Convergent evolution of plant specialized 1,4-naphthoquinones: metabolism, trafficking, and resistance to their allelopathic effects

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Abstract

Plant 1,4-naphthoquinones encompass a class of specialized metabolites known to mediate numerous plant–biotic interactions. This class of compounds also presents a remarkable case of convergent evolution. The 1,4-naphthoquinones are synthesized by species belonging to nearly 20 disparate orders spread throughout vascular plants, and their production occurs via one of four known biochemically distinct pathways. Recent developments from large-scale biology and genetic studies corroborate the existence of multiple pathways to synthesize plant 1,4-naphthoquinones and indicate that extraordinary events of metabolic innovation and links to respiratory and photosynthetic quinone metabolism probably contributed to their independent evolution. Moreover, because many 1,4-naphthoquinones are excreted into the rhizosphere and they are highly reactive in biological systems, plants that synthesize these compounds also needed to independently evolve strategies to deploy them and to resist their effects. In this review, we highlight new progress made in understanding specialized 1,4-naphthoquinone biosynthesis and trafficking with a focus on how these discoveries have shed light on the convergent evolution and diversification of this class of compounds in plants. We also discuss how emerging themes in metabolism-based herbicide resistance may provide clues to mechanisms plants employ to tolerate allelopathic 1,4-naphthoquinones.

Keywords: Allelopathy, convergent evolution, juglone, 1,4-naphthoquinone, shikonin, specialized metabolism.

Introduction

Plants produce an amazing diversity of specialized metabolites playing roles in adapting to ecological niches. Many of the studied compounds fall within the ‘major’ chemical classes of specialized metabolites: the phenylpropanoids/benzenoids (Vogt, 2010; Widhalm and Dudareva, 2015), terpenoids (Nagegowda and Gupta, 2020), and alkaloids (Ziegler and Facchini, 2008). With the explosion of functional and comparative genomics and advances in metabolomics, however, there has been major progress in understanding the evolution of lesser studied chemical classes across plant taxa (Smith et al., 2019). One such class is the specialized 1,4-naphthoquinones, which has gained interest due to the potential of its members...
to serve as novel therapeutics (Duru et al., 2014), drug scaffolds (F. Wang et al., 2019), and leads for natural product-based herbicides (Durán et al., 2018).

The 1,4-naphthoquinones are a diverse group of compounds structurally related to naphthalene. The core 1,4-naphthoquinone skeleton is comprised of a benzene ring fused to a fully conjugated cyclic diketone with para-oriented carbonyl groups (Fig. 1A). All plants synthesize phylloquinone (vitamin K₁), a methylated and phytylated 1,4-naphthoquinone (Fig. 1B) that functions as a one-electron transporter at the A₁ site of PSI and as a two-electron acceptor from a protein disulfide isomerase involved in folding plastidial proteins (van Oostende et al., 2011). While phylloquinone is anchored to thylakoid and plastoglobule membranes by a liposoluble side chain, the hundreds of known specialized 1,4-naphthoquinones collectively produced by plants are untethered, often deployed in the environment, and mediate various plant–microbial, plant–fungal, and plant–insect interactions, as well as plant–plant competition (allelopathy) (Widhalm and Rhodes, 2016). New evidence also implicates them in responding to abiotic stresses, such as iron deficiency (Rajniak et al., 2018).

The bioactivities of 1,4-naphthoquinones are derived from their chemically reactive nature in vivo. Unlike higher redox potential quinones, 1,4-naphthoquinones in partial or fully reduced forms spontaneously oxidize in the presence of oxygen. The reactive oxygen species (ROS) generated from this autooxidation oxidatively modify lipids and proteins, causing immediate damage to cells, or lead to production of more damaging free radicals that cause DNA mutations. Depending on the functional groups attached to the naphthoquinone ring, the oxidized quinone itself can form adducts with reduced glutathione (GSH), proteins, and DNA, leading to thiol depletion or macromolecule damage (Klotz et al., 2014). Most prokaryotes today still solely or conditionally rely on 1,4-naphthoquinones (menaquinones; vitamin K₂) in their anaerobic respiratory electron transport chains (Schoepp-Cothenet et al., 2013). The propensity of 1,4-naphthoquinones to react with oxygen, however, probably drove evolution from ancestral menaquinones to higher redox potential quinones in the bioenergetic chains of some prokaryote lineages following the ‘Great Oxidation Event’ 2.5 billion years ago (Hohmann-Marriott and Blankenship, 2011), including in the progenitors of modern-day mitochondria and chloroplasts (Schoepp-Cothenet et al., 2013). As a result, the present macroscopic biosphere is dominated by Eukaryotes relying on ubiquinone for aerobic respiration and/or plastoquinone for oxygenic photosynthesis to generate ATP (Bergdoll et al., 2016). Despite the shift to high redox potential quinones, many species throughout the tree of life, and in the plant kingdom in particular, appear to have convergently evolved to produce specialized 1,4-naphthoquinones that function as novel ‘chemical weapons’ in an oxygenic environment.

