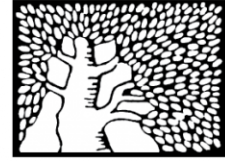


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1 **Catch-22 in Specialized Metabolism: Balancing Defense and Growth**

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11

12 **ABSTRACT**

13 Plants are unsurpassed biochemists that synthesize a plethora of molecules in response to everchanging
14 environment. The majority of these molecules considered as specialized metabolites, effectively protect the
15 plant against pathogens and herbivores. However, this defense most likely comes at a high expense, leading to
16 reduction of growth (known as the 'growth-defense tradeoff'). Plants employ several strategies to reduce the
17 high metabolic costs associated with chemical defense. Production of specialized metabolites is tightly
18 regulated by a network of transcription factors facilitating its fine-tuning in time and space. Multifunctionality
19 of specialized metabolites, their effective recycling system by re-using carbon, nitrogen and sulphur, thus, re-
20 introducing them back to the primary metabolite pool allows further cost reduction. Spatial separation of
21 biosynthetic enzymes and their substrates, sequestration of potentially toxic substances and conversion to less
22 toxic metabolite forms are plant's solutions to avoid the detrimental effects of metabolites they produce as
23 well as reduce production costs. Constant fitness pressure from herbivores, pathogens and abiotic stressors
24 lead to honing of specialized metabolite biosynthesis reactions to be timely, efficient and metabolic cost-
25 effective. In this review we assess the costs of specialized metabolites production for chemical defense and the
26 different plant mechanisms to reduce the price of of such metabolic activity in terms of self-toxicity and
27 growth.

28

29 **Key words:** Specialized metabolite, transport, self-toxicity, vacuole, growth/defense tradeoff, herbivore,
30 biosynthesis, regulation

31 **HIGHLIGHT**

32 This review is focused on the costs of plant chemical defense in terms of resource allocation and self-toxicity
33 in view of mechanisms for fine-tuning the “growth-defense tradeoff”.

34

35 **INTRODUCTION**

36 The unsurpassed ability of plants to synthesize a wide variety of low molecular weight compounds, largely
37 specialized (secondary) metabolites, facilitates their adaptation to a changing environment and battling natural
38 enemies (Wink, 2010; Kessler and Kalske, 2018). It is predicted that the plant kingdom can generate from two
39 hundred thousand to one million specialized metabolites (Wang *et al.*, 2019). These metabolites represent four
40 major structurally diverse classes; terpenoids, phenolic, alkaloids and sulphur-containing compounds
41 (Guerriero *et al.*, 2018). This huge diversity originates from basic metabolite skeletons, modified at different
42 positions through glycosylation, methylation, hydroxylation, oxidation, and additional reactions (Wang *et al.*,
43 2019). The elevation of defense responses in plants typically suppresses their growth; a phenomenon known in
44 ecology as the “growth-defense tradeoff” (Adler and Karban, 1994; Karban, 2019). In addition to metabolic
45 allocation costs (diversion of resources from growth and reproduction), a combination of other factors, such as
46 ecological, opportunity and storage costs need to be taken into account when considering growth and defense
47 (Purrington, 2000). The metabolic allocation cost in the “growth-defense tradeoff” is yet questionable as there
48 are more significant evidences for the opportunity and ecological costs and less evidence for actual tradeoffs
49 supporting metabolic costs (Agrawal *et al.*, 2012). In this review we will focus on potential metabolic costs of
50 chemical defence linking it to the penalty in terms of self-toxicity costs and mechanisms of recycling. In the
51 last decade, advances in molecular ecology facilitated validation of “growth-defence tradeoff” concept, using
52 transgenic and mutant plants, although precise calculations of metabolic costs remains elusive (Neilson *et al.*,
53 2013; Karasov *et al.*, 2017).

