

## RESEARCH ARTICLE

Functional Ecology



# Shifts in woody plant defence syndromes during leaf development

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## Abstract

1. Herbivores target young leaves in forests world-wide. How this strong and predictable selection pressure has shaped plant defence syndromes remains unclear. Specifically, whether young leaf herbivory has led to general global patterns of shifting leaf defences during leaf development (i.e. ontogenetic trajectories) remains unknown but likely.
2. Using meta-analysis, we have synthesized developmental shifts in chemical, physical and indirect defence traits, as well as leaf nutrient content, during leaf expansion and maturation across 124 woody plant species. Leaf traits were compared for immature young leaves vs. mature leaves within studies, and these developmental shifts were then compared across studies to characterize general patterns.
3. Traits shifted strongly during leaf maturation, giving rise to discrete defence syndromes, with young leaves having significantly greater nutrient and secondary chemistry concentrations but reduced toughness and indirect defence traits than mature leaves. These patterns corroborate a growing consensus on the importance of ontogeny in plant defences, illustrating ontogenetic trajectories at the scale of leaves.
4. Trait developmental shifts were stronger in species with synchronous than asynchronous leaf flushing, but whether this reflects variation across biomes remains unclear.
5. Future research on under-studied traits, such as alkaloids, cyanogenesis, leaf phosphorus content and indirect defence traits, and in a more biogeographic context will provide additional insights into the generality of shifts in defence syndromes during leaf development.

## KEYWORDS

Herbivore resistance, leaf morphogenesis, log response ratio, meta-analysis, ontogeny, physical defences, quantitative review, secondary chemistry

## 1 | INTRODUCTION

It has long been observed that many temperate and boreal insect herbivores emerge in the spring at just the right time to feed on newly flushing tree leaves (Feeny, 1970; Thomas & Packham, 2008). That

climate change disrupts this synchronous phenology (Fox, 2013; Parmesan, 2006; Renner & Zohner, 2018), with dire consequences for insects, further underscores the benefit to insect herbivores of feeding on young leaves. Tropical insect herbivores also target young leaves (Coley & Barone, 1996), suggesting that this is a global

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trend. Such intense and predictable herbivory on developing leaves indicates consistently strong selection pressure on trees to defend young leaves. While many studies have examined patterns of chemical defences across leaf ages or seasons (Coley, 1980; Feeny, 1968; Kearsley & Whitham, 1989), we still lack a comprehensive analysis of general patterns in the full spectrum of defences during the narrow window of leaf maturation. Most plants defend themselves with suites of covarying traits, resulting in defence syndromes (Agrawal & Fishbein, 2006); this further emphasizes the need to develop a leaf maturation model that incorporates simultaneous developmental shifts in nutrients, chemical, structural and indirect defence traits.

Plants should defend young leaves not only because the likelihood of herbivory is high, but also because young leaves have a greater fitness value than mature leaves (Harper, 1989). The total photosynthetic potential is much greater in young leaves, decreasing as leaves age and eventually senesce (Wiedemuth et al., 2005). Optimal defence theory thus predicts that young leaves should be well defended on the basis of both their high fitness value and high likelihood of attack (Feeny, 1976; McKey, 1979; Rhoades & Cates, 1976). Consistent with this prediction, a meta-analysis of 58 young vs. old leaf comparisons found generally higher levels of secondary chemicals in young leaves (McCall & Fordyce, 2010). However, McCall and Fordyce (2010) examined only chemical defences, which are known to be poor predictors of herbivory in many plant-herbivore interactions (Carmona, Lajeunesse, & Johnson, 2011; Smilanich, Fincher, & Dyer, 2016). A comprehensive review of chemical as well as other defence traits is needed to better understand how defence syndromes change during leaf maturation, particularly because leaves undergo dramatic shifts in anatomical and physiological composition during development which alters structural and indirect defences as well as secondary chemistry.