Convergent evolution in plant metabolism is surprisingly common (Pichersky and Lewinsohn, 2011). There are numerous examples of plants—mostly Angiosperms—belonging to different lineages that independently evolved to produce the same compound (e.g. caffeine; Huang et al., 2016) or structurally similar compounds (e.g. stilbenes; Troph et al., 1994), or of plants in disparate lineages that synthesize different compounds to fulfill the same function (e.g. floral volatiles; Dudareva et al., 2013). With recent progress in understanding plant 1,4-naphthoquinone distribution, metabolism, and function, it is becoming evident that the ability to synthesize these...
Convergent evolution of plant specialized 1,4-naphthoquinones has independently evolved several times and was facilitated by extraordinary events of metabolic innovation and/or metabolic links to respiratory and photosynthetic quinone metabolism (McCoy et al., 2018; Auber et al., 2020; Ueoka et al., 2020). In this review, we highlight advances in understanding specialized 1,4-naphthoquinone biosynthesis,
trafficking, and resistance strategies with emphasis on how these discoveries have shed light on the convergent evolution and diversification of this class of compounds in plants.

**Convergent evolution of plant 1,4-naphthoquinone biosynthesis**

*Specialized 1,4-naphthoquinones are distributed across multiple discrete plant taxa*

Plotting the distribution of detected 1,4-naphthoquinones (excluding phylloquinone) in plants on the phylogenetic reconstruction of plant metabolism provided by the Angiosperm Phylogeny Group shows that the ability to synthesize these compounds is scattered across multiple lineages of vascular plants (Fig. 2). While they appear to be predominantly produced by dicots, specialized 1,4-naphthoquinones are reported in monocots, magnoliids, and ferns. *Lygodium japonicum*, also known as ‘Japanese climbing fern’, is a true fern native to Asia that was introduced into other parts of the world as an ornamental, later escaped, and is today regarded as a pest in many non-native habitats (‘Lygodium japonicum (Thunb.) Sw’, 2019). Investigation into the chemical constituents of *Lygodium* roots, which are used in traditional Chinese medicine, revealed the presence of a new compound, 6-hydroxy-2-isopropyl-7-methyl-1,4-naphthoquinone (Chen et al., 2010) (Fig. 2). Similarly, two new 1,4-naphthoquinones, goniothalaminone A and B, were isolated from the roots of *Goniothalamus sorranchinii* (Prawat et al., 2012), a medicinal plant native to Thailand that is a member of the Magnoliales order (Fig. 2). The monocotyledonous species, *Aristea ecklonii* (Asparagales), or ‘blue-eyed iris’, is native to parts of central and southern Africa and produces plumbagin (Fig. 2) in its rhizomes (Kumar et al., 1985). Plumbagin is also produced by dicots such as *Diospyros* species in the Ebenaceae family (Ericales) as well as in members of several families in the Caryophyllales, including carnivorous plant families (*Drosophyllaceae*, *Nepenthaceae*, and *Droseraceae*) and non-carnivorous families such as the Plumbaginaceae (Hook et al., 2014). Plumbagin is not the only structurally identical 1,4-naphthoquinone detected in species belonging to distantly separated orders. Lawsons (Fig. 2), the compound responsible for the orange dying properties of henna (* Lawsonia inermis*, Myrtales), was also recently reported in root exudates of opium poppy (*Papaver somniferum*, Ranunculales) (Rajniak et al., 2018). Thus, plumbagin and lawsons are obvious examples of convergently evolved 1,4-naphthoquinones. The wide dispersal of structurally diverse 1,4-naphthoquinones (Fig. 2) and their apparent absence in intervening taxa further suggests that 1,4-naphthoquinone metabolism independently evolved multiple times in plants. However, when determining convergent evolution in plant metabolism, it is important to remember that ‘absence of evidence is not evidence of absence’ (Pichersky and Lewinsohn, 2011). It is possible that 1,4-naphthoquinones are more ubiquitously present in plants than currently understood but at levels below detection or in unexamined tissues, developmental stages, or environmental conditions.

