

ANALYSIS OF LEAF TOLERANCE TO MIDVEIN DAMAGE BY A SIMPLE MIDVEIN CUT-OFF TRIAL

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ABSTRACT

Venation type is an important trait for terrestrial plant leaves. Especially the midvein plays a crucial role in water and nutrient transportation and correlates with leaf tolerance to physical damage. In our study, we explored the leaf tolerance to midvein damages in 95 terrestrial species by a simple midvein cut-off trial. Within three months, no detectable changes of leaf intactness were found for over two-thirds of the selected species. In two species, *Acer rubrum* and *Hylotelephium erythrostictum*, over 10 % of the leaf area was damaged by the end of the experiment. The diameter of the midvein and the leaf length showed a significantly positive correlation with the ratio of lamina damaged area to the total area (RLD), but the correlation became insignificant after including the phylogenetic relationships. The RLD varied considerably within each venation type (netted or parallel) while showed no significant difference and did not differ significantly between the two venation types; however, the RLD tended to be higher in herbaceous plants than in shrubs and trees. Our results indicated that the leaf tolerance to physical damages on the midvein of terrestrial plants was a trait somehow independent of phylogenetic relationships.

Keywords terrestrial plants; *Acer rubrum*; *Hylotelephium erythrostictum*; midvein damage; leaf tolerance; venation type

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INTRODUCTION

Leaf is an essential photosynthetic organ and crucial to carbon fixation. There are two extremes on the leaf economics spectrum: one featuring thin leaves with a short life span and a high photosynthesis rate, the other featuring thick leaves with a long lifespan, but a low photosynthesis rate (Wright *et al.*, 2004).

Besides the variation in leaf economic traits in terrestrial plants, the leaf structure is also diversified, e.g. compound leaf versus a single leaf, and the vein type can be netted, parallel or radical. The leaf venation is a key factor for species taxonomy (Yu and Chen, 1991) and is a core component of water and nutrient transportation (Zwieniecki *et al.*, 2002). Furthermore, the leaf venation network plays a critical role in the leaf functional traits based on research on over 2000 species (Blonder *et al.*, 2011), and the vein density is an essential characteristic of leaf venation. Additionally, the midvein, sometimes called the major vein, has demonstrated to affect the leaf functional traits (Sack *et al.* 2012; Sack and Scofoni, 2013). The vein scaling relationship describes that the thicker the midvein, the larger the expected leaf area (Sack *et al.*, 2012).

The midvein serves as the backbone of the leaf venation network. Previous studies mainly focused on the role of the midvein in intact leaves (Wright *et al.*, 2004;

Sack *et al.*, 2012) however, in natural conditions, the leaves commonly suffer from physical damages. For example, the grazing of insects would induce significant alterations in the leaf structure, and these effects are related to leaf morphological traits (Edwards and Wratten, 1983; Brown and Lawton, 1991). The tolerance to midvein damages could be essential to leaf survival and photosynthesis. For instance, Harayama (Harayama *et al.* 2019) detected that the blockage of the major vein induced a rapid decline in stomatal conductance in the vine species *Pueraria lobata*. Here, we aim to find the responses of terrestrial plant leaves to physical midvein damage (simulated by a midvein cut-off) and to investigate the potential correlation between plant traits (such as leaf morphology and plant life forms) and leaf tolerance to external damages based on the research on over 90 terrestrial species with the hypothesis that the midvein damage would induce significantly adverse effects on leaf growth and intactness.

MATERIALS AND METHODS

Initially, 104 single leaf type species were treated in Wuhan Botanical Garden in September, 2018. A simple method was used to simulate the leaf damage: a cut-off of the midvein at ¼ of the total length near the leaf base was performed with a needle (shown in Figure

1) (Harayama *et al.* 2019). The cut was not sealed with cyanoacrylate as described in previous studies (Harayama *et al.*, 2019; Nardini and Salleo, 2003) in order to mimic physical damage in natural circumstances compared to a midvein blockage.. The damaged leaves were tagged with a plastic label. Five healthy and mature leaves of each species were randomly chosen to serve as replicates, other untreated leaves were used as controls. A weekly observation of the leaf growth was conducted. After three

months, 95 out of 104 species were selected for further analysis after discarding species affected by human disturbance (e.g. the removal of tags or the whole leaf). Those species consisted of 20 herbs, 39 shrubs and 36 trees. The plants could be divided according to the leaf venation type: 78 species had netted veins and 17 species had parallel veins. General information on the species is shown in Table 1.