54 Estimation of the price tag for specialized metabolites production under different conditions is difficult due
55 to the complexity of the metabolic network as well as our limited knowledge with respect to metabolic fluxes
56 and yet unidentified pathways and reactions. The metabolic cost for chemical defense is primarily associated
57 with assimilation of carbon and/or nitrogen into specialized metabolite molecules (Havko *et al.*, 2016). The
58 biochemical nature of plant specialized metabolites makes them not only toxic for enemies but potentially
59 harmful to the plants themselves. In other words, plants can rather not overaccumulate defensive specialized
60 metabolites, as at a certain threshold they possess a negative impact on plant fitness. To overcome the toxic
61 effect of plant’s own metabolites, these chemicals accumulate to very high levels in specific tissues, organs,

62 cells and organelles. For example, several cyanogenic plants accumulate nearly 25-50% cyanogens in the
63 seedlings only, and in *Cannabis* spp. upto 25% cannabinoids accumulate in specific glandular trichomes
64 (Adewusi, 1990; Mahlberg and Kim, 2004; Livingston *et al.*, 2019). Conversely, some defensive metabolites
65 are specifically biosynthesized only when required. For example, glucosinolates and cyanogenic glucosides
66 don't really track the optimal defense theory given that their bipartite nature, means they need to be
67 accumulated prior to pathogen attack to protect the plant (Pedras and Yaya, 2015). This adds a significant bill
68 to the cost budget as such potentially cytotoxic molecules require sequestration and effect plant development
69 and growth (Shitan, 2016; de Brito Francisco and Martinoia, 2018). In consequence, plants possess several
70 solutions to avoid the harmful effects of metabolites they produce (Figure 1). Mechanisms to avoid self-
71 toxicity in plants include (i) sequestration to either the vacuole, extracellular space or specialized cells, (ii)
72 'trapping' metabolites in metabolons, (iii) translocation between the site of production and storage site; (v)
73 conversion to less toxic metabolite forms and (vi) separation between substrates and the corresponding
74 enzyme making a toxic product (Figure 1); [Samanani and Facchini, 2006; Møller, 2010; Jørgensen *et al.*,
75 2015; Shitan, 2016; Nour-Eldin *et al.*, 2017; Payne *et al.*, 2017; Takanashi *et al.*, 2017; Shitan and Yazaki,
76 2019].

77 To interpret mechanisms of plant self-toxicity avoidance the spatio-temporal pattern of biosynthesis and
78 regulation of a given specialized metabolite in the spatiotemporal manner. While the biosynthesis of
79 specialized defense metabolites is well-studied, limited information is available with respect to fine-tuning of
80 their organelle -or tissue-specific accumulation. No information is available on the maximum capacity of
81 specialized metabolism induction in certain tissues and response to stress. There are plenty of studies reporting
82 on different modes of regulation [i.e. transcriptional, post-transcriptional (e.g. miRNAs), translational and
83 post-translational] of plant specialized metabolites. Yet, the link between those modes is barely understood.

84

85 This review is focused on specialized metabolism in the context of the plant 'growth-defense tradeoff'. We
86 discuss resource allocation, compartmentalization, phytotoxicity prevention and regulation with respect to
87 different aspects of specialized metabolites production costs.

88

89 **Cost calculation of specialized metabolite production**

90 Limited resource allocation is the main assumption for the tradeoff between biosynthesis, transport and
91 storage of specialized metabolites at the expense of plant growth and development (Stamp, 2003). Throughout
92 the years attempts were made to identify and accurately calculate the budget allocation for chemical defense

