

The Ontogeny of Plant Defense and Herbivory: Characterizing General Patterns Using Meta-Analysis

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Submitted March 17, 2009; Accepted November 2, 2009; Electronically published February 19, 2010

Online enhancements: tables. Dryad data: <http://hdl.handle.net/10255/dryad.1045>.

ABSTRACT: Defense against herbivores often changes dramatically as plants develop. Hypotheses based on allocation theory and herbivore selection patterns predict that defense should increase or decrease, respectively, across ontogeny, and previous research partly supports both predictions. Thus, it remains unclear which pattern is more common and what factors contribute to variability among studies. We conducted a meta-analysis of 116 published studies reporting ontogenetic patterns in plant defense traits and herbivory. Patterns varied depending on plant life form (woody, herbaceous, grass), type of herbivore (insect, mollusk, mammal), and type of defense trait (secondary chemistry, physical defense, tolerance). In woody plants, chemical defense increased during the seedling stage, followed by an increase in physical defenses during the vegetative juvenile stage. Mammalian herbivores showed a strong preference for mature compared to juvenile tissues in woody plants. Herbs experienced a significant increase in secondary chemistry across the entire ontogenetic trajectory, although the magnitude of increase was greatest during the seedling stage. Correspondingly, mollusks preferred young compared to older herbs. Future research investigating growth/defense trade-offs, allometry, herbivore selection patterns, and ecological costs would shed light on the mechanisms driving the ontogenetic patterns observed.

Keywords: plant-age hypothesis, growth-differentiation-balance hypothesis, plant resistance, induced defense, tolerance, seedlings.

Introduction

As plants develop and grow, they pass through a series of predictable and recognizable stages. During this progression, termed ontogeny, plants germinate into the seedling stage, pass through the vegetative juvenile stage, become mature at the onset of reproduction, and finish in senescence. Although the transition from one ontogenetic stage to the next may be difficult to pinpoint, there are generally

accepted traits that characterize each stage. For example, seedlings are defined by their dependence on stored seed reserves, with the transition to the juvenile stage occurring when seed reserves are exhausted (Hanley et al. 2004). The mature stage is marked by its reproductive status and includes plants just beginning to initiate reproduction as well as those that are flowering and fruiting.

Many plant traits vary across ontogenetic stages, including those involved in defense against herbivores. Variation among ontogenetic stages has been found in physical defenses such as trichomes (Traw and Feeny 2008), spines (Gowda and Palo 2003), and leaf toughness (Kearsley and Whitham 1989; Loney et al. 2006), as well as in chemical defenses such as alkaloids (Ohnmeiss and Baldwin 2000; Greganini et al. 2004; Elger et al. 2009), cyanogenic glycosides (Goodger and Woodrow 2002; Goodger et al. 2006), phenolics (Donaldson et al. 2006; Neilson et al. 2006; Elger et al. 2009), defensive proteins, and enzymes such as protease inhibitors (Van Dam et al. 2001; Doan et al. 2004), and various types of terpenoids (Sinclair and Smith 1984; Langenheim et al. 1986; Barton 2007). Ontogenetic shifts in defense traits may be associated with dramatic changes in levels of herbivory experienced by seedlings, juvenile, and mature plants. For example, concentrations of papyriferic acid, a triterpenoid, decrease by 96% from the juvenile to the mature stage in *Betula resinifera*, corresponding to 250 times higher consumption rates of mature birch shoots by snowshoe hares (*Lepus americanus*) in a choice experiment (Reichardt et al. 1984). Understanding the role of ontogeny in plant defense can thereby provide key new insights into observed patterns of plant resistance and herbivory.

Two existing hypotheses suggest different mechanisms behind the ontogenetic patterns in plant defenses against herbivory and offer conflicting predictions about the general directionality of those patterns. The growth-differentiation-balance hypothesis predicts that intrinsic factors, namely, acquisition and allocation of resources, limit the production of defensive secondary compounds in young

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Am. Nat. 2010. Vol. 175, pp. 481–493. © 2010 by The University of Chicago. 0003-0147/2010/17504-5115\$15.00. All rights reserved.
DOI: 10.1086/650722

plants, thereby leading to an ontogenetic increase in defense (Herms and Mattson 1992). In contrast, an aspect of the optimal-defense hypothesis that focuses specifically on ontogeny, the plant-age hypothesis (Bryant et al. 1992, *sensu* Spiegel and Price 1996), predicts that extrinsic factors, namely, selection by herbivores, lead to high levels of defense in juveniles, followed by decreases as plants mature and become less susceptible to the fitness reductions of these attacks. Numerous studies that tested the above hypotheses have documented both increases (e.g., Macedo and Langenheim 1989; Erwin et al. 2001; Schaffner et al. 2003; Elger et al. 2009) and decreases (e.g., Cipollini and Redman 1999; Fritz et al. 2001; Goodger et al. 2006) in plant defense traits during ontogeny, offering partial support for both of these hypotheses. Thus, it remains unclear which pattern is more common and what factors may contribute to variability among studies.

Several factors are likely to explain observed variability among studies in the ontogenetic patterns of plant defense. For instance, the longevity of the juvenile stage may play a key role in differentiating ontogenetic patterns of defense. Woody plants often survive for many years, even decades, as juveniles, before light gaps allow them to grow and mature, making them highly "apparent" to herbivores (*sensu* Feeny 1976). Defense during the juvenile stage is thus very important for survival and is likely to lead to higher or similar levels of defense in juveniles compared to mature woody plants. In contrast, the seedling and juvenile stages are typically short-lived in herbs and grasses because they germinate into dense communities where fast growth rates are essential for successful establishment (Berendse and Elberse 1990). Limited root and shoot tissues of seedlings and juvenile herbaceous plants further limit their acquisition and allocation of resources to defense. Therefore, in herbs and grasses, we might expect to find that defense increases from the seedling/juvenile to the mature stage.

