CONSEQUENCES AND COSTS OF CHEMICAL COMPLEXITY: THE EVOLUTIONARY ECOLOGY OF DIRECT PHYTOCHEMICAL DEFENSE AGAINST HERBIVORES

Megan Blanchard[1],* and Liza M. Holeski*

*Department of Biological Sciences and Center for Adaptable Western Landscapes, Northern Arizona University, 617 South Beaver Street, Flagstaff, Arizona 86011, USA

Editor: Cassie Majetic

Phytochemical defenses, plant-produced compounds that can deter or slow herbivores, are critical in influencing the interactions between herbivores and their host plants—some of the most diverse and abundant organisms on earth. We discuss the foundational findings and theories related to the direct defense of plants against herbivores by these compounds, as well as continuing research questions in this field. We highlight the costs associated with phytochemical defense production and variation within individual plants in the distribution of phytochemicals, both spatially and temporally. We then discuss coevolutionary theory and the adaptations of herbivores to cope with phytochemical defense compounds. Last, we delve into the incredible diversity of phytochemical compounds, the role of diversity in herbivory, and new ways of measuring and understanding phytochemical diversity.

Keywords: phytochemical defense, herbivory, allocation, phytochemical diversity.

Introduction

The ongoing conflict between plants and their herbivores involves some of the most abundant and diverse interactions on the planet. In response to millions of years of attack by animals, virtually all plants have evolved suites of defensive traits. Plants can deter attackers or slow their feeding rates with chemical and physical traits (direct defenses), attract predators or parasitoids that prey upon herbivores (indirect defenses), or avoid herbivory by growing at a time when herbivores are less abundant. Most plants can also tolerate some level of herbivory and then regrow (McNaughton 1983; Garcia and Eubanks 2019). Defensive chemical compounds are ubiquitous in the plant kingdom and present in all forms of higher plants (Fraenkel 1959; Wink 2003). Herbivores display a great variety of strategies to avoid, tolerate, or even utilize these compounds. These phytochemical defenses (see "Glossary") play a crucial role in how herbivorous insects and other animals, which represent nearly one-quarter of all species on earth, select the host plants upon which they live, feed, and grow.

The study of direct and indirect defenses, along with tolerance, are all very active areas of research (Bezerra et al. 2021; Pearse et al. 2022). One burgeoning area of indirect defense research is in the study of volatile organic compounds (VOCs), which plants produce when they are attacked by herbivores. Because these compounds are volatile, they disperse into the air and can be used for longer-distance communication. Herbivores can be attracted to or repelled by the release of VOCs (Birkett et al. 2000). Predators and parasitoids of herbivorous insects use VOCs to locate their prey, which can prove a very effective mechanism of indirect defense for plants (Turlings et al. 1995; Thaler 1999).

In this primer, we discuss the evolutionary ecology only of phytochemical defenses that act directly against herbivores, due to space limitation. We introduce the origins and mechanisms of direct phytochemical defenses. We discuss how these defenses impact different types of herbivores and the adaptations that herbivores use to circumvent or overcome such defenses. We also review patterns of phytochemical defense distribution within individual plants, within species, and within communities.

How Phytochemical Defenses Work

Phytochemical defense compounds may be toxic or interfere with the digestion of plant enemies (Mithöfer and Boland 2012). The mechanism, or the molecular interaction that allows defense compounds to affect herbivores, varies with compound type. Of the compounds for which the mechanism is known, the majority target cellular structures and processes shared by most herbivores. This makes most phytochemical defenses broadly bioactive (i.e., active against a variety of organisms). For example, cardenolides, which are most famously produced by milkweeds, bind to and inhibit the sodium-potassium pump that is used in animal cells to establish membrane potentials (Emery et al. 1998). Another group, iridoid glycosides, is broken down in the guts of herbivores into a highly reactive compound that denatures proteins and can inhibit enzymes (Konno et al. 1999). Elucidating

[1] Author for correspondence; email: megan.blanchard@nau.edu.

Glossary

Allocation of phytochemical defense. Creating chemical defense compounds is costly; therefore, the limited energy budget for production of defenses must be divided up among the tissues of the plant. The pattern of division reflects the allocation of phytochemical defenses.

Coevolution. The reciprocal process of two or more species (or groups of species) exerting natural selection upon each other that results in the evolution of both.

Diet breadth. The variety in resources that an organism exploits for food. For herbivores, this is generally defined as the variation in host plant taxa that the herbivore consumes. Herbivores fall on a gradient of diet specialization based on this variation in their host plants.

Direct costs. Trade-offs in organisms due to finite resources such that investment in one process results in a decrease in investment in another process. The production of phytochemical defenses requires resources that plants could use for other processes like growth and reproduction. A decrease in growth or reproduction as a result of producing defenses is therefore a direct cost of defense.

Ecological costs. An indirect cost, in the form of a negative interaction with another species, that results from an organism investing resources in a process. One example of an ecological cost of the production of phytochemical defenses is decreased visitation of flowers (and therefore decreased pollination rates) for plants that produce nectar that contains defensive compounds.

Ecological fitting. When traits that organisms have evolved in one ecological context prove to be beneficial in a new ecological context.

Feeding guilds. Organisms that feed upon the same resources or use similar mechanisms for feeding. For example, insects in the piercing-sucking feeding guild use needlelike mouthparts to feed from the vascular tissue within plants.

Generalists. Organisms that can utilize a great variety of food resources and/or thrive in a variety of habitats. Generalist herbivores can feed upon a large number of taxa, generally across many plant families.

Induction. Broadly, when a stimulus results in a phenotypic change in an organism. In plant defense biology, induction is a change in the production or translocation of plant defenses in response to herbivore/pathogen attack or a chemical cue from another plant.

Metabolic pathways. The series of chemical reactions that are used by organisms to produce organic compounds.

Metabolome. All of the small organic compounds found within an organism (or a part of an organism).

Ontogenetic variation. A genetically controlled change in phenotype that occurs throughout the development of an organism. Seedlings, for example, often have different phytochemical defense levels and compositions from mature plants.

Optimal defense theory (ODT). A theory that explains how plants allocate costly (and therefore limited) phytochemical defenses to their tissues, both spatially and through time. According to ODT, this allocation is determined primarily by (1) the likelihood of attack of a given tissue, (2) the value of that tissue to the fitness of the plant, and (3) the cost of defending that tissue.

Phytochemical defenses. Chemical compounds produced by plants that serve a role in increasing plant resistance against attack by herbivorous animals and/or microbial pathogens.

Plant secondary metabolites. Chemical compounds that are not involved with plant primary processes of growth, development, or reproduction.

Specialists. Organisms that require very specific resources (in terms of both food and habitat). Specialist herbivores can feed upon only a limited set of plant taxa or a limited type of plant tissue.

---

these mechanisms has allowed researchers to understand the negative effects that these defenses can have on consumers and how some herbivores have evolved to tolerate, and sometimes even utilize, these compounds. The monarch butterfly, for example, substitutes one amino acid in its sodium-potassium pump, which prevents cardenolides from binding and allows monarch caterpillars to feed upon milkweeds (Holzinger et al. 1992). Many herbivores who tolerate iridoid glycosides, on the other hand, prevent the splitting of these compounds into the reactive form, either by suppressing the enzymes responsible or by quickly transporting these compounds out of the gut (Dobler et al. 2011). The mechanism by which a compound impacts herbivores also influences the dose necessary to deter herbivores, and the impacts of defenses are concentration and herbivore dependent (Lankau 2007).

Plant defensive compounds belong to a larger group of phytochemicals termed secondary metabolites (see "Glossary"), so named because they have historically been thought to play no role in so-called primary plant physiological processes, that is, fundamentals such as growth and development (Fraenkel 1959; Berenbaum and Zangerl 2008). Not all secondary metabolites serve a biotic defensive function; for example, flavonoids act as UV protectants (Agati and Tattini 2010). Many secondary metabolites fill multiple roles for the plant; terpenes, for example, provide both protection against herbivory and pollinator attrac-

tion (Dötterl et al. 2006). As evidence has accrued that secondary metabolites fulfill multiple functional roles within plants—some of which can influence primary physiological processes, including regulation of growth, contributions to primary metabolism, and facilitation of plant acquisition of micronutrients—what was once seen as a clear distinction between primary and secondary metabolites has attracted new scrutiny (Erb and Kliebenstein 2020). Unfortunately, most metabolic pathways (see "Glossary") for phytochemical defenses are still poorly understood. Characterization of these biochemical pathways can help to reveal the evolutionary histories of these compounds within plant lineages (Theis and Lerdau 2003), as certain compounds are shown to be chemical precursors to others (fig. 1B; Damtoft 1994), and can provide insight regarding how energetically costly these chemical defenses are for plants to make and maintain (Gershenzon 1994; Neilson et al. 2013).

