Natural Compounds as Next-Generation Herbicides

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Herbicides with new modes of action (MOAs) are badly needed due to the rapidly evolving resistance to commercial herbicides, but a new MOA has not been introduced in over 20 years. The greatest pest management challenge for organic agriculture is the lack of effective natural product herbicides. The structural diversity and evolved biological activity of natural phytotoxins offer opportunities for the development of both directly used natural compounds and synthetic herbicides with new target sites based on the structures of natural phytotoxins. Natural phytotoxins are also a source for the discovery of new herbicide target sites that can serve as the focus of traditional herbicide discovery efforts. There are many examples of strong natural phytotoxins with MOAs other than those used by commercial herbicides, which indicates that there are molecular targets of herbicides that can be added to the current repertoire of commercial herbicide MOAs.

The evolutionary forces driving the survival of species include chemical interactions between organisms, which function in positive interactions such as mutualistic and symbiotic relationships and negative interactions such as competitive and parasitic relationships. These processes have led to the emergence of novel secondary metabolic pathways (often through gene duplication), producing a vast array of structurally diverse and biologically active molecules (Moore and Purugganan, 2005; Ober, 2005; Flagel and Wendel, 2009; Jiang et al., 2013). This evolutionary process is similar to a high-throughput screen. However, unlike conventional in vitro screens, which test many compounds on a single biochemical target over a very short period of time, this natural high-throughput process selects molecules based on their whole-organism activities, involving numerous chemical interactions between countless organisms and target sites over millions of years. To date, approximately 200,000 secondary metabolites have been identified (Tulp and Bohlin, 2005), with many more expected to be discovered. Few of these compounds have been examined for phytotoxicity, and the modes or mechanisms of action (MOAs) of even fewer known phytotoxins have been elucidated.

The negative chemical interactions between organisms are often characterized using anthropomorphic language, such as chemical warfare, referring to the production of phytotoxins used by plant pathogens to invade their host plants (Maor and Shirasu, 2005), and the novel weapons hypothesis, which is associated with the chemical-based advantage of some invasive plant species over native plant populations (Callaway and Aschehoug, 2000; Callaway and Ridenour, 2004; Callaway and Maron, 2006; Cappuccino and Arnason, 2006; Callaway et al., 2008). While simplistic, this terminology illustrates how these toxin-based interactions exploit biochemical weaknesses between an organism and its host or enemy/competitor to enhance its own survival (Verhoeven et al., 2009). In fact, these interactions can even be multitrophic, such as when exotic plants enhance their invasiveness by promoting the growth of certain native soil pathogens noxious to native plants (Mangla et al., 2008; Barto et al., 2011).

As humans evolved from a nomadic hunter-gatherer subsistence existence to an agricultural lifestyle, they learned to utilize certain biologically active secondary metabolites to manage agricultural pests. Indeed, the concept that nature is an excellent source of natural pesticides is captured in the following ancient Lithica poem (circa 400 B.C.): "All the pests that out of earth arise, the earth itself the antidote supplies" (Ibn et al., 1781). Less than a century later, Greek and Roman treatises described practices to control agricultural pests that include the use of essential oils. Similar documents are found in Chinese literature, such as a survey describing plant species used to control plant pests (Yang and Tang, 1988). The mid-20th century ushered in the use of synthetic pesticides, which have revolutionized agriculture. Like pharmaceuticals (Harvey, 1999, 2008; Newman and Cragg, 2012), many pesticides are based on natural compounds. However, natural products have not played a major role in herbicide discovery (Copping and Duke, 2007; Hüter, 2011).

CURRENT IMPACT OF NATURAL PRODUCTS ON HERBICIDE DISCOVERY AND DEVELOPMENT

While almost 70% of all newly registered active pesticide ingredients have their origins in natural products research, only 8% of conventional herbicides are derived from natural compounds and only 7% of biochemical biopesticides (natural compounds) approved by the U.S. Environmental Protection Agency are bioherbicides (Cantrell et al., 2012). This is remarkable, because weeds have the largest negative impact on crop productivity among pests (Pimentel et al., 2005), and the lack of weed control is the most pressing concern expressed by farmers (Stokstad, 2013). Furthermore, in the United States, herbicides are used in far larger volumes than insecticides and fungicides combined (Köhler and Triebskorn, 2013).

There is a growing need for new herbicides with safer toxicological and environmental profiles and new MOAs. This need is driven by both the loss of older herbicides due to safety issues and to the rapidly increasing evolution of resistance to herbicides and herbicide classes that remain on the market (Heap, 2014). Natural product-based herbicides are considered by the public to be generally safer than conventional synthetic herbicides, although this assumption remains to be validated. Furthermore, there is a strong rationale for examining natural products to uncover novel MOAs (Dayan et al., 2012; Gerwick and Sparks, 2014).

The U.S. Environmental Protection Agency has three categories of biopesticides: (1) microbial pesticides used as biocontrol organisms; (2) plant-incorporated protectants (PIPs), which are natural pesticides produced by crops due to the presence of transgenes (e.g. *Bacillus thuringiensis* toxin production in transformed crops); and (3) biochemical pesticides, which are naturally occurring materials (Environmental Protection Agency, 2014). This Update covers the current status of the use of natural compounds as herbicides and as starting molecules for the production of synthetic herbicides. We also discuss the promise of natural compounds for future herbicide and herbicide MOA discovery. We preface this with a brief discussion of the potential use of PIP bioherbicides.

POTENTIAL USE OF NATURAL PRODUCTS AS PIP BIOHERBICIDES

After glyphosate-resistant crops, the second most successful transgenic crops are those transformed to produce *B. thuringiensis* toxins (Duke, 2011), demonstrating that the PIP approach to biopesticides has great potential. While there are currently no successful examples of bioherbicide PIPs, the approach of enhancing the allelopathy of crops with the use of transgenes to enhance or impart allelochemical production for weed control is promising and requires further study (Duke et al., 2001; Duke, 2003; Bertin et al., 2008).

Allelopathy has a controversial past due to the plethora of less than robust studies about this topic and the often questionable claims about the role of allelochemicals in plant-plant interactions (Duke, 2010). For decades, attempts have been made to enhance the allelopathic properties of crops by conventional breeding and variety selection (Batish et al., 2011; Bertholdsson et al., 2012; Worthington and Reberg-Horton, 2013). However, to our knowledge, rice (*Oryza sativa*) is the only crop for which there are allelopathic germplasm releases produced by conventional breeding (Kong et al., 2011; Gealy and Yan, 2012; Gealy et al., 2013). Much, if not all, of the allelopathy of rice is due to momilactones that are exuded from rice roots (Kato-Noguchi, 2004; Kong et al., 2004). Rice lines with an RNA interference knockout of a gene in the momilactone pathway are unable to produce momilactones and lose their allelopathic properties (Xu et al., 2012). Allelopathic rice varieties do not provide the same level of weed control as herbicides, but the allelopathic suppression

of weeds enables reductions in the use of synthetic herbicides (Gealy et al., 2003). A higher level of allelopathy might be obtained by imparting or increasing the production of allelochemicals in crops using more advanced genetic manipulation. Such an approach can be facilitated by identifying genes that encode enzymes involved in the synthesis of potent allelochemicals and elucidating how the expression of these genes is regulated (for review, see Duke et al., 2009). Importantly, allelochemicals such as momilactone B in rice (Kato-Noguchi and Ino, 2004) and sorgoleone in *Sorghum* spp. (Dayan et al., 2010), which are produced by roots and released to the rhizosphere, reach target plants more quickly than shoot-localized phytotoxins.

