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Specificity and breadth of plant specialized metabolite–microbe interactions

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Abstract

Plant specialized metabolites shape plant interactions with the environment including plant–microbe interactions. While we often group compounds into generic classes, it is the precise structure of a compound that creates a specific role in plant–microbe or–pathogen interactions. Critically, the structure guides definitive targets in individual interactions, yet single compounds are not limited to singular mechanistic targets allowing them to influence interactions across broad ranges of attackers, from bacteria to fungi to animals. Further, the direction of the effect can be altered by counter evolution within the interacting organism leading to single compounds being both beneficial and detrimental. Thus, the benefit of a single compound to a host needs to be assessed by measuring the net benefit across all interactions while in each specific interaction. Factoring this complexity for single compounds in plant–microbe interactions with the massive expansion in our identification of specialized metabolite pathways means that we need systematic studies to classify the full breadth of activities. Only with this full biological knowledge we can develop mechanistic, ecological, and evolutionary models to understand how plant specialized metabolites fully influence plant–microbe and plant–biotic interactions more broadly.

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Abbreviations

DIMBOA, 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one; HDMBOA, 2-hydroxy-4,7-methoxy-1,4-benzoxazin-3-one.

Introduction

Plants rely on specialized metabolites to exist in the complex ever-changing environment. These compounds provide a myriad of functions including resistance to biotic attackers, tolerance to abiotic stress, physical strength, etc. [1,2]. One prominently ascribed function to plant specialized metabolites is their role in modulating plant interactions with insect herbivores (e.g. the study by Li et al. [3]). These plant metabolite–insect interactions are often been modeled as a co-evolutionary arms race model similar to the plant-microbial zigzag model that is marked by plant adaptation and ensuing attacker counter-adaptation [4,5]. However, in contrast to plant–insect interactions, the role of plant specialized metabolites in shaping the evolution of plant–pathogen interactions specifically and plant–microbial interactions more generally is relatively less studied. In this review, I will work to convey some new developments and concepts on how plant specialized metabolites are shaped by and may shape microbial interactions with an eye to how this may influence our interpretation of plant–microbe evolution. Examples from plant–insect interactions will also be used to support and illustrate how these plant–microbial examples may be reflective of more general properties of plant specialized metabolites.

Activity is specific compounds not generic compound classes

Plant specialized metabolism is defined by a pathway architecture where a common core structure is formed and then elaborated through side-chain or structural modification enzymes. This modularity allows the efficient creation of diverse metabolites from a single core structure. In biotic or abiotic roles of plant specialized metabolites studies, we use the core structure as a guide to simplify this complexity by ascribing functions to the class of compounds rather than specific compounds, e.g. "glucosinolates are anti-herbivory compounds." Focusing on metabolic groups creates circularity in the literature where the group is solely discussed rather than specific chemicals eventually leading to solely reporting the accumulation of the compound group rather than the individual structures creating the activity. While this may be a useful simplification for our generic understanding of plant defense metabolism, there is minimal support for this being a useful biological framing of plant defense

metabolism. In contrast, there is growing support that the structural modifications upon the core create a diversity of biological activities that mirrors the chemical diversity.

An example of this specific compound/specific activity link is in the convergent biological activities within indolic plant specialized metabolites. Tryptophan derived indolic moieties are at the core of a diverse array of plant specialized metabolites including benzoxazinoids and indolic glucosinolates. Within this family of compounds, the specific structural modification to the indole moiety dramatically shifts the compound's function. For instance, O-methylation of indole glucosinolates shifts the compound from having a role in aphid defense to being the key component in non-host microbial resistance and an inducer of callose formation [6,7]. Similarly, in the monocot benzoxazinoids, O-methylation from DIMBOA-Glc to HDMBOA-Glc changes the functionality of the metabolite. The DIMBOA-Glc induces callose defenses that can function against microbial attackers, while O-methylation to HDMBOA-Glc shifts the ability to resist aphids while losing the callose inducing activity [3]. It remains elusive if the defensive and signaling properties linked to this convergent O-methylation switch have a shared mechanistic basis.

