Tallow tree allocates contrasting secondary chemicals in response to varying environments along elevational gradients

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Abstract

Aims Understanding how tree species regulate multiple types of secondary chemicals along elevational gradients is critical for elucidating the physiological and ecological strategies of plants in response to varying biotic and/or abiotic environments. This study aims to examine how Chinese tallow tree (*Triadica sebifera*) allocates resources to the production of different secondary chemicals in response to varying environments across elevational gradients.

Methods We conducted field surveys of different herbivore feeding guilds and their damage rates on Chinese tallow trees along an elevational gradient in China and measured secondary chemicals (tannins and flavonoids) in damaged and undamaged leaves.

Important Findings The odds of a leaf being damaged (chewing or scarring) decreased with elevation. Flavonoid concentrations increased with elevation in undamaged leaves but decreased with elevation in damaged leaves, with quercitrin contributing most strongly to this pattern, likely as results of plant responding to changing biotic or abiotic stresses along elevational gradients. Tannin concentrations did not vary with elevation, so undamaged leaves had relatively lower tannin to flavonoid ratios at high elevation than at low elevation. Our study reveals variation in herbivory and contrasting trends in plant secondary metabolism along an elevation gradient and highlights the importance of simultaneously considering multiple types of secondary chemicals in plant physiological and ecological strategies.

Keywords: herbivory, climate, elevation, defence strategies, secondary metabolites, Chinese tallow tree

摘要：了解树木如何沿海拔梯度调节多种类型的次生代谢产物对于阐明植物如何采用生理和生态策略来应对各种生物及非生物环境变化至关重要。本研究旨在探索乌桕如何分配不同的次生代谢产物来响应海拔梯度上的环境变化。我们在中国沿海拔梯度对乌桕不同取食类型的植食性昆虫及其对乌桕叶片的危害率进行了野外实地调查，并对健康叶片和虫害叶片中的次生代谢产物(单宁和黄酮类)进行了测定分析。研究结果表明，乌桕叶片被危害(咀嚼或潜叶式危害)的可能性随海拔的升高而减小。遭受虫害叶片和未遭受虫害的健康叶片中黄酮类化合物的浓度随海拔梯度变化呈现相反的趋势，即随着海拔的升高，健康叶片和遭受虫害叶片受不同的生物和非生物因素驱动，健康叶片中黄酮类化合物浓度增加，而遭受虫害叶片中黄酮类化合物浓度下降，其中槲皮苷对黄酮类化合物随海拔梯度变化的贡献最大。单宁浓度随海拔梯度的变化未发生显著变化，高海拔地区乌桕健康叶片中单宁与黄酮类化合物的比例与低海拔相比相对较低。我们的研究揭示了沿海拔梯度昆虫植食性以及不同植物次生代谢产物的变化趋势，并强调了在理解植物的生理和生态策略中同时考虑多种次生代谢物质的重要性。

关键词：植食性，气候，海拔，防御策略、次生代谢产物，乌桕

INTRODUCTION

Environmental variations along elevational gradients have increasingly been used to assess the impacts of diverse biotic and abiotic factors on plant physiological and ecological strategies (Defossez *et al.* 2018; Körner 2007; Wang *et al.* 2018). For example, rates of herbivory can be higher at lower elevation compared with higher elevation (Rokaya *et al.* 2016), where abiotic stress is typically higher (Diffey and Larkö 1984; Green and Harding 1980). Recently, it has been proposed that specific plant syndromes occur across elevational zones, representing tradeoffs among tolerance to abiotic stress, plant growth, and plant defence against herbivores (Defossez *et al.* 2018). In response to environmental stresses, plants produce secondary metabolites to adapt to these environments (Hartmann 1996; Karppinen *et al.* 2016). Because biotic and abiotic factors may interact to shape defence production in plants, plant chemical responses to elevational gradients are complex (Pellissier *et al.* 2014). Therefore, although studies have recently examined plant responses to elevational gradient (Defossez *et al.* 2018; Galman *et al.* 2018; Pellissier *et al.* 2016), we have no general conceptual framework for how plants allocate resources to the production of different secondary chemicals across elevational gradients.

One of the most notable biotic factors that shapes plant secondary chemicals is herbivory (Coley and Barone 1996; Züst *et al.* 2012). The abundance and diversity of herbivores are generally thought to be greater at lower elevation than at higher elevation (Hodkinson 2005; Lara *et al.* 2002; Lawton *et al.* 1987); thus, plants at lower elevation may exhibit higher investment in defences against herbivores due to higher herbivory pressure (Coley and Barone 1996; Pellissier *et al.* 2012, 2014). However, previous reports on the effects of herbivory on plant secondary chemicals along elevational gradients have found inconsistent patterns (reviewed by Rasmann *et al.* 2014). For example, with leaf herbivory decreasing with increasing elevation, the level of iridoid glycosides in *Plantago lanceolata* declined with increasing elevation (Pellissier *et al.* 2014). However, when leaf herbivory increased towards higher elevations, total sesquiterpenes in *Pinus yunnanensis* needles decreased (Hengxiao *et al.* 1999), whereas phenolics in *Quercus robur* leaves increased (Abdala-Roberts *et al.* 2016b). Interactions between herbivory pressure and other factors may contribute to these contrasting patterns.