For hundreds of thousands of phytochemical defense compounds, the chemical structure (see "Glossary") has been elucidated, while many others have yet to be characterized (Wink 1988; Pichersky and Lewinsohn 2011). Plant secondary compounds are structurally very diverse and are organized into classes by chemical structure (fig. 1A). Some classes contain compounds that are quite similar in structure. Terpenes, for example, are all unsaturated hydrocarbons with multiples of five carbon and eight hydrogen atoms (Harborne et al. 1999). In contrast, alkaloids
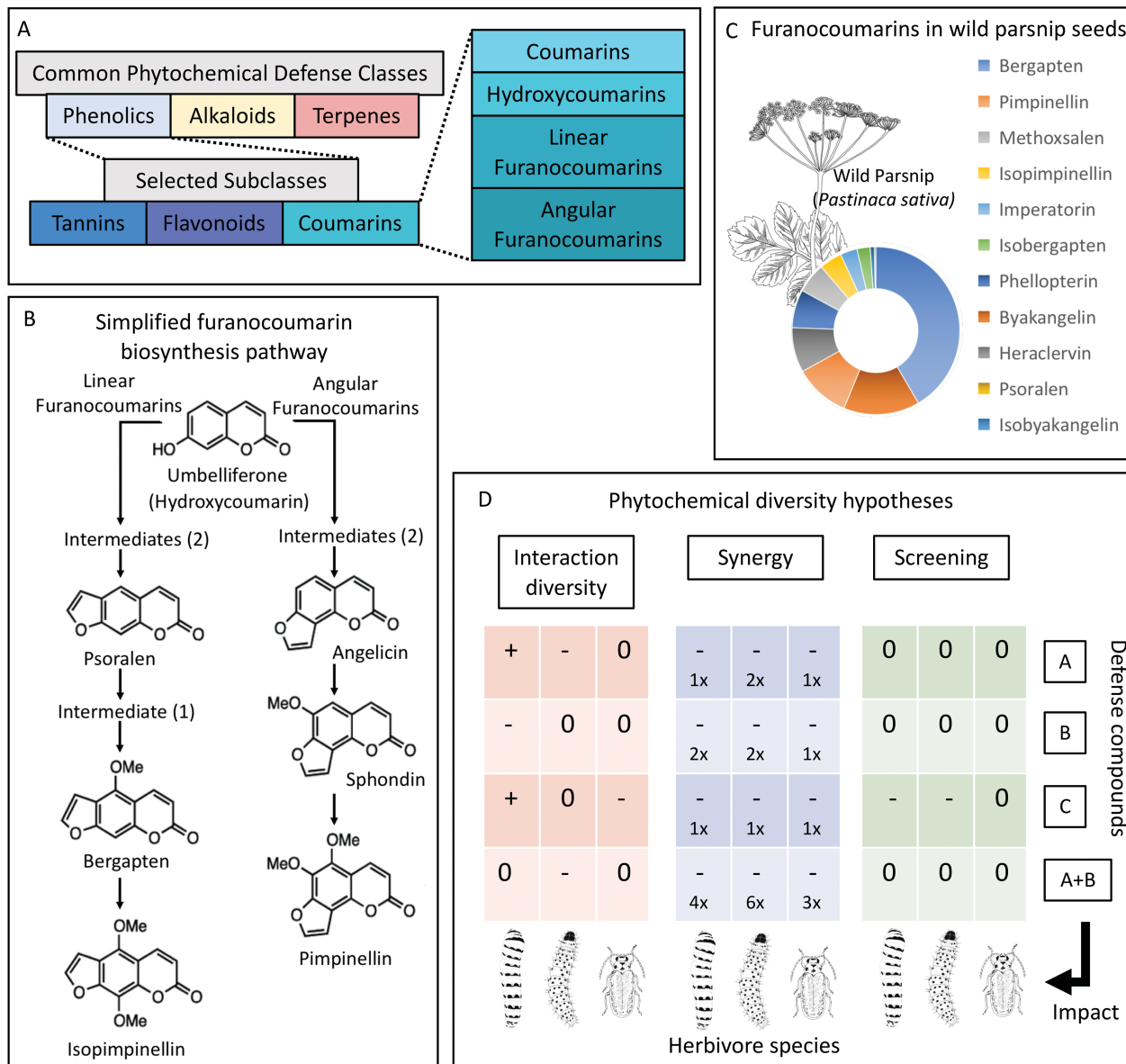
Fig. 1 Furanocoumarins (one subclass of chemical defense) illustrate general patterns of phytochemical diversity. *A*, The organization of phytochemical defense compounds is based on chemical structure, with subclasses of similar compounds falling under more general classes. *B*, An example metabolic pathway: the simplified pathway of the biosynthesis of linear and angular furanocoumarins from the hydroxycoumarin umbelliferone (modified from Bruni et al. 2019). Each pathway can give rise to many similar compounds, and there are thousands of different pathways used by plants. *C*, Wild parsnip (*Pastinaca sativa*) produces a diversity of furanocoumarins, in addition to other defense compounds (data from Kviesis et al. 2019). This represents the diversity of just one subclass of compounds found in one tissue type (seeds) in one species of plant. *D*, Three leading, non–mutually exclusive hypotheses explain why individual plants produce such a great diversity of phytochemical defense compounds. A plus sign indicates that the compound has a positive impact on the herbivore; a minus sign indicates a negative effect; 0 indicates no impact. For example, the interaction diversity hypothesis predicts that compounds will have differing effects on different herbivores; compound A has a positive effect on one caterpillar and a negative effect on the other. The synergy hypothesis predicts that the effect of the mixture of A+B has a greater effect (a negative effect of 4× on the first caterpillar) than the additive effects of the compounds individually (compound A = 1×; compound B = 2×). The screening hypothesis predicts that most compounds will have no impact, while a minority will have a negative effect on herbivores (e.g., compound C).

encompass a broad group of structurally and metabolically diverse compounds, united only by the inclusion of nitrogen in their structures (Wink 2018). Within these large chemical classes, subclasses are groups of compounds that are structurally quite similar and often share a defensive mechanism (fig. 1*A*).

Patterns of Phytochemical Defense Distribution within and across Plants

Tremendous variability in defense levels exists both within individual plants and at an intraspecies level. There are numerous

and nonexclusive drivers of this variation, including developmental and/or allocational factors as well as abiotic and biotic drivers. We discuss the impacts of some of these factors on patterns of defense below.

Allocation and Cost of Defenses

Individual plants produce phytochemical defenses in mixtures and concentrations that vary spatially within the individual plant and change through time. As a result, plants do not possess a single, static chemical defense phenotype, but instead a "dynamic mosaic phenotype" (Harder et al. 2019). Understanding what determines the distribution of chemical defenses, and the consequences for herbivores, has been a key focus of research on phytochemical defense. Central to these efforts is the idea that there is a cost to the production and maintenance of defensive compounds (Herms and Mattson 1992). Constructing any molecule comes with an energy cost; if plants have a fixed energetic budget, then producing defense compounds must come at the expense of something else (i.e., a trade-off with investment in growth or reproduction). These costs can be highly context dependent and could manifest in many ways, such as lower growth, fewer flowers, or reduced investment in physical defenses like tough leaves (Herms and Mattson 1992; Strauss et al. 2002).

Because the production of chemical defenses comes at an energetic cost, a plant that can maximize the protection benefit of these defenses while minimizing their cost should have higher fitness. The spatial and temporal mosaic of chemical defenses in plants is generally thought to be a result of this strategy. Optimal defense theory (ODT; see "Glossary") has emerged as a leading explanation for how plants might optimize their investment in defenses (McKey 1974, 1979; Rhoades 1976, 1979; Stamp 2003). The theory posits that plants allocate chemical defenses to different parts of the plant based on (1) the chance of that plant tissue being attacked by enemies, (2) how valuable that tissue is to the success of the plant, and (3) the cost of the defense. This theory has been tested many times, and as research has continued to reveal new layers of complexity in the dynamic distribution of chemical defenses within plants, ODT has remained relevant and has been used to understand these findings (Zangerl and Bazzaz 1992; Ohnmeiss 2000; Heath 2014; Gershenzon and Ullah 2022).

ODT is just one of many hypotheses that have been used to understand patterns of allocation of phytochemical defense (see "Glossary"). The growth-differentiation balance hypothesis suggests that the balance between factors limiting growth and those limiting photosynthesis determine the relative allocation to growth versus differentiation (including producing phytochemical defenses); environments that promote slow growth but ample photosynthesis will result in plants with high levels of defense, while those with fast growth relative to photosynthesis will have lower levels of defense (Loomis 1932, 1953; Herms and Mattson 1992). The resource availability hypothesis (or growth rate hypothesis) posits a negative relationship, among species, between growth rate and defense levels, such that species growing in high-resource environments will have evolved to favor faster growth and lower investment in defense relative to species in low-resource environments, which will have a greater need to protect their slow-growing tissues, the replacement of which would be challenging in conditions of scarcity

(Coley et al. 1985, 1987). The plant apparency hypothesis, which is linked with ODT, provides an alternative explanation for differences in defense levels among long- and short-lived plants. It predicts that the apparency of plants (how easy it is for herbivores to find them) determines the broad mechanisms of the chemical defenses they use. More apparent plants should invest in digestibility reducers that are less costly to produce and work against a broader range of herbivores in a very concentration-dependent manner (i.e., quantitative defenses), while less apparent plants should invest in more costly and more toxic compounds (i.e., qualitative defenses; Feeny 1976; Rhoades and Cates 1976; Massad et al. 2011). The carbon-nutrient balance hypothesis proposes that availability of carbon (due to sunlight levels) and nutrients (due to soil availability) influence allocation to defense, as there is competition for these resources between primary and secondary metabolism (Bryant et al. 1983; Tuomi et al. 1988). These theories are not mutually exclusive, and each has received support and proven useful in understanding different aspects of variation in allocation of chemical defense within the plant kingdom (Stamp 2003). ODT in particular has proven especially useful in understanding intraspecies comparisons and generating testable experimental hypotheses.