Differences in ontogenetic patterns among various plant defensive traits may be an additional source of variability among studies. The production of physical defenses is likely to exact a significant photosynthetic investment, and so we predict that leaf toughness, spines, and trichomes will increase with plant age (Hanley et al. 2007). It is difficult to generalize about ontogenetic patterns in defensive secondary chemistry because of the great diversity within and among classes of secondary compounds. Some secondary compounds require specialized cells or storage tissues not yet developed in young seedlings, leading to ontogenetic increases when these tissues become available (e.g., idioblastic cells in *Umbellularia californica*, Lauraceae; Goralka and Langenheim 1996; Goralka et al. 1996). In contrast, other specialized structures are differentiated during early seedling development (e.g., epithelial ducts,

laticifers, and glandular trichomes; Herms and Mattson 1992) and are thus unlikely to show similar ontogenetic constraints in production. Moreover, many classes of secondary compounds do not require specialized storage tissues at all and are typically produced at very low amounts (e.g., alkaloids), leading to the prediction that these compounds may be relatively more abundant in seedlings and juveniles to provide protection during this highly vulnerable but valuable plant stage (Ohnmeiss and Baldwin 2000). Furthermore, differences may occur between constitutive and induced defenses. Induction allows plants to minimize investment in defense until necessary (i.e., after herbivory) and is thus expected to be favored when resource allocation constraints limit defense (Cipollini et al. 2003; Zangerl 2003). In addition, induction is linked to actively growing and differentiating tissues, which are proportionately more abundant in juvenile plants (Karban and Baldwin 1997). Thus, we predict that induced defense would peak in seedlings and juveniles, followed by a decrease with maturity. Finally, plant tolerance to herbivory is likely to show higher levels in mature plants compared to juveniles because of limitations on resource acquisition and stored reserves in young plants (Strauss and Agrawal 1999; Kelly and Hanley 2005; Hanley and Fegan 2007).

Ontogenetic changes in plant defense traits may result in differences in herbivory levels experienced by various plant ontogenetic stages or, alternatively, be caused by differing herbivore selection pressure on those ontogenetic stages. It is therefore important to examine the degree of correspondence between the observed ontogenetic patterns in plant defense traits and ontogenetic patterns in herbivory. Such comparisons may reveal which types of defensive traits are the most important determinants of herbivory. Furthermore, it is likely that the type of herbivore (mammals vs. insects vs. mollusks vs. birds) also explains variability among studies due to differences in herbivore access to ontogenetic stages. For example, many ground-dwelling mammalian herbivores cannot access mature plant foliage and as a result may impose different selection pressure on (or show different susceptibilities to) ontogenetic patterns in plant defense traits compared to insects that have access to both juvenile and mature plants.

In this study, we review the existing literature on ontogenetic changes in plant defenses and herbivory in order to reveal general patterns and important sources of variation. We expand on two previous reviews on the same topic. The first review, by Swihart and Bryant (2001), was much narrower in focus and examined only ontogenetic patterns of winter browsing of woody plants by mammals. Using meta-analysis, Swihart and Bryant (2001) demonstrated that winter-browsing mammals in choice experiments overwhelmingly prefer tissue from mature woody plants compared to tissue from juvenile plants. Presum-

ably, this reflects a decrease in the production of chemical defenses after trees outgrow mammalian herbivores. However, Swihart and Bryant (2001) were unable to directly test this hypothesis because their review did not include studies on ontogenetic patterns in defensive traits or in other types of herbivory (e.g., by insect herbivores). The second review, by Boege and Marquis (2005), adopted a more inclusive approach, presenting a general model for the ontogeny of plant defense. On the basis of resource allocation theory, Boege and Marquis (2005) predicted that ontogenetic patterns in plant defense are nonlinear, showing an initial decline during the seedling stage, an increase from juvenile to mature stages, and another decline during senescence. The model provides many interesting predictions that can be tested, but the review of evidence was limited by its narrative format.

In this study, we increase the scope of the review to include 153 plant species and apply meta-analysis to provide a quantitative assessment of the available literature. In particular, we address the following questions: (1) Is there a general ontogenetic pattern in plant defense? Applying the Boege and Marquis (2005) model, we predict a decrease from the seedling to the juvenile stage followed by an increase from the juvenile to the mature stage and a decrease within the mature stage. (2) What factors explain the variation observed among reports on the ontogeny of plant defense and herbivory? We predict that the following factors are likely candidates to account for variability among studies: plant life form (woody vs. herb vs. grass), plant defense traits (physical traits, chemical traits, induced defenses, tolerance), and type of herbivore (mollusks vs. insects vs. mammals vs. birds). (3) Does the general pattern in the ontogeny of plant defense reflect ontogenetic patterns in herbivory? We predict that ontogenetic patterns in plant defense traits coincide with ontogenetic patterns in herbivory.

Material and Methods

Compilation of the Database

We started our search for suitable studies by examining the bibliographies of the previous reviews on this subject (Swihart and Bryant 2001; Boege and Marquis 2005). Additional studies were identified by searching the online database Web of Science, using combinations of plant defense keywords ("plant defen?e," "resistance AND herbivor*," "herbivor*," "plant chemical defen?e," "induced defen?e," "induction," "physical defen?e," "tolerance AND herbivor*," "compensation AND herbivor*") with ontogeny keywords ("ontogen\$", "plant age," "development"). Finally, studies were identified by perusing the bibliographies of all retrieved studies. To be included in the final data

set, studies had to meet the following criteria: (1) Ontogenetic stage was either explicitly reported for plants or could be inferred from the descriptions of plant ages and vegetative characteristics (number of leaves, reproductive status, etc.). We define seedlings to be plants within days or weeks (up to several months in woody plants) of germination. Because of differences in the precise stages of plants analyzed, seedling studies differed between herbs and woody plants. Studies of herbaceous seedlings primarily included comparisons of cotyledon-stage plants with older seedlings containing true leaves. In contrast, seedling studies within woody plants included comparisons of young and older seedlings, all of which already had true leaves. We call small vegetative plants "juveniles," irrespective of their chronological age. Thus, in woody plants, the juvenile stage may last for decades and includes small forest plants waiting for light gaps as well as much larger saplings. (2) Plants categorized as juveniles grew from seed or grew as ramets in clonal species (e.g., *Populus tremuloides*). This excluded studies in which juveniles resprouted from stumps or were vegetatively propagated from adult plant tissues (e.g., Bryant et al. 1985; Den Herder et al. 2004; Lindroth et al. 2007). (3) The study provided a comparison of plant defense traits or herbivore damage and performance either between two different ontogenetic stages (e.g., juvenile vs. mature plants) or at least between two time points within the same ontogenetic stage (e.g., older vs. younger seedlings). (4) Comparisons of plant defense or herbivory between or within ontogenetic stages were made among different individuals. This excluded studies that compared tissues of various ages within an individual plant ("leaf age" studies) and studies following damage within individuals across time because most such studies focused on seasonal patterns rather than ontogeny per se. (5) The experimental design was robust, avoiding such common pitfalls as pseudoreplication and confounding explanatory variables (e.g., different geographic places for ontogenetic stages; Dominy et al. 2003). (6) The data needed for calculation of effect size (sample sizes, means, standard deviations, *F*-test statistics, or r^2 values) were reported in the article, either in numerical or graphical form, or could be provided by the authors on request.