Changes in abiotic factors along elevational gradients, including climate and UV radiation, can drastically affect plant secondary metabolism (Karppinen *et al.* 2016; Ramakrishna and Ravishankar 2011; Tegelberg *et al.* 2001). Plants at higher elevations are typically exposed to more severe environmental stresses, such as lower temperatures and enhanced UV-B radiation (Körner 1999). Accordingly, various classes of plant secondary chemicals, which respond to those abiotic factors along elevational gradients, are expected to show elevational trends (Albert *et al.* 2009; Alonso-Amelot *et al.* 2007, 2004). For example, variations in total phenolics and flavonoids may indicate plant response to changes in temperature and UV-B radiation (Cotrozzi *et al.* 2018; Hectors *et al.* 2014; Mikulic-Petkovsek *et al.* 2015; Schreiner *et al.* 2014).

Plant secondary chemicals differ in their responses to multiple biotic and abiotic stresses (Bartwal *et al.* 2013; Maag *et al.* 2015; Nakabayashi and Saito 2015). Some secondary chemicals, such as tannins, can be feeding deterrents and/or toxic, negatively affecting the development and reproduction of herbivores (Barbehenn and Peter Constabel 2011; Cooper and Owen-Smith 1985; Forkner *et al.* 2004; Hughes 1990; van Hoven 1984). For example, Feeny (1968) found tannin content in oak leaves influenced the larval growth of the winter moth *Operophtera brumata*. van Hoven (1984) reported that tannin levels in *African acacias* leaves increased after being damaged by Kudu. Other chemicals, such as flavonoids, can not only protect plants from herbivores and microbial pathogens but also help plants tolerate many abiotic factors, such as UV, low temperature, and drought (Brunetti *et al.* 2018; Harborne and

Williams 2000; Jiang *et al.* 2016; Ma *et al.* 2017; Mathesius 2018; Schulz *et al.* 2015; Treutter 2005). If secondary metabolites with different functions are derived from the same biosynthetic pathway (Patra *et al.* 2013; Yonemori *et al.* 2010; Yoshida *et al.* 2015), changes in one class of chemicals that are associated with response to one kind of stress may affect another class of chemicals that are associated with other stresses. For example, previous studies showed that tannins share a biochemical synthesis pathway with flavonoids (Chen *et al.* 2009; Hichri *et al.* 2011; Vogt 2010). Thus, herbivore- or abiotic environment-induced changes of tannins may be associated with changes in flavonoids (Huang *et al.* 2013; Wam *et al.* 2017).

Because plants often simultaneously respond to biotic and abiotic stresses that vary along elevational gradients (Abdala-Roberts *et al.* 2016b; Rasmann *et al.* 2014), we predict that production of secondary chemicals will depend on the major stresses and variation of these stresses along elevation. Most previous studies, however, have focussed only on how a single class of herbivore-resistant chemicals or anti-abiotic stress chemicals change with elevational gradients (Albert *et al.* 2009; Carbonell-Bejerano *et al.* 2014, Pellissier *et al.* 2016; Sandeep *et al.* 2015; Shukla *et al.* 2016), neglecting that the effects of biotic and abiotic factors on various secondary chemicals may not be independent especially when two classes of chemicals are in the same biosynthetic pathway. Simultaneously considering how plants regulate multiple types of secondary chemicals that are closely related in function and category to respond to biotic and abiotic stresses may facilitate the explicit understanding of plant physiological and ecological changes along elevational gradients. To date, however, such studies are rare (but see Rasmann *et al.* 2014).

Here, we examine elevational patterns of secondary chemicals in Chinese tallow tree (*Triadica sebifera* (L.) Small = *Sapium sebiferum* (L.) Roxb., hereafter referred to as '*Triadica*') as responses to changes in herbivory and abiotic environments. *Triadica sebifera* is a perennial deciduous tree with a variety of medicinal, industrial and horticultural values (Rao *et al.* 2015; Wang *et al.* 2013). Previously, we have shown that various secondary chemicals in tallow tree can be induced by multiple feeding types of herbivores, with leaf-chewing and leaf-rolling herbivores increasing leaf phenolics and tannins and leaf-rolling weevils decreasing individual root flavonoids (Xiao *et al.* 2019). However, there is little known about how secondary chemical changes in tallow tree respond to abiotic stresses or combined biotic and abiotic conditions. Because tannins and flavonoids vary in their functional responses to herbivory and abiotic stresses (Barbehenn and Peter Constabel 2011; Bashandy *et al.* 2009; Forkner *et al.* 2004; Jaakola and Hohtola 2010), we predict that along elevational gradients, tannins will be more closely associated with herbivory, while flavonoids will be more affected by varying abiotic environments.