Despite decades of work on this theory, ODT continues to provide opportunity for new questions. While the three components of the theory may appear intuitive, quantifying probability of attack, tissue value, and/or costs of defense production is quite complex. Factors determining the chance of attack for a particular tissue can include apparency (how visible or conspicuous a tissue is to herbivores; Strauss et al. 2015; Smilanich et al. 2016), the nutritional value of that tissue to different herbivores (Behmer et al. 2002; Robert et al. 2012), how the feeding strategies of herbivores are influenced by their own predators and parasites (Stamp 2001; Lucas-Barbosa et al. 2014), and the complex population dynamics of herbivore communities (Underwood 1999). The fitness value of various tissues is generally determined by removing the same mass or surface area of different tissues and measuring the resulting impact on reproduction. This approach, however, may not account for nonlinear impacts such as small amounts of damage to flowers influencing rates of pollination (McCall and Irwin 2006). The third component of ODT, the cost to the plant of defending a particular tissue, is perhaps the most difficult to pin down. Though there is ample evidence for an energetic cost of production of phytochemical defense (Strauss et al. 2002), quantifying this cost has often proven notoriously difficult and is context dependent (Agrawal 2011). For example, costs may be detectable only for plants growing in resource-poor conditions, where such costs are likely to be the binding constraint. Recent findings that some secondary metabolites might be reintegrated into primary metabolism, which we discussed earlier (Erb and Kliebenstein 2020), may minimize the costs associated with producing phytochemical defenses. How ODT is relevant for understanding the distribution of phytochemical defenses along the spatial and temporal axes discussed below, and especially the intersection of these axes, is the topic of continuing research.

Spatial Patterns of Phytochemical Defense Allocation

ODT has received much attention from researchers working to understand how plants defend different types of tissues. Roots,

stems, leaves, flowers, fruits, and seeds can all contain phytochemical defenses, attract different herbivores, and differ both in the composition of compounds present for defense and in their concentrations (Keith and Mitchell-Olds 2017; De La Pascua et al. 2020). The defense of reproductive tissues involves unique trade-offs. The optimization of phytochemical defense levels in seeds may differ for maternal and paternal plants and the embryos contained in the seeds, resulting in a conflict for control of this trait (Zangerl and Nitao 1998). Chemical defense of reproductive tissues can have ecological costs (see "Glossary"), due to the detrimental impact that defenses have upon interactions with mutualists. Chemical defenses present in nectar, for example, can decrease pollination rates (Stevenson et al. 2017). It is still unclear how independent the phytochemical defenses of different plant tissues are from one another and to what extent any interconnection may limit the optimization of defense of different tissues. Changes in leaf chemistry, for example, can result in changes in fruit, which can make fruits less appealing to seed-dispersing birds (Whitehead and Poveda 2011; Cipollini et al. 2003). Despite how critical reproductive tissues are to plant success, and the often-high rates of herbivory observed on reproductive tissues, the study of phytochemical defense of foliar tissue dominates the literature.

Plants must also defend many repeated units of the same types of tissues. A mature oak tree, for example, may produce as many as half a million leaves each season. This modularity is somewhat unique to plants and results in the potential for another axis of variation in plant phytochemical defense. Though variation on this scale is often overlooked, it can be quite significant and can shape insect community diversity and population structure (Richards et al. 2015, 2016). And because most small leaf-feeding herbivores interact with only one or a few leaves, for example, variation on this scale can determine the outcomes of these interactions. One example of within-plant variation is in cottonwood (*Populus*), in which leaves in different regions of the canopy differ in phytochemical concentrations and also in resistance to a specialist aphid species (Kearsley and Whitham 1997; Holeski et al. 2009, 2012). It is still largely unknown, however, how much variation commonly exists among repeated units of plant tissues, the extent to which genetic versus environmental factors affect this variation, and whether it is adaptive in the context of herbivory.

Temporal Patterns of Phytochemical Defense Allocation

In addition to spatial variation in phytochemical defenses, individual plants often change defenses through time. Temporal variation in defenses can occur in different patterns: (1) changes in defenses in response to herbivory, (2) seasonal variation in defense levels, and (3) changes in defense levels as plants develop.

Plants can respond dynamically to the threat of herbivory by changing levels of phytochemical defenses when needed. In addition to the constitutive (i.e., baseline) levels of defense compounds found in plants in the absence of herbivory, plants can employ induction (i.e., change) of defenses following herbivory (Adler and Karban 1994; Cipollini 1998). Induction is beneficial if current herbivory levels are a reliable indicator of future herbivory levels (Karban and Baldwin 1997; Harvell and Tollrian 1999). This plastic response can occur via up- or downregulation of the production of phytochemicals or movement of phytochemicals within the plant (Karban and Baldwin 1997). Induc-

tion of defenses have an (often-short) time lag between the cue and increased levels of defense. Induction can minimize the cost of defense by increasing investment in these compounds only when needed and can enable plants to tailor defenses to particular herbivores (Karban and Baldwin 1997; Ali and Agrawal 2012). Defenses can also be transgenerationally induced, with offspring defense levels affected by herbivory levels in the parental generation (Herman and Sultan 2011; Holeski et al. 2012a). Induction of defenses in response to one herbivore can impact, either positively or negatively, plant resistance to other herbivores (Poelman et al. 2010). How plants induce defenses in response to complex communities of herbivores (Bernhardsson et al. 2013) and how plants detect and identify herbivores (Bonaventure 2012; Arimura 2021) are important continuing areas of research.

Temporal changes in defense levels also occur in predictable patterns across a growing season, following shifts in plant physiological and allocational priorities. For example, growth and defense may be prioritized early in a growing season, while reproduction is a priority later in a growing season. The production of flowers and fruits, for example, may correspond with changes in defense of vegetative tissues (Blanchard and Bowers 2020). Changes in photoperiod, temperature, precipitation, and nutrient availability can also occur seasonally, bringing corresponding changes in plant physiology and defense (Holeski et al. 2012b; Koricheva and Barton 2012). Plants that experience a predictable pattern of attack by different herbivores throughout the season may also regulate their induced responses to prepare for common attackers (Mertens et al. 2021).

Finally, levels of both constitutive and induced defenses can change over the course of plant development; this genetic-based phenomenon is generally called ontogenetic variation (see "Glossary"; Boege and Marquis 2005; Barton and Koricheva 2010; Holeski et al. 2012b). Tissue from different developmental stages, such as leaves from juvenile versus adult phases, have different values to the plant in terms of resource acquisition and metabolic potential, as well as cost of replacement. Because ontogenetic patterns of tissue value differ across taxa, patterns of ontogenetic defense levels also vary. Some species have higher levels of defense in juvenile versus adult tissues, while others have the reverse pattern (Kearsley and Whitham 1989; Karban and Thaler 1999; Cole et al. 2020).

Abiotic Factors

Physical environmental factors have a large impact on variation in the concentration of phytochemical defenses among and within plants. Light is particularly important; high-light environments can result in higher levels of defense (Burns et al. 2002; Mooney and Niesenbaum 2012). Some plants also show diurnal (day to night) fluctuations in defense levels (Palo 1984). Soil nutrient levels (Fajer et al. 1992) and temperature (Bidart-Bouzat and Imeh-Nathaniel 2008) also impact phytochemical defense concentration.