The final data set included 116 studies published in 1969–2008 and conducted on 153 plant species from 36 plant families interacting with 30 herbivore species (see metadata bibliography, <http://hdl.handle.net/10255/dryad.1046>). Many publications reported on more than two ontogenetic stages, allowing for multiple ontogenetic comparisons. For studies that included multiple measurements within stages, mean values were calculated for each stage. Likewise, when additional treatments or factors were used (e.g., nutrients, populations) and separate means were reported for similarly aged groups of plants under different

treatments or populations, overall means were calculated so that single values could be used for each ontogenetic stage in each study. The final data set included 376 ontogenetic comparisons, distributed among the following four analyses: (1) young versus old seedlings ($n = 40$), (2) within juveniles (seedlings vs. juveniles or young juvenile vs. older juvenile; $n = 88$), (3) juvenile (seedling/sapling/vegetative juvenile) versus mature plants ($n = 219$), and (4) early versus late mature plants ($n = 29$).

Variables Analyzed

The retrieved studies reported either measures of plant defense traits (plant perspective studies) or measures of herbivore preference, performance, and density (herbivore perspective studies). Response variables for the plant perspective studies included concentrations of secondary chemicals (constitutive and induced), measures of physical defense (densities of trichomes and spines, leaf toughness), and tolerance. Two variables, induced defense and tolerance, represent plant responses to herbivory and were reported as comparisons of plant traits (chemistry, physical defenses, growth, or reproduction) for plants with and without herbivores. Response variables for the herbivore perspective studies included measures of herbivore damage and preference (leaf area missing, palatability, herbivore density, etc.) as well as herbivore performance (survival, growth rate, reproduction).

Several potential explanatory variables were tested, including study perspective (plant or herbivore), plant life form (woody, herb, grass), type of defense (chemical, physical, induced defense, tolerance), phylogeny (plant family), and herbivore type (mammal, insect, mollusk, bird). Within chemical defenses, differences were tested among classes of secondary chemicals (alkaloids, cyanogenic glycosides, glucosinolates, hydroxamic acids, phenolics, defensive proteins, terpenoids). Within the herbivore perspective, differences were tested among the three main groups of response variables: herbivore density, performance, and damage.

Meta-Analysis

The meta-analysis was conducted using the MetaWin 2.1 statistical program (Rosenberg et al. 2002). For constitutive plant defenses and herbivory measures, the effect size was calculated as Hedges's d , the standardized mean difference (Gurevitch and Hedges 2001) between the older and the younger ontogenetic stage means: $d = [(\bar{X}_O - \bar{X}_Y)/s]J$, where \bar{X}_O designates the mean of plant defense trait or herbivory measure in the older plant stage (e.g., mature plant), \bar{X}_Y is the corresponding mean for the younger plant stage (e.g., juvenile), s is the pooled standard

deviation, and J is the small-sample-size bias correction factor. When the means and standard deviations needed for calculation of Hedges's d were presented in graphs, they were scanned and digitized using the TechDig 2.0 software (Jones 2007). In some studies, these data were not available, and univariate statistics (r^2 , F , or t) were converted into Hedges's d estimates (Rosenberg et al. 2002).

The analyses of ontogenetic patterns in tolerance and induced defense were conducted on Hedges's d values calculated from means, standard deviations, and sample sizes of four groups (treatment and control groups for both older and younger plants) as demonstrated in Gurevitch et al. (2000): $d_1 = \{[(\bar{X}_{OH} - \bar{X}_{OC}) - (\bar{X}_{YH} - \bar{X}_{YC})]/s\}J$, where \bar{X}_{OH} designates the mean value for the herbivore-damaged plants in the older stage, \bar{X}_{OC} designates the control plants in the older stage, \bar{X}_{YH} designates the herbivore-damaged plants in the younger stage, and \bar{X}_{YC} designates the control plants in the younger stage. This formula essentially computes the difference in the plant responses to herbivory between the older and the younger developmental stages. Because these "interaction- d " calculations differ from the remaining data, tolerance and induced defense were analyzed together in a separate set of tests.

Hedges's d and d_1 were always calculated so that a positive value reflected an ontogenetic increase in plant defense (e.g., higher levels of secondary chemicals, tolerance, or physical defenses in mature plants compared to juveniles). For studies using the herbivore perspective, the sign was reversed in order to make these studies consistent with those with the plant perspective. For example, higher levels of herbivore damage in juveniles compared to mature plants would initially give a negative Hedges's d , but because a negative ontogenetic pattern in herbivory implies a positive ontogenetic pattern in defense, the sign was changed so that the final value was positive. Thus, for the herbivore perspective studies, a positive Hedges's d indicates lower levels of herbivore performance and damage in mature plants than in juveniles.

The analyses were performed using the mixed-effects model, which assumes that studies differ because of both sampling error and random variation. Given that these sources of variation are likely to be important in ecological data, the mixed-effects model is preferred for these analyses (Gurevitch and Hedges 2001). Bias-corrected bootstrap 95% confidence intervals around the effect size were generated from 4,999 iterations (Adams et al. 1997), and effects were considered to be significant when 95% confidence intervals did not include 0. For those mean-effect sizes that were significantly different from 0, we calculated a fail-safe number (n_{fs}) using the weighted method of Rosenberg 2005. This number represents how many additional studies of null effect and mean weight would need

