ORIGINAL PAPER

A quantitative evaluation of major plant defense hypotheses, nature versus nurture, and chemistry versus ants

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Abstract Variation in plant secondary metabolite content can arise due to environmental and genetic variables. Because these metabolites are important in modifying a plant's interaction with the environment, many studies have examined patterns of variation in plant secondary metabolites. Investigations of chemical defenses are often linked to questions about the efficacies of plant defenses and hypotheses on their evolution in different plant guilds. We performed a series of meta-analyses to examine the importance of environmental and genetic sources of variation in secondary metabolites as well as the antiherbivore properties of different classes of defense. We found both environmental and genetic variation affect secondary metabolite production, supporting continued study of the carbon-nutrient balance and growth-differentiation balance hypotheses. Defenses in woody plants are more affected by genetic variation, and herbaceous plant defenses are more influenced by environmental variation. Plant defenses in agricultural and natural systems show similar responses to manipulations, as do plants in laboratory, greenhouse, or field studies. What does such variation mean to herbivores? A comparison of biotic, physical, and chemical defenses revealed the most effective defensive strategy for a plant is biotic mutualisms with ants. Fast-growing plants are most often defended with qualitative defenses and slow-growing plants with quantitative defenses, as the plant apparency and resource availability hypotheses predict. However, we found the resource availability hypothesis provides the best explanation for the evolution of plant defenses, but the fact that there is considerable genetic and environmental variation in defenses indicates herbivores can affect plant chemistry in ecological and evolutionary time.

Handling Editor: Samantha M. Cook.

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Introduction

Plants display significant intraspecific variation in defenses, including production of secondary metabolites, physical defenses, and adaptations for attracting predators and parasites of herbivores. Variation in constitutive chemical defenses affects growth, photosynthesis, reproductive fitness, herbivory, pathogen infection, herbivore-natural enemy interactions, and pollination (reviews include: Lindroth 1988; Rosenthal and Berenbaum 1991, Behmer et al. 2002, Bassman 2004, Koricheva et al. 2004). This variation can arise as a result of phenotypic plasticity in secondary metabolite production, which allows plants to respond to

variable environmental conditions, including light availability, soil nutrients, soil moisture, atmospheric CO_2, herbivory (in the case of induction), and, in a few documented cases, the ecology and physiology of neighboring plants (Baldwin and Schultz 1983; Arimura et al. 2000; Karban et al. 2000; Farmer 2001; Karban 2001; Massad and Dyer 2010). The quantitative response of plant defenses to resources in their environments has been formalized in the carbon-nutrient balance (CNB) and the growth differentiation balance (GDB) hypotheses (Bryant et al. 1983; Herms and Mattson 1992). The utility of the CNBH and its assumptions have generated considerable controversy (Hamilton et al. 2001; Lerdau and Coley 2002), however, it still succeeds in explaining variation in diverse groups of defenses (e.g., Koricheva et al. 1998; Dyer et al. 2004).

Genotype may also constrain the variability of plant secondary metabolite production (e.g., Marquis 1992; Keinanen et al. 1999; reviewed in Hamilton et al. 2001; Ivey et al. 2009). Genetic variability can be expressed as differences in the identity or concentration of plant chemicals between genotypes or as variation in phenotypic plasticity between genotypes. Although a wide range of experiments, surveys, and other studies have examined and debated the contributions of genotype and environment to variation in plant secondary metabolism, controlled experiments comparing the importance of environmental and genetic variables are rare (Karban 1992; Marquis 1992; Hamilton et al. 2001; Orians and Ward 2010). The generality of individual studies is also limited, since most experiments focus on individual defenses, using a single model species or comparing a few plant species. Light, nutrients, genotype, and other variables clearly have significant effects on plant defenses, but these effects vary between plant species, growth form, type of secondary metabolite, presence of synergy, and other factors (Koricheva 2002, 2004; Nykanen and Koricheva 2004; Dyer et al. 2004).

This immense variation in plant defense quality and quantity shapes plant herbivore interactions, and a large body of literature examines the effects of plant defenses on herbivores (reviewed by Harborne 1993; Bernays and Chapman 1994; Price 1997). Empirical studies on the efficacies of plant defenses have been guided by a strong theoretical framework (Feeny 1976; Rhoades and Cates 1976; reviewed by Price 1997; Stamp 2003), and while these studies have demonstrated effectiveness of some defenses against narrow herbivore guilds, it is clear no plant defensive strategy can be characterized as entirely successful against broad taxa or guilds of herbivores (Bernays and Graham 1988; Bernays et al. 1989a; Ayres et al. 1997; Nykanen and Koricheva et al. 2004; Cornell and Hawkins 2003). These two broad topics, controls on plant defense production and the effects of plant defenses

against herbivores, are central to the fields of chemical ecology and plant-arthropod interactions. Although other meta-analyses have examined environmental effects on defense production in woody plants (Koricheva et al. 1998) and changes in herbivory via effects of global change on defenses (Massad and Dyer 2010), we expand this work with quantitative syntheses of environmental and genetic variation in woody and herbaceous plant chemical defenses and an inclusive review of the effects of chemical, physical, and biotic defenses on generalist and specialist herbivores. Our hypotheses are guided by the theoretical framework developed in classic plant defense hypotheses: resource availability and plant apparency. Although these hypotheses are well summarized by Stamp (2003), our work provides a quantitative evaluation of their applicability and provides a thorough summary of current progress in chemical ecology and the efficacies of plant defenses.

Resource availability or growth rate hypotheses suggest that available resources direct the evolution of broad defensive classes (Janzen 1974; Grime 1977; Coley et al. 1985; Coley 1987). These hypotheses assume slow-growing plants evolved in resource poor environments via selection against a suite of fast-growth strategies. Because the production of plant tissues is costly under such conditions, slow-growing species tend to have long-lived leaves protected with expensive, quantitative defenses. Furthermore, in resource rich environments, plants evolved fast growth rates to take advantage of available nutrients, with an associated reduction in antiherbivore defenses. Thus, according to the resource availability hypothesis, slow-growing species should be better defended and suffer less herbivory than fast-growing species (Coley et al. 1985). Associated ecological hypotheses, focused on phenotypic plasticity, suggest that plants can respond to excess resources in their environments by using them to increase defenses, so that when a plant has more carbon or nitrogen than can be invested in growth, the excess can be used to augment carbon- or nitrogen-based defenses (CNBH, Bryant et al. 1983; GDBH, Herms and Mattson 1992). In addition, Bryant et al. (1983) suggest fast-growing species have greater resource mobility than slow-growing species and may easily increase their defensive levels, resulting in large decreases in herbivory.