In this study, we conducted field surveys of herbivores and their damages on tallow trees and performed chemical (tannins and flavonoids) analysis of both damaged and undamaged leaves sampled from trees growing on a mountain from 100 to 1000 m above sea level. Specifically, we asked the following questions: (i) Do the abundance and diversity of herbivores and herbivory on tallow trees decrease with increasing elevation? (ii) Do tannins and flavonoids vary along elevational gradients, and do they show similar patterns? (iii) Do secondary chemicals (tannins and flavonoids) in damaged and undamaged leaves show different patterns in response to biotic and abiotic environmental changes along elevational gradients?

MATERIALS AND METHODS

Study system and site

Triadica sebifera is widely distributed in southern China (Zhang *et al.* 1994) and mostly grows below 1000 m above sea level (Jin and Huang

1984). *Triadica* plants are usually attacked by a diverse array of specialist and generalist insect herbivores (Huang *et al.* 2014; Wang *et al.* 2009, 2012b; Zhang *et al.* 2015), including leaf chewers, leaf miners, leaf gall formers, leaf rollers and phloem suckers, which contribute to the variations in defence induction in *Triadica* plants (Huang *et al.* 2014).

We conducted an elevational survey sample collection on Tianzhu Mountain, which is located in Yichang City, Hubei Province. The highest peak of Tianzhu Mountain has a height of 1445 m above sea level and *Triadica* plants are commonly found around the mountain area.

Herbivores and damage

In July of 2015, we investigated herbivore abundance and diversity on individual *Triadica* plants growing from 128 to 910 m above sea level. We originally planned to sample at least three trees every 50 m along the elevation. However, some sampling sites had fewer than three trees. In total, we selected 22 *Triadica* plants that grew below 500 m above sea level for herbivory survey and leaf sample collection, spaced at least 10 m apart. As we found only a very small number of *Triadica* plants at an elevation of above 500 m, we investigated herbivory and collected samples for all *Triadica* plants that can be investigated and sampled at these higher elevations ($N = 3$). All the sampled trees were in similar size with the average height of 2 m (1.5–2.5 m).

To assess the abundance and diversity of herbivorous insects on *Triadica* plants along elevational gradients, we carefully visually inspected and examined the identities and quantities of all insect herbivores on each tree. For the unknown insects on each plant, we recorded the number of each species and then stored samples in 70% alcohol and sent them to taxonomists at the Institute of Zoology, Chinese Academy of Sciences for subsequent species identification. We calculated herbivore species number on each tree as the species richness of herbivores. We calculated the abundance of herbivorous insects of each tree as follows: $\sum N_i$ where, N_i = the individual number of the ith herbivore insect.

To evaluate insect herbivory and plant defence, we collected leaves from six randomly selected branches per plant and recorded the number of damaged leaves and undamaged leaves, thus calculating the total leaf numbers of each branch. Then, we collected all damaged leaves from each plant and took digital images to measure herbivory. We put all the undamaged leaves and damaged leaves of each plant into a zipper storage bag filled with silica gel. We dried the samples at room temperature for 7 days, ground them to powder and then stored them in sealed tubes at −20°C until chemical analysis.

To quantify the level of herbivory, we measured leaf damaged area and leaf damage frequency. For leaf damaged area, ImageJ software (National Institutes of Health, Bethesda, MD) was used to calculate the area removed by herbivores and the total area of each photographed leaf. When the leaf edge was affected by insect damage, a line was drawn representing the leaf perimeter to obtain an approximate value for total leaf area (Lobregat *et al.* 2018). The leaf damaged area (%) of each plant was calculated as follows: removed leaf area/total leaf area of damaged leaves × 100 × number of damaged leaves/total number of leaves. For leaf damage frequency, we first divided leaf damage into three different types (i) leaf-chewing damage (caused by leaf chewers), (ii) leaf-rolling damage (caused by leaf rollers such as weevils) and (iii) leaf feeding scars (caused by leaf miners and suckers, with symptoms including the removal of green leaf tissue, leaving a clear membrane, or with 'shot holes' in the leaf). We counted the number of leaves on each tree with and without each of these types of damage.

Chemical analyses

We analysed total tannins and the levels of five individual flavonoids (quercetin, isoquercetin, quercitrin, kaempferitrin and kaempferol) in all leaf samples. We extracted each 100-mg sample with 5 ml of methanol–0.4% phosphoric acid in aqueous solution (48:52, v:v) at 4°C for 24 h and removed insoluble material by filtering the solutions through a 0.22-μm membrane. We estimated total tannin content using a modified radial diffusion assay (Hagerman 1987) using tannic acid as the standard., High-performance liquid chromatography (HPLC) was used to determine the level of each of the five flavonoids according to the methods in Wang *et al.* (2012a). The total flavonoid content of each plant was calculated as the sum of the five individual flavonoid contents.