*Plant 1,4-naphthoquinones are biosynthesized via several different pathways*

Support for multiple events of convergent evolution becomes stronger when considering that several pathways starting from different metabolic precursors and involving different intermediates and enzymes have been implicated in plant 1,4-naphthoquinone biosynthesis. A review by Widhalm and Rhodes (2016) previously detailed numerous biochemical and tracer studies showing that specialized 1,4-naphthoquinones are synthesized via one of at least four different metabolic routes (Fig. 1B). Since then, several studies have further corroborated the existence of different 1,4-naphthoquinone pathways across plants and provided insight into their metabolic evolution (McCoy et al., 2018; Rajniak et al., 2018; S. Wang et al., 2019; Auber et al., 2020; Song et al., 2020; Ueoka et al., 2020) (Box 1). Moreover, genome assemblies from 1,4-naphthoquinone-producing species also revealed potential glimpses into the metabolic innovation that facilitated 1,4-naphthoquinone pathway evolution. De novo assembly of the red gromwell (*Lithospermum erythrorhizon*) and *Echium plantagineum* genomes indicated that retrotransposition-derived duplication (Auber et al., 2020) and whole-genome duplication (Tang et al., 2020) within the Boraginaceae contributed to establishing the shikonin/alkannin pathway. Reported genome assemblies from English walnut (*Juglans regia*) (Martínez-Garcia et al., 2016), *J. regia* × *J. microcarpa* hybrid (Zhu et al., 2019), and several other *Juglans* species (Stevens et al., 2018) underscored the importance of whole-genome duplication (Luo et al., 2015) in driving metabolic diversification in the Juglandaceae. These genome assemblies are also expected to serve as new resources for gene discovery in 1,4-naphthoquinone metabolism. In the next section, we explore newly uncovered links between 1,4-naphthoquinones and the metabolism of photosynthetic and respiratory quinones that perhaps contributed to the convergent evolution of specialized 1,4-naphthoquinone biosynthesis.

*Metabolic connections to primary quinone metabolism*

One way in which new specialized metabolic pathways evolve is when a newly acquired enzyme converts an intermediate from an existing pathway into a novel intermediate or end product (Pichersky and Gang, 2000). If that intermediate is ubiquitously present in plants and requires minimal modification to produce a biochemically active compound, it may lead to repeated evolution of functionally and/or structurally similar compounds. Recent studies point to the repeated evolution of specialized 1,4-naphthoquinones from 1,4-dihydroxynaphthoic acid.
Convergent evolution of plant specialized 1,4-naphthoquinones

(DHNA), an intermediate in the biosynthesis of phyto-
quinone (Fig. 1B). McCoy et al. (2018) demonstrated that the naphthalenoid moiety of the allelochemical juglone in black walnut (J. nigra) originates from DHNA made via the phyllo-
quinone pathway. The recent report of carboxylated lawsone (3-hydroxy-DHNA) in opium poppy root exudates suggests that like juglone in black walnut (Fagales, Fig. 2), lawsone in opium poppy (Ranunculales, Fig. 2) is synthesized from DHNA. Lawsone is also the 1,4-naphthoquinone responsible for the orange dyeing properties of henna (Lawsonia inermis, Myrtales) and was shown through classic tracer studies in Impatiens species (Ericales) to be synthesized via DHNA (see text for further details).

Box 1. Key developments in understanding metabolic innovation in the evolution of specialized plant 1,4-naphthoquinone metabolism

- Retrotransposition-derived duplication contributed to evolution of the shikonin pathway
  De novo assembly of the red gromwell (Lithospermum erythrorhizon) genome and phylogenetic analysis by Auber et al. (2020) showed that PGT genes arose in a common ancestor of modern shikonin/alkannin-producing Boraginaceae species via a retrotransposition-derived duplication event and subsequent neofunctionalization of an ancestral prenyltransferase gene.
  
- The shikonin pathway relies on GPP derived from the MVA pathway via a unique cytosolic GPPS
  Ueoka et al. (2020) showed that a histidine residue adjacent to the first aspartate-rich motif in a novel cytoplasmic farnesyl diphosphate synthase (FPSS) is responsible for the evolved geranyl diphosphate synthase (GPPPS) activity in the enzyme recruited to provide geranyl diphosphate (GPP) precursor to the shikonin pathway.

- Members of the expanded CYP76B subfamily in the Boraginaceae function in the shikonin pathway
  S. Wang et al. (2019) showed that CYP76B74, which localizes to the endoplasmic reticulum membrane, catalyzes the hydroxylation of the shikonin pathway intermediate geranylhydroquinone in Arnebia euchroma. Song et al. (2020) more recently demonstrated that A. euchroma CYP76B100 is also capable of hydroxylating geranylhydroquinone, while CYP76B101 catalyzes the three-step oxidation of geranylhydroquinone to form a 3"-carboxylic acid derivative of geranylhydroquinone. The expansion of the CYP76B subfamily in the Boraginaceae thus appears connected to evolution of shikonin biosynthesis.