Leaf maturation involves both an increase in size (growth) and the development of specialized anatomy and biochemistry (differentiation). During early phases, developing leaves are sinks, requiring influxes of water for cell expansion and carbon and nutrients for construction (Turgeon, 1989). The sink-source transition typically coincides with the end of the expansion phase, at which point photosynthetic rates peak (Coleman, 1986). Now in the source phase, the leaves undergo differentiation, with development of the midrib and major veins from the base, development of the mesophyll tissues and the minor veins from the leaf tip and finally with sclerification of sclerenchymatous tissues (Turgeon, 1989). Sclerification is a key turning point in leaf maturation as tissues are highly susceptible to damage before cells are sclerified, leading to weak physical defences against herbivores in young leaves (Coleman, 1986). Indeed, many ecologists have predicted that leaf toughness underlies lower rates of herbivory in mature compared with young leaves (Coley, 1983; Feeny, 1970; Kursar & Coley, 1991; Lowman & Box, 1983; Webber & Woodrow, 2008). Considering the anatomical constraint of young leaves to utilize toughness as a physical defence, it is likely that many species undergo developmental switches from chemical to physical defence during leaf maturation.

Plant defence traits are not the only factors underlying herbivore hostplant use, and patterns of herbivory during leaf maturation may also depend on leaf nutrient content and availability. For example, insect herbivores are often nitrogen-limited, and protein content of their hostplants may determine consumption rates (Awmack & Leather, 2002; Barbehenn, Knister, Marsik, Jahant-Miller, & Nham, 2015). Changes in available leaf nitrogen content during leaf maturation can vary among species, providing insights into herbivore selection patterns (Barbehenn et al., 2015). The role of other nutrients in patterns of herbivory during leaf maturation is less clear, but likely to be important (Awmack & Leather, 2002), particularly if considered in conjunction with developmental shifts in secondary chemicals and leaf toughness as posited by the defence syndrome concept (Agrawal & Fishbein, 2006).

Finally, even if young leaves are more palatable to herbivores due to high nutrient quality and low toughness, mature leaves may sustain greater damage given their greater availability. Young leaves are an ephemeral resource in most forests, with mature leaves being more abundant and long-lived (Hunter & Lechowicz, 1992). This greater apparency of mature leaves to herbivores may have led to significant selection for defences in mature leaves (Feeny, 1976). Phenology further contributes to the availability of young leaves for herbivores, with distinct differences between species with synchronous and asynchronous leaf flushing (Kursar & Coley, 2003). Synchronous leaf production may satiate herbivores, allowing some leaves to escape damage through the maturation process (Coley, 1980). Synchronous species may further narrow the window of vulnerability via fast expansion rates, allowing young leaves to escape herbivory (Kursar & Coley, 1991, 2003), but potentially also resulting in smaller leaves (Moles & Westoby, 2000). In contrast, asynchronous leaf production provides a continuous supply of young leaves to herbivores, which may move around the canopy to target young leaves within the mosaic of young and mature leaves (Kearsley & Whitham, 1989). Selection has probably led to high investment in chemical defence for asynchronously flushing species that experience reliably high herbivory, while synchronous species are associated with escape via herbivore satiation (Kursar & Coley, 2003). Thus, although it has been predicted that the magnitude of developmental change in secondary chemicals and nutrients is greater in species with synchronous than asynchronous leaf flushing (Coley, 1980), it remains unclear whether this is a general pattern.

We conducted a meta-analysis with the goal of characterizing the magnitude and directionality of shifts in leaf nutrient concentrations, secondary metabolite concentrations, physical defence traits and indirect defence traits during leaf maturation. Predicted sources of heterogeneity in the developmental shifts of leaf traits were tested, including biome (tropical, temperate, boreal) and leaf phenology (asynchronous vs. synchronous flushing). By expanding on previous research to include physical and nutritive traits as well as secondary metabolites (McCall & Fordyce, 2010), and a global scope (Kursar & Coley, 2003), we can develop a general model for shifts in defence syndromes during leaf maturation.

## 2 | MATERIALS AND METHODS

### 2.1 | Compiling the database

Studies to include in the meta-analysis were identified using concatenated search terms in the Web of Science Core Collection (1980–2014) with the following terms: “leaf age OR development OR stage,” “phenology,” “plant defence OR resistance,” “secondary chemistry,” “plant secondary metabolites,” “physical defence,” “indirect defence,” “herbivory,” “bioassay.” Because abiotic factors may contribute to seasonal patterns of leaf chemistry and toughness (Sampaio, Edrada-Ebel, & Da Costa, 2016), we included only those comparisons of young vs. mature leaves made within the window of leaf development (i.e. within a few days usually), and excluded comparisons of young vs. old leaves made over longer time spans such as seasons or years. Herbaceous plants have strong whole-plant ontogenetic shifts in defence traits (Barton & Koricheva, 2010), and because the time-scale of leaf ontogeny overlaps with whole-plant ontogeny, particularly in short-lived annuals, we focused this meta-analysis on woody plants only. The final plant dataset draws from 52 studies published in 1980–2014.