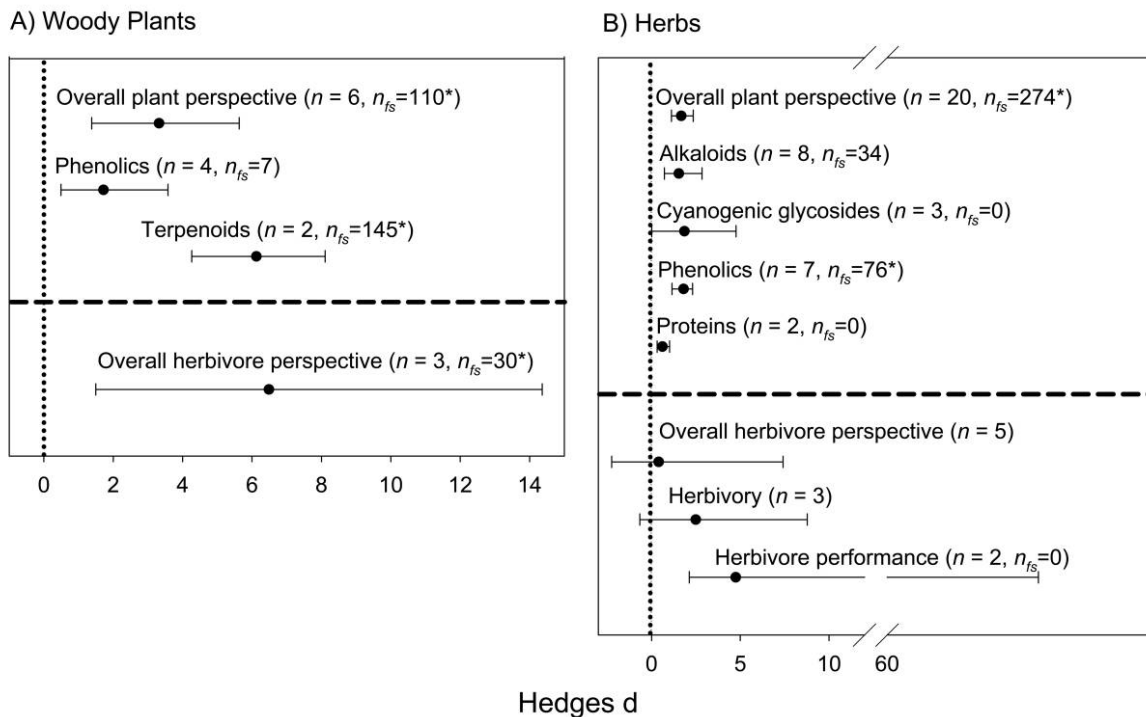


Figure 1: Mean Hedges's d effect sizes ($\pm 95\%$ bias-corrected confidence intervals), sample sizes, and fail-safe numbers (n_{fs}) for studies comparing young versus old seedlings in woody plant species (A) and herbaceous species (B). Significant patterns occur when the confidence intervals fail to include zero, which is marked with a dotted line. Asterisks indicate that the fail-safe number is robust ($> 5n + 10$).

to be added to reduce the significance level of the observed mean effect to 0.05. In general, a fail-safe number greater than $5n + 10$, where n is the original number of studies in the analysis, is considered robust against publication bias (Rosenthal 1991). In addition, we examined publication bias by means of funnel plots (Light and Pillemer 1984) and calculated rank correlation coefficients between effect size and sample size for the main study groups. Funnel plots and rank correlation coefficients revealed a low probability that publication bias affected this data set. Six potential outliers were identified (see complete meta database, <http://hdl.handle.net/10255/dryad.1047>), and their effects on the results were tested by removing them from the data and rerunning analyses. Results were similar for analyses conducted with and without the potential outliers, and so these studies were included in the final data set.

To test whether patterns differed among the explanatory variables discussed above (perspective, plant life form, herbivore type, plant family, etc.), studies were subdivided into corresponding groups, and between-group heterogeneity was examined using the χ^2 statistic Q_b (Gurevitch and Hedges 2001).

Results

Patterns within Seedlings

Both the plant and herbivore perspectives revealed an increase in defense during seedling development (study perspective effect was not significant, $P = .2284$; table A1 in the online edition of the *American Naturalist*; fig. 1). However, the pattern varied significantly among plant forms ($P = .0108$; table A1): the magnitude of increase was generally greater in woody plants compared to herbs (fig. 1), and grasses failed to show any clear pattern (table A2 in the online edition of the *American Naturalist*).

In both woody plants and herbs, levels of all examined classes of secondary compounds increased during seedling development. Although directionality of effect was similar among classes of secondary compounds, in woody plants terpenoids tended to increase more than phenolics ($P = .0656$; table A1; fig. 1A). The relatively few studies available from the herbivore perspective supported the conclusion that defense increases during seedling development in both woody plants and herbs (table A1; fig. 1).

In grasses, studies were restricted to measures of sec-

ondary compounds. Phenolic levels tended to increase through the seedling stage while concentrations of hydroxamic acids decreased (table A2), although the difference in patterns between classes of compounds was not significant ($P = .6934$).

Patterns within Juveniles

Ontogenetic patterns were weak within juvenile plants and varied among plant life forms ($P = .0438$) but not between plant and herbivore perspectives ($P = .2950$; table A3 in the online edition of the *American Naturalist*; fig. 2). In woody plants, overall measures of the herbivore and plant perspectives were not significant ($P = .3670$; fig. 2A). However, within the plant perspective, significant increases were detected for physical defenses (fig. 2A). No variation was observed for the secondary chemistry of juvenile woody plants ($P = .9440$; table A3; fig. 2A). All but one herbivore study in woody plants measured levels of insect herbivory, precluding any additional analyses of variability among herbivore types or herbivory measures.

In herbs, there was no difference between study perspectives ($P = .1354$); both perspectives indicated an ontogenetic increase in defense, although this pattern was significant only for herbivore studies (fig. 2B). Ontogenetic patterns were variable among classes of secondary com-

pounds in herbs ($P = .0474$; table A3), with significant increases observed for terpenoids but not for defensive proteins or alkaloids (fig. 2B). In herbs, all but one herbivore study reported measures of herbivore performance, again precluding additional analyses of heterogeneity.

In grasses, patterns portrayed by the plant and herbivore perspectives were marginally significantly different ($P = .0914$; table A2). Plant perspective studies were restricted to measurements of hydroxamic acids, which underwent significant declines during juvenile development (table A2). However, studies from the herbivore perspective failed to corroborate these patterns (table A2). Studies of plasticity in defense were available for herbs only, and these indicated that neither induced defense nor tolerance changes significantly during juvenile development (table A4 in the online edition of the *American Naturalist*).

Patterns across Juvenile-Mature Stage Transition

Ontogenetic patterns across the juvenile-mature plant transition were strong and varied significantly among woody plants, herbs, and grasses ($P = .0002$) and also between the herbivore and plant perspectives ($P = .0002$; table A5 in the online edition of the *American Naturalist*). Plant life form effects were confounded by type of plant defense trait ($\chi^2 = 36.411$, $df = 5$, $P < .0001$) and