- Repeated evolution of 1,4-naphthoquinone pathways from an intermediate of the phylloquinone pathway
  McCoy et al. (2018) demonstrated that biosynthesis of juglone in black walnut (Juglans nigra) relies on the phylloquinone pathway intermediate 1,4-dihydroxynaphthoic acid (DHNA; Fig. 1B). While using untargeted metabolomics to investigate compounds produced by roots in response to iron deficiency, Rajniak et al. (2018) detected lawsone and carboxylated lawsone (3-hydroxy-DHNA) in the root exudates from opium poppy (Papaver somniferum). This suggests that like juglone in black walnut (Fagales, Fig. 2), lawsone in opium poppy (Ranunculales, Fig. 2) is synthesized from DHNA. Lawsone is also the 1,4-naphthoquinone responsible for the orange dyeing properties of henna (Lawsonia inermis, Myrtales) and was shown through classic tracer studies in Impatiens species (Ericales) to be synthesized via DHNA (see text for further details).

New research also points to a metabolic connection between shikonin and the vital respiratory cofactor ubi-
quinone. The shikonin and ubiquinone pathways both start with conjugation of \( p \)-hydroxybenzoate and a polyprenyl diphosphate. Like the ubiquinone pathway (Ducluzeau et al., 2012; Liu and Lu, 2016), the prenyl diphosphate precursor for shikonin biosynthesis, geranyl diphosphate (GPP), is synthesized using the five-carbon building blocks isopentenyl diphosphate (IPP) and dimethylallyl diphos-
phate (DMAPP) derived from the mevalonic acid (MVA) pathway (Schmid and Zenk, 1971; Gaisser and Heide, 1996). The origin of MVA-derived GPP for shikonin, however, has
remained enigmatic as GPP is usually produced in plants from the condensation of IPP and DMAPP derived from the methylerythritol phosphate (MEP) pathway via plastid-localized GPP synthases (GPPSs) (Vranová et al., 2013). In a breakthrough study by Ueoka et al. (2020), it was shown that GPP used to synthesize shikonin can be produced by a novel cytoplasmic farnesyl diphasphate synthase (FPPS) that independently evolved GPPS activity. This explains how IPP and DMAPP from the MVA pathway are used to produce GPP, and further validates that shikonin and ubiquinone are derived from the same isoprenoid precursor pool. Moreover, ubiquinone polyprenyltransferases (PPTs) and shikonin p-hydroxybenzoate:geranyltransferases (PGTs), which catalyze the conjugation of p-hydroxybenzoate with their prenyl di-phosphates, were found to belong to the same orthogroup (Auber et al., 2020). Subsequent phylogenetic analysis of prenyltransferases encoded in the L. erythrorhizon genome and in other shikonin- and non-shikonin-producing species, indicated that PGT genes arose in a common ancestor of modern shikonin/alkannin-producing Boraginaceae species via a retrotransposition-derived duplication event and subsequent neofunctionalization of an ancestral prenyltransferase gene (Auber et al., 2020). Whether the duplication was from a PPT or another related prenyltransferase is unclear. Given that there are other modifications to the p-hydroxybenzoate moiety that occur in both ubiquinone and shikonin biosynthesis and a priori require the action of similar enzymes, it is possible that there are additional evolutionary linkages between the two pathways (Auber et al., 2020). Like ubiquinone and shikonin, similar connections based on shared precursor pools and metabolic modifications link the synthesis of plastoquinone and the specialized 1,4-naphthoquinone chimaphilin (Fig. 1B) (Widhalm and Rhodes, 2016).

**Plants deploy specialized 1,4-naphthoquinones in different ways**

It is increasingly apparent that plants independently evolved diverse mechanisms to release 1,4-naphthoquinones into the rhizosphere. Looking at other plant metabolites, biosynthesis of volatiles, for example, typically occurs in epidermal cells and allows for direct emission into the environment (Widhalm et al., 2015). The same biological principle seems to apply in some cases for excreting 1,4-naphthoquinones. Expression of shikonin metabolic genes is higher and shikonin pool sizes are more abundant in L. erythrorhizon root periderm compared with vascular tissue (Auber et al., 2020). This permits direct access for release into the soil. Shikonins have also been found to be more abundant in the periderm of Echium species (Durán et al., 2017), as has juglone in the roots of black walnut trees (McCoy et al., 2018). At the subcellular level, multiple mechanisms are implicated in the trafficking of naphthoquinones to the apoplast. Confocal imaging and application of membrane trafficking inhibitors to L. erythrorhizon hairy roots suggests that shikonin can be secreted to the apoplast inside endoplasmic reticulum-derived vesicles via trafficking pathways similar to those involved in protein secretion (Tatsumi et al., 2016). At the same time, genetic evidence indicates that excretion of shikonins from L. erythrorhizon hairy roots also involves plasma membrane-localized ATP-binding cassette (ABC) transporters (Zhu et al., 2017a, b). In black walnut, it is proposed that arbuscular mycorrhizal fungal hyphae contribute to transporting juglone from roots into the rhizosphere (Achatz and Rillig, 2014; Achatz et al., 2014). Interestingly, new research suggests that some plants have evolved to use aerial organs to deploy 1,4-naphthoquinones into the soil. Block et al. (2019) found that lawsone and 2-methoxy-1,4-naphthoquinone are present in the nectar excreted from Impatiens glandulifera extra-floral nectaries, which could be the conduit by which the allelochemicals are leached into the soil by rain from above-ground tissues (Ruckli et al., 2014). In Plagiobothrys arizonicas, alkannin is visible in the margins and midvein of the abaxial leaf surface and is readily exuded when the leaves are crushed (Kelley, 2012). Hook and Sheridan (2020) showed that cultured plantlets of Streptocarpus dunnii excrete a mixture of allelopathic naphtho- and anthraquinones from their fibrous roots as well as from around their petiolodes and leaf bases, which bear glandular trichomes involved in synthesizing (±)-dunnione (Hook et al., 2018). Taken together, it appears that in addition to convergent evolution of 1,4-naphthoquinone biosynthesis, plants have also independently evolved different ways to deploy the compounds into the rhizosphere.