Multiple comparisons between young and mature leaves were included from the same publication if multiple species of plants or multiple leaf traits were examined. If multiple years, sites or populations were sampled from the same species, only the contrast of greatest magnitude was extracted in order to reduce the extent of non-independence within our dataset. While this approach may not reflect average leaf developmental shifts within species, it leads to a more balanced dataset given that many studies did not report multiple measures within species and allows us to estimate the potential developmental shifts that traits may undergo during leaf maturation which can be more informative than average for comparing across biomes, trait types, etc. The final dataset included 340 effect sizes from 124 woody plant species representing 48 plant families. Leaf traits included plant secondary metabolites (various classes and subclasses, secretory structures such as glandular trichomes and pellucid dots), nutrients (water, nitrogen, phosphorus), physical/structural traits linked to defence (toughness measured with a penetrometer or as leaf mass per area, non-glandular trichomes) and indirect defence attractants (extrafloral nectaries, food bodies).

### 2.2 | Meta-analysis

Means, standard deviations and sample sizes for leaf traits were extracted from text, tables or graphs. Data were extracted from graphs using TechDig 2.0 software (Jones, 2007). As an effect size measure, we used the log response ratio which was calculated as follows:  $\ln(M_M/M_Y)$  where  $M_M$  is the mean trait value for mature leaves, and  $M_Y$  is the mean trait value for young leaves, so that positive numbers reflect increases during leaf maturation, and negative numbers represent developmental decreases. We conducted the meta-analysis using the “metaphor” package in R version 3.5.1 (Viechtbauer, 2010).

In a meta-analysis involving diverse species, residual variation in effect size may be phylogenetically structured, affecting the validity of hypothesis tests. To evaluate phylogenetic signal, we incorporated phylogenetic models of the variance–covariance matrix of a species-level random effect (Ives & Helmus, 2011). A supertree was constructed using Phylomatic (Webb & Donoghue, 2005), and branch lengths were made consistent with known node ages using Phylocom (Webb, Ackerly, & Kembel, 2008). For each of the four trait types (secondary metabolites, nutrients, physical/structural traits, indirect defence), we used Pagel's  $\lambda$  (Pagel, 1999) to test for phylogenetic signal. In all cases,  $\lambda = 0$  had a lower AIC than any positive value of  $\lambda$ , indicating no evidence of phylogenetic signal in the species random effect. As an alternative, we subsequently used plant family as a random effect, which explained significant variation in effect size in all models. The discrepancy between the taxonomic random effect and the phylogenetic model suggests that patterns of trait evolution are more complex than can be accounted for with a simple model; in other words, families differ in their mean trait values but not in such a way that trait covariance is a simple function of shared branch length across the tree.

Using mixed-effects models with the `rma.mv()` function, we tested whether leaf developmental patterns varied among the four main plant trait classes: secondary chemicals, nutrients, physical and indirect. We then subset the data to examine each of the trait classes separately in order to test for variation among (a) classes of secondary compounds; (b) nutrients (water, nitrogen, phosphorus); (c) metrics of physical defence (non-glandular trichomes, puncture tests, leaf mass per area); and (d) synchronous vs. asynchronous leaf production. In all analyses, publication and plant family were included as random factors.

Model-generated group means and 95% confidence intervals are presented in the figures. When confidence intervals do not overlap with zero, the group can be concluded to have a significant developmental shift. To facilitate interpretation, group mean log response ratios are back-transformed to % difference between young and mature leaves as follows:  $100 - 100e^{(\text{response ratio})}$ .

Publication bias occurs when studies with non-significant results are not published, leading to an overestimation of the effect size, or when effect sizes of particular directionality (i.e. in favour of a hypothesis) are published more frequently than opposite effects (Csada, James, & Espie, 1996). To test for potential publication bias in our dataset, we assessed the relationship between effect size and sample size using the funnel plot asymmetry test (Begg & Mazumdar, 1994) on the full dataset and then on datasets subsetted by trait type (nutrients, plant secondary chemistry and physical defences; there were insufficient indirect defence traits to warrant this analysis). In addition, we investigated whether effect sizes within the trait groups change significantly over time to investigate the likelihood that missed data (before 1980 or after 2014) could potentially alter the conclusions. Calculation of fail-safe numbers further examines the potential for additional studies to reduce significant patterns to null results.

