exceptions where statistical significance was not achieved.

Two potential mechanisms may explain the different traits of undamaged vs. damaged leaves 1) that insects were selecting for fundamental constitutive differences in leaf biomechanical traits that existed prior to any herbivory and preferred (i.e., found it more cost effective) to feed on leaves with certain pre-existing properties; or 2) that all leaves have similar traits prior to any insect herbivory damage and that the differences we detected were induced in response to attack by insects (Fürstenberg-Hägg et al., 2013). Induced plant defense mechanisms present the advantage of only incurring a cost to the plant when needed; however, damage will already have begun at this point.

Our findings strongly support the constitutive nature of leaf physical traits, as evidenced by two critical observations. First, we detected substantial trait variation among both damaged and undamaged leaves (Table 1), demonstrating that natural heterogeneity exists independent of herbivory. Second, and more conclusively, the frequency distributions of all measured traits showed exceptionally high overlap between damaged and undamaged leaves (Fig. 1) - with overlap coefficients exceeding 89 % for each trait. This remarkable consistency in trait distributions strongly indicates that herbivores are selectively foraging on leaves with particular pre-existing characteristics, rather than inducing trait changes through their feeding activity. These patterns align with the fundamental structural basis of key biomechanical traits like toughness, which are primarily determined by fixed developmental processes such as cell wall cellulose and lignin deposition (Onoda et al., 2011). The maintenance of similar trait distributions between damaged and undamaged leaves, despite significant mean differences, provides compelling evidence that resource allocation to mechanical resistance traits is largely constitutive and not substantially altered post-herbivory.

Indeed, plants may produce a proportion of more palatable sacrificial leaves to enable them to resist insect herbivore damage. Tougher, more robust leaves may present a mechanical constraint on insect feeding, wearing down insect mandibles (Raupp, 1985; Clissold, 2007) and exerting substantially higher energy demands on leaf-cutting ants (Roces and Lighton, 1995). The constitutive biomechanical properties of tough leaves may also indirectly deter phytophagy through the dilution of leaf nutrients; for example, proteins may be less available due to binding with lignin (Read and Stokes, 2006). In turn, this reduced leaf digestibility may affect insect diet selection (Wright and Illius, 1995; Hochuli, 1996) and slow down insect feeding rates, increasing insect vulnerability to abiotic stress and natural enemies (Hoffman and McEvoy, 1986; Clissold et al., 2009; Shelake et al., 2024). However, our results showed that leaves with greater toughness, thickness, and lower SLA were more likely to be consumed by insect herbivores, which contradicts common expectations about which leaves are most susceptible to herbivory. While thick, tough, and low-SLA leaves often have longer life spans (Reich et al., 1992; Kitajima et al., 2012; Onoda et al., 2017) that could theoretically lead to more cumulative damage over time, we emphasize that this interpretation remains speculative in our study due to the lack of direct leaf lifespan measurements. Moreover, our inability to identify the herbivore species prevents us from distinguishing between alternative explanations, such as: 1) specialized insect adaptations for processing tough leaves, or 2) herbivore foraging strategies that preferentially target longer-lived leaves independent of their mechanical properties. Notably, larger leaves were damaged more frequently than smaller leaves in our study, consistent with the apparency hypothesis (Feeny, 1976)—larger leaves are more easily detected by insects. The observed association between herbivory and leaf thickness, toughness, and SLA may simply reflect the effect of leaf size on herbivory, as all these variables were correlated with leaf size.

We acknowledge that our study has limitations that constrain the interpretation of these results. First, we did not identify the insect herbivore species, which limits our understanding of their feeding habits (e.g., generalists vs. specialists) and their potential associations with plant defense strategies. This gap hinders our ability to directly link the observed leaf trait patterns to the apparency theory (Feeny, 1976), which distinguishes between quantitative defenses (common in apparent plants) and qualitative defenses (common in unapparent plants) and their differential effectiveness against generalist vs. specialist herbivores. Second, we did not estimate leaf age of sampled