In addition to the shift of function from aphid to microbial defense, chemical modifications on specialized metabolites can influence functions even more specifically. Changing the chain length of an aliphatic glucosinolate influences the effect on both aphids and fungi, with four carbon glucosinolates providing relative resistance to the *Brevicoryne brassicae*, three carbon glucosinolate providing resistance to *Lipaphis erysimi*, and longer chains shifting to antifungal activities [8,9]. It is possible that the anti-aphid functions of these compounds are due to its action against the aphid's bacterial symbiont or microbiome. Side-chain modifications can differentially alter virulence even within individual pathogens. Single carbon or oxygen modifications to a core terpene differentially modulated *Phytophthora capsici* virulence by altering effector regulation indicating that the terpenes likely have different mechanistic targets [10]. These are but a sampling of the studies showing that apparently subtle chemical changes create dramatic shifts in biotic activity [11,12]. Further, it also illustrates how we must assume that specialized metabolites have the potential for diverse roles within an individual plant when confronting the breadth of attackers in the environment.

Diverse specialized metabolites function as two-component defense systems where the intact specialized metabolite is activated by a specific enzyme, typically a glycosyl hydrolase. Classical two-component defenses are glucosinolates and cyanogenic glycosides with recent studies identifying benzoxazinoids, triterpene saponins, and other glycosylated plant specialized metabolites as convergent members of this two-component model [13–15]. The compound and enzyme are typically stored in separate cellular compartments and come together upon tissue disruption leading to a common assumption that they are anti-herbivory compounds. However, they are also key components of anti-microbial defense as both aliphatic and indolic glucosinolates play major roles in *Arabidopsis* resistance to non-host bacterial and fungal pathogens [7,16,17]. In contrast to their anti-herbivory activity, the two-component anti-microbial role does not appear to require tissue disruption. The indole glucosinolates utilize the PEN system that provides non-host resistance to pathogens. In this system, the site of pathogen attack is targeted by active transport of the indolic glucosinolate and vesicle mediated secretion of the activating myrosinase bringing the two-components together in the apoplast [18,19]. The methionine derived aliphatic glucosinolates also contribute to non-host resistance as with non-adapted *P. syringae* but it is unclear how this occurs. Recent studies show there may be a constant pool of apoplastic aliphatic glucosinolates allowing a steady-state presence of the activated isothiocyanate [20,21]. Thus, it is possible that there is a constant level of activated glucosinolates in the apoplast either due to a plant myrosinase or potentially a microbial myrosinase [22].

Two-component systems can also create chemical diversity in addition to their storage capacity for toxic compounds. For example, there are accessory proteins that can shift the glucosinolate reactions towards nitriles, thiocyanates, and epithionitriles instead of isothiocyanates, and cyanogenic glucoside reactions to nitriles instead of cyanide [23–26]. In glucosinolates, this structural shift is predominantly considered to change the function from the isothiocyanate as a direct herbivory deterrent to an indirect nitrile-based attractant for predatory and parasitic wasps [27,28]. However, this catabolic shift likely influences plant–microbe interactions where the isothiocyanate directly inhibits bacterial type III secretion systems by covalently modifying a cysteine [29]. While conversion to nitrile abolishes the isothiocyanate modification of cysteines, a nitrile moiety can create a different covalent modification of both cysteine and serine residues but direct targets have not been assayed [30]. Similarly, a shift in the catabolic product could influence the general composition of the microbiome by altering the efficiency of different microbial nutrient acquisition pathways. A more detailed systematic survey of how individual plant specialized metabolites influence plant–microbe interactions at the species level to the entire microbiome is needed to understand how the evolution of new plant defense chemicals influences their biotic interactions.