93 during stress conditions (King *et al.*, 2006; Goodger *et al.*, 2006; Dahlgren *et al.*, 2009; Manzaneda *et al.*,
94 2010; Paul-Victor *et al.*, 2010; Penuelas *et al.*, 2010; Siemens *et al.*, 2010; Sampedro *et al.*, 2011; Züst *et al.*,
95 2011; Heinrich *et al.*, 2013). Accurate quantification of the metabolic cost possesses two major challenges: i)
96 the lack of an adequate experimental setup that can consider variations in environmental factors during the life
97 cycle of the plant, since it is increasingly difficult to dissect the role of a single environmental factor on a plant
98 fitness, at a certain time. and ii) the effect of natural selection on specialized metabolism, due to large intervals
99 of time that are necessary for evolutionary changes to occur (Strauss *et al.*, 2002; Paul-Victor *et al.*, 2010).
100 Carbon is considered a unit for metabolic cost assessment that is hypothetically associated with defense *vs.*
101 growth tradeoffs. Oversimplification of carbon budget for defense *vs.* growth dichotomy is challenging as the
102 mode of division in carbon flux to plant chemical defense pathways and growth physiologies is still unclear
103 (Weraduwage *et al.*, 2015).

104 Several methods exist that could be used to evaluate the costs of chemical defense. Proximal analysis is
105 used for cost assessment of specialized metabolites, synthesized from precursor primary metabolites (De Vries
106 *et al.*, 1974; Gershenzon, 1994). In this method, the total energy requirement for biochemical constituents of a
107 given tissue, including the cost of biosynthesis and transport is examined. Typically there is a high metabolic
108 cost for production of more reduced specialized metabolites such as triterpenoids as compared to
109 carbohydrates and phenolic compounds. It was reported that 3.1g and 1.5g glucose (in the form of acetyl-CoA,
110 ATP and NADPH) is required for the biosynthesis of the monoterpene camphor and phenol glycoside,
111 respectively (Gershenzon, 1994). Calorimetric analysis is another method for analyzing budget allocation,
112 where a calorimeter is used for the combustion of the tissue or whole plant. Although not as informative as
113 proximal analysis, it is a relatively simple approach which offers satisfactory information on total energy
114 stored in the tissue, but not on the energy contribution of each individual metabolite, thus both methods are
115 used for wide-range studies (Havko *et al.*, 2016). Miller *et al.* (2013) used calorimetric analysis to evaluate
116 alteration of carbon partitioning in lipid biosynthesis of *Arabidopsis*, yet, up to date, no report exists that
117 describes the use of this method to estimate budget allocation during growth-defense tradeoff and specialized
118 metabolism.

119 As compared to the limited use of the proximal and calorimetric methods, flux balance analysis (FBA) is
120 the most suitable and widely accepted approach for cost estimation of metabolite production. Construction of
121 *in-silico* metabolic network of an entire biochemical pathway is necessary for FBA, and is achieved by
122 combining gene expression data and information on chemical reactions from a particular biosynthetic
123 pathway. In recent years, several studies examined the use of complex network analysis to conduct predictive

124 modification of specialized metabolism and plant productivity (Mintz-Oron *et al.*, 2012; Fesenko and
125 Edwards, 2014; Liu and Stewart, 2015; Fuentes *et al.*, 2016; Töpfer *et al.*, 2017; Delfin *et al.*, 2019; Küken
126 and Nikoloski, 2019). FBA was extensively used to estimate nitrogen assimilation and allocation during
127 specialized metabolite biosynthesis. Stable isotope labeling using ¹⁵N revealed the cost of nitrogen allocation
128 in photosynthesis and specialized metabolism during growth vs. defense tradeoff (Ullmann-Zeunert *et al.*,
129 2012; 2013). Ullmann-Zeunert *et al.* (2013) reported that in *Nicotiana attenuata*, herbivory reduced nitrogen
130 assimilation in ribulose-1, 5-bisphosphate carboxylase/oxygenase (RuBisCO) and in total soluble proteins (by
131 approximately 90%). Specialized metabolite biosynthesis pathways are highly cross-connected with each other
132 by using the same precursor molecules. The major limitation of FBA is insufficient knowledge regarding the
133 function, localization and regulation of the entire set of enzymes in a single given metabolic pathway, making
134 it increasingly more difficult to link diverse pathways for specialized metabolites biosynthesis (Stavriniades *et al.*,
135 *et al.*, 2015; Wurtzel and Kutchan, 2016; Barros *et al.*, 2019). For example, phenylalanine serving as precursor
136 for numerous and diverse specialized metabolites was known for many years to be synthesized through a
137 plastidial pathway (Widhalm *et al.*, 2015). Recently, Qian *et al.* (2019) demonstrated an alternative, cytosolic
138 pathway for phenylalanine biosynthesis. A combined model using FBA with additional systems biology tools
139 [COBRA model and Systems Biology Markup Language (SBML)] (Rai *et al.*, 2017; Rowe *et al.*, 2018) as
140 well as kinetic models (Guo *et al.*, 2018) will likely provide more accurate estimation regarding the cost of
141 specialized metabolism production. Additionally, lack of fully annotated genome-scale metabolic network(s)
142 is the key problem for accurate estimation of the cost budget for specialized metabolism (Mintz-Oron *et al.*,
143 2012). For example, A genome-scale metabolic network reconstruction of tomato was developed by Yuan *et al.*,
144 *et al.* (2015) which mostly deals with photorespiratory metabolism, thus this network is inadequate for other
145 metabolic pathways. Providing a comprehensive metabolic network model that accounts for primary as well as
146 secondary metabolite pathways (ideally under changing environmental conditions) would greatly improve
147 understanding the costs associated with the use of primary megtabolites as precursors for specialized
148 metabolism.