POTENTIAL FOR THE DISCOVERY OF NEW MOAs OF HERBICIDES BASED ON NATURAL COMPOUNDS

Natural products offer an unparalleled source of structural diversity, with little overlap with synthetic compounds generated by traditional organic synthesis in the laboratory (Koch et al., 2005; Harvey, 2007; Lipkus et al., 2008). This wider structural diversity may enable natural products to have unique MOAs (Duke and Dayan, 2013).

While commercial herbicides have only approximately 20 MOAs (Duke, 2012), evidence from the natural phytotoxin literature suggests that there are many more viable MOAs. Table I summarizes the known molecular target sites of highly effective phytotoxins, along with examples of their natural compound inhibitors. Sites targeted by both synthetic and natural inhibitors are indicated in italics. Some natural compounds, such as *Alternaria alternata* ssp. *lycopersici* (AAL)-toxin and tentoxin, are active at lower concentrations than many commercial herbicides (Duke, 1993; Abbas et al., 1994). This analysis provides strong evidence that there are herbicide target site alternatives to those utilized by current commercial herbicides. The natural products discussed below illustrate the diversity of the MOAs of natural products with potential use as herbicides.

AMINO ACID SYNTHESIS

Plants synthesize all essential amino acids used to build the proteins responsible for myriad biochemical and structural functions. Some of the most successful commercial herbicides target enzymes involved in amino acid synthesis, and these pathways are also the targets of natural phytotoxins (Table I; Fig. 1).

Gln Synthetase

Gln synthetase (GS) catalyzes the ATP-dependent condensation of Glu with ammonia to yield Gln. GS holds a special place with regard to the use of natural products as herbicides. Indeed, GS is the target site of L-phosphinothricin [homoalanin-4-yl(methyl)phosphinate; Fig. 1], a natural peptide produced by several *Streptomyces* spp. (Leason et al., 1982). L-Phosphinothricin is the active

Table I. *Molecular target sites of selected natural phytotoxins*

Target sites in italics are also target sites of synthetic commercial herbicides.

MOA	Molecule	Source	Reference
Amino acid synthesis			
GS	Phosphinothricin	*Pseudomonas syringae* and *Streptomyces* spp.	Lydon and Duke (1999)
	Phosalacine		Omura et al. (1984a)
	Tabtoxinine-β-lactam	*P. syringae* pv *tabaci*	Thomas et al. (1983)
Trp synthase	5-Methyl-Trp	*Cantharellus cibarius*	Hsiao et al. (2007)
Asp transaminase (aminotransferase)	Gostatin	*Streptomyces sumanensis*	Nishino et al. (1984)
	Cornexistin	*Paecilomyces variotii*	Amagasa et al. (1994)
Orn carbamoyl transferase	Phaseolotoxin	*P. syringae* pv *phaseolicola*	Templeton et al. (2005)
β-Cystathionase	Rhizobitoxine	*Bradyrhizobium* spp.	Giovanelli et al. (1973)
Energy transfer			
CF1 ATPase	Tentoxin	*Alternaria tenuis*	Groth (2002); Meiss et al. (2008)
Photophosphorylation uncoupler	Nigericin	*Streptomyces hygroscopicus*	Shavit and San Pietro (1967)
PSI electron diverter	Pyridazocidin	*Streptomyces* spp.	Gerwick et al. (1997)
PSII electron transport	Sorgoleone	*Sorghum bicolor*	Gonzalez et al. (1997)
	Cyanobacterin	*Scytonema hofmanni*	Lee and Gleason (1994)
	Fischerellin A	*Fischerella muscicola*	Hagmann and Juettner (1996)
	Stigmatellin	*Stigmatella aurantica*	Oettmeier et al. (1985)
	Aurachins	*S. aurantica*	Oettmeier et al. (1990)
Photosynthetic pigment synthesis			
Tyr aminotransferase	Cineole analog		Grossmann et al. (2012)
p-Hydroxyphenylpyruvate dioxygenase	Leptospermone	*Leptospermum scoparium*	Dayan et al. (2007a)
Deoxyxylulose-5-phosphate reductase	Fosmidomycin	*Streptomyces lavendulae*	Kuzuyama et al. (1998)
Glu-1-semialdehyde aminotransferase	Gabaculine	*Streptomyces toyacaenis*	Kahn and Kannangara (1987)
Aminolevulinic dehydratase	Gabaculine	*S. toyacaenis*	Kedy et al. (1994)
Protoporphyrinogen oxidase	Cyperin	*Preussia fleischhakii* and others	Harrington et al. (1995)
Lipid synthesis			
ENR	Cyperin	*P. fleischhakii* and others	Dayan et al. (2008)
β-Ketoacyl-ACP synthase	Thiolactomycin	*Norcardia* and *Streptomyces* spp.	Price et al. (2001)
	Cerulenin	*Cephalosporium cerulens*	Feld et al. (1989)
Ceramide synthase	AAL-toxins	*Alternaria alternata*	Abbas et al. (1994)
	Fumonisins	*Fusarium* spp.	Abbas et al. (1994)
Membrane functions and lipid stability			
H+-ATPase	Sorgoleone	*S. bicolor*	Hejl and Koster (2004b)
	Juglone	*Juglans* spp.	Hejl and Koster (2004a)
	Prehelminthosporol	*Bipolaris sorokiniana*	Olbe et al. (1995)
NADH oxidase	Glaucarubolone	*Castela polyandra*	Morré and Grieco (1999)
	Fusicoccin	*Fusicoccum amygdali*	Gomarasca et al. (1993)
	Simalikalactone D	*Quassia africana*	Morré and Grieco (1999)
Membrane destabilizers	Syringomycin	*P. syringae*	Schagina et al. (1998)
	Beticolins	*Cercospora beticola*	Goudet et al. (1998)
	T-toxins	*Bipolaris maydis*	Levings et al. (1995)
	Cercosporin	*Cercospora kikuchii*	Daub (1982)
Cuticle destabilizers	Pelargonic acid	*Pelargonium* spp.	Coleman and Penner (2006)
	Sarmentine	*Piper longum*	Huang et al. (2010)
Gene expression and regulation			
Adenylosuccinate synthase	Hydantocidin	*Streptomyces hygroscopicus*	Siehl et al. (1996)
	Ribofuranosyl triazolone		Schmitzer et al. (2000)
Isoleucyl tRNA synthase	Pseudomonic acids	*Pseudomonas fluorescens*	Clinch (1996)
Peptide deformylase	Actinonin	*Actinomyces* sp. MG848-hF6	Hou et al. (2007)
Ser/Thr protein phosphatases	Cantharidin	*Epicauta* spp.	Bajsa et al. (2011a)
RNA polymerase	Tagetitoxin	*P. syringae* pv *tagetis*	Mathews and Durbin (1990)
Aminopeptidase	Bestatin	*Actinomycetes* spp.	Umezawa et al. (1976)

(*Table continues on following page.*)

**Table I.** (*Continued from previous page.*)

MOA	Molecule	Source	Reference
Lys deacetylases	*Helminthosporium carbonum*-toxin	*Cochliobolus carbonum*	Meeley and Walton (1991)
AMP deaminase	Carbocyclic coformycin	*Saccharothrix* spp.	Dancer et al. (1997)
Calmodulin	Ophiobolin A	*Helminthosporium oryzae*	Leung et al. (1985)
Hormonal regulation			
Jasmonate mimic	Coronatine	*P. syringae*	Block et al. (2005)
	Cinnacidin	*Nectria* sp. DA060097	Irvine et al. (2008)
Auxin signaling	Toyocamycin	*Streptomyces toyocansis*	Hayashi et al. (2009)
	Terfestatin A	*Streptomyces* sp. F40	Hayashi et al. (2008)
ACC synthase	Rhizobitoxine	*Bradyrhizobium elkanii*	Yasuta et al. (1999)
GA mimic	GA$_3$	*Gibberella fujikuroi*	Hedden et al. (2001)
GA oxidase	Myrigalone	*Myrica gale*	Oracz et al. (2012)
Cytokinin mimic	Cytokinin	*Agrobacterium tumefaciens*	Jameson (2000)
Macrostructure			
Microtubule polymerization	Citral	*Cymbopogon citratus*	Chaimovitsh et al. (2010)
Cellulose synthesis	Thaxtomin	*Streptomyces scabies*	Scheible et al. (2003); Bischoff et al. (2009)
Golgi assembly	7-Dehydrobrefeldin A	*Alternaria carfhami*	Driouich et al. (1997)
Plant cell cycle			
DNA polymerase α and δ	Aphidicolin	*Phoma betae*	Ikegami et al. (1978)
Ribonucleotide reductase	Mimosine	*Mimosa pudica*	Perennes et al. (1993)
Proteasome interference	Lactacystin	*Streptomyces* spp.	Planchais et al. (2000)

ingredient of glufosinate (sold under several trade names, including Basta, Ignite, and Liberty; Lydon and Duke, 1999). Glufosinate also contains an equivalent amount of the inactive D-enantiomer. Glufosinate is the only commercial herbicide with this MOA.