Data analyses

A set of regressions (that varied in the model types depending on the response variable—detailed below) were used to examine how the abundance and species richness of herbivores and leaf area damaged depended on elevation. Logistic regression was used to examine the odds of a leaf being damaged (probability of being damaged/probability of not being damaged) on elevation (overall, and by each type of herbivory damage) (using proc glimmix, logit link, binary variable). Because tannin and flavonoid concentrations appeared to have non-linear relationships with elevation, we fit linear regressions (separate intercept and slopes, plant as a random factor to control for non-independence in the data) and power functions (separate constant and exponents, plant as a random factor). In every case, power function had lower Akaike's information criterion scores (and so we used those). Another mixed model regression was used to examine whether the ratio of tannins to flavonoids (relative change in tannins and flavonoid) varied with elevation, damage or their interaction in a model that included plant as a random factor to control for non-independence in the data (proc mixed). For variables with relationships with altitude that differed in sign (i.e. one is positive and so significantly >0 and the other is negative and so significantly <0) for damaged vs. undamaged leaves, we inferred the relationships were different by transitive reasoning (i.e. whenever $x > z$ and $y < z$, then also $x > y$). All analyses were conducted using SAS version 9.4.

RESULTS

Herbivores and herbivory along the elevational gradient

In total, we found 16 herbivore species on *Triadica* across the elevational gradient, including 4 specialist herbivores and 12 generalist herbivores (Table 1). Visual inspection appeared to show that the abundance and diversity of herbivores and the percentage of leaf area damaged decreased with elevation (Supplementary Fig. S1). However, the abundance ($F_{1,23} = 0.12$, $P = 0.727$) and diversity ($F_{1,23} = 3.03$, $P = 0.095$) of herbivores and leaf area damaged ($F_{1,23} = 2.55$, $P = 0.124$) did not depend significantly on elevation. The odds of a leaf being damaged (overall Fig. 1a, chewing Fig. 1b, scarring Fig. 1c, but not rolling Fig. 1d) decreased with elevation.

Plant secondary chemicals along elevations

Tannin concentrations appeared to decrease with elevation, especially for damaged leaves, but these patterns were not significant (Fig. 2a). Flavonoid concentrations in damaged and undamaged leaves showed opposite trends: with increasing elevation, flavonoid concentration increased in undamaged leaves but decreased in damaged leaves (Fig. 2b). The main component of flavonoids, quercitrin (46.67%), showed the same elevational pattern as total leaf flavonoids in both damaged and undamaged leaves (Fig. 3a), but the other individual flavonoids did not show similar elevational patterns (Fig. 3b–e).

The ratio of tannins to flavonoids (relative change in tannins and flavonoid) showed a pattern opposite to that of flavonoids with elevation causing a relative decrease in undamaged leaves and a relative increase in damaged leaves (Fig. 4).

Table 1: Herbivores found on *Triadica sebifera* plants classified by host specificity, damage type and distribution along elevations

Herbivore	Family	Host specificity[a]	Damage type	Distribution along elevations (m)
Lasioptera sp.	Cecidomyiidae	Specialist	Leaf scarring	100–400
Heterapoderopsis bicallosicollis	Attelabidae	Specialist	Leaf rolling	100–600
Bikasha collaris (Walker)	Chrysomelidae	Specialist	Leaf chewing	100–400
Gadirtha inexacta (Walker)	Noctuidae	Specialist	Leaf chewing	100–150
Clania variegata Snellen	Psychidae	Generalist	Leaf chewing	100–350
Gryllotalpa africana Palisot et Beauvois	Grylloidea	Generalist	Leaf chewing	100–150
Cnidocampa flavescens (Walker)	Eucleidae	Generalist	Leaf chewing	100–350
Gatesclarkeana idia Diakonoff	Olethreutidae	Generalist	Leaf chewing	100–500
Biston marginata Shiraki	Geometridae	Generalist	Leaf chewing	100–150
Mimastra cyanura Hope	Chrysomelidae	Generalist	Leaf chewing	300–350
Atractomorpha sinensis Bolvar	Locustoidea	Generalist	Leaf chewing	300–350
Unknown	Geometridae	Generalist	Leaf chewing	300–350
Geisha distinctissima (Walker)	Flatidae	Generalist	Leaf sucking	800–850
*Ricania speculum (*Walker)	Ricaniidae	Generalist	Leaf sucking	100–600
Tricentrus aleuritis Chou	Membracidae	Generalist	Leaf sucking	200–450
Unknown	Cicadellidae	Generalist	Leaf sucking	450–500