### 3 | RESULTS

Leaf developmental shifts varied significantly among different leaf trait classes ( $Q_M = 4,428.05$ ,  $df = 3$ ,  $p < 0.0001$ ). Concentrations of secondary metabolites and nutrients significantly declined during leaf maturation, while physical traits and indirect defence traits significantly increased (Figure 1).

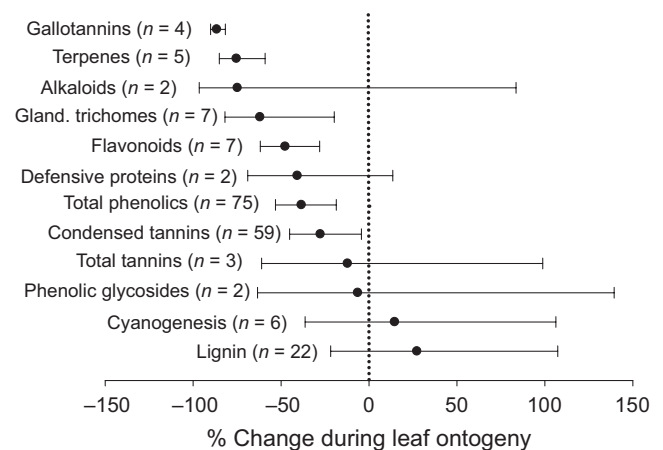
Significant variation in leaf developmental trajectories was detected among classes of plant secondary compounds ( $Q_M = 50.32$ ,  $df = 5$ ,  $p < 0.0001$ ). Variation among phenolic groups was also detected ( $Q_M = 575.24$ ,  $df = 6$ ,  $p < 0.0001$ ). Young leaves had significantly greater concentrations of gallotannins, terpenes, flavonoids, total phenolics and condensed tannins than mature leaves (Figure 2). Some of these developmental declines were dramatic, such as the nearly 100% decline detected for gallotannins. The density of glandular trichomes was also significantly higher on young than mature leaves (Figure 2). Other classes of secondary compounds, sampled in fewer studies, had no clear trend during leaf maturation, including alkaloids, defensive proteins, total tannins, phenolic glycosides, cyanogenesis and lignin (Figure 2).

Leaf developmental patterns varied significantly in magnitude, but not direction, among nutrient types ( $Q_M = 12.73$ ,  $df = 2$ ,  $p = 0.0017$ ). Overall, young leaves are more nutritious than mature leaves, with greater concentrations of nitrogen, phosphorus and water (phosphorus was excluded from Figure 3 due to sample size of  $n = 1$ ).

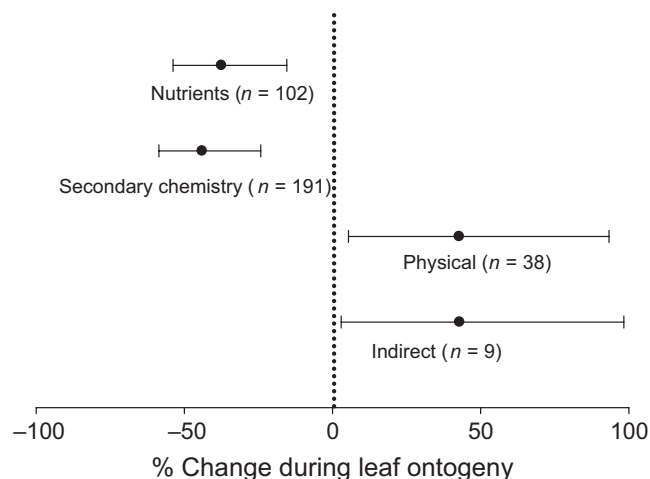
For physical defences, leaf developmental patterns differed significantly between measures of leaf toughness and non-glandular trichome densities ( $Q_M = 137.99$ ,  $df = 1$ ,  $p < 0.0001$ ). While non-glandular trichome density significantly declined during leaf maturation (consistent with the developmental pattern of glandular trichomes; Figures 2 and 4), leaf toughness was greater in mature than young leaves (Figure 4). While both metrics of leaf toughness

increase during leaf maturation (Figure 4), puncture tests generally depict a much stronger increase than measures of leaf mass per area ( $Q_M = 113.06$ ,  $df = 1$ ,  $p < 0.0001$ ).