Inhibition of GS causes the accumulation of toxic levels of ammonia as well as the inhibition of photorespiration due to reduced levels of amino acid donors (for review, see Lydon and Duke, 1999; Duke and Dayan, 2015). Glufosinate is a broad-spectrum herbicide that kills weeds more quickly than glyphosate [*N*-(phosphonomethyl)glycine], the most widely used herbicide worldwide (Duke and

Powles, 2008). Transgenic, glufosinate-resistant crops have been commercialized (for review, see Duke, 2014), but they are not as widely used as glyphosate-resistant crops. However, the rapidly increasing evolution of glyphosate-resistant weeds has intensified the adoption of glufosinate-resistant crops and the introduction of crops that have transgenes for resistance to both herbicides in the same crop variety.

Streptomyces hygroscopicus and *Streptomyces viridochromogenes* also synthesize the tripeptide L-alanyl-L-alanyl-phosphinothricin (bialaphos, also known as bilanofos), a proherbicide that releases L-phosphinothricin in planta
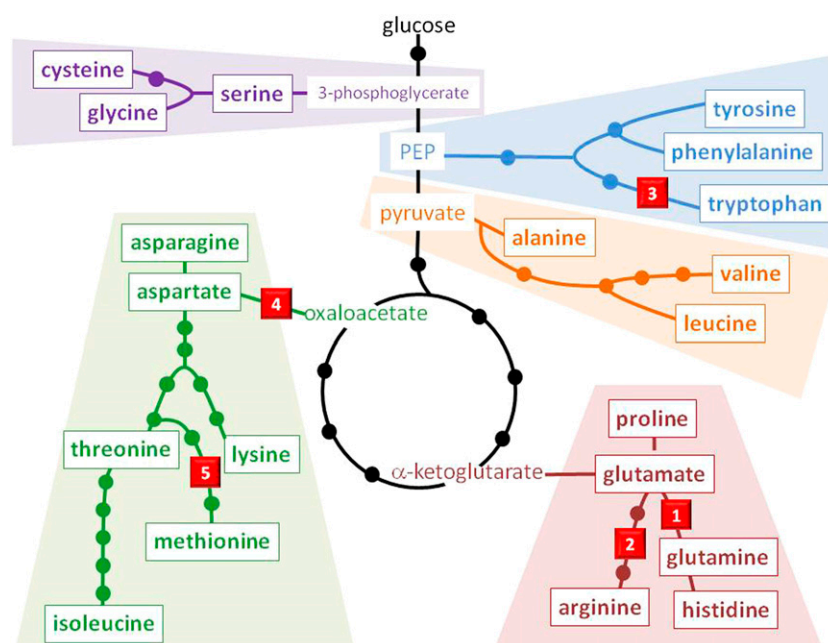
Figure 1. Amino acid biosynthesis pathways showing the enzymatic target sites of natural compound phytotoxins. 1, GS, the target site of phosphinothricin; 2, Orn carbamoyl transferase, the target site of phaseolotoxin; 3, Trp synthase, the target site of 5-methyl-Trp; 4, Asp transaminase, the target site of gostatin; 5, β-cystathionase, the target site of rhizobitoxine. PEP, Phospho*enol*pyruvate.

(Tachibana and Kaneko, 1986; Tachibana et al., 1986; Wild and Ziegler, 1989). Bialaphos is sold in Japan as a minor bioherbicide. Other natural product GS inhibitors such as tabtoxinine-β-lactam, phosalacine, and oxetin have not been commercialized (Thomas et al., 1983; Omura et al., 1984a, 1984b; Duke and Dayan, 2015).

Orn Carbamoyl Transferase

Orn carbamoyl transferase, a key enzyme in the urea cycle that converts Orn and carbamoyl phosphate to citrulline, is the target site of phaseolotoxin, a sulfodiaminophosphinyl peptide produced by *P. syringae* pathovars that are responsible for halo blight (Fig. 1). While phaseolotoxin is a competitive inhibitor of Orn carbamoyl transferase, it is converted in planta to octicidine, which in turn is an irreversible inhibitor of Orn carbamoyl transferase and is the predominant form of the toxin in infected tissues. No commercial herbicides have been developed to target this enzyme (Turner, 1986; Bender et al., 1999; Templeton et al., 2005).

Trp Synthase

Trp synthase catalyzes the last two steps in the synthesis of Trp (Fig. 1). This pathway does not exist in the animal kingdom, making it an interesting target site for herbicide development. 5-Methyl-Trp is an indole compound in the fruiting bodies and mycelia of fungi such as *C. cibarius* (Muszyńska et al., 2013). The levels of this compound are generally low (approximately 1 mg 100 g^{-1} dry weight). Initial studies on the bacteriostatic activity of 5-methyl-Trp have suggested that this antimetabolite inhibits an early step of Trp synthesis (Moyed, 1960), and it was later confirmed that it inhibits Trp synthase in plants (Hsiao et al., 2007).

Asp Transaminase

Asp transaminase, also called Asp aminotransferase, is an important pyridoxal phosphate-dependent enzyme in amino acid metabolism (Fig. 1). This enzyme catalyzes the reversible transfer of an α-amino group interconverting Asp and α-ketoglutarate to oxaloacetate and Glu. A number of phytotoxic natural products such as gostatin (5-amino-2-carboxy-4-oxo-1,4,5,6-tetrahydro-pyridine-3-acetic acid), produced by *S. sumanensis* (Nishino et al., 1984), and cornexistin, produced by *P. variotii* (Nakajima et al., 1991; Amagasa et al., 1994), target this enzyme. Gostatin is a slow-binding inhibitor (time dependent) that acts as a suicide substrate (mechanism-based inhibitor). On the other hand, the mechanism of inhibition of Asp aminotransferase by cornexistin is not well characterized, but it appears that cornexistin may undergo in planta metabolic bioactivation.

β-Cystathionase

β-Cystathionase, also called cystathionine β-lyase, catalyzes a pyridoxal phosphate-dependent elimination reaction where L-cystathionine and water are converted to L-homocysteine, NH_3, and pyruvate (Fig. 1). This reaction is important for several metabolic pathways (i.e. Met metabolism, Cys metabolism, selenoamino acid metabolism, nitrogen metabolism, and sulfur metabolism). Therefore, its inhibition by the microbial metabolite rhizobitoxine [2-amino-4-(2-amino-3-hydropropoxy)-transbut-3-enoic acid] causes phytotoxicity, resulting in the accumulation of homoserine, and plants become chlorotic (Giovanelli et al., 1971, 1973; Okazaki et al., 2007).