Plant apparency theory, which was originally developed by a number of papers proposing complimentary hypotheses (Feeny 1976; Rhoades and Cates 1976; Rhoades 1979; reviewed by Stamp 2003), posits that long-lived, apparent plants contain quantitative defenses (usually C-based compounds) that function by reducing digestion and nutrient uptake. These defenses are effective against specialist and generalist herbivores, and specialists are predicted to be less abundant on these plants. Consequently, specialists are found more often on herbaceous plants with

qualitative defenses (both N-based and more toxic classes of C-based compounds), which are defined as having toxic effects on herbivores. Many specialist herbivores adapt to these qualitative defenses and are often capable of sequestering them to use in defense against their own predators and parasites (Rhoades and Cates 1976; Montllor et al. 1990; Dobler and Rowell-Rahier 1994; Theodoratus and Bowers 1999; Zalucki and Malcolm 1999; Dyer 1995; Engler-Chaouat and Gilber 2007; but see Agrawal and Kurashige 2003). Generalist herbivores, in contrast, are not as likely to be adapted to toxic defenses, and feed broadly on more apparent plants with dose-dependent, C-based defenses (Rhoades and Cates 1976; Rhoades 1979). Here, we focus on generalist and specialist herbivore responses to distinct secondary metabolites and plant guilds (*sensu* Kindscher and Wells 1995).

We examine predictions generated from these plant defense hypotheses, using meta-analysis to quantify changes in chemical defenses in different plant guilds. We then explore the effects of defenses on herbivores. We reviewed hundreds of papers in our analyses and summarize classes of defense employed by different plant guilds to evaluate whether defensive strategies are represented in plant guilds as predicted by plant defense theory (Table 1). Because there are many independent studies focusing on plant defenses and herbivores, meta-analysis is an effective method to summarize support for these hypotheses. Meta-analysis is a statistical tool for the quantitative comparison of multiple experiments addressing similar questions; it is a well-developed technique that has been successfully used in a wide range of fields (Cooper et al. 2009).

Predictions for sources of plant chemical variation

Environmental and genetic factors account for a significant proportion of the variation in plant secondary metabolite content. Based on the CNBH, GDBH, and other hypotheses linking growth rate, nutrient availability, and defense

(Bryant et al. 1983; Coley et al. 1985; Tuomi et al. 1988; Herms and Mattson 1992), we predict that herbaceous and woody plants that produce carbon versus nitrogen based secondary metabolites will respond differently to environmental manipulations, such as CO_2 enrichment and soil nutrient supplements, since these treatments affect the C/N balance of the plant. Likewise, we expect C- and N-based secondary metabolites will differ in the magnitude and direction of their responses to environmental manipulations. We also predict that experiments in controlled environments (e.g. laboratories and greenhouses) will have larger effect sizes than those taking place in the field, where it can be more difficult to control all sources of experimental error. For similar reasons, we predict that agricultural study systems will have larger effect sizes than natural systems.

Predictions for herbivore response

We hypothesize that if herbivore pressure influences the evolution of different classes of plant defenses in the manner proposed by Rhoades and Cates (1976), then qualitative, nitrogen-containing metabolites should be more deterrent to generalist herbivores, while digestibility-reducing defenses should limit both specialists and generalists. Second, if specialists are more adapted to qualitative than quantitative defenses, then increases in defenses of late successional plants should be more deterrent to specialist herbivores than increases in defenses of fast-growing and herbaceous species. Third, if generalists lack such adaptations to qualitative defenses, then they should be deterred more by increases in secondary metabolites in fast-growing plants because of their toxic effects. Biotic and structural defenses may have equivalent impacts on both types of herbivores. Fourth, we hypothesize that if fast-growing and herbaceous plants have more resource mobility (as suggested by Bryant et al. 1983), then these plants may increase their levels of defense more effectively

Table 1 Predictions of the plant apparency vs. the resource availability hypotheses

	Predictions of the plant apparency hypothesis (based on Feeny 1976 and Rhoades and Cates 1976)		Predictions of the resource availability hypothesis (based on Coley et al. 1985)	
Evolutionary constraints	Specialist herbivore pressure on apparent plants	Generalist herbivore pressure on unapparent plants	Resource rich environment	Resource poor environment
	↓	↓	↓	↓
Plant defense response	Quantitative defenses evolved in apparent plants	Qualitative defenses evolved in unapparent plants	Fast-growing plants evolved qualitative defenses	Slow-growing plants evolved quantitative defenses
	↓	↓		
Herbivore response	Specialists are more deterred by quantitative defenses	Generalists are deterred by qualitative and quantitative defenses	No predictions; relies solely on the plant's resource environment to explain patterns of defense	

than slow-growing plants, resulting in stronger herbivore responses.

Methods

We performed mixed model meta-analyses on data collected from articles in peer-reviewed journals published between 1975 and 2005. Meta-analysis allows quantitative comparison of studies that address similar questions but differ in methods, study systems, locations, and scale (Gurevitch and Hedges 2001). For each study, an effect size (d_{si}) and 95% confidence intervals were calculated with the means, standard deviations, and sample sizes of control and experimental treatments. Data were gathered from tables, text, figures, or calculated from other statistics. The effect size for each experiment indicates the magnitude of the response of the experimental samples, as compared to the control samples and is independent of sample size. Effect sizes from many studies can be combined to determine whether a given treatment category has an effect that is significantly different from zero and whether treatment categories differ from each other. We considered any effect size greater than 1.0 to be large (Gurevitch and Hedges 2001). Differences between effect sizes were tested using the between class heterogeneity statistic, Q_B, which is distributed approximately as χ^2, with degrees of freedom equal to the number of classes minus one (Gurevitch and Hedges 2001).

A potential problem with meta-analysis is publication bias, which omits non-significant results from the data synthesis. Two common statistical methods are used to test for publication bias in meta-analysis datasets, funnel plots (Cooper et al. 2009) and fail-safe numbers (Rosenberg 2005). We used SAS to calculate funnel plot symmetry (Rendina-Gobioff and Kromrey 2006) with $P > 0.05$ as our critical β; $P > 0.05$ indicates our data had an unbiased distribution. We calculated fail-safe numbers with Rosenberg's fail safe calculator to produce the number of studies with effect sizes equal to zero that would have to be added to our dataset to make Q_B values non-significant (Rosenberg 2005).

Plant defense methods

Appropriate studies were acquired using Web of Science searches with the terms chem* AND defense, herbiv*, plant AND chem*, secondary AND chem*, secondary AND metab*, genetic variation, phenotypic plasticity, and nutrient AND availability. A thorough examination of all issues through 2005 of the Journal of Chemical Ecology was also completed to find relevant papers. We included only terrestrial experiments clearly reporting means, standard deviations and sample sizes, or reporting some combination of statistics from which these values could be calculated. To ensure that all values included in the analyses were independent, we accepted only one effect size per independent experiment, for a maximum of three experiments per paper. Individual effect sizes and experiments were randomly selected from each paper. When a series of measurements were taken over time, we used data from the final sampling date. Only chemical defenses were included for this part of the study; other types of plant defense, including ant associations, toughness, and mechanical defense, were not examined.

To examine sources of variation among studies, we extracted a variety of information from each study, including the latitude of the place of origin of study plants (tropical or temperate), whether plants were wild or agriculturally cultivated, the location of the study site (greenhouse, laboratory, or field), and the type of secondary metabolite measured (C- or N-based). Additionally, plants were grouped into two types: herbaceous plants (annuals and perennials) and woody plants. Common garden experiments conducted out-of-doors were considered to be field experiments. Induction of secondary metabolite production by herbivores, artificial damage, and chemical means was not addressed in this analysis.