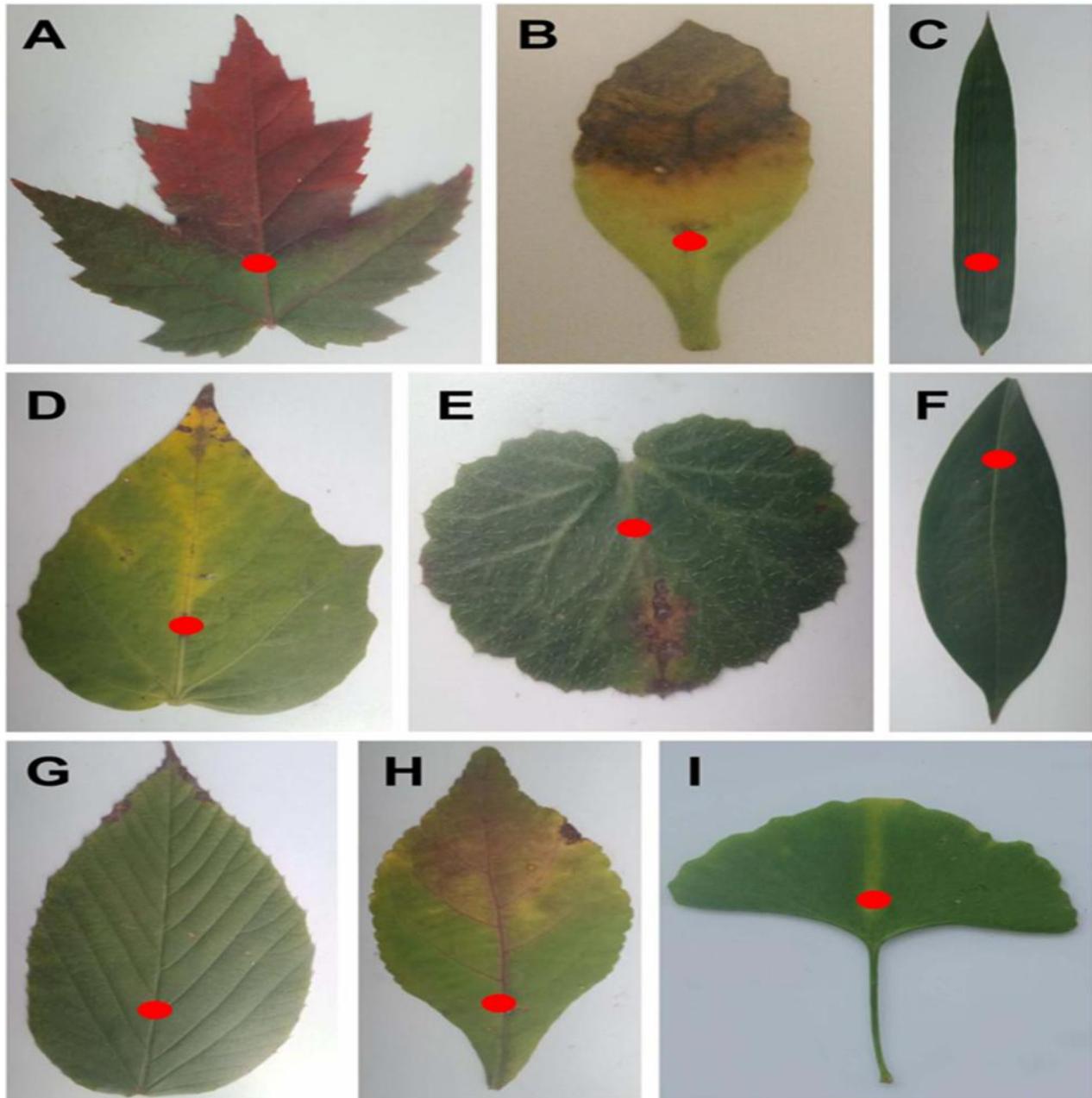


Figure 1 An example of leaves showing insignificant and serious leaf damage symptoms after the midvein cut-off. (A) *Acer rubrum*, (B) *Hylotelephium erythrostictum*, (C) *Bambusa chungii*, (D) *Hibiscus moscheutos*, (E) *Saxifraga stolonifera*, (F) *Illicium dunnianum*, (G) *Corylopsis multiflora*, (H) *Callicarpa bodinieri*, (I) *Ginkgo biloba*. The cut-off point is marked with a red dot.