Biotic Factors

Plants and their herbivores exist in complex communities of organisms, and this context shapes their interactions. In addition to the varying impacts of different herbivores through induction, microbes also affect the chemical defenses of plants. Attack by pathogens causes plants to change their defense levels (Felton
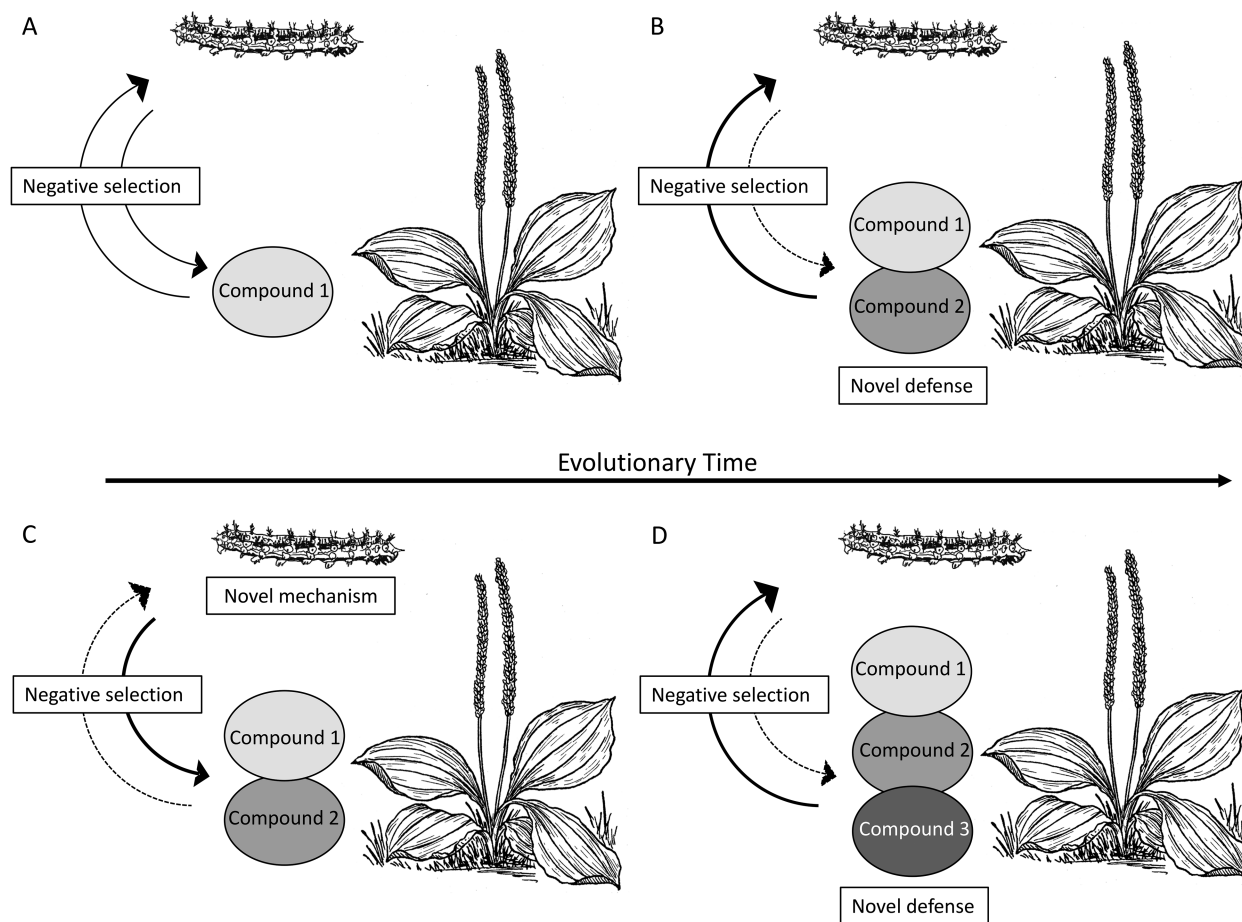
Fig. 2   *A*, The coevolutionary arms race between plants and herbivores requires reciprocal natural selection exerted on each species by the other. *B*, The evolution of a novel chemical defense by the plant makes the plant a lower-quality food for the herbivores and results in a decrease in the selective pressure exerted on the plant by the herbivore (dashed line) and an increase in the selective pressure exerted on the herbivore by the plant (solid line). *C*, In response to this selection, the herbivore evolves novel mechanisms that allow it to feed more effectively on the plant. This results in a decrease in the selective pressure exerted by the plant on the herbivore and an increase in the selective pressure exerted by the herbivore on the plant. *D*, This stepwise pattern of escalating defensive and tolerance traits in plants and herbivores continues through evolutionary time.

and Korth 2000). Symbionts, such as mycorrhizal fungi in the soil and endophytic fungi in leaves, can deliver nutrients needed for phytochemical synthesis and even directly produce toxic compounds (Cheplick and Clay 1988; Vannette et al. 2013).

Relationship between Herbivores and Plant Defenses

Coevolution

In 1964, Ehrlich and Raven proposed that plants and herbivores evolve together in a stepwise fashion (fig. 2). Plants produce novel compounds that allow them to reduce or escape herbivory, and thus increase plant fitness, herbivores evolve mechanisms to tolerate or avoid those compounds in response, and then the cycle repeats (fig. 2). This process of reciprocal natural selection and coadaptation between two species (or groups of species, as discussed later) is called coevolution (see "Glossary"). The evolution of new defense compounds is thought to often co-occur with the evolution of new plant species, which has resulted in an increase in phyto-chemical diversity as plant lineages have radiated over time (Becerra et al. 2009). Within the study of phytochemical defense, the metaphor of a coevolutionary "arms race," in which plants and herbivores take turns evolving escalating counterdefenses (Ehrlich and Raven 1964; Janzen 1980; Thompson 1994), has been a foundational concept and has been used to explain the codiversification of flowering plants and their herbivores.

There is an abundance of empirical evidence for coevolutionary theory (Mérillon and Ramawat 2020). Classic studies that support this theory include investigations of chemical defense in wild parsnip (*Pastinaca sativa*) of the carrot family (Apiaceae). Many plants in the carrot family (including wild parsnip) produce linear furanocoumarins, compounds that can bond with DNA in the presence of UV light, with highly toxic results (Berenbaum 1978). Furanocoumarins are modified from hydroxycoumarins, compounds that are found in many other members of the carrot family as well as many other plant families (fig. 1*C*; Berenbaum 1983). The existence of these derived compounds in a limited lineage suggests that furanocoumarins

represent the evolution of novel chemical defense. Some herbivores, such as the southern armyworm caterpillar (*Spodoptera eridania*; Noctuidae), commonly feed upon carrot family species that contain hydroxycoumarins but cannot tolerate linear furanocoumarins, which suggests that this novel defense has allowed these plants to escape from herbivory by these insects. Evidence for an herbivore evolutionary response to the novel defense comes from the black swallowtail caterpillar (*Papilio polyxenes*; Papilionidae), which feeds successfully upon furanocoumarin-containing plants (Berenbaum 1983).

Though coevolution is an important process in plant-herbivore interactions, not every plant-produced compound that affects herbivores has arisen through direct coevolution with herbivores. As discussed earlier, many compounds that play a defensive role also serve other functions for the plant. Phenolic compounds may have evolved in response to the need for protection from UV radiation as plants transitioned to land; their role in influencing herbivory may be secondary (Close and McArthur 2002). When a trait evolves in response to one set of selective pressure but proves adaptive in the context of others, it is called ecological fitting (see "Glossary"; Janzen 1985). Flavonoids, a type of phenolic that impact herbivores, may have evolved partially to mediate the interaction of plants with arbuscular mycorrhizal fungi (Nascimento and Tattini 2022). Coevolution is also often more complicated than one herbivore species and one plant species exerting reciprocal selection on one trait in each species. As plants and herbivores are embedded in communities, diffuse coevolution can occur among groups of interacting species (Janzen 1980; Strauss et al. 2005). Because a plant species' range may include significant variation in environmental factors that influence both plants and herbivores as well as variation in herbivore community members, the strength and nature of coevolution can vary spatially (geographic mosaic theory of coevolution; Thompson 1994). Evidence that a chemical defense helps to reduce herbivory and that an herbivore possesses a trait to tolerate this defense does not necessarily mean direct coevolution.

Adaptations of Herbivores

The ubiquity and diversity of plant chemical defenses are reflected in the strategies that herbivores employ to circumvent these defenses. Herbivores are generally selective in their host plants and can detect chemical defenses. Some herbivores are able to avoid or minimize consuming defensive compounds in their hosts by modifying their feeding behavior. Most herbivores are also able to consume some amount of chemical defenses while minimizing the negative physiological impacts.

Herbivorous animals are an incredibly large and diverse group that vary greatly in size and taxonomy (e.g., from tiny insects like thrips to giant mammals like elephants). One axis of variation that has proven important for understanding herbivore adaptation to chemical defense is diet breadth (see "Glossary"; the number of different host plant species that an herbivore utilizes). Diet breadth influences the array of chemical defenses that an herbivore faces, which in turn is thought to influence how herbivores cope with phytochemical defenses. Dietary specialist (see "Glossary") herbivores feed on a narrower range of plant taxa that are generally closely related and/or share phytochemical compounds, while generalists (see "Glossary") feed on a larger phylogenetic diversity of host taxa that are also diverse phytochemically

(Fraenkel 1959; Loxdale and Harvey 2016). Herbivores exist on a gradient of specialization ranging between narrow specialization and broad generalization.

More specialized herbivores have evolved mechanisms that are specific to the compounds found in their limited group of host plants. Behavioral modifications can allow specialists to feed on highly toxic plants while receiving low doses of toxins. Beetles and caterpillars that feed on milkweeds, for example, avoid consuming the toxic latex that their host plant produces by first severing the leaf veins that contain the latex before feeding on leaf tissue (Dussourd and Eisner 1987). Other herbivores rapidly excrete phytochemicals to minimize exposure (Self et al. 1964). Specialists also employ physiological changes. These include site insensitivity; alterations in the molecular targets of phytochemicals that prevent binding, as discussed earlier for monarchs (Holzinger et al. 1992); and detoxification of phytochemicals via specialized enzymes (Ratzka et al. 2002). Some specialists (and even a small number of generalists) can actually accumulate the phytochemical defenses that they consume in their own bodies in order to become protected from predators, in a process known as sequestration (Nishida 2002). Though specialists are often still negatively impacted by high levels of chemical defense, these mechanisms generally result in specialists having higher tolerance to low levels of chemical defenses than generalists (Ali and Agrawal 2012).