We tested for differences in effect size between environmental and genetic influences on plant chemical content. Additional analyses using subsets of the data allowed us to compare effect sizes between plants producing C- or N-based secondary metabolites, studies in agricultural and natural systems, studies taking place in the field and under controlled conditions (laboratory or greenhouse) and manipulations of different environmental variables, including light availability, water availability, soil nutrient availability, and atmospheric CO_2 content. For some of these subsets of data, the number of studies using particular predictor variables was small. Therefore, a factorial combination of all possible analyses of each data subset for each predictor variable was not possible.

For most questions, we examined differences between the magnitudes of effect sizes. Therefore, for each study, we used the absolute value of the effect size in the analysis. When comparing the effects of different environmental variables on C- and N- based secondary metabolites, we calculated the sign and the magnitude of each effect size. We hypothesized that these two groups of secondary metabolites would differ in the sign of their responses to manipulations of light, fertilizer, and CO_2.

Herbivore response methods

We performed a broad literature search as described in the 'Plant defense methods' section and evaluated 1875

relevant papers for data on herbivory, consumption of artificial diet, or insect herbivore growth as affected by plant defenses. We calculated a total of 550 effect sizes from 295 appropriate articles. Changes in herbivory, consumption of artificial diets or leaves with chemicals painted on them, and herbivore growth were the dependent variables analyzed as responses to plant defenses. When these dependent variables were combined as a single response in analyses, they are referred to as 'herbivore activity.' Since effect sizes do not have units, meta-analyses typically combine such response variables (Cooper et al. 2009). A positive effect size indicates the treatment reduced herbivory, diet consumption, or insect growth. In additional analyses, specialist and generalist herbivore responses were examined separately. Diet breadth categories were determined either from the study producing the data or from other published sources on the focal herbivores. For most studies, specialists were either monophagous or fed on plants within one family while generalists consumed plants in multiple families (*sensu* Bernays and Chapman 1994).

Broad categories of chemical, biotic, and physical plant characteristics that may have defensive value were utilized in the analysis: N-containing compounds, mutualistic ants, architecture, enzymes, leaf structure, phenolics, terpenoids, and toughness. The architecture category included leaf symmetry, plant branching, and habitat complexity. The leaf structure category included epiphyll coverage, latex, pubescence, trichomes, and waxes. More refined analyses were done within the N-containing compounds, phenolics, and terpenoids to consider the potency of specific defenses. These analyses combined all responses of herbivory, growth, and consumption to provide balanced sample sizes.

Plant guilds were also used as independent variables, and effect sizes were compared between: (1) herbaceous plants, (2) fast-growing angiosperm trees, (3) moderately growing angiosperm trees (4) slow-growing angiosperm trees (5) fast-growing gymnosperm trees (6) moderately growing gymnosperm trees (7) slow-growing gymnosperm trees (8) trees that could not be resolved into a finer grouping (9) shrubs (10) C3 grasses, and (11) C4 grasses. Trees described as pioneers or light-demanding were classified as fast-growing. Moderately-growing trees were those described as moderate, fast to moderate, moderate to slow, or midsuccessional. Shade tolerant and canopy trees were classified as slow-growing. In temperate zones, these growth rates generally translate to: fast stem height gain ≥25" per year, medium height gain = 13–24" per year, and slow height gain ≤12" per year (Dirr 1998). We had about three times more temperate than tropical entries in our dataset. If the articles themselves did not categorize the plant guild, other articles and websites were consulted for growth form information, particularly http://www.na.fs.fed.us/pubs/silvics_manual/volume_* and http://hort.ufl.edu/trees/*. Analyses compared herbivore responses to defenses in different plant guilds.

Results

Publication bias

Funnel plot results for data of environmental versus genetic variation in plant defense show a small publication bias ($P = 0.04$), which supports any instances where the null hypothesis is accepted but suggests that more studies are necessary to support weak patterns uncovered by the meta analysis. On the other hand, the nonparametric trim and fill test suggests a lack of bias as do Begg Rank Corrleation tests. In either case, accepting the null hypothesis (e.g., no difference between environmental versus genetic effects) is justified.Rosenberg's random-effects fail-safe number for the environmental/genetic variation dataset was 0. Funnel plot results for defensive efficacy response variables (herbivory, growth, and consumption) were non-significant ($P \geq 0.5$), indicating an absence of publication bias in this dataset. Rosenberg's fail-safe number for all dependent variables combined was 31 studies; for herbivory alone it was 0 (the Q_B value for herbivory was already non-significant; see results below); for growth it was 17; for consumption it was 13.

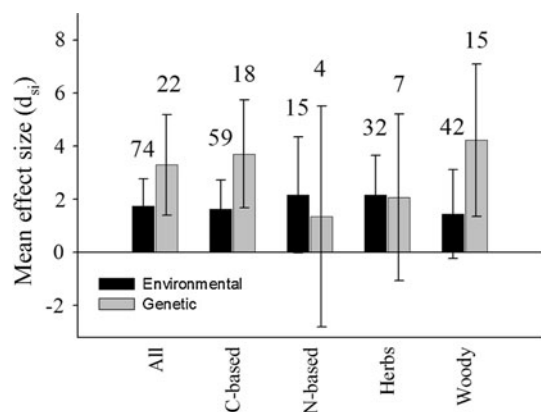### Genetic vs. environmental sources of variation in plant defenses

We utilized data from 96 experiments in 74 publications encompassing 21 families and 36 genera of plants. The majority of studies took place in temperate study systems. Only six studies in tropical or subtropical plant systems met the criteria to be included in our data set. Response variables included concentrations of a wide variety of types of secondary metabolites, including alkaloids, cyanogenic glycosides, condensed and hydrolysable tannins, flavonoids, furanocoumarins, glucosinolates, iridoid glycosides, terpenes, organic acids, phenolics, phenolic glycosides, polyacetylenes, proteins and enzymes, saponins, and trypsin inhibitors. Environmental manipulations included supplemental fertilizers, increased atmospheric CO_2 concentrations, variations in water availability, increases in temperature, changes in total light, UV-A and UV-B availability, and the presence or absence of worms (Online Appendix 1). Studies comparing the secondary metabolite concentrations of different genotypes used either clones, distinct populations grown in common gardens, or half-siblings.

Effect sizes for all studies examining genetic and environmental causes of variability in plant secondary metabolites were significantly greater than zero, as indicated by

their 95% CI (Fig. 1). The effect sizes of experiments that manipulated genetic sources of variation were not different from the effect sizes of those that manipulated environmental sources ($Q_B = 0.5$, $df = 1$, $P > 0.1$). Both environmental and genetic manipulations had significant effects on the concentrations of C-based secondary metabolites (Fig. 1), but these two sources of variation did not differ in the magnitude of their effects ($Q_B = 1.0$, $df = 1$, $P > 0.1$). For experiments examining the response of N-based plant chemicals, neither environmental nor genetic manipulations had any effect on secondary metabolite content (Fig. 1). Environmental, but not genetic, manipulations had strong effects on the secondary metabolites of herbaceous plants (Fig. 1). The magnitude of the effect sizes of these two types of manipulations did not differ ($Q_B = 0.004$, $df = 1$, $P > 0.1$). Woody plant secondary metabolites responded to genetic, but not environmental manipulations (Fig. 1), and the magnitude of the effect sizes of environmental and genetic manipulations were significantly different ($Q_B = 4.2$, $df = 1$, $P < 0.05$).