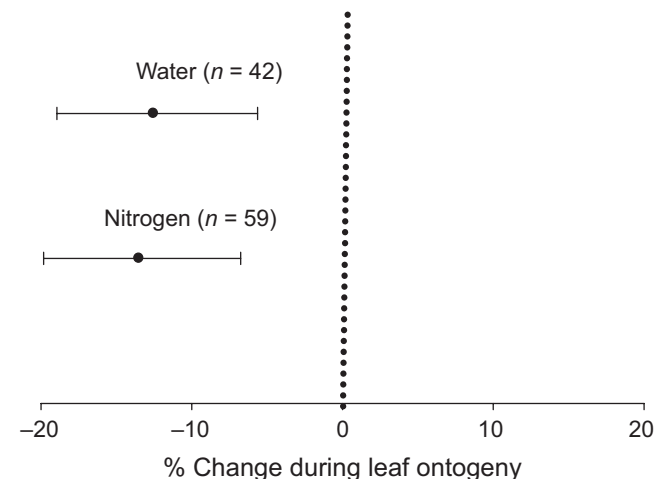
Because biome and leaf phenology were highly confounded ( $\chi^2 = 147.32$ ,  $p < 0.0001$ ), it was not informative to analyse both factors. In nearly all tropical species examined, leaves were flushed asynchronously, while temperate species were quite equally represented by synchronous and asynchronous leaf flushing. Although there was no main effect of leaf phenology on trait differences globally ( $Q_M = 2.93$ ,  $df = 3$ ,  $p = 0.4024$ ), nor in temperate species alone ( $Q_M = 1.76$ ,  $df = 3$ ,  $p = 0.6231$ ), we detected a significant interaction between trait and phenology using log-likelihood model comparison ( $\chi^2 = 139.74$ ,  $df = 9$ ,  $p < 0.0001$ ). Within synchronous flushing species, developmental trends were strong, with significant declines



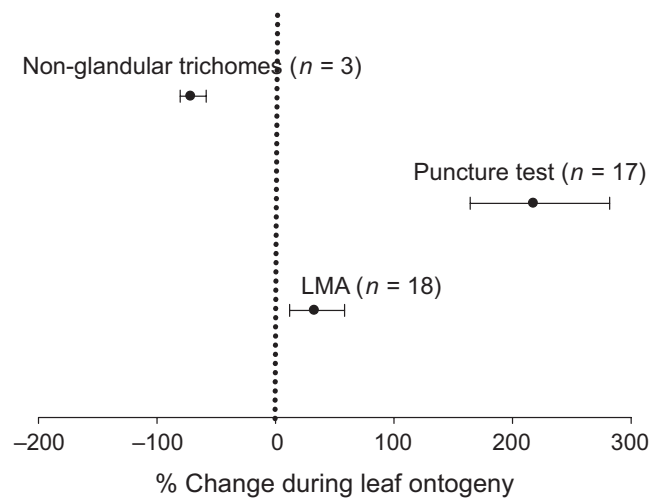
**FIGURE 2** Per cent difference in classes of plant secondary chemicals between young and mature leaves. Calculated from log response ratios, with bias-corrected 95% confidence intervals. Sample sizes are in parentheses



**FIGURE 1** Per cent difference in leaf traits between young and mature leaves. Calculated from log response ratios, with bias-corrected 95% confidence intervals. Sample sizes are in parentheses



**FIGURE 3** Per cent difference in nutrient concentration between young and mature leaves. Calculated from log response ratios, with bias-corrected 95% confidence intervals. Sample sizes are in parentheses



**FIGURE 4** Per cent difference in physical defence traits between young and mature leaves. Calculated from log response ratios, with bias-corrected 95% confidence intervals. Sample sizes are in parentheses

in nutrients and secondary chemicals, and significant increases in physical and indirect defence traits during leaf maturation (Figure S1). In contrast, asynchronously flushing species demonstrated much weaker trends during leaf maturation, with significant declines detected only for secondary chemicals (Figure S1).