[a]Host specificity of the species was obtained from Wang *et al.* (2009); Wang *et al.* (2012b); Huang *et al.* (2014); Zhang *et al.* (2015).
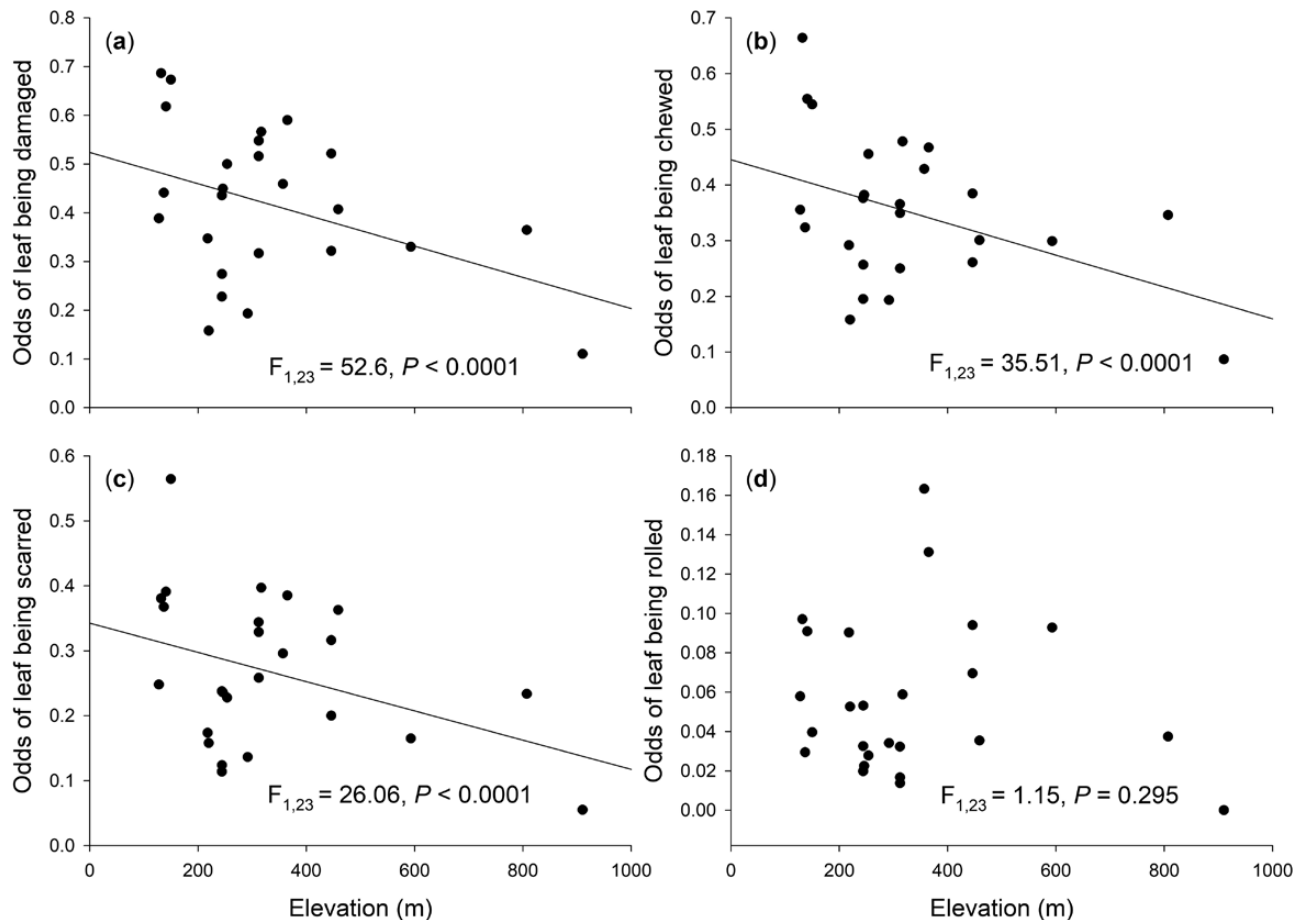

Figure 1: Relationship between elevation and (**a**) the odds of a leaf being damaged by any type of herbivore feeding (probability of being damaged/ probability of not being damaged), (**b**) the odds of a leaf being damaged by a chewing herbivore, (**c**) the odds of a leaf being damaged by scarring herbivore damage and (**d**) the odds of a leaf being damaged by a leaf-rolling herbivore. McFadden's pseudo-R^2 values [1 − ln (likelihood of full model)/ln (likelihood of model with only intercept)] were 0.16, 0.18, 0.14 and 0.01, respectively.
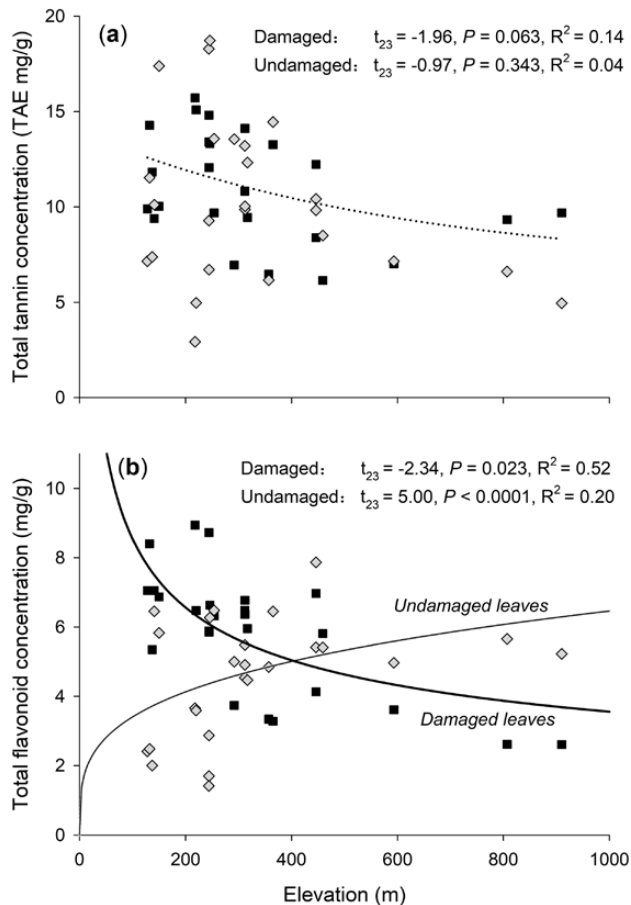
Figure 2: Effects of elevation on (**a**) a leaf tannin concentration in damaged (black squares) and undamaged (grey diamonds) leaves and (**b**) leaf flavonoid concentration in damaged (black squares) and undamaged (grey diamonds) leaves of *Triadica* plants. Solid fitted curves indicate a significant relationship ($P < 0.05$), and the dotted curve indicates a marginally significant relationship ($0.05 \leq P < 0.10$).