Specific compounds: broad interactions

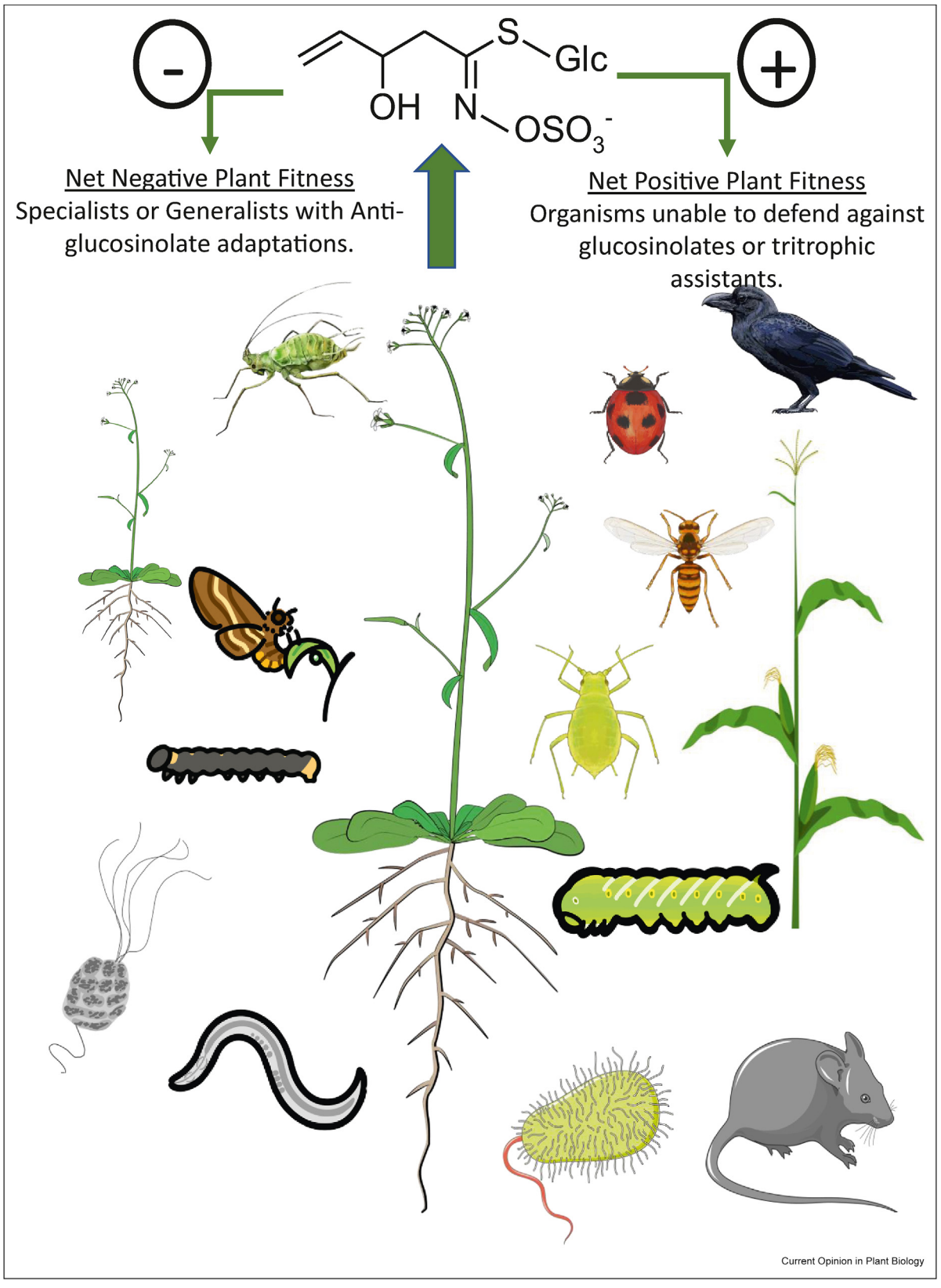
Plant co-evolution with microbial attackers is often considered on a pairwise or limited species basis where the host and pathogen evolve in response and subsequent counter-response, frequently involving specific protein–protein interactions [31]. This constrained model, while being helpful at mechanistic insights, does have significant limitations in a broader environmental context [32]. One complication for co-evolutionary models is that plants interact with an unknown but massive diversity of biotic attackers. For the majority of natural systems, it is not clear how often a singular interaction is a dominant sustained driver of both the plant and attacker's fitness. If plant specialized metabolites had highly specific interactions, this would alleviate this complication as the metabolite would be the focus of the specific interaction. This would be similar to effector mediated resistance where the pathogen produces a specific protein that is recognized by a specific host protein creating a molecular focus for the interaction. However, the full breadth of plant specialized metabolite literature argues against this specificity and suggests that individual plant specialized metabolites influence broad swaths of a plant's biotic interactions.