## ENERGY TRANSFER

Coupling Factor1 ATPase

Coupling Factor1 (CF1) ATPase, the chloroplastic ATP synthase producing the ATP required for the light-independent reactions, is located within the thylakoid membrane, with its CF1 part extending into the stroma (Fig. 2). Tentoxin, a cyclic tetrapeptide from the plant pathogen *A. tenuis* (Saad et al., 1970), inhibits chloroplast development (Halloin et al., 1970), interacting directly with chloroplast CF1 ATPase (Pinet et al., 1996; Groth, 2002; Meiss et al., 2008). This compound acts like a selective herbicide (Durbin and Uchytil, 1977; Lax et al., 1988) and has been the focus of a combinatorial synthesis program based on its unique structural scaffold (Jiménez et al., 2003).

Photophosphorylation Uncoupler

The electron transport chain and oxidative phosphorylation are coupled by the proton gradient across the thylakoid membrane of the chloroplast. This proton gradient, which is generated by the splitting of water by the light reaction of photosynthesis, is required for oxidative phosphorylation of ADP to ATP through the action of ATP synthase (Fig. 2), and uncoupling these processes is herbicidal. Nigericin, a metabolite from *S. hygroscopicus*, is a strong uncoupler of photophosphorylation (Shavit and San Pietro, 1967).

PSII

PSII catalyzes the energy-demanding, light-driven splitting of water, which releases oxygen and provides the reducing equivalents (electrons) required for the conversion of CO_2 into chemical energy (Fig. 2). A key step in this process that is sensitive to inhibition is the transfer of electrons from the secondary electron-acceptor (Qb) binding site to plastoquinone. Sorgoleone, a plastoquinone analog produced by sorghum roots, is a very potent inhibitor of photosynthesis (Gonzalez et al., 1997; Rimando et al., 1998; Dayan et al., 2003, 2007b; Dayan, 2006). Several microbial metabolites, such as cyanobacterin from *S. hofmanni* (Lee and Gleason, 1994), fischerellin A from *F. muscicola* (Hagmann and Juettner, 1996; Srivastava et al., 1998), and stigmatellin from *S. aurantica* (Oettmeier
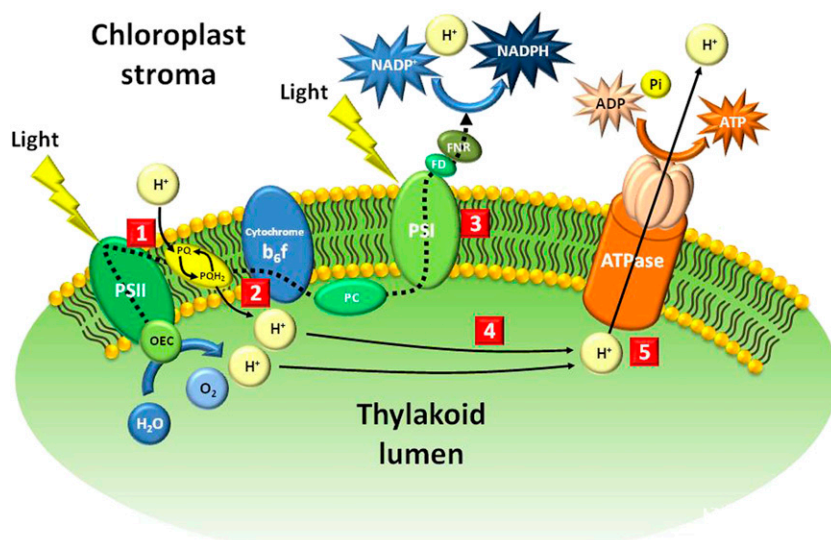
Figure 2. Various target sites of natural products that interfere with various aspects of the light reaction of photosynthesis within the thylakoid membrane. 1, The Q_B binding site of plastoquinone, the target site of sorgoleone; 2, electron flow between PSII and the cytochrome b_6/f complex, the target site of aurachins; 3, pyridazocidin diverts electrons from PSI; 4, the target site of nigericin, which uncouples photophosphorylation; 5, chloroplast CF1 ATPase, the target site of tentoxin. The dotted black line illustrates the flow of electrons from the oxygen-evolving complex to ferredoxin-NADP$^+$ reductase. Solid black lines illustrate the accumulation of the proton gradient inside the lumen and its use by CF1 ATPase to synthesize ATP. FD, Ferredoxin; FNR, ferredoxin-NADP$^+$ reductase; OEC, oxygen-evolving complex; PC, plastocyanin; Pi, inorganic phosphate; PQ, plastoquinone; PQH$_2$, reduced PQ.

et al., 1985), also inhibit PSII. Aurachins are metabolites from *S. aurantica* that act downstream from the Q_B binding site by interfering with the photosynthetic electron flow between PSII and the cytochrome b_6/f complex (Oettmeier et al., 1990).

PSI

PSI is a key component of the photosynthetic electron transport chain (Fig. 2). A number of synthetic herbicides (e.g. bipyridiliums) divert electrons from PSI and prevent the subsequent conversion of NADP into NADPH (Hess, 2000; Trebst, 2007). In addition, these herbicides are dications that become highly reactive free radicals upon acceptance of electrons from PSI, generating reactive oxygen species that cause extensive and very rapid desiccation of foliage. Pyridazocidin is a cationic phytotoxin produced by some *Streptomyces* spp. that acts by the same mechanism as bipyridilium herbicides in both broadleaf and grass weeds (Gerwick et al., 1997).

PHOTOSYNTHETIC PIGMENT SYNTHESIS

Carotenoids and chlorophylls are essential for photosynthesis and other important biochemical processes (Cazzonelli, 2011; Dayan and Dayan, 2011). These pathways are targeted by several classes of herbicides (Dayan and Duke, 2003, 2010). While no natural products that directly interfere with the carotenoid and chlorophyll synthesis pathways have been developed as herbicides, several products act by targeting key enzymes in these pathways.

Carotenoid Synthesis

Tyr Aminotransferase

Tyr aminotransferase (or Tyr transaminase) catalyzes the conversion of Tyr to *p*-hydroxyphenylpyruvate (the

first step in plastoquinone and tocopherol synthesis; Fig. 3). Inhibition of Tyr aminotransferase ultimately affects carotenoid synthesis because plastoquinone is a necessary cofactor of the enzyme phytoene desaturase (PDS; Norris et al., 1995). The herbicidal activity of cinmethylin, a 1,4-cineole derivative, is reported to be due to its inhibition of Tyr aminotransferase (Grossmann et al., 2012). If this finding is confirmed, it would represent the most recent discovery of a new commercial herbicide target site (Grossmann et al., 2012). While many cineoles (e.g. 1,4-cineole and 1,8-cineole) are phytotoxic (Romagni et al., 2000a), their MOA has not been elucidated. The presence of an epoxide ring is required for the biological activity of these monoterpenes.

p-Hydroxyphenylpyruvate Dioxygenase

p-Hydroxyphenylpyruvate dioxygenase (HPPD) is a ubiquitous Fe(II)-containing nonheme oxygenase that catalyzes the conversion of *p*-hydroxyphenylpyruvate into homogentisate (Fig. 3). Inhibition of HPPD leads to the depletion of plastoquinone, a cofactor for PDS. The bleaching symptoms caused by inhibition of PDS are similar to those caused by herbicides that inhibit PDS directly. The triketone HPPD inhibitor class of herbicides was derived from a natural β-triketone, leptospermone. The clue leading to the discovery of the triketones came from the observation that leptospermone, a natural compound from the allelopathic bottlebrush plant (*Callistemon citrinus*), is phytotoxic (Lee et al., 1997). The herbicidal activity of leptospermone is also due to its inhibition of *p*-hydroxyphenylpyruvate dioxygenase (Dayan and Duke, 2003; Dayan et al., 2007a; Owens et al., 2013). Inhibition of HPPD was the last herbicide MOA introduced for major commercial herbicides in the past 25 years (Duke, 2012). Other natural products from plants, such as usnic acid from lichens and sorgoleone from *Sorghum* spp., are also HPPD inhibitors