Table 1. The basic information of the chosen 95 species based on APG III classification system.

Species	Family	Life form	Venation type
<i>Acer rubrum</i>	Aceraceae	tree	netted
<i>Actinodaphne forrestii</i>	Lauraceae	tree	netted
<i>Annona squamosa</i>	Annonaceae	tree	netted
<i>Arundo donax</i>	Poaceae	herb	parallel
<i>Aspidistra elatior</i>	Asparagaceae	herb	parallel
<i>Bambusa chungii</i>	Poaceae	herb	parallel
<i>Buxus henryi</i>	Buxaceae	shrub	netted
<i>Callicarpa cathayana</i>	Lamiaceae	shrub	netted
<i>Camellia cuspidate</i>	Theaceae	shrub	netted
<i>Camellia grijsii</i>	Theaceae	shrub	netted
<i>Camellia japonica</i>	Theaceae	shrub	netted
<i>Camellia oleifera</i>	Theaceae	shrub	netted
<i>Camellia sasanqua</i>	Theaceae	tree	netted
<i>Canna indica</i>	Cannaceae	herb	netted
<i>Celastrus hindsii</i>	Celastraceae	shrub	netted
<i>Celtis julianae</i>	Cannabaceae	tree	netted
<i>Celtis sinensis</i>	Cannabaceae	tree	netted
<i>Chimonanthus praecox</i>	Calycanthaceae	shrub	netted
<i>Cinnamomum camphora</i>	Lauraceae	tree	netted
<i>Cinnamomum burmanii</i>	Lauraceae	tree	netted
<i>Corylopsis sinensis</i>	Hamamelidaceae	shrub	netted
<i>Disporopsis fuscopicta</i>	Asparagaceae	herb	netted
<i>Distylium buxifolium</i>	Hamamelidaceae	shrub	netted
<i>Distylium chinense</i>	Hamamelidaceae	shrub	netted
<i>Distylium racemosum</i>	Hamamelidaceae	shrub	netted
<i>Elaeagnus lanceolate</i>	Elaeagnaceae	shrub	netted
<i>Elaeagnus pungens</i>	Elaeagnaceae	shrub	netted
<i>Eriobotrya japonica</i>	Rosaceae	tree	netted
<i>Euonymus alatus</i>	Celastraceae	shrub	netted
<i>Euonymus fortune</i>	Celastraceae	shrub	netted
<i>Fatsia japonica</i>	Araliaceae	shrub	netted
<i>Ficus tikoua</i>	Moraceae	shrub	netted
<i>Flueggea suffruticosa</i>	Phyllanthaceae	shrub	netted
<i>Gardenia jasminoides</i>	Rubiaceae	shrub	netted
<i>Gardenia jasminoides</i> var. <i>radicans</i>	Rubiaceae	shrub	netted
<i>Hainania trichosperma</i>	Malvaceae	shrub	parallel
<i>Hedera nepalensis</i> var. <i>sinensis</i>	Araliaceae	shrub	netted
<i>Hibiscus moscheutos</i>	Malvaceae	herb	netted
<i>Hosta ventricosa</i>	Asparagaceae	herb	parallel
<i>Hydrangea macrophylla</i>	Saxifragaceae	shrub	netted
<i>Hylotelephium erythrostictum</i>	Crassulaceae	herb	netted
<i>Ilex cornuta</i>	Aquifoliaceae	shrub	netted
<i>Ilex corunata</i> var. <i>fortune</i>	Aquifoliaceae	shrub	netted
<i>Ilex integra</i>	Aquifoliaceae	tree	netted
<i>Ilex latifolia</i>	Aquifoliaceae	tree	netted
<i>Illicium dunnianum</i>	Schisandraceae	shrub	netted
<i>Illicium henryi</i>	Schisandraceae	shrub	netted
<i>Indocalamus tessellatus</i>	Poaceae	herb	parallel
<i>Kolkwitzia amabilis</i>	Caprifoliaceae	shrub	netted
<i>Lindera megaphylla</i>	Lauraceae	tree	netted
<i>Liriodendron chinense</i>	Magnoliaceae	tree	netted
<i>Liriope muscari</i>	Asparagaceae	herb	parallel