More generalized herbivores will likely face a greater diversity of compounds and so employ mechanisms that allow them to consume a variety of chemicals. It has been proposed that generalists dilute toxins in their diet by consuming a variety of plants (Bernays et al. 1994; meta-analysis by Lefcheck et al. 2013) and/or may vary their diet based on physiological needs (Singer and Bernays 2009). Expansion and diversification of detoxification enzymes that are important to specialists seem to be important for broad generalists (Rane et al. 2019; Breeschoten 2022). For example, the enzyme family cytochrome P450 is important for detoxification of phytochemicals in many herbivores regardless of diet breadth (Feyereisen 1999). The specialist black swallowtail (*Papilio polyxenes*), which we discussed earlier, can detoxify furanocoumarins using a small group of P450 enzymes (Cohen et al. 1989). The broad generalist corn earworm (*Helicoverpa zea*) also utilizes P450 but produces a more diverse set of enzymes (Li et al. 2002).

It has also been predicted that plants should respond to attack by specialists and generalists with differences in defense induction. Some herbivores can also suppress the induced responses of their host plants (Ballaré 2001; Musser et al. 2002); this ability may be influenced by diet breadth (Eichenseer et al. 2010). However, degree of herbivore specialization has still not been shown to correspond generally with phytochemical induction, perhaps partially due to methodological issues that do not allow for the separation of the impact of diet breadth and other factors such as feeding guild (discussed below; Ali and Agrawal 2012).

Herbivores are further classified into feeding guilds (see "Glossary"), based on their mode of feeding (e.g., chewing or sucking, leaf miners, gall makers) and the types of plant tissue that they feed upon (leaves, flowers, fruits, roots, etc.; Simberloff and Dayan 1991; Novotny et al. 2010). Herbivory from different feeding guilds has differential fitness consequences for plants and thus variable ecological and evolutionary significance (Eisenring et al. 2018). Likewise, the effects of secondary compounds

on herbivores from different feeding guilds vary. For example, chewing herbivores ingest all cellular contents, while piercing/sucking herbivores ingest only xylem or phloem content, which influences the concentration and composition of phytochemical defenses that they must cope with. Herbivores that are more generalized in the tissue types that they consume will be exposed to a greater diversity of phytochemical defenses.

The feeding guild of herbivores has a strong influence on the induced defensive response of plants, which can result in trade-offs for plants attacked by multiple herbivores. In response to attack by piercing-sucking insects, plants usually upregulate the salicylic acid metabolic pathway, which is also induced by pathogens (Walling 2000). Attack by chewing herbivores, on the other hand, generally results in induction of the jasmonic acid or ethylene pathways, which can yield a different phytochemical response (Kessler and Baldwin 2002). Induction of the milkweed *Asclepias syriaca* by the aphid *Aphis nerii*, for example, results in increased susceptibility of plants to attack by monarch caterpillars (*Danaus plexippus*; Ali and Agrawal 2014). Investigations into plant-mediated interactions among herbivore guilds are yielding new insights into how plants balance these trade-offs in the context of complex herbivore communities (de Bobadilla et al. 2022).

Diversity of Phytochemical Defense Compounds

Plants present an incredible diversity of phytochemical defense compounds, with most species producing a unique mixture of chemicals. Many species produce multiple classes of defense compounds, and most plant species have an array of similar compounds within a particular subclass that share a primary structure but have small structural differences that can cause varying levels of toxicity in herbivores (fig. 1B; Bowers and Puttick 1988; Rotter et al. 2018 [which features work by undergraduate researcher J. Garcia]). Our efforts to understand the evolutionary origin and function of this great diversity have long been driving questions in the study of plant chemical defense against herbivores.

Intraspecific Patterns of Phytochemical Diversity

Though coevolution may explain the great diversity of phytochemical defenses displayed across the plant kingdom, explanations for the diversity of compounds found within species are more tenuous. Wild parsnip, for example, contains many different furanocoumarins, which function similarly but vary in their ability to bind DNA (fig. 1C; Berenbaum et al. 1991). This apparent redundancy has long perplexed researchers. Three leading hypotheses (reviewed in Wetzel and Whitehead 2020) have emerged for this pattern of unexplained diversity: (1) the interaction diversity hypothesis, in which a diverse community of herbivores is thought to maintain a diversity of phytochemical defenses within a species because each compound is particularly effective against a different herbivore; (2) the synergy hypothesis, namely, that mixtures of compounds interact to produce greater toxicity than would be found through the additive effects of individual compounds; and (3) the screening hypothesis, which holds that plants that utilize metabolic pathways that can, with small variations in enzyme activity, produce a diversity of products are more likely to contain defensive compounds that are effective against unknown herbivores, in addition to many non-adaptive compounds, and therefore persist and radiate (fig. 1D).

These hypotheses are not mutually exclusive, and evidence has been presented that supports each.

Due to limitations imposed by the methods and equipment used to extract and quantify phytochemical compounds, most research on phytochemical defenses has focused on single subgroups of compounds within particular taxa or suites of similar compounds produced by individuals. To measure the impact of phytochemical defense compounds on plant resistance to herbivores, researchers have used a mix of correlative and experimental approaches. Correlative approaches that leverage natural variation in phytochemistry include quantifying phytochemistry along with the amount of plant tissue removed due to herbivory across populations and/or genotypes of a plant species grown in a common garden (Bidart-Bouzat and Kliebenstein 2008) and measuring the preference and performance of herbivores in the laboratory when fed different populations or species (Kelly and Bowers 2016). Experimental methods include measuring caterpillar response when fed an artificial diet containing different isolated compounds (Bowers and Puttick 1988) or when fed plants genetically engineered to alter their chemical defenses (Hunziker et al. 2021).

These investigations have provided in-depth understanding of how specific compounds or groups of compounds mediate plant interactions with insects. Some studies have shown that individual compounds can have different impacts on different herbivores, with some compounds even having positive effects on some herbivores and negative on others (Roslin and Salminen 2008; Rotter et al. 2018). This lends credence to the interaction diversity hypothesis that the production of diverse suites of compounds has evolved in response to a diverse community of herbivores. Other intraspecific studies have been key in supporting the importance of synergy among related compounds in herbivore defense (Richards et al. 2012).

More recently, to gain a more complete view of phytochemical diversity, researchers are extracting and quantifying the multiple classes of compounds found within species. For example, a recent study experimentally tested for support of the three leading hypotheses maintaining phytochemical diversity. Researchers assessed the response of four different herbivores to apple tree phenolics belonging to four different subclasses (which represent ~66% of apple tree phenolic diversity), individually and in mixtures that varied in complexity (Whitehead et al. 2021). Herbivores were not generally more negatively impacted by chemical mixtures (lack of support for synergy hypothesis) and key compounds did not exist that drove herbivore effect across species (lack of support for screening hypothesis). Instead, more diverse chemical mixtures affected more herbivores, and the effect of each compound varied by herbivore species, which supports the interaction diversity hypothesis. Future studies that take a similarly broad approach to chemical diversity might help reveal whether these drivers of phytochemical diversity are specific to a given system or whether greater generalizations about the evolution of phytochemical diversity can be made.

Interspecific Patterns of Phytochemical Diversity and Metabolomics

Closely related plant taxa (or related groups) often show a phylogenetic signal, or consistency in broad patterns of presence/absence of particular compounds or classes of compounds

Table 1

Common Methods Used to Quantify and/or Identify Phytochemical Defense Compounds

Method	Gas chromatography and liquid chromatography	Nuclear magnetic resonance
Extraction and purification	Targeted	General
Compound identification	If coupled with mass spectrometry and known mass spectra	Very useful for compound identification
Strengths	Quantification of a subset of compounds with known biological relevancy	Ability to quickly quantify a near-complete plant metabolome
Weaknesses	Missing compounds that may be relevant for herbivores	Datasets require a large amount of computing power; may include many compounds that do not influence herbivores

(Liscombe et al. 2005; Agrawal and Fishbein 2008; Züst et al. 2020). For example, almost all members of the genus *Lupinus* (Fabaceae) that have been studied produce quinolizidine alkaloids, which are also found in closely related genera such as *Thermopsis* and *Baptisia* (Ohmiya et al. 1995). Distantly related plant taxa may also contain phytochemical compounds of the same class; this may result from convergent evolution and/or differential expression of genes that evolved prior to the divergence of these groups (Wink 2003; Agrawal et al. 2009, 2012; Bauer et al. 2010).

The role of phytochemical diversity has recently been explored on the scale of communities and across landscapes, resulting in new insights into the ecological relevance of the staggering variety of defense compounds presented by the plant kingdom (Richards et al. 2015; Hunter 2016). To facilitate these investigations, some chemical ecologists have partnered with organic chemists to attempt to measure the complete chemical diversity of plant samples, or metabolome (see "Glossary"; table 1). Because metabolomics attempts to capture a snapshot of all plant-produced small molecules present in a plant, analysis of metabolomics data requires a great deal of computing power, and interpreting the ecological significance of such complex patterns is not intuitive. Researchers have used metrics of diversity borrowed from community ecology to characterize and compare phytochemical diversity across populations or taxa (reviewed by Wetzel and Whitehead 2020).