Manipulations caused large and significant increases in total secondary metabolite concentrations of woody and herbaceous plant species (Fig. 2), but these plant categories did not differ from each other ($Q_B = 0.0004$, $df = 1$, $P > 0.1$). Carbon-based defenses increased significantly in woody plants but not herbaceous plants (Fig. 2), and woody and herbaceous plants did not differ from each other in their responses ($Q_B = 0.06$, $df = 1$, $P > 0.1$). Effect sizes of N-based defenses were not significantly different from zero (Fig. 2). Carbon-based secondary metabolites

did not exhibit significant effect sizes in response to manipulations of atmospheric CO_2 content, soil nutrients, additional light, or additional water. Experiments with individual environmental variables were too rare for N-based metabolites to permit a similar analysis.

The magnitudes of effect sizes of studies in agricultural and natural settings were both significantly different from zero (Fig. 3). Effect sizes of agricultural studies did not differ from effect sizes of studies in natural settings ($Q_B = 0.6$, $df = 1$, $P > 0.1$). When analyses were limited to studies that manipulated environmental variables to measure their effects on plant secondary metabolite content, once again the magnitudes of the effect sizes of agricultural and natural system studies were significantly greater than zero (Fig. 3) but were not different from each other ($Q_B = 0.3$, $df = 1$, $P > 0.1$). Studies that manipulated genetic sources of variation in secondary metabolite content were too few to permit analysis of differences between the effect sizes of studies in agricultural and natural systems. When we examined only studies in which CO_2 or fertilizer was manipulated, effect sizes (taking into account magnitude and direction of effects) were not different from zero for studies in agricultural or natural systems (Fig. 3). In agricultural systems, experiments that manipulated light showed a significant increase in production of secondary metabolites (Fig. 3). In this case, the effect sizes of agricultural experiments were greater than those of natural experiments ($Q_B = 7.1$, $df = 1$, $P < 0.01$). Although studies conducted in the field and the greenhouse/lab showed significant responses of secondary metabolites to manipulations, effect sizes were not different in these two locations ($Q_B = 0.04$, $df = 1$, $P > 0.1$).

Fig. 1 Means and 95% confidence intervals for the effect size (d_{si}) of studies manipulating potential environmental or genetic sources of variation in secondary metabolite content. Values are shown for (*left* to *right*)—all studies, experiments in which C-based or N-based metabolites were the response variable, and experiments with herbaceous versus woody plant species. Numbers above each column represent the number of studies analyzed. Overall, there were significant effects of genotype and environment on plant defensive compounds, and these effects did not vary systematically by type of defense. Woody plant secondary metabolites were affected by genetic, but not environmental manipulations

Fig. 2 Means and 95% confidence intervals for the effect size (d_{si}) of studies of the secondary metabolite production of woody and herbaceous plants for (*left* to *right*) all studies and studies that measured C- and N-based secondary metabolites as the response variable. Numbers above each column represent number of studies analyzed. Manipulations had large effects on chemical defenses of woody and herbaceous plant species
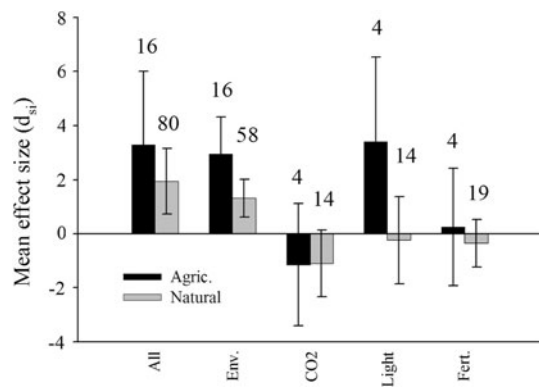
Fig. 3 Means and 95% confidence intervals for the effect size (d_{si}) of studies in agricultural (Agric.) or natural systems. Effect sizes are for (*left* to *right*) all studies, studies with all environmental variables combined (Env.), and studies of CO_2, light, and fertilizer (Fert.). Numbers above each column represent the number of studies analyzed. Large effect sizes were recorded for studies in both agricultural and natural settings

Herbivore responses

Effects of all plant defenses

There were no differences between effect sizes of the broad defense categories, but some defenses had significant effects on response variables. When herbivory, growth, and consumption were combined into one response variable, chemical defenses (N-containing compounds, phenolics, and terpenoids) limited herbivore activity. These three classes of defense also had the largest sample sizes (N-containing compounds, $N = 68$; phenolics, $N = 229$; terpenoids, $N = 108$). Generalists were limited by the same defenses, but there were no significant effects of defenses on specialists (Fig. 4). When herbivory was analyzed alone, ant mutualists were the only defense to significantly reduce herbivory. Sample sizes between classes were more balanced in this analysis (Fig. 5). There were no significant effects of plant defenses on herbivory when generalist and specialist herbivores were analyzed independently. Leaf structures (epiphyll coverage, latex, pubescence, trichomes, and waxes) and phenolics both caused significant decreases in herbivore growth (Fig. 6a). Generalist growth was also inhibited by phenolics (Fig. 6b). Terpenoids and phenolics were significantly effective defenses against consumption of artificial diet by all herbivores combined and generalists alone (Figs. 7a and 7b). Specialist consumption was reduced only by diets high in phenolics (Fig. 7c).

Specific chemical defenses

The analyses of effects of specific N-containing compounds, phenolics, and terpenoids utilized a combination of all herbivore responses as a single response variable
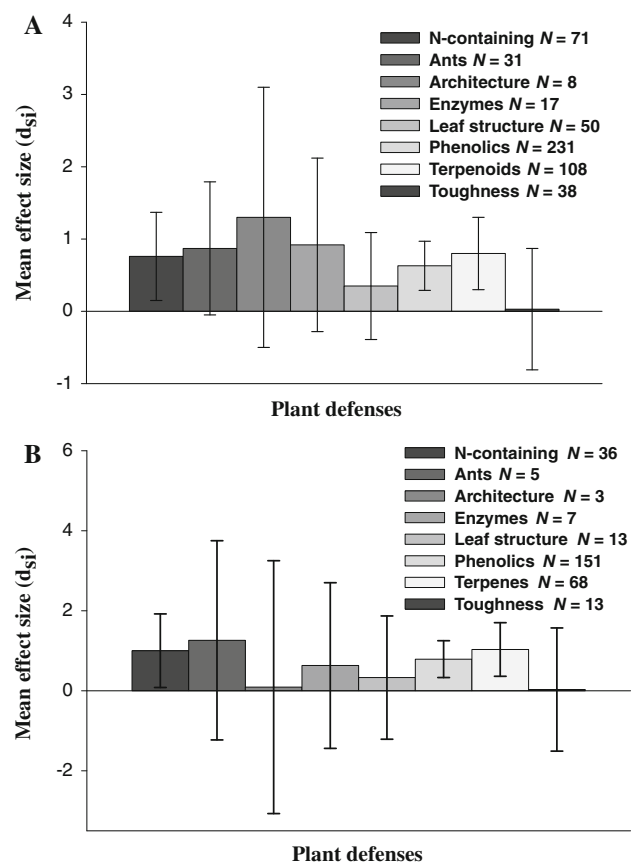
Fig. 4 a Mean effect sizes (d_{si}) with 95% confidence intervals for impacts of defensive classes on all herbivore activity (herbivory, growth, and consumption combined). *Error bars* that cross zero indicate the magnitude of the effect size is not significantly different from zero. Terpenoids suppressed herbivore activity more than any other defensive class. N-containing compounds and phenolics were the only other classes to significantly limit herbivores. There is no difference in between class efficacy ($Q_B = 0.35$, $df = 7$, $P > 0.995$). **b** Mean effect sizes and 95% confidence intervals for defensive classes tested on generalist herbivores with all response variables combined. Generalists were limited by the same defenses as all herbivores together. The strongest effect was from terpenoids followed by N-containing compounds and phenolics. The effect sizes were statistically indistinguishable ($Q_B = 1.63$, $df = 7$, $P = 0.95$)