<i>Litsea coreana</i> var. <i>sinensis</i>	Lauraceae	tree	netted
<i>Machilus ichangensis</i>	Lauraceae	tree	netted
<i>Machilus salicina</i>	Lauraceae	tree	netted
<i>Machilus velutina</i>	Lauraceae	tree	netted
<i>Magnolia biondii</i>	Magnoliaceae	tree	netted
<i>Manglietia patungensis</i>	Magnoliaceae	tree	netted
<i>Manglietia szechuanica</i>	Magnoliaceae	tree	netted
<i>Manglietia yuyuanensis</i>	Magnoliaceae	tree	netted
<i>Meliiodendron xylocarpum</i>	Styracaceae	tree	netted
<i>Michelia figo</i>	Magnoliaceae	shrub	netted
<i>Michelia martinii</i>	Magnoliaceae	tree	netted
<i>Mussaenda esquirolii</i>	Rubiaceae	shrub	netted
<i>Myrica rubra</i>	Myricaceae	tree	netted
<i>Nageia nagi</i>	Podocarpaceae	tree	parallel
<i>Osmanthus fragrans</i>	Oleaceae	tree	netted
<i>Osmanthus yunnanensis</i>	Oleaceae	shrub	netted
<i>Parakmeria omeiensis</i>	Magnoliaceae	tree	netted
<i>Phoebe bournei</i>	Lauraceae	tree	netted
<i>Phoebe faberi</i>	Lauraceae	tree	netted
<i>Phoebe nanmu</i>	Lauraceae	tree	netted
<i>Photinia davidsoniae</i>	Rosaceae	tree	netted
<i>Phyllostachys bambusoides</i>	Poaceae	herb	parallel
<i>Phyllostachys dulcis</i>	Poaceae	herb	parallel
<i>Phyllostachys aureosulcata</i> cv. ' <i>Spectabilis</i> '	Poaceae	herb	parallel
<i>Pittosporum tobira</i>	Pittosporaceae	shrub	netted
<i>Pleioblastus oleosus</i>	Poaceae	herb	parallel
<i>Pollia japonica</i>	Commelinaceae	herb	parallel
<i>Reineckea carnea</i>	Asparagaceae	herb	parallel
<i>Rubus parvifolius</i>	Rosaceae	shrub	netted
<i>Ruellia brittoniana</i>	Acanthaceae	herb	netted
<i>Sapium sebiferum</i>	Euphorbiaceae	tree	netted
<i>Setaria palmifolia</i>	Poaceae	herb	parallel
<i>Shibataea chinensis</i>	Poaceae	herb	parallel
<i>Sinojackia xylocarpa</i>	Styracaceae	tree	netted
<i>Symplocos tetragona</i>	Symplocaceae	tree	netted
<i>Ternstroemia gymnanthera</i>	Theaceae	shrub	netted
<i>Trachelospermum jasminoides</i>	Apocynaceae	shrub	netted
<i>Ulmus parvifolia</i>	Ulmaceae	tree	netted
<i>Viburnum brachybotryum</i>	Adoxaceae	shrub	netted
<i>Viburnum odoratissimum</i>	Adoxaceae	shrub	netted
<i>Viburnum rhytidophyllum</i>	Adoxaceae	shrub	netted
<i>Zelkova schneideriana</i>	Ulmaceae	tree	netted
<i>Zingiber officinale</i>	Zingiberaceae	herb	parallel

A digital camera (Sony ILCE-6000, Tokyo, Japan) was used to capture the form of the leaves. The images were analyzed with SC-E software (Hangzhou Wanshen Detection Technology Co., Ltd., Hangzhou, China) to measure the length, width, perimeter and area of the lamina (excluding the petiole) of ten randomly chosen healthy and mature leaves of each species (Fang *et al.* 2015). The thickness of the leaf's edge and midvein was measured by a Vernier caliper, and the dry leaf weight was measured after being oven-dried at 80 °C for 24 h. Five additional healthy and mature leaves were harvested per species and an electric body fluorescence

microscopy imaging system (Nikon SMZ25, Tokyo, Japan) was used for further analysis of the leaf vein structure (Li. 2017). The leaf vein density of only 36 species was obtained by PhenoVein following the instructions of Bühler (Bühler *et al.*, 2015). For the species that displayed almost no changes in leaf intactness at the end of the experiment, only one leaf was harvested. For the species that displayed significant leaf damage, two or more leaves were harvested. After harvest, the ratio of the damaged area (leaf damage ratio) was determined using the method mentioned above and by separately measuring the total and damaged area of

the lamina. The ratio of lamina damaged area to the total area (RLD) was also calculated. The mean values were further used in the study.