**Metabolism-based resistance to allelopathic 1,4-naphthoquinones in plants**

Just as plants independently evolved biosynthesis pathways for 1,4-naphthoquinones and strategies to deploy them, species producing allelopathic 1,4-naphthoquinones must have evolved mechanisms to resist their herbicidal effects. How this is accomplished is poorly understood for specialized 1,4-naphthoquinone metabolism, and allelopathy in general. One potential place to look for clues is in the numerous weed species that evolved resistances to synthetic herbicides (Heap, 2020). Seventy years of synthetic herbicide use has created strong selection pressure to drive evolution of target site resistance (TSR) and non-target site resistance (NTSR). A recent review by Gaines et al. (2020) provides an excellent update on the contributions of TSR, NTSR, and the combination of both to the evolutionary resilience of weed populations to herbicides.

Given the widespread chemical reactivity of 1,4-naphthoquinones in vivo, resistant plants might employ NTSR-like strategies, also known as metabolism-based resistance mechanisms (Hatzios, 2004). Catabolic detoxification of herbicides...
Convergent evolution of plant specialized 1,4-naphthoquinones via NTSR strategies in plants broadly mirrors elimination of xenobiotics or drugs in animals. The process occurs over multiple phases through the sequential action of enzymes from a handful of major classes (Riechers et al., 2010). Phase I reactions introduce or unmask reactive functional groups, such as hydroxyls, and often rely on cytochrome P450 enzymes. Phase II metabolism results in the addition of a larger polar group such as glutathione S-transferases (GSTs) or UDP-dependent glycosyl transferases (UGTs). If a sufficiently reactive and available functional group(s) is present on the compound, the detoxification process may not require a phase I enzyme. During phase III, the conjugated compound is shuttled and transported, often via ABC transporters, into the vacuole for storage and/or further degradation, or is incorporated into the cell wall (Gaines et al., 2020).

Similarly to other defensive compounds, such as glucosinolates (Halkier, 2016) and benzoxazinoids (Zhou et al., 2018), 1,4-naphthoquinones are often found stored in their glucosylated forms. In their reduced forms (naphthohydroquinones), the quinone ring of 1,4-naphthoquinones becomes aromatized while its carbonyl groups become hydroxyls available for conjugation with sugars. Juglans species, including black and English walnut (Müller and Leistner, 1978), and pecan (Carya illinoensis) (Hedin et al., 1980; Gueldner et al., 1994) produce hydrojuglone glucoside. Roots from I. glandulifera contain 1,2,4-trihydroxynaphthalene-1-O-glucoside, which is the glucosylated form of reduced lawsone (Tříška et al., 2013). Reduced and glucosylated forms of plumbagin and 7-methyljuglone were reported from the common sundew species Drosera rotundifolia (Budzianowski, 1996). A Thai medicinal plant, Diospyros mollis, produces Makluoside B, which is a glycosylated 1,4-naphthoquinone dimer (Suwama et al., 2018). Thus, just as 1,4-naphthoquinone production evolved independently multiple times, so too must have the glucosylation of reduced forms. This phase II-like process requires the action of oxidoreductases to reduce oxidized quinones and UGTs that remain to be identified. Plants that do not synthesize 1,4-naphthoquinones but that are resistant to their allelopathic effects could theoretically use the same approach. Detection of β-glucosidase activity with high specificity in English walnut husks that catalyzes the release of juglone from its glucosylated form (Duroux et al., 1998) also suggests that 1,4-naphthoquinone-producing plants evolved deglycosylation mechanisms for deploying their stored allelochemicals.