Using three different approaches, we detected little evidence for publication bias in our dataset. First, funnel plot asymmetry tests were not significant when inspecting the full plant dataset ( $t = 1.0013$ ,  $df = 337$ ,  $p = 0.3174$ ; Figure S2), nor when subsetting the data into secondary chemistry ( $t = -0.3493$ ,  $df = 189$ ,  $p = 0.7272$ ; Figure S3), nutrients ( $t = 1.9437$ ,  $df = 100$ ,  $p = 0.0547$ ; Figure S4) or physical ( $t = -1.0140$ ,  $df = 36$ ,  $p = 0.3173$ ; Figure S5) traits. Funnel plots did reveal two outliers with strongly negative effect sizes (Figure S3), which are shifts in cyanogenesis in two species of *Grevillea* (Lamont, 1993), but because removal of these data did not alter the significance nor directionality of mean patterns, they were retained for all relevant analyses. Second, regression analyses revealed no significant shifts in log response ratios over time for plant secondary chemistry ( $R^2 = 0.0003$ ,  $n = 191$ ,  $p = 0.4828$ ) or nutrients ( $R^2 = 0.0063$ ,  $n = 102$ ,  $p = 0.4274$ ). However, we did detect a significant negative temporal pattern for physical defence traits ( $R^2 = 0.1356$ ,  $n = 38$ ,  $p = 0.0211$ ), suggesting that recent studies report greater magnitude reductions in physical defence traits during leaf ontogeny. Finally, calculation of Rosenberg fail-safe numbers (Rosenberg, 2005), the required non-significant effect sizes to make the results null, indicates that our results are robust to publication bias. For all trait groups, the Rosenberg fail-safe numbers far exceeds the recommended threshold of  $5N + 10$ , in which  $N$  is the number of effect sizes (Rosenthal, 1991): secondary chemistry Rosenberg fail-safe number is 296,918 (threshold  $5N + 10 = 965$ ), nutrient Rosenberg number is 45,690 (threshold  $5N + 10 = 520$ ), and physical traits Rosenberg number is 24,901 (threshold  $5N + 10 = 200$ ).

## 4 | DISCUSSION

Using meta-analysis, we determined that plant defence syndromes shift dramatically during leaf maturation in highly consistent ways across species, suggesting distinct defence syndromes in young vs. mature leaves. In general, immature and unexpanded leaves have higher nutrient concentrations and higher secondary metabolite concentrations, whereas fully expanded mature leaves are tougher and have higher levels of indirect defences.

Although secondary chemicals tend to be more highly concentrated in young than mature leaves, we did uncover significant variation among classes of secondary chemicals in the magnitude of this developmental shift. The strongest declines were detected for gallotannins, terpenes, flavonoids and total phenolics. Classes of secondary compounds with no clear shifts across leaf maturation include alkaloids, defensive proteins, phenolic glycosides and cyanogenesis, although all of these are represented by very few studies. It is clear that phenolics have been the focus of most leaf maturation studies, likely due to early studies that emphasized the importance of phenolics in spring herbivory of temperate trees (Feeny, 1970; Rhoades, 1979). For secondary chemicals with large structures that are not recyclable, such as phenolics, the declines in concentration during leaf expansion may reflect dilution as leaf biomass increases (Koricheva, 1999), or transformation into other compounds of a shared biosynthetic pathway (Salminen et al., 2004). In contrast, secondary chemicals with smaller, more mobile structures may undergo more dynamic patterns during leaf maturation. For example, pyrrolizidine alkaloids are translocated from senescing leaves into young leaves so that shifts in alkaloid concentration during leaf maturation reflect translocation and not synthesis or dilution (Van Dam & Hartmann, 1993). Considering that much of the internal leaf anatomy and specialized biochemistry do not differentiate until after the leaf expansion phase (Coleman, 1986), high concentrations of secondary chemicals in immature and unexpanded leaves likely reflect patterns of translocation and not synthesis. Future studies characterizing underlying mechanisms of developmental shifts in secondary chemicals, and chemical profiles (Wait, Jones, & Coleman, 1998), are needed to more fully understand variation among classes of secondary chemicals during leaf maturation.