Figure 3. Simplified pathways of chlorophyll (green) and carotenoid (orange) biosynthesis. These two pathways are connected via the phytyl tail, originating from the chloroplastic isoprenoid geranylgeranyl pyrophosphate (PP). The pathway of prenylquinones, shown in purple, is also important for carotenoid biosynthesis because plastoquinone is an essential cofactor for PDS activity. Solid lines represent single reactions, and the dotted line represents many steps. 1, Glu-1-semialdehyde aminotransferase, the target site of gabaculine; 2, protoporphyrinogen oxidase, one of the target sites of cyperin; 3, deoxyxylulose-5-phosphate reductase, the target site of fosmidomycin; 4, p-hydroxyphenylpyruvate dioxygenase, the target site of natural β-triketones such as leptospermone; 5, tyrosine aminotransferase, the target site of the 1,4-cineole-derived herbicide cinmethylin.
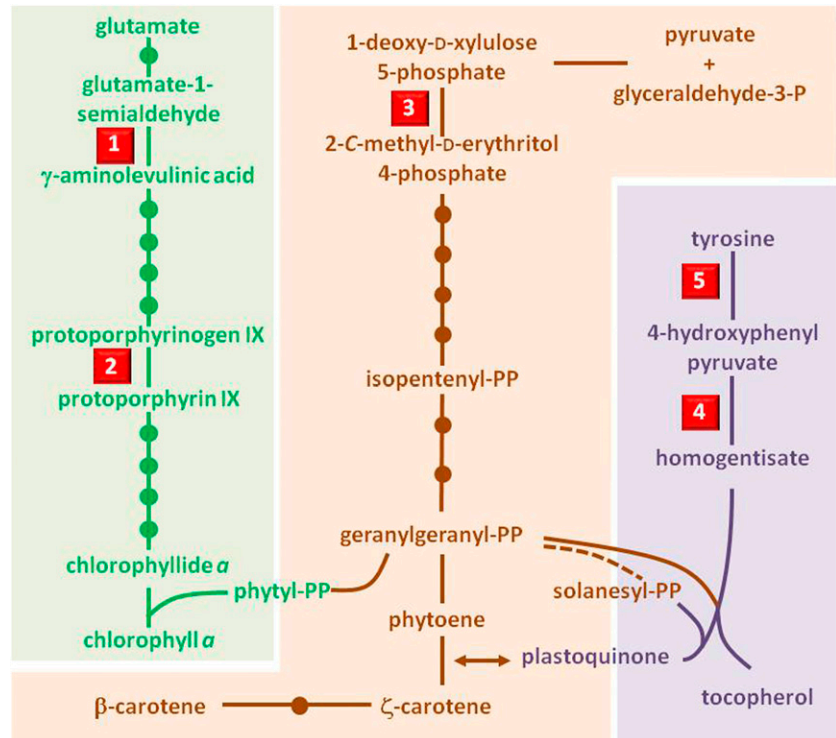
(Romagni et al., 2000b; Meazza et al., 2002), although sorgoleone has other target sites that appear to be more important for its phytotoxicity (Dayan et al., 2010).

Deoxyxylulose-5-Phosphate Reductase

Deoxyxylulose-5-phosphate reductase catalyzes the second step of the nonmevalonate pathway to terpenoid synthesis (Fig. 3). Inhibition of deoxyxylulose-5-phosphate reductase causes bleaching of green tissues due to a reduction in carotenoid content. Fosmidomycin, a phytotoxic metabolite from *S. lavendulae*, is a potent inhibitor of this enzyme, with I_{50} in the nanomolar range (Kuzuyama et al., 1998).

Chlorophyll Synthesis

Glu-1-Semialdehyde Aminotransferase

Glu-1-semialdehyde aminotransferase (also called Glu-1-semialdehyde 2,1-aminomutase) catalyzes the last step of γ-aminolevulinic acid synthesis, a key step in chlorophyll synthesis (Fig. 3). Therefore, the inhibition of this enzyme is lethal to plants. Gabaculine (3-aminobenzoic acid), a toxin produced by *S. toyacaenis*, is a potent inhibitor of Glu-1-semialdehyde aminotransferase (Kannangara and Schouboe, 1985) and represses ALA synthesis in plants (Flint, 1984). Gabaculine-resistant green algae have overcome the effect of this phytotoxin by overexpressing the target enzyme (Kahn and Kannangara, 1987), whereas *Synechococcus* spp. have developed resistance following

the deletion of a tripeptide near the NH_2 terminus of the enzyme and an Met-248-to-Ile substitution, which reduces the specific activity of the enzyme but increases resistance to gabaculine 100-fold (Grimm et al., 1991). Gabaculine-resistant Glu-1-semialdehyde aminotransferases from both microbial and plant sources have been used as selectable markers in plants (Gough et al., 2001; Ferradini et al., 2011).

Aminolevulinic Acid Synthesis

Aminolevulinic dehydratase, the enzyme catalyzing the step following Glu-1-semialdehyde aminotransferase activity in chlorophyll synthesis, is affected by gabaculine, but it was later shown that this is only a secondary effect associated with the inhibition of the synthesis of aminolevulinic acid (Kedy et al., 1994).

Protoporphyrinogen Oxidase

Protoporphyrinogen oxidase (PPO) catalyzes the last step in common between heme and chlorophyll synthesis in plants (Fig. 3). PPO is a well-known target site for herbicides whose action results in the rapid light-dependent peroxidation of membranes. Many of the commercial herbicides affecting this enzyme are diphenyl ethers. The natural diphenyl ether cyperin, produced by a number of plant pathogens, is moderately active against PPO (Harrington et al., 1995), but its phytotoxicity is light independent, suggesting that it has a different MOA. This notion was later confirmed (see below).

LIPID SYNTHESIS

β-Ketoacyl-Acyl Carrier Protein Synthase

β-Ketoacyl-acyl carrier protein (ACP) synthase (also called 3-oxoacyl-ACP synthase) is a key enzyme of the dissociated fatty acid biosynthesis complex in plants and bacteria (Fig. 4). Thiolactomycin, a metabolite from *Norcardia* and *Streptomyces* spp., and cerulenin, a metabolite from *C. cerulens*, are potent inhibitors of the plant enzyme involved in de novo fatty acid synthesis (Nishida et al., 1986; Feld et al., 1989). A study elucidating the structure of bacterial β-ketoacyl-ACP synthase crystallized with thiolactomycin revealed the essential enzyme-ligand binding interactions and established the existence of hydrophobic and pantetheine-binding pockets (Price et al., 2001).