Climatic data was collected from a local weather station from 26 September to 8 December 2018. The data are shown in Figure S1 in the appendix.

The phylogenetic relationship between the chosen species based on APG III classification system was obtained using the 'PhyloMatic' function in the package 'brranching' in R 3.5.1, and the phylogeny tree from Zanne (Zanne *et al.* 2014) was used. The phylogenetic similarities between the leaf traits and the RLD of the assayed species were analyzed with the function 'multiPhylosignal' in the package 'picante'. The traits were considered phylogenetically conservative when the K-values were close to 1 or the p-values < 0.05. The correlation between the RLD and indicators of the leaf morphology, such as plant life form or venation type, was analyzed with/without the phylogenetic data. When the phylogenetic data were included, the following formula was applied: `model<-gls (damage_ratio ~ indicators, correlation = corBrownian(value = 1,phy), data = traits)`, based on the 'picante' package. The data were log-transformed to satisfy the assumption of the analysis.

RESULTS

For the 95 selected species, the lamina area ranged from 5.5 to 552.1 cm², and the leaf midvein diameter ranged from 0.376 to 5.100 mm (Table 2). A total of 73 species showed negligible changes (less than 0.1% of the lamina area) in leaf intactness after the midvein cut-off. The leaf vein density ranged from 1.92 to 9.13 mm mm⁻². The four species with an RLD over 5% at the end of the experiment are *Hosta ventricosa* (6.1%), *Hibiscus moscheutos* (6.4%), *Acer rubrum* (14.9%), and *Hylotelephium erythrostictum* (17.4%).

Leaf traits, including area, perimeter, length, width, dry weight, edge thickness, and midvein diameter, did not show significant phylogenetic signals (K < 0.25

and p > 0.05). However, the RLD displayed significant phylogenetic signals (K values > 0.3 and p < 0.01) as shown in Figure 2.

Many plant species exhibited contrasting responses after cutting off the midveins as shown in Figure 1. Results showed lamina length and midvein diameter were significantly related to the RLD when the phylogenetic data were not included, while no leaf trait was significantly correlated when the phylogenetic data were included (Table 3).

Of the 36 species with their vein density determined, five species (*Sinojackia xylocarpa*, *Meliiodendron xylocarpum*, *Magnolia biondii*, *Euonymus alatus*, and *Celtis julianae*) were found with detectable changes (> 0.1% lamina area) in leaf damage, and all had a leaf vein density < 5 mm mm⁻².

The correlation analysis of leaf venation type and plant life form revealed an insignificant effect of leaf vein types (parallel and netted) on RLD, independent of the phylogenetic data (F < 2.3, p > 0.05). Meanwhile, the RLD differed among the three plant life forms when the phylogenetic data were not included (F= 3.79, p < 0.05): the herbaceous plants had a higher RLD than the two other life forms (Figure 3). However, plant life forms no longer significantly affected the RLD when the phylogenetic data were included (F= 1.42, p > 0.05).

Table 2 The values of leaf traits (morphology and venation type) and the ratio of lamina damaged area to the total area (RLD).

Indicators	Range (median)
Lamina area (cm ²)	5.5~552.1 (114.6)
Lamina perimeter (cm)	12.9~386.8 (81.0)
Lamina length (cm)	5.7~51.9 (30.7)
Lamina width (cm)	1.2~34.6 (11.4)
Leaf dry weight (g)	0.033~4.239 (0.249)
Leaf edge thickness (cm)	0.097~0.706 (0.258)
Leaf midvein diameter (mm)	0.38~5.10 (0.90)
Leaf vein density (mm mm ⁻²)	1.92~9.13 (4.97)
RLD (%)	0~17 (0)