Numerous studies have documented declines in leaf nitrogen and water content during maturation. From these patterns, it is clear that young leaves are more nutrient-dense and therefore likely to be more nutritious for herbivores. Although a few studies have documented similar declines in leaf phosphorus and potassium during leaf maturation (Ho et al., 1984; Mauffette & Oechel, 1989), there is insufficient evidence to conclude whether these trends are general. High nutrient content of young leaves, though costly to plants due to increased attractiveness to herbivores, plays an important role in leaf development. Developing leaves are strong sinks, requiring carbohydrates and nutrients for construction of leaf anatomy and to promote high metabolic rates necessary for expansion and differentiation (Coleman, 1986; Turgeon, 1989). Although nitrogen content (% total N/dry weight) may



indicate nutritional quality of leaves for herbivores (Wait et al., 1998), measures of protein content and protein assimilation rates, as well as soluble nitrogen as amino acids, are often more informative (Barbehenn et al., 2015; Cizek, 2005). Moreover, high protein content may not necessarily promote herbivore performance if it coincides with high secondary chemical content, which is the case for young leaves. Thus, the ratio of protein to secondary chemical content of leaves is likely to correlate most strongly with herbivore performance (Lambdon & Hassall, 2005) and should be the focus of future studies.

Despite the generality of high nutrients in young leaves to support leaf development, some plant species have been selected for low nitrogen content in young leaves, resulting in the 'delayed greening' syndrome (Kursar & Coley, 2003). Delayed greening is generally associated with low nitrogen concentrations and fast expansion rates, and is a particularly common syndrome in tropical plants that flush leaves synchronously, providing a mechanism by which young leaves escape from herbivory (Kursar & Coley, 2003). The prediction that synchronous leaf flushing is associated with stronger developmental shifts in secondary chemistry and nutrients than asynchronous leaf flushing (Coley, 1980) was supported by our meta-analysis. How these different ontogenetic trajectories relate to patterns of herbivory and whether synchronous leaf development is linked with delayed greening syndromes remain unclear as most studies did not specifically identify species with delayed greening. Moreover, because leaf phenology was confounded with biome in our meta-dataset, with nearly all represented tropical species flushing leaves asynchronously while temperate species expressed both synchronous and asynchronous leaf phenology, it is difficult to characterize global patterns in leaf developmental defences. Recent studies have countered early hypotheses about latitudinal gradients in plant defence and herbivory (Anstett, Ahern, Johnson, & Salminen, 2018; Moles, Bonser, Poore, Wallis, & Foley, 2011; Moles & Ollerton, 2016), although none of these explicitly considered the importance of ontogeny in explaining global patterns. Whether tropical trees demonstrate less variation in defence traits during leaf maturation than temperate trees, due to higher frequencies of asynchronous leaf phenology in the tropics, would require more comprehensive community surveys than what can be gleaned from compiling species-specific data for meta-analysis.

Many authors have suggested that intensive herbivory on young leaves reflects the effective deterrence of toughness in mature leaves (Feeny, 1970; Kursar & Coley, 2003; Peeters, 2002; Raupp & Denno, 1983). High levels of secondary chemicals in young leaves are presumed to be the consequence of strong selection for herbivory, but they are apparently not as deterrent to insect herbivores as toughness. This has led to the proposal that insect herbivores face a brief phenological window when young leaves are available and upon which their development depends (Hunter & Lechowicz, 1992; Martel & Kause, 2002). Indeed, we detected that leaf toughness increases strongly during leaf maturation across a diverse species pool. Developmental increases in toughness reflect anatomical

constraints that prevent cell sclerification during the expansion phase, making young leaves vulnerable to physical damage and pathogen infection as well as herbivory (Coleman, 1986). We found that the magnitude of developmental increase depended on the metric of leaf toughness used in studies, consistent with previous biophysical studies (Read & Stokes, 2006). Puncture tests revealed stronger increases in toughness during leaf maturation than studies that quantified toughness as leaf mass per area. However, the ease with which leaf mass per area is measured, and its consistency with puncture test results, may argue for its general use (Hanley, Lamont, Fairbanks, & Rafferty, 2007).

Both glandular and non-glandular trichomes decrease in density during leaf maturation. While these shifts likely confer greater defence in young vs. mature leaves, it is unlikely that selection pressure has necessarily driven this developmental pattern. Trichomes differentiate early in leaf development when epidermal cell density is high (Szymanski, Lloyd, & Marks, 2000). Leaf expansion leads to a passive dilution of trichomes, driving this decrease in trichome density during leaf maturation.