Metabolomics studies have revealed new patterns of phytochemical diversity. The chemical diversity of species from the tropical genus *Inga*, for example, does not show a phylogenetic signal (as predicted by the theory of coevolution); instead, species that grow together in communities are more dissimilar. This may indicate that diversity in chemical defense influences or is influenced by community structure, with phytochemical diversity among species creating variable niches within the community and allowing for the great diversity of plants found in the tropics (Kursar et al. 2009). *Inga* has high rates of co-occurrence of con-

specifics, and it is unknown whether similar patterns occur outside of hyperdiverse tropical ecosystems. Other researchers have leveraged metabolomics to use phylogenetics and abiotic factors to predict landscape-scale phytochemical diversity (Defossez et al. 2021). Future research on diversity in phytochemical defense will likely continue to develop theory on how to best utilize metrics of diversity and understand the role of diversity of defense across ecological scales, from plants to across landscapes (Wetzel and Whitehead 2020) as well as how to integrate metabolomics with a more mechanistic understanding of the impacts of specific compounds.

Conclusion

Inspired by the incredible array of plant secondary metabolites and the diverse behavioral and physiological adaptations of herbivores to cope with these defense compounds, researchers have developed theory that has proven crucial for discoveries in many areas of the ecology and evolution of species interactions. Foundational ideas such as optimal defense theory continue to inspire investigations that reveal the complex factors underlying defense production and allocation and the implications for herbivores. Today, increasingly powerful techniques for the quantification of secondary metabolites are aiding exploration of decades-long questions, such as why diversity in phytochemical defense exists.

Acknowledgments

Literature Cited

Adler FR, R Karban 1994 Defended fortresses or moving targets? another model of inducible defenses inspired by military metaphors. Am Nat 144:813–832

Agati G, M Tattini 2010 Multiple functional roles of flavonoids in photoprotection. New Phytol 186:786–793.

Agrawal AA 2011 Current trends in the evolutionary ecology of plant defence. Funct Ecol 25:420–432.

Agrawal AA, M Fishbein 2008 Phylogenetic escalation and decline of plant defense strategies. Proc Natl Acad Sci USA 105:10057–10060.

Agrawal AA, M Fishbein, R Halitschke, AP Hastings, DL Rabosky, S Rasmann 2009 Evidence for adaptive radiation from a phylogenetic study of plant defense. Proc Natl Acad Sci USA 106:18067–18072.

Agrawal AA, G Petschenka, RA Bingham, MG Weber, S Rasmann 2012 Toxic cardenolides: chemical ecology and coevolution of specialized plant-herbivore interactions. New Phytol 194:28–45.

Ali JG, AA Agrawal 2012 Specialist versus generalist insect herbivores and plant defense. Trends Plant Sci 17:1360–1385.

——— 2014 Asymmetry of plant-mediated interactions between specialist aphids and caterpillars on two milkweeds. Funct Ecol 28:1404–1412.

Arimura G 2021 Making sense of the way plants sense herbivores. Trends Plant Sci 26:288.

Ballaré CL 2001 Smart plants or stealthy bugs? Trends Plant Sci 6:142.

Barton KE, J Koricheva 2010 The ontogeny of plant defense and herbivory: characterizing general patterns using meta-analysis. Am Nat 175:481–493.

Bauer P, J Munkert, M Brydziun, E Burda, F Muller-Uri, H Groger, YA Muller, W Kreis 2010 Highly conserved progesterone 5 beta-reductase genes (P5 beta R) from 5 beta-cardenolide–free and 5 beta-cardenolide–producing angiosperms. Phytochemistry 71:1495–1505.

Becerra JX, K Noge, DL Venable 2009 Macroevolutionary chemical escalation in an ancient plant–herbivore arms race. Proc Natl Acad Sci USA 106:18062–18066.

Behmer ST, SJ Simpson, D Raubenheimer 2002 Herbivore foraging in chemically heterogeneous environments: nutrients and secondary metabolites. Ecology 83:2489–2501.

Berenbaum M 1978 Toxicity of a furanocoumarin to armyworms: case of biosynthetic escape from insect herbivores. Science 201:532–534.

——— 1983 Coumarins and caterpillars: a case for coevolution. Evolution 37:163–179.

Berenbaum MR, JK Nitao, AR Zangerl 1991 Adaptive significance of furanocoumarin diversity in Pastinaca sativa (Apiaceae). J Chem Ecol 17:207–215.

Berenbaum MR, AR Zangerl 2008 Facing the future of plant-insect interaction research: le retour à la "raison d'être." Plant Physiol 146:804–811.

Bernays EA, KL Bright, N Gonzalez, J Angel 1994 Dietary mixing in a generalist herbivore: tests of two hypotheses. Ecology 75:1997–2006.

Bernhardsson C, KM Robinson, IN Abreu, S Jannson, BR Albrectsen, PK Ingvarsson 2013 Geographic structure in metabolome and herbivore community co-occurs with genetic structure in plant defence genes. Ecol Letters 16:791–798.

Bezerra RHS, L Sousa-Souto, AEG Santana, BG Ambrogi 2021 Indirect plant defenses: volatile organic compounds and extrafloral nectar. Arthropod-Plant Interact 15:467–489.

Bidart-Bouzat MG, DJ Kliebenstein 2008 Differential levels of insect herbivory in the field associated with genotypic variation in glucosinolates in Arabidopsis thaliana. J Chem Ecol 34:1026–1037.

Birkett MA, CA Campbell, K Chamberlain, E Guerrieri, AJ Hick, JL Martin, M Matthes, et al 2000 New roles for cis-jasmone as an insect semiochemical and in plant defense. Proc Natl Acad Sci USA 97:9329–9334.

Blanchard M, MD Bowers 2020 Critical phenological events affect chemical defense of plant tissues: iridoid glycosides in a woody shrub. J Chem Ecol 46:206–216.

Boege K, RJ Marquis 2005 Facing herbivory as you grow up: the ontogeny of resistance in plants. Trends Ecol Evol 20:441–448.

Bonaventure G 2012 Perception of insect feeding by plants. Plant Biol 14:872–880.

Bowers MD, GM Puttick 1988 Response of generalist and specialist insects to qualitative allelochemical variation. J Chem Ecol 14:319–334.

Breeschoten T, CF van der Linden, VI Ros, ME Schranz, S Simon 2022 Expanding the menu: are polyphagy and gene family expansions linked across Lepidoptera? Genome Biol Evol 14:evab283.

Bruni R, D Barreca, M Protti, V Brighenti, L Righetti, L Anceschi, L Mercolini, S Benvenuti, G Gattuso, F Pellati 2019 Botanical sources, chemistry, analysis, and biological activity of furanocoumarins of pharmaceutical interest. Molecules 24:2163.

Bryant JP, FS Chapin III, DR Klein 1983 Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. Oikos 40:357–368.

Burns AE, RM Gleadow, IE Woodrow 2002 Light alters the allocation of nitrogen to cyanogenic glycosides in Eucalyptus cladocalyx. Oecologia 133:288–294.

Cheplick GP, K Clay 1988 Acquired chemical defences in grasses: the role of fungal endophytes. Oikos 52:309–318.

Cipollini D, CB Purrington, J Bergelson 2003 Costs of induced responses in plants. Basic Appl Ecol 4:79–89.

Cipollini DF 1998 Induced defenses and phenotypic plasticity. Trends Ecol Evol 13:200.

Close DC, C McArthur 2002 Rethinking the role of many plant phenolics: protection from photodamage not herbivores? Oikos 99:166–172.

Cohen MB, MR Berenbaum, MA Schuler 1989 Induction of cytochrome P450-mediated detoxification of xanthotoxin in the black swallowtail. J Chem Ecol 15:2347–2355.

Cole CT, CJ Morrow, HL Barker, KF Rubert-Nason, JFL Riehl, TG Kollner, ND Lackus, RL Lindroth 2020 Growing up aspen: ontogeny and trade-offs shape growth, defence, and reproduction in a foundation species. Ann Bot 127:505–517.

Coley PD 1987 Interspecific variation in plant anti-herbivore properties: the role of habitat quality and rate of disturbance. New Phytol 106:251–263.

Coley PD, JP Bryant, FS Chapin III 1985 Resource availability and plant antiherbivore defense. Science 230:895–899.

Damtoft S 1994 Biosynthesis of catalpol. Phytochemistry 35:1187–1189.

Defossez E, C Pitteloud, P Descombes, G Glauser, PM Allard, TM Walker, P Fernandez-Conradi, JL Wolfender, L Pellissier, S Rasmann 2021 Spatial and evolutionary predictability of phytochemical diversity. Proc Natl Acad Sci USA 118:e2013344118.

de Bobadilla MF, A Vitiello, M Erb, EH Poelman 2022 Plant defense strategies against attack by multiple herbivores. Trends Plant Sci 27:528–535.

De La Pascua DR, C Smith-Winterscheidt, JA Dowell, EW Goolsby, CM Mason 2020 Evolutionary trade-offs in the chemical defense of floral and fruit tissues across genus Cornus. Am J Bot 107:1260–1273.

Dobler S, G Petschenka, H Pankoke 2011 Coping with toxic plant compounds: the insect's perspective on iridoid glycosides and cardenolides. Phytochemistry 72:1593–1604.

Dötterl S, A Jurgens, K Seifert, T Laube, B Weissbecker, S Schutz 2006 Nursery pollination by a moth in Silene latifolia: the role of odours in eliciting antennal and behavioural responses. New Phytol 169:707–718.

Dussourd DE, T Eisner 1987 Vein-cutting behavior: insect counterploy to the latex defense of plants. Science 237:898–901.