Enoyl-ACP Reductase

Enoyl-ACP reductase (ENR), a component of type II fatty acid synthase in plants and prokaryotes, catalyzes a critical step in fatty acid elongation (Fig. 4). This enzyme is sensitive to triclosan, a synthetic diphenyl ether (McMurry et al., 1998; Roujeinikova et al., 1999) commonly used in antibacterial soaps. As mentioned above (inhibitors of PPO), cyperin is a natural diphenyl ether produced by several plant pathogens that has some inhibitory activity against PPO, but its phytotoxicity is not light dependent. Inhibition of ENR alters fatty acid synthesis and leads to a rapid light-independent destabilization of membrane integrity. Cyperin's mechanism is consistent with that of triclosan, as it inhibits plant ENR (Dayan et al., 2008).
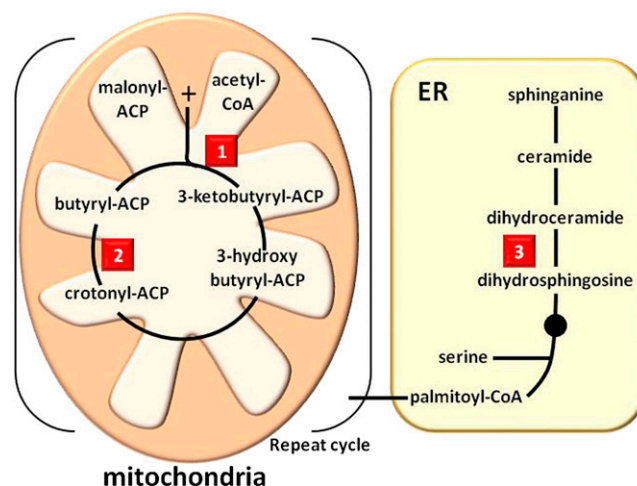
Ceramide Synthase

Ceramide synthases are integral membrane proteins in the endoplasmic reticulum that catalyze the synthesis of ceramide via the acylation of sphinganine to a long-chain fatty acid (Fig. 4). AAL-toxins and fumonisins, produced by *A. alternata* and *Fusarium* spp., respectively, inhibit ceramide synthases at submicromolar concentrations (Abbas et al., 1994, 1998). These toxins cause the rapid accumulation of sphingolipid precursors and the subsequent rapid loss of plasma membrane integrity. Phytosphingosine, one of the precursors that accumulate after AAL-toxin exposure, is highly phytotoxic (Tanaka et al., 1993) and may account for most of the herbicidal activity. A survey of the responses of 88 plant species to AAL-toxin revealed that some plants are highly sensitive to this toxin, whereas others are unaffected (Abbas et al., 1995). Unfortunately, no AAL-toxin or fumonisin analog with high phytotoxicity and low mammalian toxicity has been found (Abbas et al., 2002).

MEMBRANE FUNCTIONS AND LIPID STABILITY

H$^+$-ATPase

Plasma membrane H$^+$-ATPase and associated membrane proteins play essential roles in maintaining water

Figure 4. Simplified pathways of lipid biosynthesis, showing target sites of natural phytotoxins. Fatty acid synthesis initiates in the mitochondria. The biosynthesis of ceramide, which leads to the production of cerebrosides, is localized in the endoplasmic reticulum (ER). 1, β-Ketoacyl-ACP synthase, the target site of thiolactomycin; 2, ENR, one of the target sites of cyperin; 3, ceramide synthase, the target site of AAL-toxins and fumonisins.

uptake and cell turgor, which are essential for plant growth and development (Fig. 5). Inhibition of H$^+$-ATPase reduces mineral and water uptake by the roots, resulting in stomata closure, which consequently negatively affects respiration, photosynthesis, and other processes. A number of natural plant products, such as sorgoleone from *Sorghum* spp. (Hejl and Koster, 2004b), juglone from *Juglans* spp. (Hejl and Koster, 2004a), and prehelminthosporol from *B. sorokiniana* (Olbe et al., 1995), interfere with plant growth by inhibiting H$^+$-ATPase.

NADH Oxidase

Auxin-induced NADH oxidase is responsible for proton extrusion associated with cell elongation in response to auxin (Fig. 5). This process is also accompanied by the acidification of the cytoplasm (Morré et al., 1986). Stimulation of elongation by auxin normally occurs after a lag of several minutes. However, fusicoccin, a product from *F. amygdali*, triggers the same response without delay (Cleland, 1976). As a consequence, plants exposed to fusicoccin quickly wilt due to their inability to close their stomata (Gomarasca et al., 1993). Glaucarubolone, a quassinoid extracted from the root bark of *C. polyandra*, inhibits auxin-induced plasma membrane NADH oxidase in many plant species when used at nanomolar concentrations (Morré and Grieco, 1999). The quassinoid simalikalactone D, on the other hand, inhibits constitutive NADH oxidase activity. From a structural standpoint, quassinoids that possess an oxymethylene bridge tend to have higher phytotoxic activities than those without this structure (Dayan et al., 1999).

Membrane and Cuticle Destabilizers

In addition to the cell wall, the plasma membrane plays a critical role in keeping the entire cellular structure intact, providing an environment suitable for all physiological and biochemical processes that occur in the cytoplasm. The plasma membrane is also at the interface between the cell and its environment. Destabilization of the plasma membrane has deleterious effects on plants, and a number of natural products act by interfering with the integrity of membranes. Syringomycin, a metabolite produced by *P. syringae*, is an example of a large amphiphilic lipodepsinonapeptide that creates pores in the plasma membrane when assembled into large macromolecules. The loss of membrane integrity results in a loss of electrolytes and rapid necrosis in plant tissues (Backman and DeVay, 1971; Schagina et al., 1998; Bender et al., 1999; Malev et al., 2001). The nonpeptide fungal toxin beticolin is produced by *C. beticola* (Ducrot et al., 1996). This toxin can also assemble into channel-like structures, which leads to the loss of electrolytes and rapid cell death (Goudet et al., 1998; Fig. 5). Similarly, the *B. maydis* metabolite T-toxins cause rapid loss of membrane integrity by binding to the plant mitochondrial receptor URF13, thus changing its conformation and forming a pore comprising at least six transmembrane α-helices (Levings et al., 1995).

Cercosporin, produced by *C. kikuchii*, as with many perylenequinones, can be photoactivated and generate large amounts of reactive oxygen species (both singlet oxygen and superoxide ions), leading to the peroxidation of membrane lipids (Fig. 5; Daub, 1982; Daub and Hangarter, 1983; Daub et al., 2005).

Pelargonic acid (nonanoic acid) occurs naturally as esters in the oil of *Pelargonium* spp. This compound is used as a burndown herbicide, which acts by stripping the cuticle from the leaf surface, resulting in rapid,
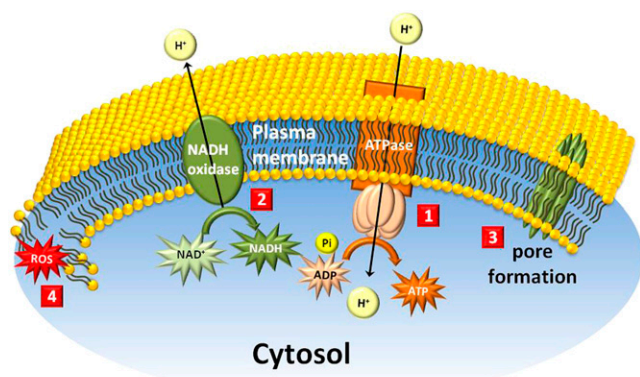
Figure 5. Membrane functions and lipid stability are affected by natural products. 1, Plasma membrane H^+-ATPase, the target site of sorgoleone and juglone; 2, auxin-induced and constitutive NADH oxidases, the target sites of fusicoccin and simalikalactone D, respectively; 3, loss of membrane integrity by the self-assembly of large amphiphilic molecules (such as syringomycin) to form pores; 4, loss of membrane integrity by natural products such as cercosporin that generate large amounts of reactive oxygen species (ROS). Pi, Inorganic phosphate.

uncontrolled tissue desiccation (Lederer et al., 2004; Coleman and Penner, 2006, 2008). Sarmentine is a lipophilic pyrrolidine (isolated from *P. longum*) that has a similar MOA to that of pelargonic acid (Huang et al., 2010).

GENE EXPRESSION AND REGULATION

Adenylosuccinate Synthase

Adenylosuccinate is a ubiquitous enzyme that plays a key role in purine biosynthesis. This enzyme catalyzes the GTP-dependent conversion of IMP and L-Asp to GDP, phosphate, and N(6)-(1,2-dicarboxyethyl)-AMP. Hydantocidin, a metabolite produced by *S. hygroscopicus*, is activated in planta via phosphorylation to form an IMP analog, which is a potent inhibitor of adenylosuccinate synthetase. The use of hydantocidin and its structural analogs as an herbicide has been studied extensively (Heim et al., 1995; Cseke et al., 1996; Fonné-Pfister et al., 1996; Poland et al., 1996; Siehl et al., 1996). Ribofuranosyl triazolone, another natural phytotoxin that targets adenylosuccinate synthetase upon phosphorylation, is readily obtained by synthetic means, making it a more suitable starting backbone for developing new herbicides than hydantocidin (Schmitzer et al., 2000).