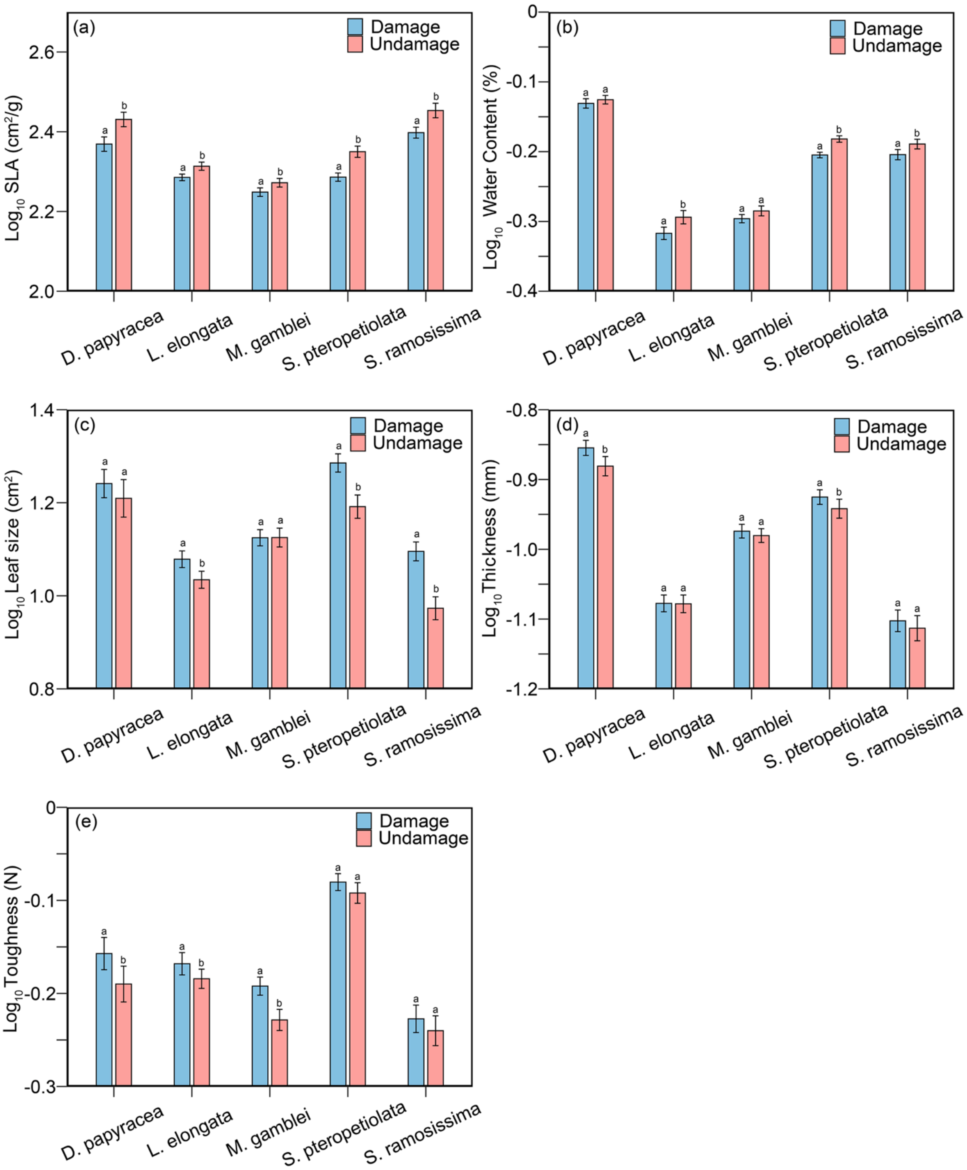

Fig. 3. Comparisons of the leaf size (a), leaf thickness (b), leaf toughness (c), water content (d), and SLA(e) between damaged and undamaged leaves across five more abundant (>30 individuals) species (*Daphne papyracea, Litsea elongata, Machilus gamblei, Stewartia pteropetiolata*, and *Symplocos ramosissima*). Mean ± SE.

leaves, which could influence trait variation and herbivory risk. Leaf age is often correlated with biomechanical traits, e.g., increasing toughness with maturity (Onoda et al., 2011), and could independently affect herbivore preferences, potentially confounding the relationships we observed. Future studies that incorporate insect species identification, leaf age estimation, and leaf positional data would greatly enhance our understanding of the mechanisms driving intra-individual variation in herbivory.

Five leaf traits were selected in this study; however, notable within-individual variation has also been detected in many other leaf traits, such as leaf morphology (Herrera, 2017), leaf nutrient composition (Wetzel et al., 2016), condensed tannins (Eisenring et al., 2021), and trichome density (Wetzel and Meek, 2019). Similar substantial within-individual variation in functional traits also occurs in other plant organs, such as flowers, fruits, and seeds (Shimada et al., 2015; Dai et al., 2016; Palacio et al., 2017; Valdés and Ehrlén, 2024), indicating that within-individual trait variation may be a general phenomenon. This type of intrinsic heterogeneity in anatomical and life-history traits is strongly associated with population resilience under changing selection pressure (Westerband et al., 2021), where plants show considerable (and variable) capacity for phenotypic adjustment of mechanical traits, potentially allowing phenotypic flexibility in both time and space.

Furthermore, our findings offer valuable insights for future research on herbivory, particularly in terms of experimental design. Simply assessing the overall insect herbivory intensity of a plant by randomly selecting leaves and calculating the average may yield biased results, as herbivory levels can vary significantly among leaves within the same plant. Therefore, a more comprehensive approach is needed, incorporating additional metrics such as the proportion of leaves with herbivory to the total number, median herbivory intensity, range, coefficient of variation, and skewness coefficient of the herbivory intensity distribution. Additionally, the variability in leaf traits within an individual plant should not be overlooked. Leaf traits themselves exhibit considerable variation, and thus similar considerations should be given to them in experimental design. Many studies examining the impact of leaf traits on herbivory intensity rely on measuring traits from leaves that have not been eaten by insects. However, our results reveal significant differences in traits between damaged and intact leaves. Therefore, relying solely on traits from intact leaves may compromise the reliability of study results, potentially leading to conflicting conclusions. The traits of damaged leaves should also be taken into account to ensure the accuracy and robustness of herbivory research.

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CRediT authorship contribution statement

Yang Liu: Writing – original draft, Visualization, Software, Investigation, Formal analysis, Data curation. **Jifa Cui:** Writing – review & editing, Writing – original draft, Formal analysis. **Bo Wang:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Bo Wang reports financial support was provided by National Natural Science Foundation of China (32171533). Bo Wang reports financial support was provided by National Natural Science Foundation of China (31971444). Bo Wang reports financial support was provided by Anhui Provincial Natural Science Foundation (2208085J28). If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.flora.2025.152857.

Data availability

Data will be made available on request.

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