('herbivore activity'). The only N-containing compounds to significantly deter herbivore activity were alkaloids, which limited all herbivores together and generalists alone (Table 2). For both generalist herbivores and all herbivores combined, the effects of different phenolic compounds were significantly different from each other (generalists: $Q_B = 520.9$, $df = 13$, $P < 0.005$; all: $Q_B = 70.6$, $df = 14$, $P < 0.005$). The only specific types of phenolics significantly affecting these groups were sulfur-containing polyynes from the Asteraceae and coumarins (Table 2). Specialists were not significantly affected by any phenolics. There were significant differences in effect sizes of terpenoids for all herbivores and for specialists (all: $Q_B = 35.9$, $df = 14$, $P < 0.005$; specialists: $Q_B = 14.8$,
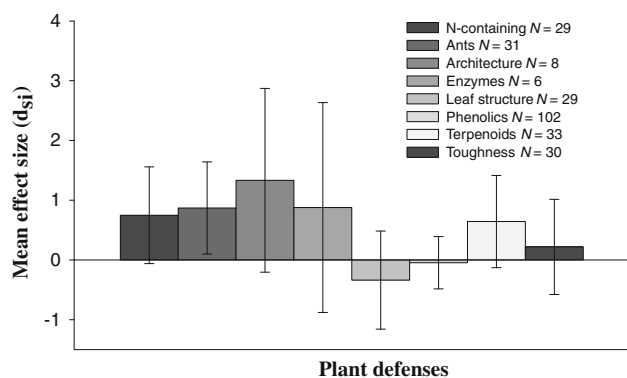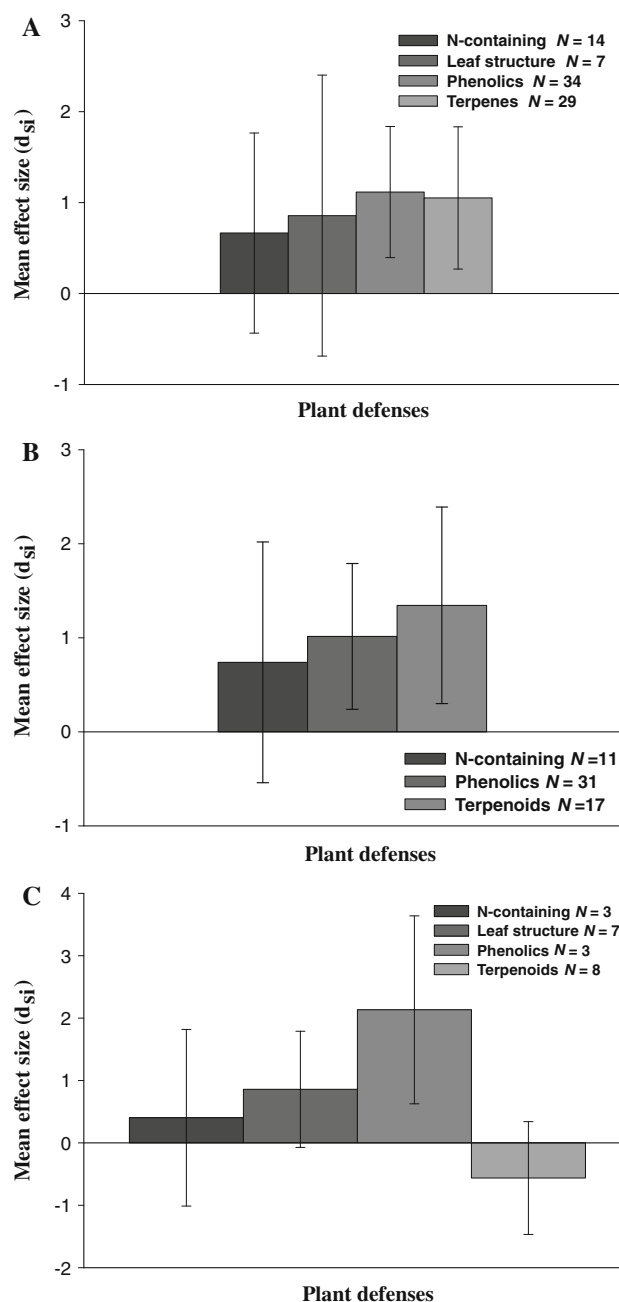
Fig. 5 Mean effect sizes with 95% confidence intervals for defensive classes affecting herbivory. Only ants had a significant negative effect. Negative d_{si} values indicate an increase in herbivory with defense. There were no differences between classes ($Q_B = 0.55$, $df = 7$, $P > 0.995$)
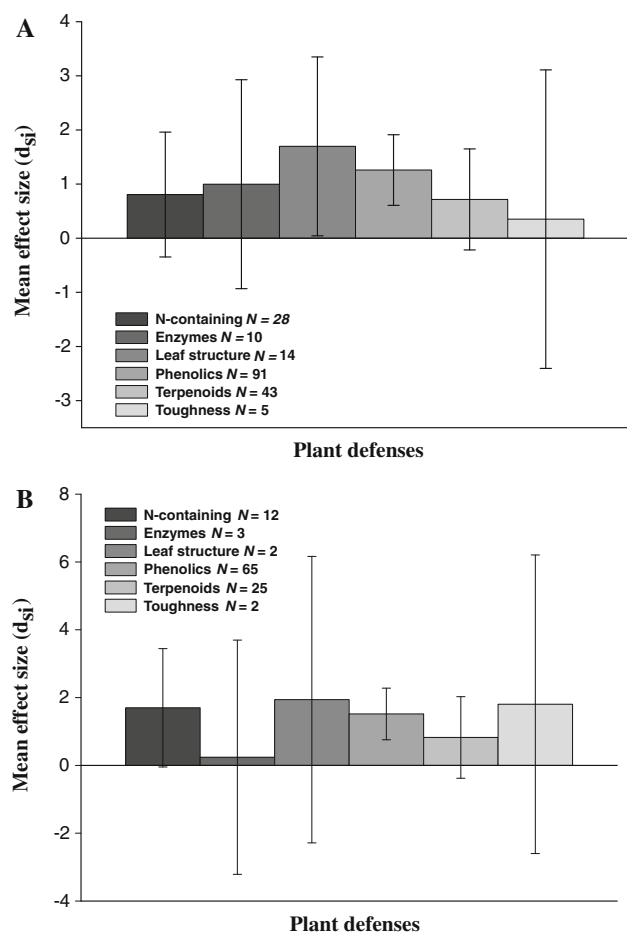
Fig. 6 a Mean effect sizes (d_{si}) and 95% confidence intervals for herbivore growth as affected by defensive classes. Leaf structure and phenolics significantly inhibit growth. **b** Mean effect sizes (d_{si}) and 95% confidence intervals for generalist herbivore growth. Growth is only inhibited by phenolics ($d_{si} = 1.47$, 95% CI = 0.76)