Isoleucyl tRNA Synthetase

Pseudomonic acids A and C, produced by *P. fluorescens*, are inhibitors of isoleucyl tRNA synthetase. Isoleucyl tRNA synthetase, a class I aminoacyl tRNA synthetase, catalyzes the attachment of Ile to its cognate tRNA molecule. The mechanism of inhibition of pseudomonic acids involves the interaction of its long side chains with both the Ile and ATP binding sites on the enzyme (Hughes and Mellows, 1978; Clinch, 1996).

Peptide Deformylase

Peptide deformylase is a critical enzyme that initiates protein translation in prokaryotes by removing the N-formyl group from N-formyl Met. This enzyme is the target of actinonin, a peptide-like hydroxamic acid produced by soil actinomycetes. Actinonin has been patented for herbicide use but has not been developed as a commercial product. Actinonin functions in plants by inhibiting the prokaryote-like plastid peptide deformylase, leading to stunting, bleaching, and necrosis in a wide range of agriculturally relevant weed species (Hou et al., 2006; Fernández-San Millán et al., 2011).

Ser/Thr Protein Phosphatases

Protein phosphatases act in concert with their protein kinase counterparts to modulate the phosphorylation status of proteins involved in such functions as signal

transduction pathways and the regulation of gene expression. Therefore, the inhibition of protein phosphatases affects a large number of molecular and physiological processes. Cantharidin is a potent toxin produced by *Epicauta* spp. and *Lytta vesicatoria* insects. Cantharidin and its analogs are strong inhibitors of plant Ser/Thr protein phosphatases (Bajsa et al., 2011b). Endothall is a commercial herbicide that is structurally similar to cantharidin and also inhibits plant Ser/Thr protein phosphatases. This herbicide is primarily used for aquatic weed control (Netherland et al., 2000; Bajsa et al., 2011a).

RNA Polymerase

Tagetitoxin, a metabolite produced by *P. syringae* pv *tagetis*, inhibits RNA synthesis directed by RNA polymerases in both chloroplasts and *Escherichia coli* (Langston-Unkefer et al., 1984; Mathews and Durbin, 1990). Plants treated with tagetitoxin do not accumulate plastid 70S ribosomes. Consequently, none of the plastid-encoded polypeptides translated by chloroplastic ribosomes are produced (Lukens et al., 1987).

Aminopeptidase

Aminopeptidases are ubiquitous proteolytic enzymes that hydrolyze single amino acids at the N termini of peptidic substrates. These enzymes are involved in physiological processes such as mitosis, angiogenesis, and regulation of the cell oxidation state (Lowther and Matthews, 2002). Natural inhibitors of plant aminopeptidases, such as bestatin produced by actinomycetes (Umezawa et al., 1976), may provide relatively simple structural backbones for new herbicides (Oszywa et al., 2013). Bestatin was recently used to dissect aspects of jasmonate signaling in Arabidopsis (*Arabidopsis thaliana*; Zheng et al., 2006).

Lys Deacetylases

H. carbonum (HC)-toxin, a host-selective cyclic peptide from *C. carbonum* (Meeley and Walton, 1991), acts as an inhibitor of Lys deacetylase (previously known as histone deacetylase; Walton, 2006). Lys deacetylase removes acetyl groups from ε-N-acetyl-Lys amino acids on histones, allowing the histones to wrap the DNA more tightly, while inhibition of this step destabilizes DNA (Abbas et al., 2001; Walton, 2006).

AMP Deaminase

AMP deaminase catalyzes the deamination of AMP to produce IMP and NH_3. This irreversible reaction essentially removes AMP from the adenylate pool and drives the equilibrium of the reaction catalyzed by adenylate kinase toward ATP synthesis. Carbocyclic coformycin is a potent phytotoxin whose primary MOA involves the inhibition of AMP deaminase following phosphorylation of the 5'-hydroxyl group (Dancer et al., 1997; Lindell et al., 1999; Riley et al., 1999).

Calmodulin

Calmodulin is a multifunctional, calcium-binding, intermediate messenger protein found in all eukaryotic cells. Calmodulin transduces calcium signals by binding calcium ions and then modifying its interactions with various target proteins. Ophiobolin A is a fungal phytotoxin produced by *H. oryzae* that inactivates calmodulin by reacting with Lys residues in calmodulin (Leung et al., 1985; Kong Au and Chow Leung, 1998).

HORMONAL REGULATION

Plant hormones affect virtually all aspects of plant growth and development. Consequently, many plant pathogens produce compounds that either mimic plant hormones or interfere with endogenous hormone synthesis in order to gain the upper hand in natural plant-pathogen interactions. Synthetic auxin mimics such as 2,4-dichlorophenoxyacetic acid are widely used as herbicides.

Jasmonate Mimics

Certain pathovars of *P. syringae* produce coronatine, a jasmonic acid mimic (Ichihara et al., 1977; Koda et al., 1996; Block et al., 2005). Coronatine suppresses natural salicylic acid-dependent plant defense mechanisms. This compound also induces the opening of stomata, which may also help the invading organism gain access to the apoplast (Jones and Dangl, 2006). Cinnacidin, a microbial product isolated from a fungal fermentation extract of *Nectria* sp. DA060097, has a promising herbicidal activity profile. Foliar application of cinnacidin causes stunting and chlorosis. Both coronatine and cinnacidin act by mimicking the role of jasmonic acid (Irvine et al., 2008).

Auxin Signaling

Toyocamycin, produced by *S. toyocansis*, inhibits auxin-responsive gene expression and blocks the auxin-enhanced degradation of the auxin/indole-3-acetic acid (IAA) repressor modulated by the SCF[TIR1] ubiquitin proteasome pathway (Hayashi et al., 2009). However, toyocamycin does not affect the proteolytic activity of the proteasome. Toyocamycin acts on the ubiquitination process regulated by SCF[TIR1]. Terfestatin A, a molecule produced by *Streptomyces* sp. F40, also interferes with auxin signaling by inhibiting the expression of auxin-inducible genes (Hayashi et al., 2008).

Auxin Functions

IAA, the primary auxin in plants, is an important hormone that regulates a large number of growth and developmental processes. Patten and Glick (1996) estimated that up to 80% of microbes isolated from the rhizosphere produce IAA, and many of these microorganisms highjack the functions of IAA to promote their interactions with plant tissues (Duca et al., 2014).

Ethylene Synthesis

Ethylene is a potent modulator of plant growth and development, affecting many aspects of the plant life cycle, including seed germination, root hair development, root nodulation, flower senescence, abscission, and fruit ripening. In higher plants, 1-aminocyclopropane-1-carboxylase (ACC) synthase is the rate-limiting enzyme that functions in the biosynthesis of ethylene from Met. Rhizobitoxine, a *B. elkanii* product, acts as a competitive inhibitor of ACC synthase (Yasuta et al., 1999). This pathway is related to *β*-cystathionase, the other known target site of rhizobitoxine.

GA Overload

GAs are tetracyclic diterpenoid acid plant hormones that regulate growth and developmental processes such as stem elongation, germination, dormancy, flowering, and leaf and fruit senescence. To date, 126 GAs have been identified. The first identified GA was isolated from the fungal pathogen *G. fujikuroi* and is responsible for foolish seedling disease in rice. The synthesis of GAs may be advantageous to pathogens, and these compounds have been developed as plant growth regulators, but whether GAs can serve as structural leads for potential herbicides is unclear.