Given the propensity of some 1,4-naphthoquinones to react with free thiols (Kot et al., 2010), it can be hypothesized that conjugation with GSH and/or free cysteine might also be a strategy plants use to cope with 1,4-naphthoquinone allelochemicals. Free GSH supplied with juglone in vitro is sufficient to reduce the growth inhibitory effects of juglone on A. thaliana seedlings (Fig. 3). Thus, even in the absence of a GST, elevated production of GSH in vivo could counteract juglone or other structurally similar 1,4-naphthoquinones via direct conjugation for subsequent storage, detoxification, or exudation (Schröder et al., 2007) and/or for neutralizing the effects of any imposed redox stress. Insects (Piskorski and

![Fig. 3. Reduced glutathione (GSH) diminishes the growth inhibitory effects of juglone. Three-day-old wild-type Arabidopsis thaliana (Columbia-0) seedlings germinated on 1/2 Murashige and Skoog (MS) medium were transferred to 1/2 MS plates containing 0 µM juglone (top), 10 µM juglone (middle), or 10 µM juglone+10 µM GSH (bottom) for 3 d. Given that GSH spontaneously reacts with juglone to form adducts (Kot et al., 2010), these data indicate that juglone–GSH conjugates are not phytotoxic and/or that they are not taken up by Arabidopsis roots. Therefore, it can be hypothesized that similar to evolved metabolism-based detoxification of certain herbicides (Gaines et al., 2020), conjugation with GSH is one mechanism plants can employ to tolerate the effects of allelopathic 1,4-naphthoquinones such as juglones. Grid squares are 12.5 mm².](image)
Dorn, 2011; Piskorski et al., 2011), bacteria (Schmidt, 1988), and other organisms have also found ways to detoxify plant 1,4-naphthoquinones so they too may hold clues to mechanisms that resistant plants could employ to manage exposure to the compounds. Bacteria (Lowe et al., 2015) and fungi (Morrisset and Osbourn, 1999) also encounter and detoxify other types of plant allelochemicals, and thus may offer additional lines of investigation.

Concluding remarks and future directions

Integration of systems biology and functional studies in the context of robust phylogenetic frameworks has shed light on metabolic evolution and plant diversification (Smith et al., 2019). This is perhaps nowhere more apparent than in the recent large-scale biology studies that have revealed insight into the metabolic innovation underlying the evolution of plant 1,4-naphthoquinone pathways. This extraordinary class of compounds arose through both convergent evolution via different pathways and through repeated evolution from the phylloquinone pathway intermediate DHNA. Plants have also evolved a diverse range of mechanisms to deploy 1,4-naphthoquinones into the soil. While it is still not well understood how target plants respond to 1,4-naphthoquinones in the rhizosphere, many plants producing 1,4-naphthoquinones appear to have converged on a strategy to keep the compounds in reduced and glucosylated forms for storage and protection from their allelopathic effects.

Given the propensity of 1,4-naphthoquinones to function as novel chemical weapons in an oxygenic environment and the number of times that they were selected for in nature, there is seemingly great potential for 1,4-naphthoquine-based applications in agriculture. Juglone’s toxicity and impairment of plasma membrane H+-ATPase (Rudnicka et al., 2014), for example, gives it a novel herbicidal mode of action unlike any existing synthetic commercial herbicides. Plant Physiology 166, 76–82.

Auber RP, Suttiyut T, McCoy RM, Ghaste M, Crook JW, Pendleton AL, Widhalm JR, Wiseacver JH. 2020. Hybrid de novo genome assembly of red gromwell (Lithospermum erythrorhizon) reveals evolutionary insight into shikonin biosynthesis. Horticulture Research 7, 82.

Bergdoll L, Ten Brink F, Nitschke W, Picot D, Baymann F. 2016. From low- to high-potential bioenergetic chains: thermodynamic constraints of respiratory or photosynthetic metabolism. Biochimica et Biophysica Acta 1857, 1569–1579.

Block AK, Yakubova E, Widhalm JR. 2019. Specialized naphthoquinones present in Impatiens glandulifera nectaries inhibit the growth of fungal nectar microbes. Plant Direct 3, e00132.

Budzanowski J. 1996. Naphthoquinone glucosides of Drosera rotundifolia and D. intermedia from in vitro cultures. Phytochemistry 42, 1145–1147.

Chen L, Zhang G, He J, Guan J, Pan C, Mi W, Wang Q. 2010. New naphthoquinone from the root of Lygodium japonicum (Thunb). Sw. Journal of Natural Medicines 64, 114–116.

Chung D, Maier UH, Inouye H, Zenk MH. 1994. Different mode of incorporation of o-succinylbenzoic acid into the naphthoquinones juglone and lawsone in higher plants. Zeitschrift für Naturforschung C 49, 885–887.