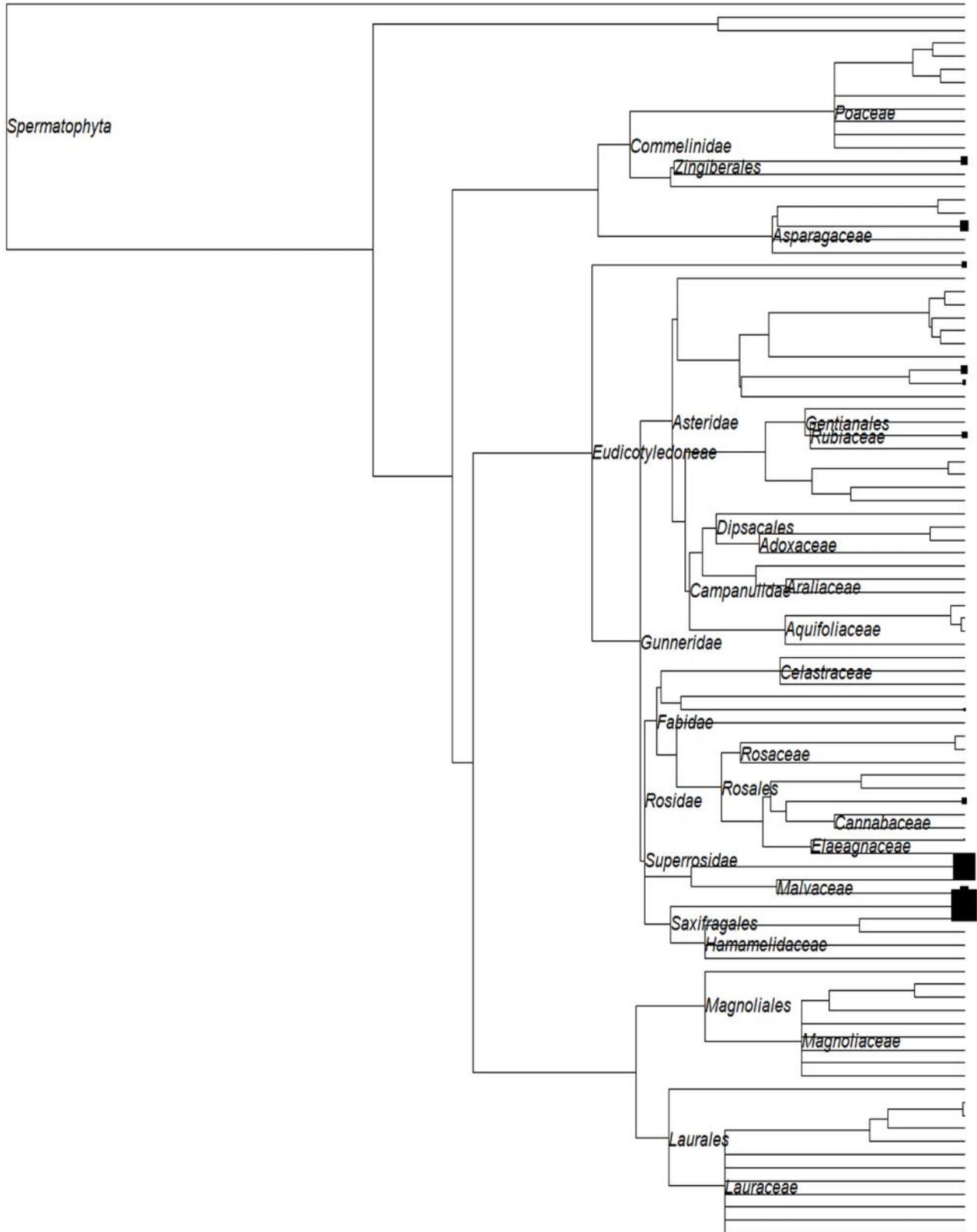
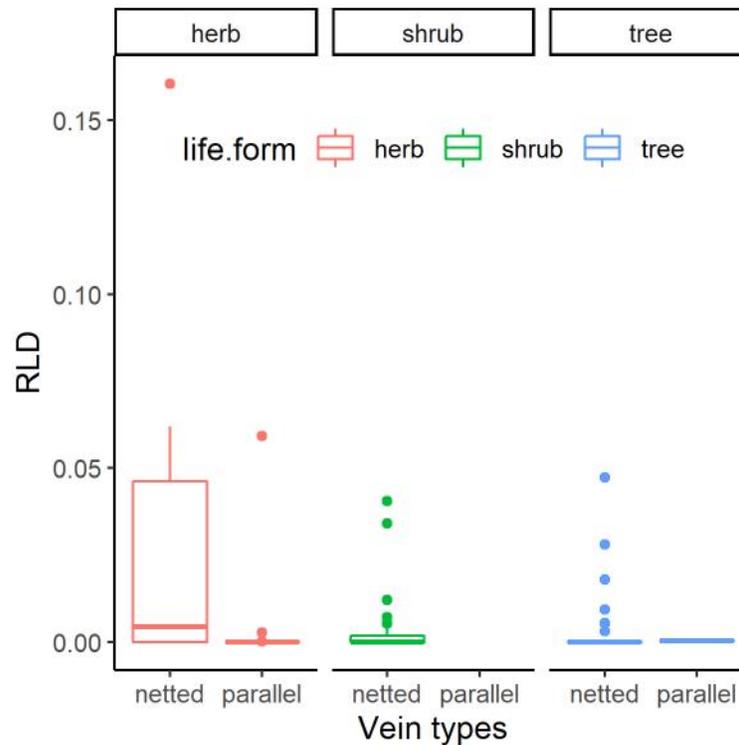