149

150 **Logistics of specialized metabolism as a mechanism to reduce production costs by avoiding self-toxicity** 151 **and fine-tuning production in time and space**

152 Plants produce an array of active specialized metabolites that represent a ‘chemical language’ mediating
153 interaction with the environment (Isah, 2019; Tohge and Fernie, 2020; Erb and Kliebenstein, 2020). It is likely
154 that most of these metabolites are toxic to plant cells at certain concentration and as noted above regulated

155 compartmentalization is required for preventing such harmful effects. In addition to preventing toxicity,
156 regulated spatial and temporal localization of metabolites represents the means by which production and
157 accumulation is controlled and costs of production are reduced. Regulating the levels of metabolites in specific
158 compartments is complemented by localization of enzymes, protein complexes as well as their metabolite
159 precursors.

160 Localization to the vacuole was shown for several groups of potentially toxic specialized metabolites; e.g.
161 alkaloids (nicotine and berberine), glycoalkaloids, saponins, cyanogenic glycosides, and glucosinolates
162 (Saunders and Conn, 1978; Alcantara *et al.*, 2005; Otani *et al.*, 2005; Sirikantaramas *et al.*, 2007; Mylona
163 *et al.*, 2008; Payne *et al.*, 2017; Kazachkova *et al.*, 2021). However, only a handful of proteins were
164 experimentally shown to be associated with transport of self-toxic specialized metabolites across the tonoplast
165 membrane. To date NPF (nitrate-peptide transporter) (Payne *et al.*, 2017; Kazachkova *et al.*, 2021) and MATE
166 (multidrug and toxic compound extrusion) (Shoji *et al.*, 2009; Morita *et al.*, 2009) family transporters were
167 shown to participate in specialized metabolite transport to the vacuole (Shitan and Yazaki, 2019). Extensive
168 experimental data and reviews are available on the mechanisms of flavonoid transport (Goodman *et al.*, 2004;
169 Poustka *et al.*, 2007; Zhao and Dixon, 2010; Francisco *et al.*, 2013; Chanoca *et al.*, 2015; Behrens *et al.*,
170 2019). Though, no definite information is available on flavonoid self-toxic effects on plant cells. In addition to
171 transporter-mediated vacuolar import, ER-associated vesicular transport to the vacuole was shown for the
172 alkaloid sanguinarine in cultured opium poppy (*Papaver somniferum*) cells in response to elicitor treatment
173 (Alcantara *et al.*, 2005). Inability to sequester self-toxic specialized metabolites to the vacuole might result in
174 toxicity. The steroidal glycoalkaloid (SGA) exporter *GORKY* (*SINPF1.5*), excludes the tomato α -tomatine and
175 its derivatives from the vacuole to the cytosol during ripening, facilitating the conversion of the entire α -
176 tomatine pool to non-toxic and non-bitter forms (Kazachkova *et al.*, 2021). Overexpression of *GORKY* in
177 tomato leaves resulted in the accumulation of SGA pathway intermediates in the cytosol causing severe
178 toxicity symptoms in the plant. This observation clearly indicated that sequestration of SGAs in the vacuole
179 serves as a self-protection mechanism (Kazachkova *et al.*, 2021). Severe and largely similar phenotypes were
180 observed in tomato plants accumulating tomatidine (the α -tomatine aglycon) following down-regulation of
181 GLYCOALKALOID METABOLISM 1 (*GAME1*); a UDP-glycosyltransferase that carries out tomatidine
182 galactosylation (Itkin *et al.*, 2011).