Dayan FE, Duke SO. 2014. Natural compounds as next-generation herbicides. Plant Physiology 166, 1090–1105.

Ducluzeau AL, Wamboldt Y, Elowsky CG, Mackenzie SA, Schuurink RC, Bassett GJ. 2012. Gene network reconstruction identifies the authentic trans-prenyl diphosphate synthase that makes the solanesyl moieties of ubiquinone-9 in Arabidopsis. The Plant Journal 69, 366–375.
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Dudareva N, Klimpien A, Muhlemann JK, Kaplan I. 2013. Biosynthesis, function and metabolic engineering of plant volatile organic compounds. New Phytologist 198, 16–32.

Durán AG, Chinchilla N, Molinillo JM, Macías FA. 2018. Influence of lipophilicity in O-acyl and O-alkyl derivatives of juglone and lawsone: a structure–activity relationship study in the search for natural herbicide models. Pest Management Science 74, 682–694.

Durán AG, Gutiérrez MT, Rial C, Torres A, Varela RM, Valdivia MM, Molinillo JMG, Skonczyn D, Weston LA, Macías FA. 2017. Bioactivity and quantitative analysis of isoxenynaphthazarins in root periderm of two Echium spp.: E. plantagineum and E. gaditanum. Phytochemistry 141, 162–170.

Douroux L, Delmote FM, Lancelin JM, Kéravis G, Jay-Allemand C. 1998. Insight into naphthoquinone metabolism: beta-glucosidase-catalysed hydrolysis of hydrojuglone beta-D-glucopyranoside. The Biochemical Journal 333, 275–283.

Duru N, Gernapudi R, Zhou Q. 2014. Chemopreventive activities of shikonin in breast cancer. Biochemical Pharmacology 83, e163.

Gaines TA, Duke SO, Morrán S, Rigon CAG, Tranjel PJ, Kupper A, Dayan FE. 2020. Mechanisms of evolved herbicide resistance. Journal of Biological Chemistry 295, 10305–10320.

Gaisser S, Heide L. 1996. Inhibition and regulation of shikonin biosynthesis in suspension cultures of Lithospermum. Phytochemistry 41, 1065–1072.

Gangopadhyay M, Dwaneeja S, Bhattacharya S. 2011. Enhanced plumbagin production in elicited Plumbago indica hairy root cultures. Journal of Bioscience and Bioengineering 111, 706–710.

Gueldner RC, Yates IE, Reilly CC, Wood BW, Smith MT. 1994. Levels of a hydrojuglone glucoside in developing pecan leaves in relation to scab susceptibility. Journal of the American Society for Horticultural Science 119, 498–504.

Halkier BA. 2016. General introduction to glucosinolates. Advances in Botanical Research 80, 1–14.

Hatzios KK, Burgos N. 2004. Metabolism-based herbicide resistance: regulation by safeners. Weed Science 52, 454–467.

Heap I. 2020. Current status of the international herbicide-resistant weed database. http://www.weedsscience.weedsscience.org/Home.aspx

Hedin PA, Collum DH, Langhans VE, Graves CH. 1980. Distribution of juglone and related compounds in pecan and their effect on Fusarium effusum. Journal of Agricultural and Food Chemistry 28, 340–342.

Henry LK, Thomas ST, Widhalm JR, Lynch JH, Davis TC, Kessler SA, Bohlmiller J, Nandi JP, Durán AG. 2011. Contribution of isopenylphosphate to plant terpenoid metabolism. Nature Plants 4, 721–729.

Hohmann-Marriott MF, Blankenship RE. 2011. Evolution of photosynthesis. Annual Review of Plant Biology 62, 515–548.

Hook I, Mills C, Sheridan H. 2014. Bioactive naphthoquinones from higher plants. Studies in Natural Products Chemistry 584, 148–153.

Hook I, Sheridan H. 2020. Effects of (±)-dunnione and quinone-containing extracts from in vitro cultured plantlets of Streptocarpus dunnii Hook. f. and a hybrid ‘Ruby’ on seed germination. South African Journal of Botany 131, 1–11.

Hook I, Sheridan H, Reid C. 2018. Trichomes and naphthoquinones protect Streptocarpus dunnii Hook.f. against environmental stresses. South African Journal of Botany 119, 193–202.

Huang R, O'Donnell AJ, Barbolina J, Barkman TJ. 2020. Quinone perception in plants via leucine-rich-repeat receptor-like kinases. Nature doi: 10.1038/s41586-020-2655-4.

Huo J, Lu S. 2016. Plastoquinone and ubiquinone in plants: biosynthesis, physiological function and metabolic engineering. Frontiers in Plant Science 7, 1788.