DISCUSSION

Shifting secondary chemical responses across elevations

The strength of plant–herbivore interactions is generally predicted to increase towards lower elevations (Pellissier *et al.* 2012; Rodríguez-Castañeda *et al.* 2010), while the impacts of climatic factors on plant traits are expected to be greater towards higher elevations (Abdala-Roberts *et al.* 2016b). Thus, changes in biotic and abiotic factors along elevational gradients may lead to variation in multiple types of defence compounds in plants (Defossez *et al.* 2018; Rasmann *et al.* 2014). In our study, we found variation among multiple secondary chemicals in *Triadica* plants along an elevational gradient, and those responses may have been driven by herbivory and abiotic environment. *Triadica* plants allocated a greater amount of secondary metabolites (tannins plus flavonoids) to damaged leaves than undamaged leaves under the higher herbivory pressures at low elevations, whereas at high elevations, plants allocated more secondary metabolites (flavonoids) to undamaged leaves (Fig. 2). These results indicate that plants may adopt an allocation strategy that is dependent on environmental variation along elevational gradients.

Response of flavonoids across elevations

In addition to defending against herbivory, flavonoids have also been reported to play a major role in helping plants to tolerate many abiotic stresses, especially UV-B and low-temperature stress (Bashandy *et al.* 2009; Jaakola and Hohtola 2010; Righini *et al.* 2019; Schulz *et al.* 2015; Treutter 2005). The stronger UV-B or colder temperatures at high elevations could induce more flavonoids in plants (Majuakim *et al.* 2014; Popović *et al.* 2018). It is important to note that we did not directly measure abiotic stress in this study. However, previous literatures have reported that as elevation increases, temperature decreases (Green and Harding 1980), while UV radiation intensity increases (Diffey and Larkö 1984); thus, change in abiotic conditions along elevational gradients is expected (Bilger *et al.* 2007; Körner 2007). The increase in flavonoids in undamaged leaves at high elevations could suggest that *Triadica* are responding to more intense abiotic stresses by producing protective flavonoids, however, flavonoids in damaged leaves decreased (Fig. 2b), which could be explained by the decreasing herbivore damage level and corresponding reduction of herbivore-induced defences as elevation increased. The contrasting trend in flavonoids in damaged and undamaged leaves at high elevations vs. low elevations probably reflects the variable roles of flavonoids in response to different environmental stresses along elevational gradients, i.e., plants may produce more flavonoids in response to stronger UV-B and harsher climates at high elevation, where there are also fewer herbivores. We note that, of the five component flavonoids, the major one, quercitrin showed the same pattern with total flavonoids (Figs 2 and 3). Likely playing a key role in responding to the abiotic stress along elevation. As reported by previous studies, quercitrin could exhibit high UV-B protective properties (dos Santos Nascimento *et al.* 2015; Nenadis *et al.* 2015).