Ehrlich PR, PH Raven 1964 Butterflies and plants: a study in coevolution. Evolution 18:586–608.

Eichenseer H, MC Mathews, JS Powell, GW Felton 2010 Survey of a salivary effector in caterpillars: glucose oxidase variation and correlation with host range. J Chem Ecol 36:885–897.

Eisenring M, G Glauser, M Meissle, J Romeis 2018 Differential impact of herbivores from three feeding guilds on systemic secondary metabolite induction, phytohormone levels and plant-mediated interactions. J Chem Ecol 44:1178–1189.

Emery AM, PF Billingsley, PD Ready, MBA Djamgoz 1998 Insect Na^+/K^+-ATPase. J Insect Physiol 44:197–209.

Erb M, DJ Kliebenstein 2020 Plant secondary metabolites as defenses, regulators, and primary metabolites: the blurred functional trichotomy. Plant Physiol 184:39–52.

Fajer ED, MD Bowers, FA Bazzaz 1992 The effect of nutrients and enriched CO_2 environments on production of carbon-based allelochemicals in Plantago: a test of the carbon/nutrient balance hypothesis. Am Nat 140:707–723.

Feeny P 1976 Plant apparency and chemical defense. Pages 1–40 in JW Wallace, RL Mansell, eds. Biochemical interaction between plants and insects. Springer, Boston.

Felton GW, KL Korth 2000 Trade-offs between pathogen and herbivore resistance. Curr Opin Plant Biol 3:309–314.

Feyereisen R 1999 Insect P450 enzymes. Annu Rev Entomol 44:507–533.

Fraenkel GS 1959 The raison d'être of secondary plant substances. Science 129:1466–1470.

Garcia LC, MD Eubanks 2019 Overcompensation for insect herbivory: a review and meta-analysis of the evidence. Ecology 100:e02585.

Gershenzon J 1994 Metabolic costs of terpenoid accumulation in higher plants. J Chem Ecol 20:1281–1328.

Gershenzon J, C Ullah 2022 Plants protect themselves from herbivores by optimizing the distribution of chemical defenses. Proc Natl Acad Sci USA 119:e2120277119.

Harborne JB, H Baxter, GP Moss 1999 Phytochemical dictionary: a handbook of bioactive compounds from plants. 2nd ed. CRC, Boca Raton, FL.

Harder LD, MM Strelin, IC Clocher, MW Kulbaba, MA Aizen 2019 The dynamic mosaic phenotypes of flowering plants. New Phytol 224:1021–1034.

Harvell CD, R Tollrian 1999 Why inducible defenses? Pages 3–9 in R Tollrian, CD Harvell, eds. The ecology and evolution of inducible defenses. Princeton University Press, Princeton, NJ.

Heath JJ, A Kessler, E Woebbe, D Cipollini, JO Stireman 2014 Exploring plant defense theory in tall goldenrod, *Solidago altissima*. New Phytol 2014:1357–70.

Herman J, SE Sultan 2011 Adaptive transgenerational plasticity in plants: case studies, mechanisms, and implications for natural populations. Front Plant Genet Genom 2:102.

Herms DA, WJ Mattson 1992 The dilemma of plants: to grow or defend. Q Rev Biol 67:283–335.

Holeski LM, ML Hillstrom, TG Whitham, RL Lindroth 2012b Relative importance of genetic, ontogenetic, induction, and seasonal variation in producing a multivariate defense phenotype in a foundation tree species. Oecologia 170:695–707.

Holeski LM, G Jander, AA Agrawal 2012a Transgenerational defense induction and epigenetic inheritance in plants. Trends Ecol Evol 27:618–626.

Holeski LM, MJC Kearsley, TG Whitham 2009 Separating ontogenetic and environmental variation in resistance to herbivory in cottonwood. Ecol 90:2969–2973.

Holzinger K, M Zink 1992 Mediation of cardiac glycoside insensitivity in the monarch butterfly (*Danaus plexippus*): role of an amino acid substitution in the ouabain binding site of Na$^+$, K$^+$-ATPase. J Chem Ecol 22:1921–1937.

Hunter MD 2016 The phytochemical landscape: linking trophic interactions and nutrient dynamics. Monographs in Population Biology 56. Princeton University Press, Princeton, NJ.

Hunziker P, SK Lambertz, K Weber, C Crocoll, BA Halkier, A Schulz 2021 Herbivore feeding preference corroborates optimal defense theory for specialized metabolites within plants. Proc Natl Acad Sci USA 118:e2111977118.

Janzen DH 1980 When is it coevolution? Evolution 34:611–612.

——— 1985 On ecological fitting. Oikos 45:308–310.

Karban R, IT Baldwin 1997 Induced responses to herbivory. University of Chicago Press, Chicago.

Karban R, JS Thaler 1999 Plant phase change and resistance to herbivory. Ecol 80:510–517.

Kearsley MJ, TG Whitham 1989 Developmental changes in resistance to herbivory: implications for individuals and populations. Ecology 70:422–434.

——— 1997 The developmental stream of cottonwoods affects ramet growth and resistance to galling aphids. Ecology 79:178–191.

Keith RA, T Mitchell-Olds 2017 Testing the optimal defense hypothesis in nature: variation for glucosinolate profiles within plants. PLoS ONE 12:e0180971.

Kelly CA, MD Bowers 2016 Preference and performance of generalist and specialist herbivores on chemically defended host plants. Ecol Entomol 41:308–316.

Kessler A, IT Baldwin 2002 Plant responses to insect herbivory: the emerging molecular analysis. Annu Rev Plant Biol 53:299–328.

Konno K, C Hirayama, H Yasui, M Nakamura 1999 Enzymatic activation of oleuropein: a protein crosslinker used as a chemical defense in the privet tree. Proc Natl Acad Sci USA 96:9159–9164.

Koricheva J, KE Barton 2012 Temporal changes in plant secondary metabolite production. Pages 34–55 in GR Iason, M Dicke, SE Hartley, eds. The ecology of plant secondary metabolites: from genes to global processes. Ecological Reviews. Cambridge University Press, Cambridge.

Kursar TA, KG Dexter, J Lokvam, RT Pennington, JE Richardson, MG Weber, et al 2009 The evolution of antiherbivore defenses and their contribution to species coexistence in the tropical tree genus *Inga*. Proc Natl Acad Sci USA 106:18073–18078.

Kviesis J, I Kļimenkovs, L Arbidans, A Podjava, M Kļaviņš, E Liepiņš 2019 Evaluation of furanocoumarins from seeds of the wild parsnip (*Pastinaca sativa* L. sl). J Chromatogr B 1105:54–66.

Lankau RA 2007 Specialist and generalist herbivores exert opposing selection on a chemical defense. New Phytol 175:176–184.

Lefcheck JS, MA Whalen, TM Davenport, JP Stone, JE Duffy 2013 Physiological effects of diet mixing on consumer fitness: a meta-analysis. Ecology 94:565–572.

Li X, MR Berenbaum, MA Schuler 2002 Plant allelochemicals differentially regulate *Helicoverpa zea* cytochrome P450 genes. Insect Mol Biol 11:343–351.

Liscombe DK, BP MacLeod, N Loukanina, OI Nand, PJ Facchini 2005 Evidence for the monophyletic evolution of benzylisoquinoline alkaloid biosynthesis in angiosperms. Phytochemistry 66:1374–1393.

Loomis WE 1932 Growth-differentiation balance vs. carbohydrate-nitrogen ratio. Proc Am Soc Hortic Sci 29:240–245.

——— 1953 Growth and differentiation: an introduction and summary. Pages 1–17 in WE Loomis, ed. Growth and differentiation in plants. Iowa State College Press, Ames.

Loxdale HD, JA Harvey 2016 The "generalism" debate: misinterpreting the term in the empirical literature focusing on dietary breadth in insects. Biol J Linn Soc 119:265–282.

Lucas-Barbosa D, EH Poelman, Y Aartsma, TA Snoeren, JJ van Loon, M Dicke 2014 Caught between parasitoids and predators: survival of a specialist herbivore on leaves and flowers of mustard plants. J Chem Ecol 40:621–631.

Massad TJ, RM Fincher, AM Smilanich, L Dyer 2011 A quantitative evaluation of major plant defense hypotheses, nature versus nurture, and chemistry versus ants. Arthropod-Plant Interact 5:125–139.

McCall AC, RE Irwin 2006 Florivory: the intersection of pollination and herbivory. Ecol Lett 9:1351–1365.

McKey D 1974 Adaptive patterns in alkaloid physiology. Am Nat 108:305–320.

——— 1979 The distribution of secondary compounds within plants. Pages 55–134 in GA Rosenthal, DH Janzen, eds. Herbivores: their interaction with secondary plant metabolites. Academic Press, New York.

McNaughton SJ 1983 Compensatory plant growth as a response to herbivory. Oikos 40:329–336.

Mérillon JM, KG Ramawat, eds 2020 Co-evolution of secondary metabolites. Springer, Cham.

Mertens D, MF de Bobadilla, Q Rusman, J Bloem, JC Douma, E Poelman 2021 Predictability of biotic stress structures plant defence evolution. Trends Ecol Evol 36:444–456.

Mithöfer A, W Boland 2012 Plant defense against herbivores: chemical aspects. Annu Rev Plant Biol 63:431–450.