183 In *Nicotiana tabacum*, nicotine transport at the tonoplast membrane is mediated by the MATE family
184 transporters NtJAT1, NtJAT2, NtMATE1, and NtMATE2 (Shoji *et al.*, 2009; Morita *et al.*, 2009). In addition
185 to the intracellular compartmentalization of nicotine, upon wounding or herbivore attack, its biosynthesis is

186 induced in root tissues and transported via xylem to leaves where it serves as an insecticide due to its
187 neurotoxic properties (Baldwin, 1989; Steppuhn *et al.*, 2004; Shitan *et al.*, 2014) (Figure 1). Berberine, a
188 renown alkaloid from *Coptis japonica*, is produced in the roots and translocated to rhizomes where it is
189 localized to the vacuole by the MATE family transporter, *CjMATE1* (Takanashi *et al.*, 2017). Several ABC
190 (B-type) transporters were shown to participate in relocation of berberine from roots to rhizomes via xylem
191 transport and through cytosolic membranes of the rhizome cells (Yazaki *et al.*, 2001; Shitan *et al.*, 2003, 2013;
192 de Brito Francisco and Martinoia, 2018).

193 *Catharanthus roseus* produces monoterpene indole alkaloids via an intricate pathway that comprises
194 multiple reactions distributed between several cell types and involving intra- and intercellular translocation of
195 pathway intermediates (Verma *et al.*, 2012; Courdavault *et al.*, 2014; De Luca *et al.*, 2014; Qu *et al.*, 2019).
196 To date, close to 30 genes involved in the pathway are known (Qu *et al.*, 2019), including several transporters.
197 *CrTPT2* encodes a plasma membrane exporter that re-locates catharanthine to the leaf surface (Yu and De
198 Luca, 2013). *CrNPF2.4*, *CrNPF2.5* and *CrNPF2.6* showed transport activity in *Xenopus laevis* oocytes against
199 the iridoid glycosides and are localized to the plasma membrane suggesting a function in intercellular
200 transport from the apoplast to the cytosol (Larsen *et al.*, 2017). The *CrNPF2.9* transporter was shown to export
201 indole alkaloid strictosidine from leaf vacuoles (Payne *et al.*, 2017). Down-regulation of *CrNPF2.9* using
202 virus-induced gene silencing (VIGS) lead to increased accumulation of strictosidine in the vacuole and
203 simultaneous decrease in downstream alkaloids. *NPF2.9*-silenced leaves showed symptoms of toxicity and
204 cell death indicating that disproportionate accumulation of strictosidine may be self-toxic to the plant (Payne
205 *et al.*, 2017) (Figure 1).

206 Several cannabinoids in *Cannabis sativa*, including tetrahydrocannabinolic acid (THCA), cannabigerolic
207 acid (CBGA) and cannabichromenic acid (CBCA) were shown to induce cell death of tobacco BY-2 and
208 *Cannabis* leaf cells causing severe mitochondrial damage (Sirikantaramas *et al.*, 2007; Morimoto *et al.*, 2007).
209 Therefore to avoid self-damage, cannabinoid biosynthesis is exclusively reserved to glandular trichomes
210 (Sirikantaramas *et al.*, 2005). Moreover, extracellular production of toxic THCA in the storage cavity of
211 glandular trichome acts as a mechanism to avoid self-toxicity (Sirikantaramas *et al.*, 2007).