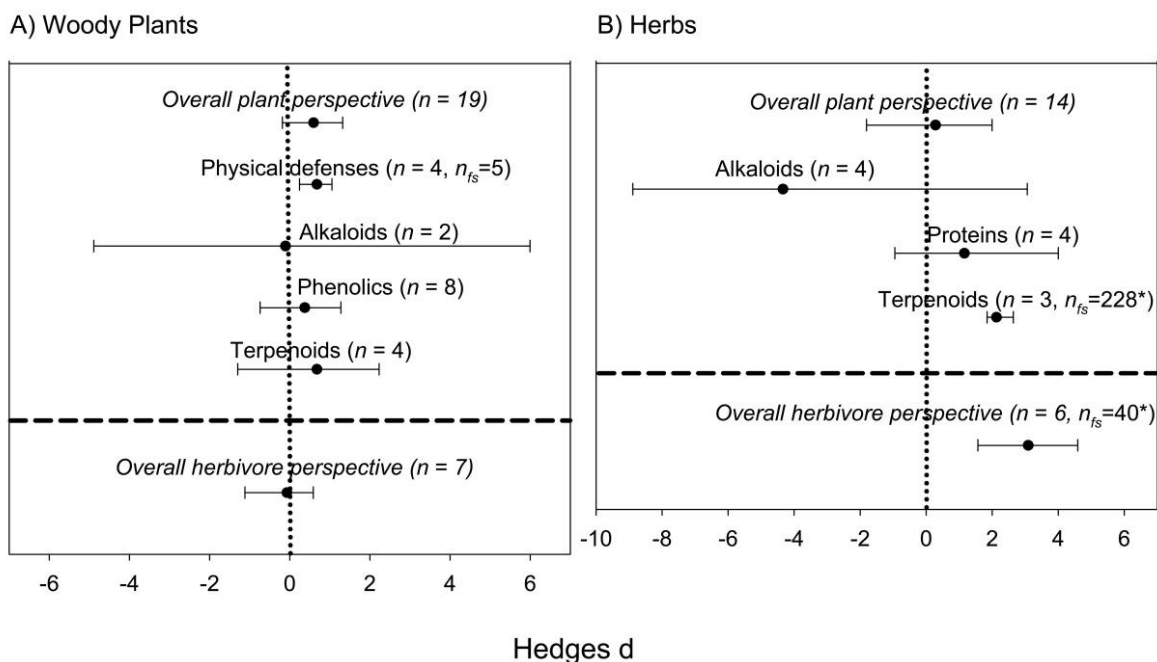


Figure 2: Mean Hedges's d effect sizes ($\pm 95\%$ bias-corrected confidence intervals), sample sizes, and fail-safe numbers (n_{fs}) for studies comparing young versus old juvenile plants in woody plant species (A) and herbaceous species (B). Significant patterns occur when the confidence intervals fail to include zero, which is marked with a dotted line. Asterisks indicate that the fail-safe number is robust ($> 5n + 10$).

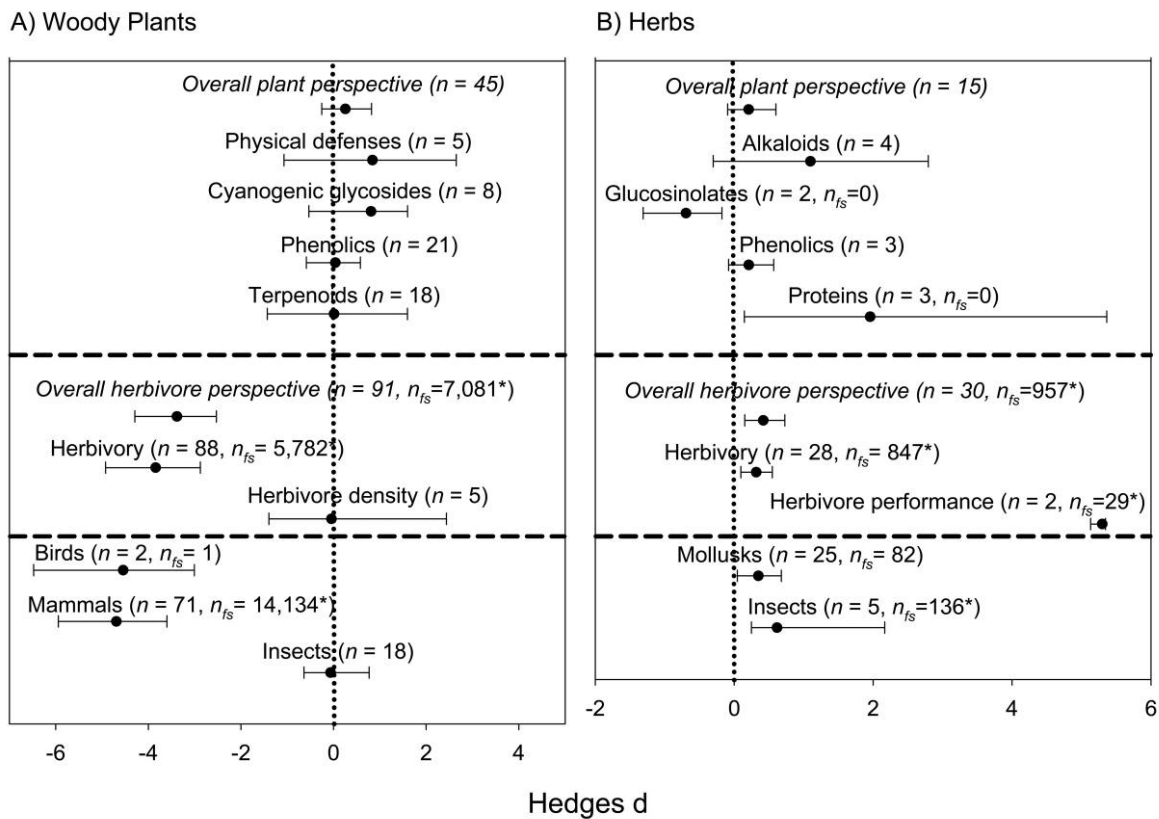


Figure 3: Mean Hedges's d effect sizes ($\pm 95\%$ bias-corrected confidence intervals), sample sizes, and fail-safe numbers (n_{fs}) for studies comparing juvenile versus mature plants in woody plant species (A) and herbaceous species (B). Significant patterns occur when the confidence intervals fail to include zero, which is marked with a dotted line. Asterisks indicate that the fail-safe number is robust ($> 5n + 10$).

herbivore type ($\chi^2 = 123.04$, $df = 4$, $P < .0001$), making it difficult to distinguish among causes of variability among studies. Studies on mammalian and bird herbivores were restricted entirely to woody plants, and studies on mollusks were restricted to herbs and grasses. Insect herbivores were represented on all plant groups, thereby providing a robust analysis of variability among plant life forms. Focusing on studies with insect herbivores only, we no longer detected significant variation among woody plants, grasses, and herbs ($P = .1620$; table A5), suggesting that differences among types of herbivores are responsible at least in part for differences detected among plant life forms.

We detected a significant difference between the two forms of phenotypic plasticity, induced defense and tolerance ($P = .0214$; table A4). Induced defense was generally higher in juveniles than in mature plants, whereas tolerance showed no ontogenetic pattern in any plant group (table A4). The overall negative pattern for induced defense was driven by the significant negative pattern for

herbs, while the two studies conducted on woody plants suggest that the pattern was actually in the opposite direction for woody plants (table A4).

In woody plants, significantly different patterns were portrayed by the two study perspectives: plant perspective studies showed no ontogenetic trend, while herbivore studies revealed a dramatic decrease in defense from the juvenile to the mature stage ($P = .0002$; fig. 3A; table A5). To determine whether the significant difference between herbivore and plant perspectives in woody plants was robust with respect to confounding differences among studies, we contrasted plant and herbivore effects within only those studies reporting both and found a similar, albeit weaker, difference between the two perspectives ($P = .1356$; table A6 in the online edition of the *American Naturalist*).