$df = 6$, $P < 0.025$). None of the terpenoids had significant individual effects on specialists, but for all herbivores together, plant hormones, saponins, triterpenes and

Fig. 7 a Mean effect sizes (d_{si}) and 95% confidence intervals for herbivore consumption. Terpenoids ($d_{si} = 1.05$, 95% CI = 0.78) and phenolics ($d_{si} = 1.12$, 95% CI = 0.72) reduced consumption. **b** Mean effect sizes (d_{si}) and 95% confidence intervals for generalist herbivore consumption. Terpenoids ($d_{si} = 1.34$, 95% CI = 1.04) and phenolics ($d_{si} = 1.01$, 95% CI = 0.78) also reduced generalist consumption. **c**. Mean effect sizes (d_{si}) and 95% confidence intervals for specialist herbivore consumption. It was reduced only by phenolics ($d_{si} = 2.13$, 95% CI = 1.51)

sesquiterpenes significantly reduced herbivore activity (Table 2). Contrasts between different terpenoids were not significant for generalist herbivore activity, but individually plant hormones, triterpenes, iridoids, sesquiterpenes,

Table 2 Effect sizes and 95% confidence intervals resulting from increases of defenses within specific chemical classes

	Effect sizes (95% CI)	
	All herbivores	Generalist herbivores N-containing
N-containing compounds		
Alkaloids[a]	1.53 (1.2); $N = 9$	3.61 (1.35); $N = 4$
Amides	NS; $N = 4$	NS; $N = 1$
Amines	NS; $N = 3$	NS; $N = 3$
Cyanogenic glycosides	NS; $N = 7$	NS; $N = 5$
Fungal alkaloids	NS; $N = 4$	NS; $N = 3$
Glucosinolates	NS; $N = 4$	
Hydroxamic acid	NS; $N = 3$	
Indole alaloids	NS; $N = 5$	NS; $N = 2$
Pyridine alkaloids	NS; $N = 8$	
Pyrrolizidine alkaloids	NS; $N = 11$	NS; $N = 7$
Quinolizidine alkaloids	NS; $N = 5$	NS; $N = 5$
Tropane alkaloids	NS; $N = 3$	NS; $N = 3$
Phenolics		
Aster sulfur compounds	7.37 (2.6); $N = 3$	13.53 (3.81); $N = 2$
Catecholic phenolics	NS; $N = 9$	NS; $N = 4$
Condensed tannins	NS; $N = 38$	NS; $N = 26$
Coumarins	1.57 (1.23); $N = 13$	1.86 (1.60); $N = 10$
Flavone glycosides	NS; $N = 3$	NS; $N = 3$
Flavonoids	NS; $N = 13$	NS; $N = 10$
Hydrolyzable tannins	NS; $N = 7$	NS; $N = 7$
Juglone	NS; $N = 3$	NS; $N = 3$
Lignan	NS; $N = 4$	
Phenol glycosides	NS; $N = 8$	NS; $N = 7$
Phenolics	NS; $N = 31$	NS; $N = 20$
Phenolics and anthocyanins	NS; $N = 3$	NS; $N = 3$
Phenolics and tannins	NS; $N = 30$	NS; $N = 18$
Salicylates	NS; $N = 14$	NS; $N = 4$
Tannins	NS; $N = 34$	NS; $N = 17$
Terpenoids		
MeJA	NS; $N = 5$	NS; $N = 3$
Alkylfurans	NS; $N = 2$	NS; $N = 2$
Diterpenes	NS; $N = 3$	
Hormones	3.33 (3.23); $N = 2$	3.33 (2.98); $N = 2$
Iridoids	NS; $N = 18$	1.51 (1.36); $N = 10$
Limonoids	NS; $N = 10$	NS; $N = 7$
Monoterpenes	NS; $N = 8$	
Oils	NS; $N = 5$	NS; $N = 5$
Saponins	2.01 (1.87); $N = 6$	NS; $N = 5$
Sesquiterpenes	1.16 (1.14); $N = 16$	1.49 (1.28); $N = 11$
Terpenes	NS; $N = 7$	NS; $N = 4$
Terpene aldehydes	NS; $N = 3$	NS; $N = 3$
Tetracyclic terpenes	NS; $N = 2$	
Triterpenes	2.22 (2.08); $N = 6$	2.12 (1.94); $N = 6$
Volatiles	NS; $N = 12$	NS; $N = 6$

Blank cells had no data in the analysis. Within the N-containing defenses, between effects differences were non-significant. There were significant differences among the phenolics for all herbivores and generalists alone. There were also significant differences among the terpenoids for all herbivores and for specialists alone, but specialists had no significant individual effect sizes

[a] Defenses not described beyond the term 'alkaloid' in the source article

and triterpenes caused significant decreases in generalist activity (Table 2).

Plant guilds

The efficacy of defenses in reducing herbivory did not vary between plant guilds. Generalist herbivory was, however, significantly limited by defenses in herbaceous plants (Table 3). For insect growth, there were no significant differences between effect sizes for different plant guilds, although defenses in fast-growing angiosperm trees, herbaceous plants and shrubs significantly reduced growth (Table 3). Generalist herbivores were also limited in their growth by defenses in fast-growing trees and herbs (Table 3). No effects of defense on herbivory or growth were found for specialists.

Insect consumption of artificial diets containing differing levels of plant extracts or leaves painted with doses of extracts was significantly different across plant guilds ($Q_B = 24.9$, $df = 5$, $P < 0.005$). The result was also significant for generalist herbivores alone ($Q_B = 38.4$, $df = 5$, $P < 0.005$); specialists could not be tested because the relevant studies used only herbaceous plant extracts. Herbaceous plant and tree defenses decreased all herbivore and generalist herbivore consumption of artificial diets, but for trees, the result is based on just two studies of coumarins (Table 3).