GA 3-Oxidase

GA biosynthesis is usually restricted to actively growing and elongating tissues, and GA 3-oxidase catalyzes the final step of the biosynthetic pathway that produces physiologically active GAs. Myrigalone, a natural *β*-triketone produced by the plant *M. gale*, interferes with GA metabolism and signaling by inhibiting GA 3-oxidase and by disrupting the GA signaling pathway. These processes are important for endosperm weakening and embryo growth (Oracz et al., 2012).

Cytokinins

Cytokinins, another class of phytohormones that participate in the complex regulatory network of plant hormones, promote cell division, or cytokinesis. Cytokinins are primarily involved in cell growth and differentiation. Cytokinin is produced by many pathogens to promote their infection by retarding senescence in infected leaf tissue. For example, the virulence of *A. tumefaciens*, the organism responsible for crown gall formation, is associated with the integration of the fungal genes for cytokinin and auxin production into the plant genome (Jameson, 2000). Like GAs, cytokinins are unlikely to serve as effective herbicides.
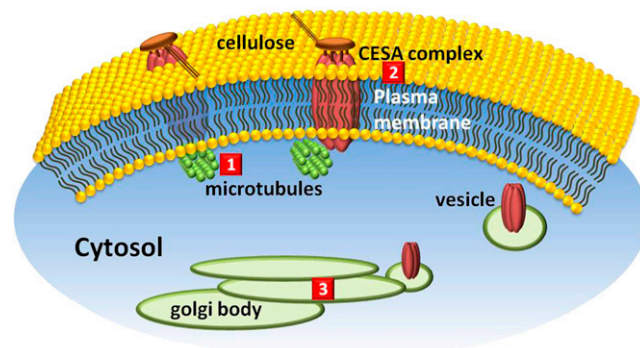
MACROSTRUCTURE

Cell wall formation requires cellulose synthesis. This process is complex and is still not fully understood. However, it is known that the direction of cellulose deposition by the cellulose synthase complex, which originates from the Golgi bodies, is intimately connected to the direction of skeletal microtubules. These processes are the targets of a number of phytotoxins.

Microtubule Polymerization

Microtubule polymerization is an essential process in all eukaryotic plants that is involved in cell growth and cellulose deposition, cytokinesis, mitosis, and vesicular transport (Fig. 6; Hashimoto, 2013). Some of the most effective mitotic inhibitors discovered to date are natural products, such as colchicine from crocus (*Colchicum* spp.) bulbs, vinblastine from *Vinca rosea* (*Catharanthus roseus*), and taxol from *Taxus* spp. (Vaughn and Vaughan, 1988). These compounds are growth inhibitors that either destabilize or hyperstabilize microtubules. Citral, a monoterpene produced by many plants such as *C. citratus*, disrupts microtubule polymerization within minutes after exposure. The inhibition of microtubule polymerization by citral has been confirmed by in vitro assays (Chaimovitsh et al., 2010).

Cellulose Synthesis

Cellulose is the major component of the plant cell wall, providing strength to the plant architecture and protecting cells against pathogens, dehydration, and

Figure 6. Cell macrostructures affected by natural products. 1, Microtubule polymerization is affected by citral; 2, cellulose synthase (CESA) is the target site of thaxtomin; 3, Golgi body structural integrity is disrupted by brefeldin A.

other abiotic factors. Cellulose deposition is carried out by the cellulose synthase complex (at least three different cellulose synthase enzymes and other associated proteins), which is tightly associated with the cortical microtubule cytoskeleton (Fig. 6). Thaxtomin A, a phytotoxic cyclic dipeptide analog produced by *S. scabies* and other species, inhibits cellulose synthesis by interfering with the formation of the cellulose synthase complexes on the outside of the cell (King et al., 2001; Scheible et al., 2003; Bischoff et al., 2009; King and Calhoun, 2009). Thaxtomin has been developed as a bioherbicide.

Golgi Assembly

Brefeldin A and its hydroxylated 7-dehydrobrefeldin A analog are produced by *A. carfhami*, a fungal pathogen of safflower (*Carthamus tinctorius*). Both of these compounds cause a cis-to-trans breakdown of the Golgi stacks in plant cells (Driouich et al., 1997) and block the secretion of cell wall polysaccharides and proteins as well as the transport of soluble proteins to the vacuole (Fig. 6; Ritzenthaler et al., 2002). The molecular target of these compounds is unknown.

PLANT CELL CYCLE

DNA Polymerase α and δ

Several natural products interfere with the plant cell cycle (Planchais et al., 2000). For example, aphidicolin analogs are phytotoxins from *P. betae* (Ichihara et al., 1984) that reversibly inhibit DNA polymerase α and δ and interrupt the G1/S progression (Ikegami et al., 1978).

Ribonucleotide Reductase

Ribonucleotide reductase catalyzes the formation of deoxyribonucleotides from ribonucleotides, which in turn are used in the synthesis of DNA. Mimosine is a nonprotein amino acid (β-3-hydroxy-4 pyridone) phytotoxin produced by *M. pudica* (Reigosa and Malvido-Pazos, 2007; Williams and Hoagland, 2007). Mimosine inhibits ribonucleotide reductase at the G1 stage, before the initiation of replication (Perennes et al., 1993).

Proteasome Interference

A major step in proteolysis by the proteasome is catalyzed by the anaphase-promoting complex (a multimeric ubiquitin ligase). This complex targets B-type cyclins and other regulatory proteins to the 26S proteasome for degradation, allowing exit from mitosis. Some *Streptomyces* spp. produce lactacystin (Omura et al., 1991). This bacterial metabolite interferes with the function of the proteasome by specifically inhibiting the anaphase-promoting complex, thus preventing the destruction of cyclins and causing the cells to escape from mitosis (Planchais et al., 2000).

SUMMARY

Herbicides with new MOAs that can be used as biochemical bioherbicides are badly needed for both conventional and organic agriculture. The structural diversity and evolved biological activities of natural compounds offer opportunities for the development of biochemical bioherbicides and synthetic herbicides based on the structures of natural phytotoxins. Natural phytotoxins are also a source of discovery of new herbicide target sites that can serve as the focus of traditional herbicide discovery efforts. The array of target sites of potent phytotoxins listed in Table I indicates that there may be no preferred target sites for phytotoxins in nature. The currently available information is probably skewed toward known target sites that were more easily determined. For example, it is very simple to determine if a compound inhibits PSII, but it is usually quite challenging to discover a new MOA. There are many natural phytotoxins for which the MOA is unknown. For example, the metabolomic profile in plants elicited by ascaulitoxin aglycone does not match any of the profiles elicited by phytotoxins with a broad array of known targets, indicating that this phytotoxin has a different, still unknown target (Duke et al., 2011). Thus, without considerably more information, no conclusions can be drawn about whether phytotoxins for particular targets are more common in nature.

MOA work with natural products can lead to the production of new tools for studying plant physiology and biochemistry (Dayan et al., 2010). For example, natural phytotoxins that interfere with different targets in mitosis have been invaluable for probing different aspects of this process (Vaughn and Vaughan, 1988; Planchais et al., 2000). Few natural phytotoxins have physicochemical properties that are optimal for direct application to weeds or soil in which weed seeds germinate (Tice, 2001), but there are exceptions, such as L-phosphinothricin and leptospermone. Structural modification of a natural compound can often improve its activity at the target site as well as the physicochemical properties required for adequate uptake, translocation, and environmental half-life. In most cases, the natural phytotoxins that we have discussed will kill plants at low doses, making it clear that there are herbicide target sites that can be added to the current repertoire of commercial herbicide molecular targets, provided that safe, efficacious, economical compounds can be found that target these sites.

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