An example of this potential for a compound to influence diverse interactions is found in the reliance of *Brassicas* on glucosinolates, both individual compounds and the class, to modulate the interaction with nearly every potential biotic interaction. Glucosinolates have long been known to be critical determinants of anti-insect defenses ranging from lepidopteran, aphid, mites, and nearly everything in between [33]. This breadth of activity includes individual glucosinolates like 2-hydroxy-but-3-enyl glucosinolate that directly impacts mammalian, avian, and insect interactions [11,34]. In this example, the mammalian and avian interactions are altered by inhibiting thyroid function but the mechanistic effect on insects is unknown. 3-hydroxypropyl glucosinolate has the ability to alter growth in at least plants and fungi by inhibiting the TOR pathway, although a in planta role in direct biotic interactions has yet to be tested [35]. Similarly, numerous glucosinolates have been linked to resistance against multiple eukaryotic and prokaryotic pathogens with a diversity of potential mechanisms [16,29,36–42]. Finally, allyl glucosinolate mediates both within and between species plant competition by an unknown mechanism(s) [43]. These activities are due to a blend of direct activity against the pathogen and signaling effects altering the plant's own defense response that are dependent on the side chain structure and not explained by the common isothiocyanate formation potential [7,35,39,44]. This suggests that some individual metabolites may influence different biotic attackers sometimes via disparate mechanisms that are lineage specific in the attacker and at other times the mechanism may be conserved across attackers.

Broad arrays of targets occur beyond the glucosinolates and may be a ubiquitous property of key plant specialized metabolites. For example, the benzoxazinoids in monocots influence aphid resistance, mite resistance, callose deposition by the plant, iron chelation, and insect attractancy [3,45,46]. Benzoxazinoid content and structure also leads to specific shifts in microbiome membership indicating potential antimicrobial roles [47,48]. Camalexin is an Arabidopsidae (*Arabidopsis thaliana* containing tribe) specific defense metabolite that illustrates the complexity in assigning biological activities. Classically, camalexin was thought to predominately control resistance to generalist fungal pathogens [49,50]. This has extended to camalexin influencing plant bacterial interactions involving both pathogens and growth promoting members of the rhizosphere [51]. The rhizosphere studies illustrate a key difficulty in conducting cause and effect studies in plant specialized metabolites, as the rhizosphere effects are caused by shoot produced camalexin that is transported to the root [52]. This tissue malleability of synthesis and effect was solved using genetic mutants, illustrating the critical need for biosynthetic mutants to identify the array of biotic or abiotic effects caused by even a single specialized metabolite.

If broad activities are a general hallmark of specialized metabolites, this could change co-evolutionary models. Instead of modeling effects on one host and one attacker, we should shift to model the fitness derived from a specialized metabolite as a vector of effects across all possible interactors multiplied by the matrix containing all possible environments in which that organism would exist. Further, this suggests that plant specialized metabolites can create evolutionary/ecological/mechanistic linkages between plant–insect and plant–pathogen interactions (Figure 1). If the plant uses a specialized metabolite for both interactions, then the plant cannot evolve the different interactions independently. While this shift to modeling matrices appears intractable, the identification of metabolic pathways in combination with plant genome engineering creates the ability to develop defined plant genotypes that differ in only the production of specific defense compounds. This would create a defined genotypic matrix that could be used in a community phenotyping effort blending lab bioassays against all feasible biotic and abiotic stresses in combination with field trials that would begin to develop the matrix of estimated effects. Comparing the lab biotic/abiotic measures of these compounds' effects with the estimated field fitness of the same genotypic matrix would enable us to test if this comparison is feasible and if so,

Figure 1



Diversity of interactions mediated by plant specialized metabolites that influence plant–pathogen interactions.
The figure is a cartoon representation of the diversity of positive and negative interactions linked to an individual compound from a single plant species to illustrate the complexity of estimating the fitness or role of a single plant specialized metabolite. Any resemblance to exact species is purely coincidental and not an intended specific example.

what parts of the matrix are more or less important. While not easy, it is feasible with combined effort.