Data gathered for these analyses were summarized to look for patterns in defensive strategies employed by different plant guilds (Fig. 8), and results supported generally accepted predictions of plant defense theory. Ants were most often found in fast-growing trees and shrubs. Phenolics were most evenly spread across plant guilds, but were proportionately highest in studies of slow-growing trees. Studies of herbaceous plants were also most often focused on phenolics, but studies of terpenoids and N-containing defenses were best represented in this group as well. The highest diversity of defensive strategies was found in

Table 3 Effects sizes and 95% confidence intervals resulting from changes in defensive levels within plant guilds

Plant guild	Response variable				
	Generalist herbivory	Herbivore growth	Generalist growth	Consumption	Generalist consumption
C3 grass	NS	NS	NS	NS	NS
	$N = 2$	$N = 9$	$N = 4$	$N = 2$	$N = 2$
C4 grass	NS	NS		NS	NS
	$N = 2$	$N = 2$		$N = 3$	$N = 3$
Fast conifers		NS			
		$N = 4$			
Fast trees	NS	1.06 (0.87)	1.26 (1.15)	NS	NS
	$N = 42$	$N = 46$	$N = 31$	$N = 10$	$N = 7$
Herbs	0.97 (0.81)	0.99 (0.84)	1.74 (1.37)	0.80 (0.61)	1.23 (1.13)
	$N = 37$	$N = 49$	$N = 22$	$N = 33$	$N = 14$
Moderate conifers		NS			
		$N = 2$			
Moderate trees	NS	NS	NS		
	$N = 8$	$N = 7$	$N = 3$		
Shrubs	NS	1.29 (1.12)	NS	NS	NS
	$N = 11$	$N = 28$	$N = 16$	$N = 3$	$N = 3$
Slow conifers		NS	NS		
		$N = 5$	$N = 4$		
Slow trees	NS	NS	NS		
	$N = 12$	$N = 4$	$N = 2$		
Trees	NS	NS	NS	4.44 (2.94)	4.62 (3.36)
	$N = 3$	$N = 4$	$N = 4$	$N = 2$	$N = 2$
Vines		NS			
		$N = 3$			

Between-effects differences were non-significant. Blank cells had no data in the analysis. 'Tree' refers to angiosperm trees, and the growth form simply called 'tree' is comprised of trees for which data on successional status were not found. Consumption refers to consumption of artificial diets or leaves painted with chemicals
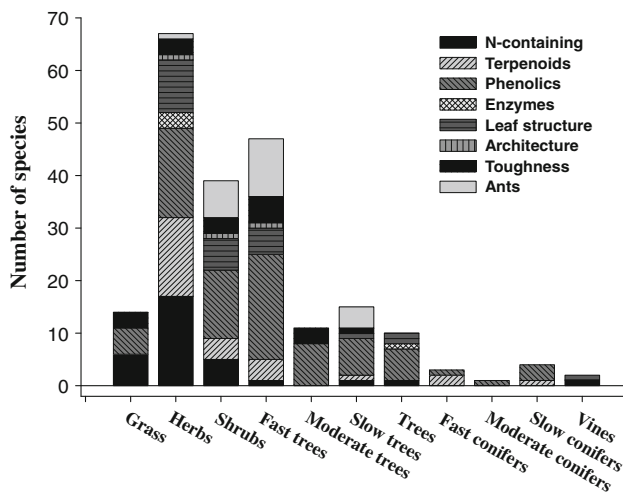
Fig. 8 Summary of categories of defenses found in different plant guilds. Guilds are on the *X*-axis, and the number of species examined with these different classes of defense are shown on the *Y*-axis

fast-growing plants, including leaf structural defenses, which were most often examined in this group.

Discussion

A quantitative synthesis of the large number of studies on causes and consequences of variation in plant defenses allows for generalizations to be made in a field full of empirical contradictions. It also highlights the complex and often species-particular nature of plant-insect interactions. As predicted, plant secondary metabolites exhibited large significant responses to environmental and genetic sources of variation. The magnitudes of the effect sizes of these two sources were very similar, supporting the idea that genetic and environmental factors have similar effects on plant defense production. Both woody and herbaceous species responded to environmental manipulations, supporting hypotheses of phenotypic plasticity in defenses, the carbon-nutrient balance hypothesis (Bryant et al. 1983), and the growth-differentiation balance hypothesis (Herms and Mattson 1992), and suggesting that these hypotheses are still useful (Hamilton et al. 2001; Lerdau and Coley 2002). The combined herbivory results support the resource availability hypothesis as the most parsimonious hypothesis for the evolution of plant defenses. These data also highlight the high effectiveness of biotic defenses for plants.

Environmental and genetic effects on plant defenses

Environmental manipulations had strong effects on the secondary metabolites of herbaceous plants, while genetic manipulations had strong effects on woody plants. This

pattern is driven by two readily-cloned, woody tree species (*Populus tremuloides* and *Betula pendula*) that comprised 80 percent of the experiments on variation among genotypes of woody plants. Thus, it is difficult to generalize from this collection of experiments about the importance of genotype for chemical defense in other woody plant species.

The strong effects of environmental manipulations on herbaceous plants fit predictions of the resource availability/growth rate hypothesis (Coley et al. 1985), which proposes that fast-growing plants like herbs should have a high degree of plasticity in defense in response to changing environmental conditions and changing herbivore pressure. This allows plants with high potential growth rates to maximize growth and competitive ability when nutrients are abundant or when herbivores are absent, while retaining the ability to defend their tissues against herbivores when favorable environmental conditions or the presence of herbivores lowers the cost to benefit ratio of defense. It also supports earlier work suggesting that plants investing less in structural material are physiologically able to increase defenses when resources allow (Bryant et al. 1983, 1987). This was corroborated by the strong response of herbivores to changes in defense of fast-growing plants.

Secondary metabolites in herbaceous and woody plants responded equally to manipulations. However, when C-based metabolites were analyzed separately, only woody plants showed a strong experimental response. This is consistent with assumptions of the plant apparency hypothesis (Feeny 1976; Rhoades and Cates 1976), which suggests that slow-growing, perennial plants that are predictable in time and space should benefit from increased investment in quantitative, C-based defenses that act to reduce the digestibility of plant material. In contrast, fast-growing annuals should produce low concentrations of toxic, N-based defenses. This result is also consistent with assumptions of the resource availability hypothesis because slower growing plants utilize quantitative, C-based defenses (Coley et al. 1985).

Agricultural versus natural systems

Plants in agricultural and natural systems both responded with similar magnitude to experimental manipulations, similar to findings from another meta-analysis (Massad and Dyer 2010). Carbon dioxide and fertilizer had no measurable effects on agricultural or natural plants, in contrast to results for only C-based defenses in woody plants (Koricheva et al. 1998). The diversity of defenses and plant types in our analysis may account for this difference. For example, light had a strong effect on agricultural plants, which are usually adapted to grow in open, high-light areas, while our non-agricultural plant group included both

shade- and sun-tolerant plants. Shade-tolerant plants have lower potential maximum photosynthetic rates, so even if they are exposed to light levels above their optimum level, they cannot dramatically increase their rate of carbon fixation (Lambers et al. 1998). Therefore, agricultural plant defenses are more responsive to manipulations of light availability than non-agricultural plants collectively.

Contrary to our predictions, we uncovered no differences between effect sizes of experiments in controlled greenhouse or laboratory settings and in field settings. This indicates that complex abiotic and biotic interactions in field or common-garden studies are not confounding or obscuring the results of manipulative field experiments.

Herbivore response

Nitrogen-containing compounds, phenolics, and terpenoids all limited herbivore activity, supporting the general hypothesis that secondary metabolites reduce herbivory and insect herbivore growth. The inhibitory effects of chemical defenses were always greater for generalists versus specialists, in contrast to predictions of plant apparency theory (Rhoades and Cates 1976; Rhoades 1979; Stamp 2003). Thus, the literature synthesized here supports the plant apparency hypothesis in terms of patterns of plant defense production among plant types but not in terms of the roles of herbivores in driving the evolution of these defenses. The apparent resistance of specialists to plant chemistry may reflect their ability to metabolize or sequester plant secondary metabolites (e.g., Montllor et al. 1990; Dobler and Rowell-Rahier 1994; Zalucki and Malcolm 1999; Dyer 1995; Engler-Chaouat and Gilber 2007).