The general patterns of simultaneous increases in leaf toughness and decreases in secondary chemical concentration and nutrients during leaf maturation indicate defence syndromes that involve an ontogenetic switch from defence based on secondary chemistry in young leaves to defence based on toughness and low nutritional quality in mature leaves. Ontogenetic switches in defence across whole-plant ontogeny have been described and attributed to allocation costs (Boege, Dirzo, Siemens, & Brown, 2007). For leaf ontogeny, switches in defence are more likely due to anatomical constraints limiting toughness in young leaves combined with strong selection pressure from herbivores. Ontogenetic switches involving indirect defence are also likely, but less well studied. For example, mature leaves produce more extrafloral nectar and food bodies in *Mallotus* spp (Euphorbia) in Japan, leading to an ontogenetic switch from defence based on trichomes and pellucid dots on young leaves to indirect defence on mature leaves (Yamawo, Tagawa, & Suzuki, 2014). Considering the specialized anatomical structures required for secretions to attract natural enemies, it is likely that many species express ontogenetic switches involving indirect defence traits during leaf development.

Consequences of shifts in defence and nutrient traits during leaf maturation will vary among different herbivores. For example, small insects are particularly sensitive to developmental increases in leaf toughness, strongly preferring young leaves, while large insects show less preference with respect to leaf age (Cizek, 2005). In addition to size, diet breadth is likely to be important, due to differences between specialists and generalists in their sensitivities to plant secondary compounds (Raupp & Denno, 1983). Specialists are predicted to prefer leaves that have the highest nutrient content and lowest physical defences regardless of secondary chemical content due to their capacity to tolerate and detoxify secondary chemicals. Generalists, in contrast, should be more sensitive to secondary chemicals and may select tissues with low secondary chemical content regardless

of nutrient content or physical defences. Feeding guild may also matter, with developmental shifts in leaf toughness contributing more to feeding patterns of chewing or galling insects than those with piercing-sucking mouthparts. Future work synthesizing variation among herbivores in feeding patterns and performance on young vs. mature leaves would complement the general plant patterns described here, shedding additional light on this narrow but important temporal dynamic.

This study contributes to a growing understanding of the important and widespread role ontogeny plays in plant defences and herbivory. The magnitude of trait variation across leaf development described here is of similar magnitude to that of whole-plant ontogenetic variation and of variation among species (Barton & Koricheva, 2010; Boege & Marquis, 2005), despite occurring at a much smaller temporal and spatial scale. While constraints of size or age may contribute to ontogenetic trajectories in defence, leading to selectively neutral 'ontogenetic drift' (Evans, 1972; Peng, Niklas, Reich, & Sun, 2010), the general patterns across leaf ontogeny strongly suggest an important role of selection by herbivores driving these trajectories. This sheds light on pressing questions about ontogenetic trajectories in defence, specifically what factors drive these trajectories and what factors explain variability across species (Barton & Boege, 2017). For leaf ontogenetic trajectories, persisting gaps in our knowledge relate largely to understanding variation among species and clarifying how these plant trait shifts link to patterns of herbivory. Specifically, future studies should (a) rigorously examine global patterns in leaf phenology (synchronous vs. asynchronous) and test for latitudinal gradients; (b) quantify shifts in secondary chemicals beyond phenolics, such as terpenes, alkaloids, defensive proteins and cyanogenesis; (c) determine whether decreases in secondary chemicals during leaf development result from biochemical activity, dilution or translocation; (d) test whether inducibility of secondary compounds shifts during leaf maturation; (e) investigate indirect defence traits in more species; and (f) examine fitness consequences of herbivory across leaf maturation in order to assess potential role of natural selection in driving these ontogenetic trajectories. Such future research directions will provide a more mechanistic and evolutionary understanding of a widespread and common ecological pattern that has fascinated biologists for decades.

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## AUTHORS' CONTRIBUTIONS

K.E.B. and J.K. developed the framework for this research; K.E.B. extracted data and conducted the analyses with K.F.E.; and K.E.B. wrote the manuscript with input from J.K. and K.F.E.

## DATA AVAILABILITY STATEMENT

Data are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.j2n1vq2> (Barton, Edwards, & Koricheva, 2019).

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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