Figure 2. The phylogenetic signals of the ratio of lamina damaged area to the total area (RLD) among the selected species.

Table 3 Summary of the correlation analysis between the ratio of lamina damaged area to total area (RLD), and leaf traits with or without the phylogenetic data.

	Not including phylogenetic relationship		Including phylogenetic relationship	
	F value	Sig.	F value	Sig.
Lamina area	0.57	0.45	0.31	0.58
Lamina perimeter	3.07	0.08	0.47	0.49
Lamina length	6.99	< 0.01, **	1.55	0.21
Lamina width	0.66	0.42	0.21	0.65
Leaf dry weight	2.09	0.15	0.30	0.59
Midvein diameter	4.86	0.03, *	2.45	0.12

**Figure 3** The ratio of lamina damaged area to the total area (RLD) between the three plant life forms and two venation types.

DISCUSSION

In contrast to our hypothesis, over two-thirds of the 95 selected species did not show significant leaf damages three months after the midvein cut-off, though there was a strong variation in the response of the remaining 22 species. Previous studies on the species *Prunus laurocerasus* revealed that the leaf water potential did not drop after the midvein cut-off, though a severe decrease of leaf hydraulic conductance indicated a physiological homeostatic maintenance (Nardini and Salleo, 2003). Furthermore, it was predicted that a trade-off existed between vein architecture at a macroscopic scale and cell-to-cell pathway at a microscopic scale for water transportation based on a similar midvein cut

experiment (Harayama *et al.* 2019). The leaf venation network is complex and consists of different sizes of areoles (Blonder *et al.* 2011). These small connected structures probably take over the function of nutrient and water transportation after the midvein cut-off.

The correlation between RLD and lamina length or leaf midvein diameter was significant when the phylogenetic data were not included in our study. The vein scaling relationship revealed that larger leaves require more physical supports from the midvein, and thus, the diameter of the midvein strongly and positively correlated with the leaf size (Sack *et al.* 2012). It is reasonable to assume more extent of leaf damage after midvein cut-off for larger leaves with thicker midveins, as was demonstrated in our study. Usually, the texture of the leaves was closely linked with leaf thickness, and our

results suggested that the thicker succulent leaves would be more vulnerable to midvein damages. Meanwhile, a study on nine aquatic species found that the species with parallel veins were more tolerant to the midvein cut-off than the other two venation types (Pan *et al.* 2019). This could be resulted by the compensation of other parallel veins that were not damaged. In contrast, no significant differences were found between the species with parallel veins and netted veins in our study. The lack of a significant difference between venation types probably reflected a high species variation within each venation type due to a high species abundance compared to Pan *et al.* (2019).

There are also other potentially important factors that play a role in affecting the response of leaf to midvein cutoff. Defoliation and evergreen also affect the physiological activity of leaves (Gaxiola and Armesto, 2015; Qin and Shangguan, 2019), which could strongly interact with the treatment of midvein damage. Wright *et al.* (2004) stated that the leaf life span is a key plant trait for leaf economics spectrum, and leaves with short lifespan would be expected to have lower construction cost and thus be prone to decay after damages. However, the leaf lifespan is unfortunately not measured in this study.