Lowe TM, Ailloud F, Allen C. 2015. Hydroxycinnamic acid degradation, a broadly conserved trait, protects Ralstonia solanacearum from chemical plant defenses and contributes to root colonization and virulence. Molecular Plant-Microbe Interactions 28, 286–297.

Luo MC, You FM, Li P, Wang JR, Zhu T, Dandekar AM, Leslie CA, Aradhyä M, McGuire PE, Dvorak J. 2015. Synteny analysis in Rosids with a walnut physical map reveals slow genome evolution in long-lived woody perennials. BMC Genomics 16, 707.

Lygodium japonicum (Thunb.) Sw. 2019. EPPO Bulletin 49, 261–266. https://onlinelibrary.wiley.com/doi/epdf/10.1111/epp.12523

Martínez-García PJ, Crepeau MW, Puiu D, et al. 2016. The walnut (Juglans regia) genome sequence reveals diversity in genes coding for the biosynthesis of non-structural polyphenols. The Plant Journal 87, 507–532.

McCoy RM, Utturkar SM, Crook JW, Thimmappuram J, Widhalm JR. 2018. The origin and biosynthesis of the naphthalenoid moiety of juglone in black walnut. Horticulture Research 5, 67.

Morrissey JP, Osbourn AE. 1999. Fungal resistance to plant antibiotics as a mechanism of pathogenesis. Microbiology and Molecular Biology Reviews 63, 708–724.

Müller-W, Leistner E. 1978. Metabolic relation between naphthalene derivatives in Juglans. Phytochemistry 17, 1735–1738.

Nagegowda DA, Gupta P. 2020. Advances in biosynthesis, regulation, and metabolic engineering of plant specialized terpenoids. Plant Science 294, 110457.

Nett RS, Lau W, Sattely ES. 2020. Discovery and engineering of colchicine alkaloid biosynthesis. Nature 584, 148–153.

Pichersky E, Gang DR. 2000. Genetics and biochemistry of secondary metabolites in plants: an evolutionary perspective. Trends in Plant Science 5, 439–445.

Pichersky E, Lewinsohn E. 2011. Convergent evolution in plant specialized metabolism. Annual Review of Plant Biology 62, 549–566.

Piskorski R, Dorn S. 2011. How the oligophagous codling moth Cydia pomonella survives on walnut despite its secondary metabolite juglone. Journal of Insect Physiology 57, 744–750.

Piskorski R, Ineichen S, Dorn S. 2011. Ability of the oriental fruit moth Grapholitha molesta (Lepidoptera: Tortricidae) to detoxify juglone, the main secondary metabolite of the non-host plant walnut. Journal of Chemical Ecology 37, 1110–1116.

Prawat U, Chaimanee S, Butsuri A, Salae AW, Tuntiwachwuttikul P. 2012. Bioactive styryllactones, two new naphthoquinones and one new styrallactone, and other constituents from Goniothalamus scortechinii. Phytochemistry Letters 5, 529–534.

Rajniak J, Giehl RFH, Chang E, Murgia I, von Wirén N, Sattely ES. 2018. Biosynthesis of redox-active metabolites in response to iron deficiency in plants. Nature Chemical Biology 14, 442–450.

Riechers DE, Kreuz K, Zhang Q. 2010. Detoxification without intoxication: herbicide safeners activate plant defense gene expression. Plant Physiology 153, 3–13.

Rockli P, Hesse K, Glauser G, Rusterholz HP, Baur B. 2011. How the oligophage codling moth Grapholitha molesta (Lepidoptera: Tortricidae) to detoxify juglone, the main secondary metabolite of the non-host plant walnut. Journal of Chemical Ecology 40, 371–378.

Rudnicka M, Polak M, Karcz W. 2014. Cellular responses to naphthoquinones: juglone as a case study. Plant Growth Regulation 72, 239–248.
A cytosol-localized geranylhydroquinone during Shikonin biosynthesis. Phytochemistry 175, 2022.

Song W, Zhuang Y, Liu T. 2020. Potential role of two cytochrome P450s in catalyzing the oxidation of geranylhydroquinone during Shikonin biosynthesis. PloS One 15, e124375.

Meyer et al. 2019. Synthesis, biological function and evaluation of shikonin in cancer therapy. Fitoterapia 134, 665–700.

Zhang Y, Chu S-J, Luo Y-L, Qi J-L, Yang Y-H. 2017b. Involvement of LeMDR, an ATP-binding cassette (ABC) transporter protein gene, in shikonin transport and biosynthesis in Lithospermum erythrorhizon. BMC Plant Biology 17, 198.

Ziegler J, Facchini PJ. 2008. Alkaloid biosynthesis: metabolism and trafficking. Annual Review of Plant Biology 59, 735–769.