Within woody plants, neither physical defense traits nor secondary chemistry showed significant ontogenetic trends (fig. 3A). Moreover, analyses revealed no differences among classes of secondary compounds ($P = .5104$; table

A5). Studies from the herbivore perspective revealed that herbivory was greater on mature plants than on juveniles, while herbivore density showed no clear pattern, leading to a significant effect of herbivore measure ($P = .0576$; table A5; fig. 3A). Further heterogeneity was detected among types of herbivores feeding on woody plants ($P = .0040$; table A5). Mammals and birds showed significant preference for mature versus juvenile plants, while insects showed no clear preference (fig. 3A).

Many of the herbivore studies on woody plants were conducted in boreal forest systems. We conducted additional analyses to investigate whether these studies differed from the studies on other woody plants. Herbivory was significantly greater on mature than on juvenile plants in boreal systems but showed no pattern in nonboreal plants, leading to a significant difference between the two groups of plants ($P = .0004$; table A6). Few studies were conducted using the plant perspective in boreal systems, but nonetheless, there was a marginally significant difference between the two groups of plants: boreal plants had significantly higher levels of defense traits in juveniles than in mature stages, while nonboreal plants tended to show the opposite pattern ($P = .0882$; table A6).

In herbs, results did not differ between study perspectives ($P = .1354$), even though significant increases were detected from measures of herbivory but not from studies of plant defense traits (fig. 3B; table A5). There were no studies on herbs that reported results for both the plant and herbivore perspectives, preventing us from further investigating the correspondence between these two general approaches.

Only one study compared physical defense in juvenile versus mature herbs, reporting an increase in trichomes (Traw and Feeny 2008). Although ontogenetic patterns appeared to vary somewhat among classes of secondary compounds (table A5; fig. 3B), difference among classes was not significant ($P = .2618$), and all analyses suffered from low sample sizes, so these patterns should be considered preliminary.

Herbivore perspective studies in herbs revealed that both herbivory and herbivore performance were greater on juveniles than on mature plants, although the magnitude of difference between ontogenetic stages was greater for herbivore performance than for herbivore damage ($P = .0026$; table A5; fig. 3B). Herbivore type did not contribute to heterogeneity among studies of herbivory in herbs ($P = .4344$; table A5). Both mollusks and insects showed significant preferences for juveniles compared to mature plants (fig. 3B).

In grasses, study perspectives were marginally significantly different ($P = .0914$; table A2). Within plant perspective studies, we detected a significant decrease in secondary chemistry across the maturity transition (table A2).

Herbivore studies conducted on grasses provided mixed evidence: measures of herbivore damage indicated significantly greater defense in mature than in juvenile plants, while studies of herbivore performance suggested the opposite pattern ($P = .0236$; table A2). Herbivore type was not a significant source of heterogeneity in studies of grasses ($P = .3238$), although mollusks tended to prefer juvenile compared to mature plants, while preferences among insects were unclear (table A2). However, caution should be exercised in interpreting these results, given the low sample sizes.

Seventeen plant families had sufficient replication to allow for a phylogenetic analysis. For studies focusing on the plant perspective, there was no detectable variation among plant families ($P = .5186$; table A6). In contrast, for studies focusing on the herbivore perspective, we detected significant variation due to phylogeny ($P = .0034$; table A6). However, these patterns are confounded with plant life form and herbivore type. The only plant family showing a significant decrease in herbivory across ontogeny was Asteraceae (table A6), and all of the studies of Asteraceae were conducted with insect herbivores. No studies were conducted from the plant perspective on Asteraceae, so how ontogenetic patterns in insect damage relate to plant traits in this family remains unclear. Woody plant families interacting with mammalian herbivores showed the reverse pattern in herbivory (table A6). Given the nonindependence among explanatory variables, it is impossible to determine whether variation among plant families is due to phylogeny, life history strategy, or herbivore type.

Patterns within Mature Plants

We detected weak overall patterns within the mature plant stage (fig. 4), and neither plant life form ($P = .2866$) nor study perspective ($P = .5142$) contributed to heterogeneity among studies (table A7 in the online edition of the *American Naturalist*). Our analysis of phenotypic plasticity in mature plants was limited to a nonsignificant negative trend for induced defense within herbs (table A4). There were no studies on grasses within the mature stage.

Within woody plants, both the plant and herbivore perspectives indicated that defense decreases during the reproductive stages of woody plants, and there was no difference between the two study perspectives ($P = .9140$; table A7), although this pattern was significant only for the plant perspective (fig. 4A). Small sample sizes prevented further analyses of woody plants.

Analyses of herbs were restricted to the plant perspective, where no significant patterns were detected (fig. 4B). Although classes of secondary compounds appear to differ

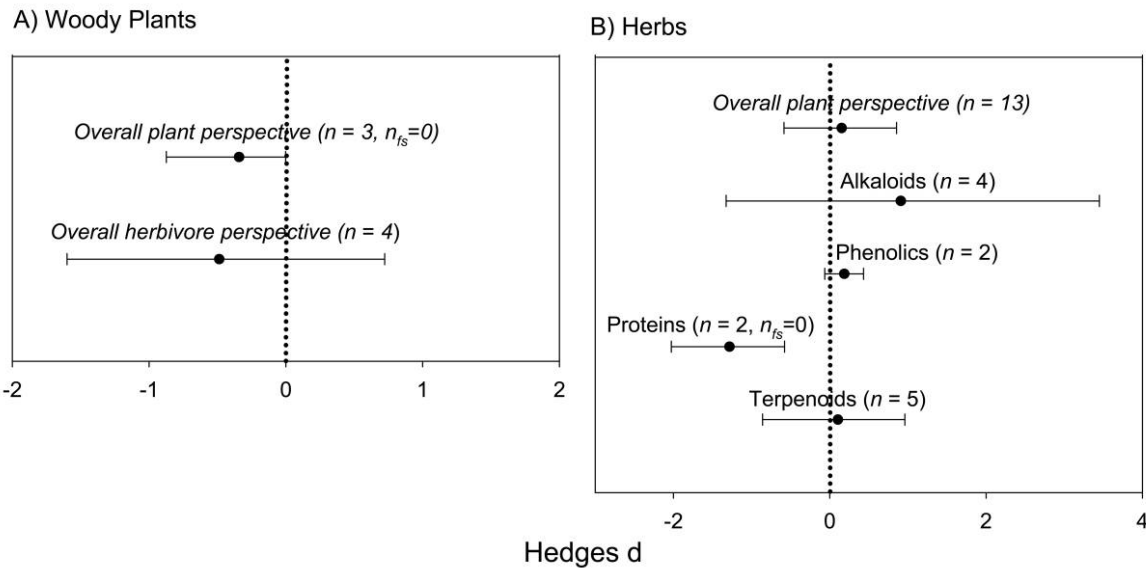


Figure 4: Mean Hedges's d effect sizes ($\pm 95\%$ bias-corrected confidence intervals), sample sizes, and fail-safe numbers (n_{fs}) for studies comparing young versus old mature plants in woody plant species (A) and herbaceous species (B). Significant patterns occur when the confidence intervals fail to include zero, which is marked with a dotted line.

in their ontogenetic patterns, these differences were not statistically significant ($P = .5042$; fig. 4B; table A7).