Response of tannins across elevations

Plant–herbivore interactions should be stronger under warmer and more stable climatic conditions; thus, plants are expected to invest more resources in defence against herbivory under such conditions (Benevenuto *et al.* 2020; Schemske *et al.* 2009). In general, plants growing at lower elevation are exposed to greater herbivore diversity and abundance, which may cause higher leaf damage, thus producing higher levels of defensive chemicals (Callis-Duehl *et al.* 2017; Pellissier *et al.* 2016). In this study, although the sampled abundance and diversity of herbivores on *Triadica* did not vary with elevation, leaf herbivory significantly decreased with increasing elevation (Fig. 1). As we sampled insect herbivores only at one time period, it may be that leaf herbivory is a more enduring signature of herbivory pressure than a single insect survey. Or, it could be that herbivores feed at different rates dependent on elevational variation in both abiotic factors (e.g. temperature) and corresponding changes to plant primary and secondary compounds (Rasmann *et al.* 2014). As a defence class, tannins are generally expected to be more important in defence against herbivores rather than abiotic stress (but see Gourlay and Constabel 2019); however, we did not detect significant variation in tannins across different elevations. This suggests that either tannins were unresponsive to herbivory levels and differences in abiotic stress (e.g. UV and temperature) across elevation or that the tannins responded to abiotic and biotic stress and that these potentially simultaneous but opposite responses masked one another (Fig. 2a) (e.g. at high elevations, plants may produce fewer tannins due to less herbivory, however, the lower temperature and/or stronger UV may result in more tannins). Of course, demonstrating an anti-herbivore effect of tannins or other chemicals in this or other observational studies is not feasible, but we assume that chemicals that have been shown to have such effects in experimental studies with *Triadica* (Huang *et al.* 2014; Wang *et al.* 2012a) are likely to have similar functions in this system.
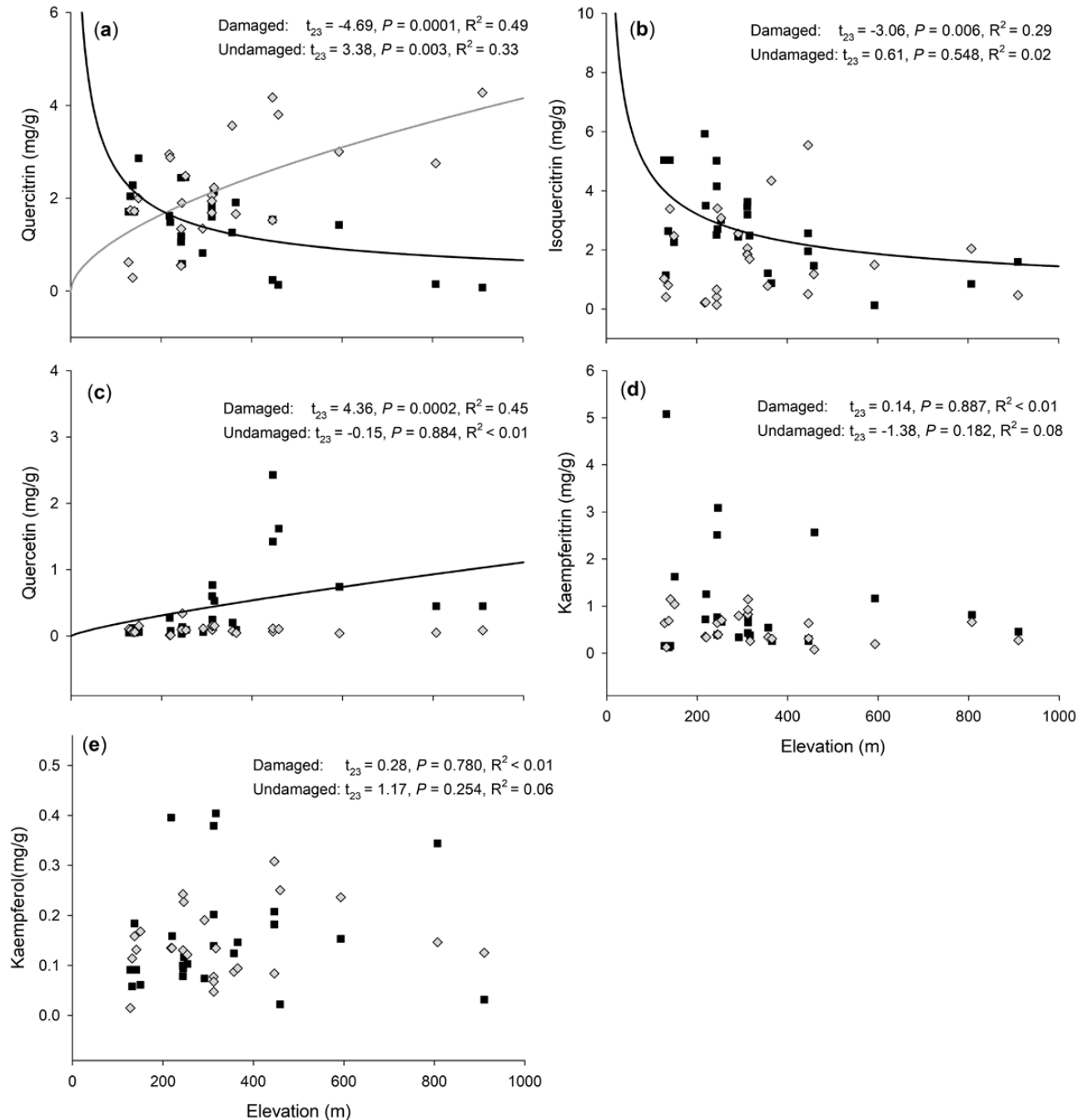
Figure 3: Relationship between elevation and the concentration of each flavonoid: (**a**) a leaf quercitrin concentration in damaged (black squares) and undamaged (grey diamonds) leaves, (**b**) a leaf isoquercetin concentration in damaged (black squares) and undamaged (grey diamonds) leaves, (**c**) a leaf quercetin concentration in damaged (black squares) and undamaged (grey diamonds) leaves, (**d**) a leaf kaempferitrin concentration in damaged (black squares) and undamaged (grey diamonds) leaves and (**e**) a leaf kaempferol concentration in damaged (black squares) and undamaged (grey diamonds) leaves of *Triadica* plants. Fitted curves indicate a significant relationship ($P < 0.05$).