212 Assembly of biosynthetic enzymes in metabolons serves as additional mechanism of avoiding cytotoxicity
213 of metabolic pathway intermediates (Winkel, 2004; Laursen *et al.*, 2016; Obata, 2019). Rapid channeling of
214 reaction products between catalytic sites of enzymes united into a metabolon facilitates their accumulation in
215 higher concentrations and quick turnover to more stable and less toxic metabolites without damaging the cells
216 (Winkel, 2004). It is anticipated that biosynthetic enzymes of many specialized metabolites such as alkaloids,

217 flavonoids, cyanogenic glycosides and possibly others are organized in metabolons (Hemm *et al.*, 2003;
218 Winkel, 2004; Jørgensen *et al.*, 2005; Møller, 2010; Weis *et al.*, 2014). Biosynthesis of dhurrin, the
219 tyrosine-derived cyanogenic glucoside from *Sorghum bicolor* seedlings, is carried out in a metabolon,
220 facilitating rapid conversion of labile highly toxic aldoxime intermediates to more stable and less toxic ones
221 (Jørgensen *et al.*, 2005; Sakurada *et al.*, 2009; Laursen *et al.*, 2016; Obata, 2019). Clustering of dhurrin
222 enzymes into metabolon facilitated engineering of the whole pathway in Arabidopsis without significant
223 adverse effects on the plant (Tattersall *et al.*, 2001; Kristensen *et al.*, 2005) indicating that metabolon
224 formation effectively encapsulates toxic intermediates in heterologous systems.

225 Another mechanism to avoid self-toxicity is separation of potentially self-toxic metabolites and the
226 enzymes that can cause metabolite breakdown to toxic products. However, when plant cells are wounded or
227 attacked by herbivores, cellular compartmentalization disintegrates; the enzymes and the substrate(s) come in
228 contact and finally release toxic defense compounds. For example, dhurrin, stored in the vacuoles in intact
229 tissues is hydrolyzed by a chloroplastic β -glucosidase (dhurrinase), forming a cyanohydrin that is further
230 cleaved by the cytosolic hydroxynitrile lyase to form aldehyde or ketone and highly toxic HCN (Morant *et al.*,
231 2008; Clausen *et al.*, 2015; Laursen *et al.*, 2016). Similarly in the case of glucosinolates, thioglucosides found
232 in Brassicaceae, and their hydrolytic enzymes, myrosinases (specific class of β -thioglucosidases), are stored in
233 separate cells. Upon wounding, myrosinases come in contact with glucosinolates, resulting in the production
234 of biologically active isothiocyanates, nitriles, and thiocyanates (Rask *et al.*, 2000; Halkier and Gershenzon,
235 2006; Winde and Wittstock, 2011) (Figure 1).

236

237 **Reduce, reuse and recycle in plant specialized metabolism.**

238 Biosynthesis of a specialized metabolites requires significant investment in energy and resources (Neilson *et*
239 *al.*, 2013). Thus, in addition to tight regulation of production, recycling of superfluous specialized metabolites
240 back into the primary metabolite pool, minimizes production costs (Neilson *et al.*, 2013; Erb and Kliebenstein,
241 2020). Throughout the life cycle, the demand of plants for defensive specialized metabolites is dynamically
242 changing and remobilization into the primary metabolite pool serves as a strategy to reuse the available
243 resources and reduce energy costs. Although evidence exists that plants recycle specialized metabolites,
244 through channeling of degradation products into the primary metabolite pool, it is difficult to estimate the
245 contribution of these degradation products to the primary metabolite content. To date, cyanogenic glucosides
246 recycling and reuse as a nitrogen source (without release of toxic HCN), is probably the best studied example
247 (Selmar *et al.*, 1988; Jenrich *et al.*, 2007; Pičmanová *et al.*, 2015) (Figure 2). Comparative metabolite profiling