Specific plant compound attack: general microbial countermove?

By shaping the plant's microbial interactions, plant specialized metabolites also alter the microbes' evolutionary trajectory. This counter-evolutionary impact is well known in plant-insect interactions where insects adapt to plant specialized metabolites. This occurs by changing the protein/gene targeted by the metabolite or by evolving new mechanisms to detoxify the metabolite. For example, insects specialized to cardenolide containing plants typically evolved tolerance mutations in the NA+/K + -ATPase that is the target of the cardenolide toxin [53]. Alternatively, insects that specialize on glucosinolate containing plants evolved detoxifying enzymes [54,55].

Recent efforts are showing that pathogen/microbial resistance to plant specialized metabolites is also a broad feature of plant−microbe interactions in both bacteria and fungi. Further, pathogen resistance to compounds may play a central role in determining host/non-host relationships [16,56,57]. These resistance mechanisms are frequently polymorphic within a pathogen species and can alter host range [16]. Critically, resistance mechanisms are not constrained to specialist pathogens, as generalist pathogens have resistance mechanisms that target phylogenetically limited plant specialized metabolites. For example, the generalist pathogens, *Sclerotinia sclerotiorum* and *Botrytis cinerea* have distinct processes to detoxify glucosinolate produced isothiocyanates. *S. sclerotiorum* hydrolyzes the compound while *B. cinerea* transports the compound out of the cell. Generalist pathogens may rely on multiple resistance mechanisms for individual compounds, as *B. cinerea* has multiple independent enzymes and transporters to detoxify the phylogenetically limited camalexin [40,58−61].

While these detoxification genes are critical for the microbe's ability to invade the host, it is unlikely that limited microbial genomes would contain narrow and specific detoxification mechanisms against all possible compounds. This suggests that these detoxification mechanisms may work against a broader array of compounds that share structural similarity, e.g. camalexin enzymes may work more broadly against any indole containing compound. An alternative model is that there is an existing body of genes within the microbiome metagenome that can provide resistance to nearly any plant metabolite. This creates a standing pool of genes allowing microbes that undergo a host shift to adapt to the new host's metabolites via horizontal gene transfer [62]. Understanding how plant pathogens adapt to plant specialized metabolites will be a key question going forward for both synthetic biology efforts to move compounds from one plant species to another and efforts to understand the processes involved in pathogen movement to new hosts.

Conclusion

Plant specialized metabolites play a wide array of highly intertwined roles, even within a single metabolite. The cited and numerous studies not cited due to space limitations are just beginning to reveal how these metabolites evolve within the plant and shape microbial interactions. The numbers of these citations are vastly outweighed by the studies on plant-microbe evolution that focus on effector−receptor interactions even though it is likely the metabolites provide the critical resistance output controlled by upstream signals. As such, we honestly don't know the relative level of plant−microbial interactions/evolution in the wild that are shaped predominantly by effector−receptor interactions versus plant specialized metabolism. This is begging to be addressed by the vast explosion in the rate of pathway identification for plant specialized metabolites, although there is a relative lack of studies on biological roles or mechanistic targets for these metabolites. To address how plant specialized metabolites shape plant−microbial interactions, we need to begin systematic surveys of all the biological effects of at least some model specialized metabolites in the lab and field to understand their true influence on plant−microbial interactions and plant−biotic interactions more broadly with an ultimate goal of describing their full contribution to plant fitness in the wild.

Author contribution

DJK wrote the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

Data availability

No data was used for the research described in the article.

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