Discussion

Using meta-analysis, we were able to uncover significant, complex, and nonlinear patterns in the ontogeny of plant defense and herbivory. Our main findings are as follows: (1) there is no single general pattern in the ontogeny of plant defense. Instead, (2) ontogenetic patterns of plant defense differ among types of plants and types of defenses (fig. 5). (3) The directionality and magnitude of change across ontogenetic stages were variable, leading to nonlinearity in the patterns observed. (4) Ontogenetic patterns in plant defense traits are not consistent with ontogenetic patterns in herbivory for woody plants, although the two are consistent in herbaceous species. Below we describe the detected patterns in more detail and consider possible explanations for them as well as suggest further directions for research on the ontogeny of plant defenses.

Ontogenetic Patterns in Plant Defenses

One of the clearest patterns detected by the meta-analysis was a dramatic increase in constitutive levels of all classes of chemical defenses through the seedling stage in both woody plants and herbs (fig. 5). For herbs, the pattern contradicts the model by Boege and Marquis (2005), which

predicts that a decrease in defenses from the cotyledon to the seedling stage as maternal resources are depleted, followed by a period of low defense production as plants allocate resources preferentially to growth. The observed increase in defenses through the seedling stage might indicate that seedlings are able to produce their own defenses even before they use all the seed reserves and that defense levels increase rapidly as seedlings grow. This would make sense from the adaptive point of view because larger seedlings are more conspicuous to herbivores, and therefore, without increased defensive investments, they might suffer more herbivory than younger, smaller seedlings. For woody plants, the seedling stage included only plants with true leaves, and so the observed increase in chemical defense further contradicts the Boege and Marquis (2005) model, which predicts low levels of chemical defenses throughout the seedling stage, when photosynthetic area is limited. Studies investigating the cotyledon stage in woody plants are needed to determine how plant defense changes during this earliest ontogenetic stage.

Trajectories of changes in plant defenses through the juvenile stage depended on the type of defenses as well as the type of plant and were not as strong as those during the seedling stage. Increases in defenses through the juvenile stage are predicted by the Boege and Marquis (2005) model on the basis of a decrease in root : shoot ratio, which allows plants to acquire more resources to use for defenses. However, we observed variable patterns among woody

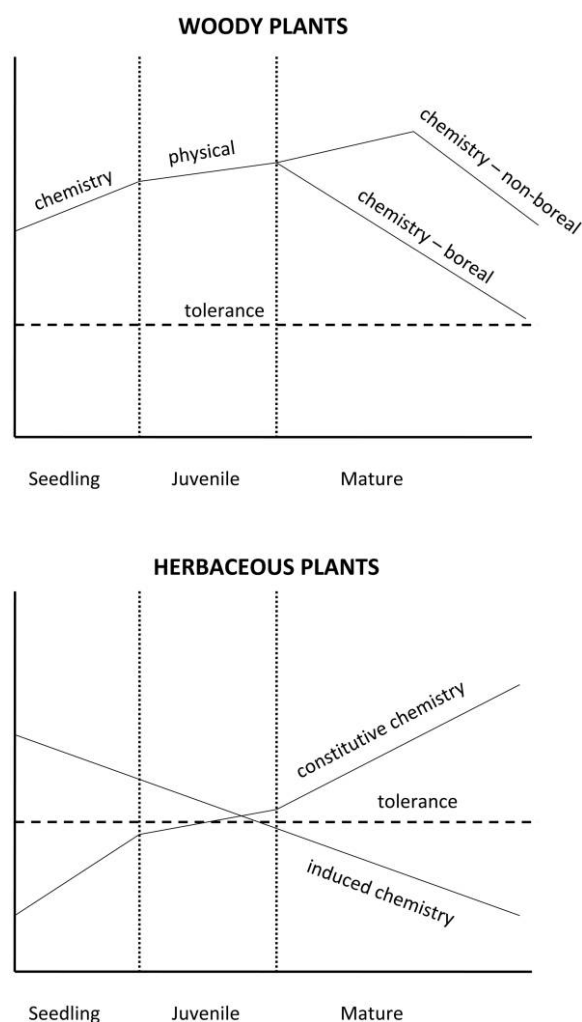


Figure 5: Summarized ontogenetic changes detected in plant defense traits (constitutive secondary chemistry, induced secondary chemical defense, physical traits, and tolerance) for woody and herbaceous plant species. Differences in patterns for boreal and nonboreal woody plants are shown for the mature stage. Grasses are omitted because small sample size constrained analyses from identifying general patterns.

plants (increase in physical defenses), herbs (increases in some secondary chemicals, decreases in others), and grasses (decrease in hydroxamic acids). Clearly, ontogenetic patterns of defense depend on plant life-history strategy and defense traits. Furthermore, the traits undergoing significant patterns in juveniles differed from those in seedlings, suggesting possible switches among key defense traits. For example, woody plants showed an increase in secondary chemicals for seedlings, followed by an increase in physical defenses for juvenile plants.

Transition from the juvenile to the mature stage had no significant effect on physical defenses, constitutive chem-

ical defenses, or tolerance in woody plants, despite reasonably large sample sizes. This reflects heterogeneity among studies due to unidentified variables and may indicate the importance of biological idiosyncrasies of plant species. In herbs, the direction of change in defenses during the transition from juvenile to mature stage depended on the type of defense: concentrations of glucosinolates decreased while those of defensive proteins increased. In addition, induced defenses in herbs decreased from juvenile to mature plants (fig. 5). This result is in accordance with our predictions based on lower costs of induced defense (Cipollini et al. 2003; Zangerl 2003) and their dependence on actively growing and differentiating tissues (Karban and Baldwin 1997).

Too few studies on grasses were available to detect a reliable pattern, but available studies seem to suggest that secondary chemistry is reduced during the transition from juvenile to mature stage. Whether this pattern results from selection pressure by herbivores, reflects reallocation of secondary chemicals from vegetative tissues to reproductive organs, or occurs as part of an ontogenetic switch with other types of defense, such as silica, which has been shown to increase from the juvenile to the mature stage in *Lolium multiflorum* (Moore 1984), requires further investigation.