248 of three plant species producing cyanogenic glycosides (i.e. almond, cassava and sorghum), pointed to a
249 similar turnover pathway leading to re-assimilation of nitrogen and carbon into the primary metabolite pool
250 (Pičmanová *et al.*, 2015). In *Sorghum bicolor*, three members of the NITRILASE 4 (NIT4) family, NIT4A,
251 NIT4B1, and NIT4B2 were shown to form heterodimeric complexes that can effectively detoxify dhurrin
252 degradation products thereby replenishing the pool of nitrogen in the plants and simultaneously avoiding
253 formation of toxic HCN (Jenrich *et al.*, 2007) (Figure 2). Similarly in Arabidopsis, three nitrilase isoenzymes
254 are postulated to participate in glucosinolate turnover by conversion of nitriles to carboxylic acids (Vorwerk *et*
255 *al.*, 2001). Sulphur-containing glucosinolates could potentially serve as an additional source of sulphur for
256 plants. Arabidopsis *glucosinolate transporters (gtr1gtr2)* double knockout plants fail to transport
257 glucosinolates to reproductive tissues and the seedlings exhibit lower biomass under sulphur-deficient
258 conditions (Nour-Eldin *et al.*, 2012, 2017). Legumes accumulate a non-protein amino acid, *L*-canavanine, that
259 is degraded during seed germination, providing the developing seedling with additional source of carbon and
260 nitrogen (Rosenthal, 1990).

261 Anthocyanin biosynthesis is well studied, however, not much is known about anthocyanin degradation and
262 its potential role as an additional carbon source for plants (Liu *et al.*, 2018). In *Brunfelsia calycina* flowers are
263 rapidly changing color from purple to white in course of time due to anthocyanin degradation, that was shown
264 to require *de novo* mRNA and protein synthesis (Vaknin *et al.*, 2005). However, it remains elusive whether
265 and to what extent such anthocyanin degradation products get re-incorporated into the primary metabolite pool
266 in anthocyanin producing plants.

267

268 **How much is too much? Constitutive vs. induced specialized metabolism**

269 During their life cycle plants withstand a remarkable diversity of potential threats, of both biotic and
270 abiotic origin; specialized metabolite production is one of the strategies they employ to survive in this rapidly
271 changing environment (Karban and Baldwin, 1997; Heil and Baldwin, 2002). Specialized metabolites perform
272 their key function in protection from pathogens and herbivores through several strategies including i) direct
273 toxicity to herbivores and pathogens ii) attraction of natural enemies of the herbivores, iii) repellent action and
274 iv) reduction of tissue digestibility and nutritional profile (Kessler, 2015). Their production can be either
275 constitutive, irrespective of herbivore threat presence, or inducible, when biosynthesis occurs only in response
276 to herbivory (Heil and Baldwin, 2002). In response to the stressor, changes in both primary and specialized
277 metabolism occur that not only induces plant resistance against the herbivore, but also simultaneously
278 transmits the information to the neighboring organisms, mostly by volatile organic compounds (Heil and

279 Baldwin, 2002; Heil and Karban, 2010; Kessler and Kalske, 2018). Interestingly, plants that were not
280 previously exposed to the herbivores had a lower baseline of specialized metabolite production levels
281 (Agrawal *et al.*, 2012). Although direct comparison of metabolic costs upon constitutive *vs.* induced
282 specialized metabolism is difficult to perform, several studies have shown that the costs of constitutive
283 production of defense compounds is rather similar to retaining early herbivory endogenous recognition
284 signaling pathways that mediate herbivore-induced metabolic responses (Steppuhn *et al.*, 2008; Abreu and
285 Munné-Bosch, 2009). Additional factors need to be taken into account in such calculations; such as the
286 multiple roles of plants specialized metabolites (Erb and Kliebenstein, 2020). For instance, glucosinolates and
287 benzoxacioids, well-known defense compounds, were shown to have a role in regulating accumulation of
288 other classes of specialized metabolites (Kim *et al.*, 2015; Zhou *et al.*, 2018; Li *et al.*, 2018). The possible
289 mechanism of crosstalk between the glucosinolate and phenylpropanoid pathways is related to the degradation
290 of the enzyme phenylalanine ammonia-lyase catalyzing the first step of the phenylpropanoid biosynthesis
291 pathway (Kim *et al.*, 2020).