In conclusion, we found that the variation of leaf tolerance to mechanical damages for different species was somehow independent of their phylogenetic relationship. It is also implied that herbaceous plants or leaves with low leaf vein density more seriously suffered after the midvein cut-off, which fits well with physical feature of leaf structure. As a shortcoming, the study was performed in a relatively short term and with no recording of changes of physiological indicators; especially the latter, which should provide better understanding of the leaf tolerance to external damages.

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Hourly climate data including air quality index (AQI), air temperature (Temp), relative moisture (RM) and precipitation (Prec): The air quality index was relatively low during the experiment (except for short-term high values near the end of the experiment). There were a few mildly rainy days during our experimental experiment, and the relative moisture fluctuated. The air temperature gradually decreased and reached < 10 °C at the end of the experiment.

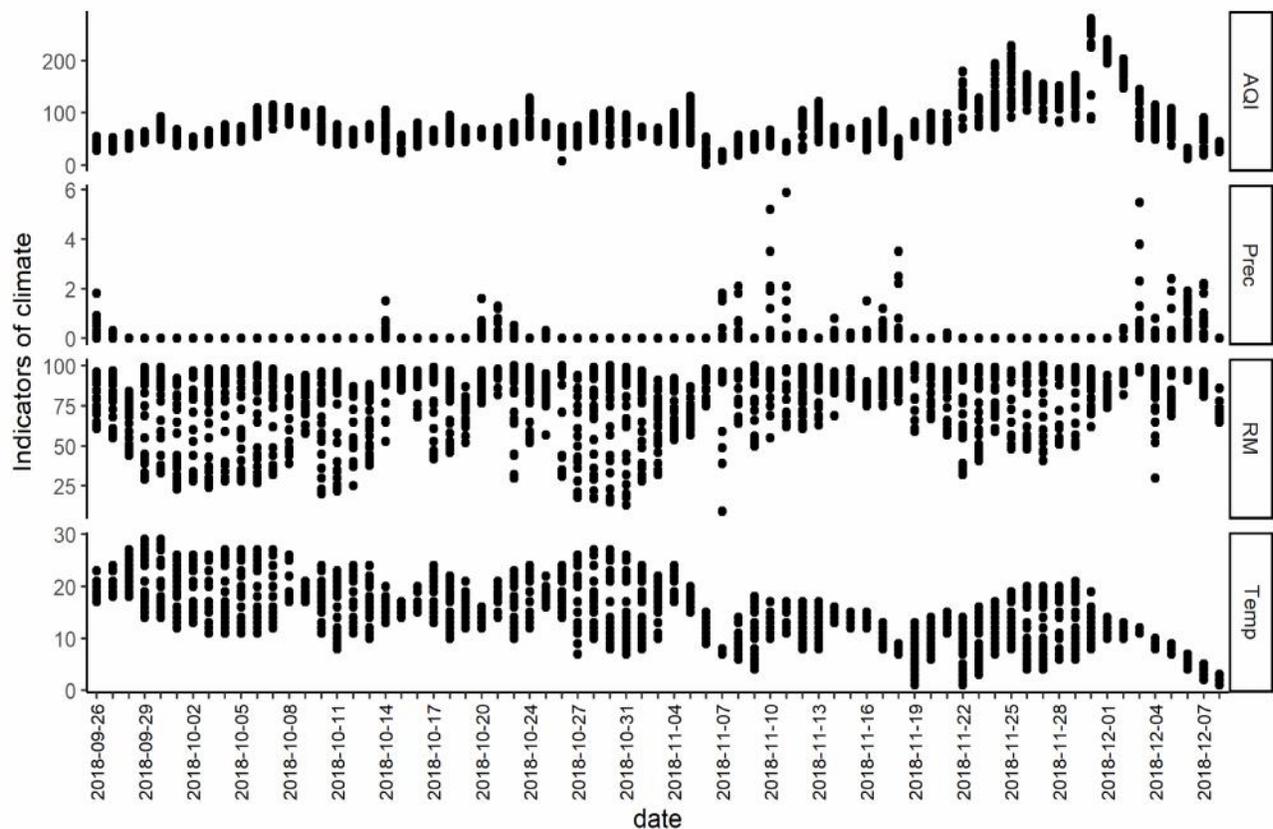


Figure S1 Hourly climate data including air quality index (AQI), air temperature (Temp, unit: °C), relative moisture (RM) and precipitation (Prec, unit: mm) from 26th September to 8th December, 2018.