Mooney EH, RA Niesenbaum 2012 Population-specific responses to light influence herbivory in the understory shrub *Lindera benzoin*. Ecology 93:2683–2692.

Musser RO, SM Hum-Musser, H Eichenseer, M Peiffer, G Ervin, JB Murphy, GW Felton 2002 Caterpillar saliva beats plant defences. Nature 416:599–600.

Nascimento LBDS, M Tattini 2022 Beyond photoprotection: the multifarious roles of flavonoids in plant terrestrialization. Int J Mol Sci 23:5284.

Neilson EH, JQ Goodger, IE Woodrow, BL Møller 2013 Plant chemical defense: at what cost? Trends Plant Sci 18:250–258.

Nishida R 2002 Sequestration of defensive substances from plants by Lepidoptera. Annu Rev Entomol 47:57–92.

Novotny V, S Miller, L Baje, S Balagawi, Y Basset, L Cizek, K Craft, et al 2010 Guild-specific patterns of species richness and host specialization in plant-herbivore food webs from a tropical forest. J Anim Ecol 79:1193–1203.

Ohmiya S, K Saito, I Murakoshi 1995 Lupine alkaloids. Pages 1–114 in GA Cordell, ed. The alkaloids: chemistry and pharmacology. Vol 47. Academic Press, London.

Ohnmeiss TE, IT Baldwin 2000 Optimal defense theory predicts the ontogeny of an induced nicotine defense. Ecology 81:1765–83.

Palo RT 1984 Distribution of birch (Betula spp.), willow (Salix spp.), and poplar (Populus spp.) secondary metabolites and their potential role as chemical defense against herbivores. J Chem Ecol 10:499–520.

Pearse IS, E LoPresti, RN Schaeffer, WC Wetzel, KA Mooney, JG Ali, PJ Ode, MD Eubanks, JL Bronstein, MG Weber 2022 Generalising indirect defence and resistance of plants. Ecol Lett 23:1137–1152.

Pichersky E, E Lewinsohn 2011 Convergent evolution in plant specialized metabolism. Annu Rev Plant Biol 62:549–566.

Poelman EH, JJ Van Loon, NM Van Dam, LE Vet, M Dicke 2010 Herbivore-induced plant responses in Brassica oleracea prevail over effects of constitutive resistance and result in enhanced herbivore attack. Ecol Entomol 35:240–247.

Rane RV, AB Ghodke, AA Hoffmann, OR Edwards, TK Walsh, JG Oakeshott 2019 Detoxifying enzyme complements and host use phenotypes in 160 insect species. Curr Opin Insect Sci 31:131–138.

Ratzka A, H Vogel, DJ Kliebenstein, T Mitchell-Olds, J Kroymann 2002 Disarming the mustard oil bomb. Proc Natl Acad Sci USA 99:11223–11228.

Rhoades DF 1979 Evolution of plant chemical defense against herbivores. Pages 3–54 in GA Rosenthal, DH Janzen, eds. Herbivores: their interaction with secondary plant metabolites. Academic Press, New York.

Rhoades DF, RG Cates 1976 Toward a general theory of plant antiherbivore chemistry. Pages 168–213 in JW Wallace, RL Masell, eds. Biochemical interaction between plants and insects. Springer, Boston.

Richards LA, LA Dyer, ML Forister, CS Jeffrey 2015 Phytochemical diversity drives plant-insect community diversity. Proc Natl Acad Sci USA 112:10973–10978.

Richards LA, AE Glassmire, KM Ochsenrider, AM Smilanich, CD Dodson, CS Jeffrey, et al 2016 Phytochemical diversity and synergistic effects on herbivores. Phytochem Rev 15:1153–1166.

Richards LA, EC Lampert, MD Bowers, CD Dodson, AM Smilanich, LA Dyer 2012 Synergistic effects of iridoid glycosides on the survival, development and immune response of a specialist caterpillar, Junonia coenia (Nymphalidae). J Chem Ecol 38:1276–1284.

Robert CA, N Veyrat, G Glauser, G Marti, GR Doyen, N Villard, MD Gaillard, et al 2012 A specialist root herbivore exploits defensive metabolites to locate nutritious tissues. Ecol Lett 15:55–64.

Roslin T, JP Salminen 2008 Specialization pays off: contrasting effects of two types of tannins on oak specialist and generalist moth species. Oikos 117:1560–1568.

Rotter MC, JJ Couture, EM Rothwell, J Garcia, LM Holeski 2018 Evolutionary ecology of plant resistance traits across the herbivore diet spectrum: a test in the model plant Mimulus guttatus. Evol Ecol Res 19:423–440.

Self LS, FE Guthrie, E Hodgson 1964 Metabolism of nicotine by tobacco-feeding insects. Nature 204:300–301.

Simberloff D, T Dayan 1991 The guild concept and the structure of ecological communities. Annu Rev Ecol Syst 22:115–143.

Singer MS, EA Bernays 2009 Specialized generalists: behavioral and evolutionary ecology of polyphagous woolly bear caterpillars. Pages 83–101 in WE Conner, ed. Tiger moths and woolly bears: behavior, ecology, and evolution of the Arctiidae. Oxford University Press, Oxford.

Smilanich AM, RM Fincher, LA Dyer 2016 Does plant apparency matter? thirty years of data provide limited support but reveal clear patterns of the effects of plant chemistry on herbivores. New Phytol 210:1044–1057.

Stamp N 2001 Enemy-free space via host plant chemistry and dispersion: assessing the influence of tri-trophic interactions. Oecologia 128:153–163.

——— 2003 Out of the quagmire of plant defense hypotheses. Q Rev Biol 78:23–55.

Stevenson PC, SW Nicolson, GA Wright 2017 Plant secondary metabolites in nectar: impacts on pollinators and ecological functions. Funct Ecol 31:65–75.

Strauss SY, NI Cacho, MW Schwartz, AC Schwartz, KC Burns 2015 Apparency revisited. Entomol Exp Appl 157:74–85.

Strauss SY, JA Rudgers, JA Lau, RE Irwin 2002 Direct and ecological costs of resistance to herbivory. Trends Ecol Evol 17:278–285.

Strauss SY, H Sahli, JK Conner 2005 Toward a more trait-centered approach to diffuse (co) evolution. New Phytol 165:81–90.

Thaler JS 1999 Jasmonate-inducible plant defences cause increased parasitism of herbivores. Nature 399:686–688.

Theis N, M Lerdau 2003 The evolution of function in plant secondary metabolites. Int J Plant Sci 164(suppl):S93–S102. https://doi.org/10.1086/374190.

Thompson JN 1994 The coevolutionary process. University of Chicago Press, Chicago.

Tuomi J, P Niemelä, FS Chapin III, JP Bryant, S Sirén 1988 Defensive responses of trees in relation to their carbon/nutrient balance. Pages 57–72 in WJ Mattson, ed. Mechanisms of woody plant defenses against insects: search for pattern. Springer, New York.

Turlings TC, JH Loughrin, PJ Mccall, US Röse, WJ Lewis, J Tumlinson 1995 How caterpillar-damaged plants protect themselves by attracting parasitic wasps. Proc Natl Acad Sci USA 92:4169–4174.

Underwood N 1999 The influence of induced plant resistance on herbivore population dynamics. Pages 211–230 in AA Agrawal, S Tuzun, E Bent, eds. Inducible plant defenses against pathogens and herbivores: biochemistry, ecology, and agriculture. American Phytopathological Society, St. Paul, MN.

Vannette RL, MD Hunter, S Rasmann 2013 Arbuscular mycorrhizal fungi alter above- and below-ground chemical defense expression differentially among Asclepias species. Front Plant Sci 4:361.

Walling LL 2000 The myriad plant responses to herbivores. J Plant Growth Regul 19:195–216.

Wetzel WC, SR Whitehead 2020 The many dimensions of phytochemical diversity: linking theory to practice. Ecol Lett 23:16–32.

Whitehead SR, E Bass, A Corrigan, A Kessler, K Poveda 2021 Interaction diversity explains the maintenance of phytochemical diversity. Ecol Lett 24:1205–1214.

Whitehead SR, K Poveda 2011 Herbivore-induced changes in fruit-frugivore interactions. J Ecol 99:964–969.

Wink M 1988 Plant breeding: importance of plant secondary metabolites for protection against pathogens and herbivores. Theor Appl Genet 75:225–233.

——— 2003 Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. Phytochemistry 64:3–19.

——— 2018 Plant secondary metabolites modulate insect behavior: steps toward addiction? Front Physiol 9:364.

Zangerl AR, FA Bazzaz 1992 Theory and pattern in plant defense allocation. Pages 363–391 in RS Fritz, EL Simms, ed. Plant resistance to herbivores and pathogens: ecology, evolution, and genetics. University of Chicago Press, Chicago.

Zangerl AR, JK Nitao 1998 Optimal defence, kin conflict and the distribution of furanocoumarins among offspring of wild parsnip. Evol Ecol 12:443–457.

Züst T, SR Strickler, AF Powell, ME Mabry, H An, M Mirzaei, T York, et al 2020 Independent evolution of ancestral and novel defenses in a genus of toxic plants (Erysimum, Brassicaceae). Elife 9:e51712.