Tolerance did not differ between juveniles and mature plants, regardless of the type of plant (fig. 5). This contradicts predictions that juveniles would be less tolerant of herbivory than are mature plants because of resource allocation and architectural constraints (Strauss and Agrawal 1999; Haukioja and Koricheva 2000; Kelly and Hanley 2005). Combining this result with the increase in defense during the seedling stage, it appears that allocation costs have little explanatory power in the ontogeny of defense, emphasizing the need for new mechanistic hypotheses of plant defense.

During the mature stage, the limited data suggest that defenses decrease in woody plants and vary among classes of secondary compounds in herbs, offering partial support of the prediction from Boege and Marquis (2005) that defenses decrease during the mature stage.

Ontogenetic Patterns in Herbivory

We predicted that ontogenetic patterns in herbivory would correspond to ontogenetic patterns in plant defense traits. This prediction is based on the assumption that plant resistance to herbivory can be measured in terms of a single or a few specific traits. However, we detected good correspondence between ontogenetic patterns in plant defense and herbivory only for some ontogenetic stages, plant types, and herbivore types. Insects and mollusks are the main herbivores during the seedling stage, and good correspondence between plant defense and herbivory patterns

during the seedling stage was observed: an increase in plant defenses was accompanied by a decrease in herbivory, which was particularly pronounced in herbs.

During the juvenile stage, there was also a good correspondence between the increase in chemical defenses and the significant decrease in herbivory in herbs. However, no clear patterns in herbivory during the juvenile stage were found in woody plants or grasses. This is despite the increase in physical defenses in woody plants and the decrease in hydroxamic acids in grasses. The lack of clarity in the herbivore studies from the juvenile stage likely results from the focus of these studies on insect herbivores. Among all three plant groups, all but one study measured insect herbivores. Indeed, across all ontogenetic stages, insects generally failed to show reliable patterns in herbivory. This could result from variability among insects in their preferences for ontogenetic stages of their host plants (Kearsley and Whitham 1989). Considering the great diversity of insect species and insect herbivore feeding guilds, it is not surprising that a general pattern in insect herbivory failed to emerge.

Patterns of herbivory across the juvenile-mature plant transition differed significantly between woody plants and herbs. In herbs, studies focused on mollusks, which generally showed significant preference for juveniles compared to mature plants. This corresponds to the increase observed in herb defense traits. In contrast, studies of woody plants focused on mammals, and patterns of herbivory across the juvenile-to-mature plant transition appear to vary geographically. Most of these studies (all but four) were conducted in boreal forest systems, examining winter browsing by small mammals, such as hares (*Lepus* spp.) and voles (*Microtus* spp., *Clethrionomys rutilus*), that showed dramatic preference for mature compared to juvenile plant foliage. Under most circumstances, these herbivores do not have access to mature plant foliage, with the exception of fallen branches or trees. Thus, we would predict that selection pressure for defense against mammalian herbivores is strong in juvenile plants and weak or absent in mature plants. Indeed, when we restricted our analysis of plant chemical traits to boreal species, it revealed higher defenses in juvenile boreal plants than in mature trees. Good correspondence between ontogenetic patterns in plant defense and herbivory in boreal woody plants highlights the importance of mammalian herbivores as selective agents in this system and supports the plant-age hypothesis (Bryant et al. 1992). Nonboreal herbivores also displayed higher preference for mature plants, although the difference was not as dramatic as for boreal herbivores, supporting the conclusion by Swihart and Bryant (2001) that plants grown in areas with harsh winter conditions elicit greater discrimination of juvenile and mature woody plant stages by mammalian herbivores than

do plants grown in more moderate climates. It is still unclear why there is no correspondence between mammalian herbivory and ontogenetic patterns of defenses in tropic and temperate ecosystems, although it may be that other herbivores, especially insects, drive ontogenetic patterns in temperate and tropical forests.

Several mutually nonexclusive mechanisms may explain the lack of correspondence between ontogenetic patterns in plant defense and herbivory. Researchers could be measuring the wrong plant traits (i.e., not those of importance to herbivores), or, more likely, defense is more complex than single-trait studies assume. Expanding our view of plant defense to consider suites of traits (or “defense syndromes,” sensu Agrawal and Fishbein 2006) might reveal that synergism among traits plays an important role in plant defense against herbivory. Moreover, consideration of ontogenetic shifts in nutrient profiles (Erwin et al. 2001; Ishida et al. 2005) and tritrophic interactions (Boege 2005; Boege and Marquis 2006) could provide greater predictability of ontogenetic patterns of herbivory.

Conclusions

Our synthesis of the literature on the ontogeny of plant defense and herbivory reveals that previous attempts to develop general models of the ontogeny of defense are too simplistic and overlook biologically meaningful variability among studies. We detected significant variation among plant life forms, among herbivore types, and among plant defense traits, allowing us to describe patterns for each of these subgroups. However, our synthesis revealed several gaps in studies of the ontogeny of plant defense and herbivory, which would provide fruitful areas for future research. For example, grasses are understudied in all regards, and more research is needed on the ontogeny of different kinds of plant traits (e.g., silica) and on patterns of herbivory in grasses. Ontogenetic patterns in physical defenses, including trichomes, spines, thorns, and leaf toughness, are poorly understood in all plant groups (Hanley et al. 2007). Other kinds of defense traits, specifically biotic defense (i.e., ants, other predators, and parasitoids) and escape, were omitted from our analysis altogether because of small sample sizes. More studies of insect herbivores, particularly nonfolivores (e.g., phloem and xylem feeders, root herbivores), are needed to determine how ontogenetic patterns in plant defense traits influence temporal patterns in insect herbivory. Finally, despite the great number of studies documenting ontogenetic patterns in plant defense traits and herbivory, the mechanisms driving these patterns and the consequences for community dynamics and seasonal and yearly trends remain unclear. Identifying which intrinsic factors (i.e., allocation constraints, allometry, autotoxicity, architecture, and phylog-

eny) and which extrinsic factors (i.e., ecological trade-offs, and herbivore selection) drive ontogenetic patterns in plant defense should be a priority of future research and will provide key new insights into this fundamentally important aspect of plant evolutionary ecology.

Acknowledgments

We thank K. Boege, M. Hanley, Y. Linhart, and one anonymous reviewer for insightful comments on the draft of this manuscript and J. Gurevitch and G. Stewart for advice on the design of the meta-analysis. Many authors of studies included in the meta-analysis graciously provided data on request, which was greatly appreciated. Financial support was provided by a Natural Environment Research Council Postdoctoral Fellowship to K.E.B. (NERC NE/E012418/1).

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Associate Editor: Tia-Lynn Ashman
Editor: Donald L. DeAngelis



Left, 2-week-old seedling; center, 4-week-old juvenile; and right, 6-week-old mature plant of *Plantago lanceolata* growing in experimental garden in Boulder, Colorado. Photographs by Kasey E. Barton.