Optimal defence resource allocation across elevations

Several classical theories seek to explain the quantitative and qualitative patterns of plant defence, such as the optimal defence hypothesis (Rhoades 1979), the resource availability hypothesis (Coley *et al.* 1985) and the environmental constraint hypothesis (Bryant *et al.* 1988; Tuomi *et al.* 1988). Plants growing along environmental gradients experience varying biotic and abiotic stresses; thus, the type and amount of plant defence may represent optimization given available resources (Coley *et al.* 1985; Herms and Mattson 1992). Generally, plants at lower elevations are more strongly affected by biotic factors, suffering more herbivore damage (Coley and Barone 1996; Pellissier *et al.* 2012, 2014), while plants at higher elevations are more strongly influenced by hasher abiotic conditions, such as lower temperatures and stronger UV-B (Körner 1999). The different strengths of biotic and abiotic interactions thus alter plant phenotype, and the complex selective pressures on plants could shape the allocation of resource to abiotic and biotic
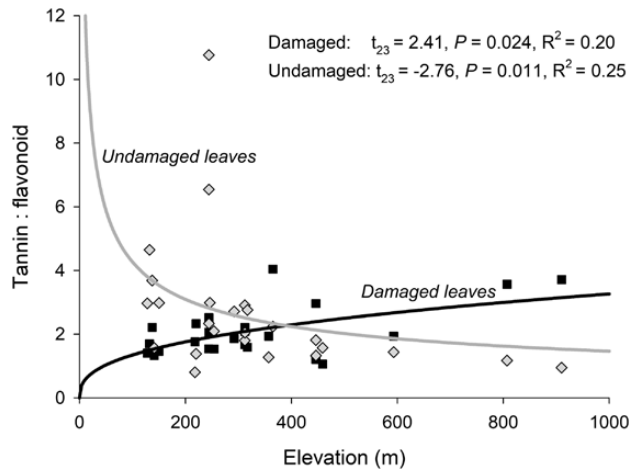
Figure 4: Effects of elevation on leaf tannin to flavonoid ratio in damaged (black squares) and undamaged (grey diamonds) leaves of *Triadica* plants. Fitted curves indicate a significant relationship ($P < 0.05$).

stressors (Abdala-Roberts *et al.* 2016b; Defossez *et al.* 2018; Pellissier *et al.* 2012; Rodríguez-Castañeda *et al.* 2010). In our study, *Triadica* plants showed different allocation patterns of secondary chemicals along an elevational gradient, with flavonoid allocation relatively greater at high elevations for undamaged leaves and relatively greater at low elevations for damaged leaves compared to tannin allocation (Fig. 4) (mostly due to changes in flavonoid levels, Fig. 2). Such contrasting flavonoid allocation patterns may imply lower defence to abiotic stresses for damaged leaves but higher defence to abiotic stresses for undamaged leaves at high elevations. However, the main limitation of our study is that we did not explicitly test these mechanisms by measuring plant fitness and abiotic conditions across the elevational gradient. Although we expect that, in general, abiotic stresses increase at higher elevations, we cannot relate specific chemical responses to specific abiotic stressors. Moreover, to tease apart whether these contrasting responses of tallow trees to biotic and abiotic environments along elevation gradients are due to adaptation (i.e. evolution) or phenotypic plasticity, reciprocal transplant common gardens along elevation gradients should be considered in future work (Bakhtiari *et al.* 2019; Buckley *et al.* 2019).

Defence resource allocation between damaged and undamaged leaves

Secondary chemicals in plants may represent either constitutive defences, which are always expressed in a plant, or inducible defences, which are synthesized or mobilized in response to a stimulus, such as herbivore damage (Karban *et al.* 1997, 1999; Rhoades 1979). In our study, the secondary metabolites in healthy leaves may generally represent the constitutive response of plants to environments. Notably, in our study, as the elevation increased, the degree of leaf damage decreased (Fig. 1), resulting in a weaker effect on the synthesis of secondary metabolites in healthy leaves. Therefore, at high elevations, the flavonoid content in healthy leaves can largely reflect the response of plants to abiotic stresses. In our study, sampling both damaged and undamaged leaves allowed us to tease apart constitutive and induced defences, facilitating an explicit understanding how plants balance multiple chemical responses to different environmental stresses. However, the content of these chemicals in healthy leaves may also be affected by herbivory because insect damage to plant leaves not only causes local defence induction in the damaged leaves but can also cause systemic defence induction in neighbouring leaves (Abdala-Roberts *et al.* 2016a). In this regard, future manipulative experiments controlling herbivores along the elevation are needed to rule out the

possibility that herbivores preferentially consumed leaves with lower tannin concentrations.

Because many secondary chemicals have multiple functions in plant response to biotic and abiotic stresses, our study indicates that the consideration of plant chemical response to varying environments may facilitate an explicit understanding of the role of secondary chemicals in responding to a specific type of stress. Also, the potential interactive effects of these chemicals including synergistic and/or antagonistic effects of chemicals as well as non-independence in the production or allocation of chemicals may need to be considered.

Author Contributions

J.D. conceived the project and designed the experiments. L.X. and J.Z. carried out the field surveys. J.C. and E.S. analysed the data with interpretation from all authors. L.X. wrote the first draft of the manuscript, and all authors revised subsequent versions.

Conflict of interest statement. The authors declare that they have no conflict of interests.

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