As predicted, statistical differences between the effects of broad classes of plant defenses were greater for specialists than generalists (using herbivory, growth, and consumption as response variables). Specialists should be unaffected by the defenses with which they may have coevolved and can also sequester defensive compounds. On the other hand, compounds to which specialists are not adapted should have large negative effects. Thus, the potential for both positive and negative responses to defenses leads to significant differences in specialists' responses to classes of defense. Generalists, in contrast, were predicted to be more uniformly affected by defenses, since they are usually not adapted to specific classes of compounds (see Hartmann et al. 2004 for exceptions). However, we did not find consistent support for the approach of classifying compounds based on their efficacy against specialists versus generalists, as generalists were more inhibited by all classes of defense, and specialist herbivory was not affected by quantitative defenses, again in contrast to expectations of the plant apparency hypothesis.

Phenolics were hypothesized to be effective against all herbivores and more effective against specialists than qualitative defenses (e.g., Nichols-Orians 1991 and Green et al. 2003 for generalists; see Huang and Renwick 1995 for specialists on a novel host). However, we found no significant effects of phenolics on either generalist or specialist herbivory. Far fewer studies tested the effects of phenolics on specialist versus generalist herbivory (14 vs. 53 studies). This may be because fewer specialists are actually found feeding on plants defended by phenolics or may be due to investigator bias. Phenolics did limit herbivore growth, which supports hypotheses that phenolics function as an indirect defense by prolonging herbivore development and thereby potentially increasing predation and parasitism rates (Price et al. 1980; but see Clancy and Price 1987 and Lill and Marquis 2001 for contrary results).

Biotic defense, in the form of ant mutualists, was the most effective protection against herbivory, which corroborates numerous studies showing ants are an important, often tightly coevolved defense (reviewed by Heil and McKey 2003). While many herbivores have been able to adapt to plant toxins (Ehrlich and Raven 1964; Berenbaum 1983; Cornell and Hawkins 2003; Engler-Chaouat and Gilber 2007), ants provide a successful, active barrier to herbivory (Heil and McKey 2003; Rosumek et al. 2009). Studies of ant defenders were most frequently found in systems with fast-growing tree species or shrubs—plants often lacking effective antiherbivore chemical defenses. If this relationship is not simply due to investigator bias (e.g., only looking for myrmecophytic associations in fast-growing plants), this pattern of biotic defenses in plants without strong chemical defense is consistent with studies that show trade-offs between biotic and chemical defenses (Dyer et al. 2001; reviewed by Heil and McKey 2003; but see Heil et al. 2002).

While classic plant defense paradigms were only somewhat supported by examining broad categories of defense, looking in detail at sub-groups within these categories proved more informative. Terpenoids varied the most in their abilities to affect herbivory and had the strongest negative effects on herbivores. They are the most diverse class of known secondary metabolites (Bernays and Chapman 1994; Croteau et al. 2000), but only 105 effect sizes were found for terpenoids as opposed to 215 effect sizes for phenolics. Most of the studies of phenolics were of tannins, which are often poor defenses or have no antiherbivore value (Coley 1983; Martin et al. 1987; Bernays et al. 1989a; Ayres et al. 1997). Synergy between individual compounds may help explain the apparent lack of defensive properties. Many compounds work in concert to produce deterrent effects (Harborne 1993; Stermitz et al. 2000; Calcagno et al. 2002; Dyer et al. 2003), thus, tests of antiherbivore activity of specific plant secondary metabolites

should be supplemented with appropriate tests of pertinent mixtures and whole plant extracts (e.g., Jones 1998; Nelson and Kursar 1999). Widespread synergy may explain why mixtures of compounds within a single plant species are the rule and not the exception and is likely responsible for what some have termed "redundancy" in chemical defenses (Romeo et al. 1996; Williamson 2001; Challis and Hopwood 2003).

Analyses of the plant guilds generally supported our hypotheses as defenses were most plastic in fast-growing plants, and generalists were most affected by changes in these plants. The potency of changes in fast-growing plants may be due to greater increases in defenses because these plants invest less in long-lived leaves or woody material (Bryant et al. 1983, 1987). Patterns of toxic versus digestibility-reducing defenses fit the predictions of resource availability (Coley et al. 1985; Coley 1987) and corroborate the paradigm of unapparent plants being defended by qualitative compounds and apparent plants utilizing dose-dependent, quantitative chemical defenses (as predicted by Feeny 1976, Rhoades and Cates 1976 and phylogenetically supported by Silvertown and Dodd 1996).

Conclusions

How do the results of this meta-analysis change the way we think about plant antiherbivore defenses? The concept that both genetic variation and phenotypic plasticity are responsible for considerable variation in plant secondary metabolites is not at all novel (e.g., Abrahamson et al. 1988), but the fact that both sources of variation are similar in magnitude is relevant to studies of plant defense. Most of the studies included in our quantitative summary manipulated genotype or environment and measured the response of plant chemistry, but the environmental manipulations have not always controlled for genotype and vice versa. Thus the error variance in these studies is likely to be considerably inflated, resulting in poor power for detecting differences in manipulated variables. For example, the lack of a nutrient effect on C-based defenses, alkaloids, or other defenses (Hamilton et al. 2001; Palumbo et al. 2007; Orians and Ward 2010) could be due to a failure to detect biologically significant differences in defense because genotype was not controlled and error variance in plant defense was high. Failure to detect effects of nutrients on chemical defense or similar negative results contributes to the well-known problem that it is difficult to make generalizations about these gross categories of defense (Bernays et al. 1989b; Ayres et al. 1997; Smilanich 2008). It will be informative to continue with studies that manipulate and control both environmental and genetic sources of variation in plant defense (Osier and Lindroth 2006; Donaldson and Lindroth 2007; Arany et al. 2009).

Such manipulations, combined with observational data, will help make progress in defense theory and to clear-up issues that have been muddy for quite some time (Hamilton et al. 2001; Stamp 2003; Koricheva et al. 2004).

Several interesting generalities emerge from our synthesis of herbivore responses to defenses. The most striking pattern is that ant mutualists are more effective defenses than physical plant properties or secondary metabolites, supporting top-down ecological (e.g., Dyer 1995) and tritrophic evolutionary (Singer and Stireman 2005) perspectives of plant-animal interactions. Another strong pattern is that generalist herbivores are always affected more by plant secondary metabolites than specialists, regardless of the class of compounds. The distribution of defenses among plant guilds supports both the plant apparency and resource availability hypotheses. However, the lack of effect of quantitative phenolics on specialists is inconsistent with plant apparency and suggests the resource availability hypothesis is the simplest explanation for patterns of plant defense. Overall, by analyzing both plant defense and herbivore responses to genetic and environmental variation, we found support for the continued study of the carbon-nutrient balance and the growth-differentiation balance hypotheses, and we can also conclude the resource availability hypothesis is the most likely explanation for interspecifc patterns of plant defense.

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