292 To date, three plant defense theories exist that provide theoretical background explaining specialized
293 metabolites accumulation patterns for plant fitness. The moving target hypothesis indicates that the change in
294 specialized metabolite production in response to herbivore attack leads to a change of the plant biochemical
295 phenotype which itself serves as a defense strategy (Adler and Karban, 1994; Kessler, 2015). The information
296 transfer hypothesis suggests that herbivore-induced specialized metabolite production also serves as chemical
297 cues that plants transmit to their environment (neighboring plants, insects, bacteria, etc) (Kessler, 2015). The
298 optimal defense hypothesis states that in the absence of herbivores, plant overall specialized metabolite
299 accumulation should be low, therefore allowing the plants to produce costly metabolites only when needed
300 (McKey, 1974; Denno, 2012). Optimal defense theory is used to predict the spatial distribution of specialized
301 metabolite levels in plant tissues, depending on how valuable certain tissues are to the plant as a whole. Plant
302 reproductive tissues, like fruit and flowers typically accumulate significant amounts of specialized metabolites
303 (Howe and Jander, 2008). Similarly young leaves are more valuable to the plant therefore contain higher
304 levels of protective metabolites (Darrow and Bowers, 1999; Kozukue *et al.*, 2004). Several studies also
305 compared the spatial allocation of specialized metabolite induction in response to above- and belowground-
306 herbivores (Bezemer *et al.*, 2004; Bezemer and van Dam, 2005; Kaplan *et al.*, 2008). Intuitively, specialized
307 metabolites are likely to accumulate in specific tissues based on the possible risk of future attack. Indeed, in *N.*
308 *tabacum*, leaf-chewing insects elicited strong response in leaf tissues but had no impact on roots (Kaplan *et*

309 *al.*, 2008). Interestingly plant responses to root herbivory did not follow the same pattern; root nematodes
310 caused changes in specialized metabolome of both, roots and shoots in tobacco plants.

311 In addition to the biotic stress, abiotic factors also have a significant impact on the accumulation of several
312 classes of specialized metabolites (Dixon and Paiva, 1995; Yang *et al.*, 2018; Li *et al.*, 2020). A number of
313 studies focused on the alteration to individual specialized metabolite or an isolated class of molecules in
314 response to one or several changing environmental conditions (Yang *et al.*, 2018; Li *et al.*, 2020). Although
315 these studies provide valuable information, they have significant limitations since analyzing changes in
316 controlled environment may not provide adequate representation of the processes occurring in a rapidly
317 changing natural environment (Mittler, 2006; Zandalinas *et al.*, 2020). Harmonized regulation of resource
318 allocation between plant growth and defense is crucial for plant fitness nature and are primarily carried out by
319 phytohormone crosstalk.

320 **Controlling the balance between growth and defense**

321 The activation of multifaceted immunity demands nutrient resources to support the biosynthesis of
322 defense metabolites; such resource utilization frequently suppresses normal growth and development. To
323 ensue such trade-offs between growth and defense, plants control carbon fluxes between primary and
324 specialized metabolism (Cheyner *et al.*, 2013). Radiolabeling studies showed that biotic stress alters
325 normal metabolic flux of carbon or nitrogen to allow the integration of these resources into defense
326 specialized metabolites (Engelsdorf *et al.*, 2013; Ullmann-Zeunert *et al.*, 2013). The molecular
327 mechanisms controlling this trade-off involve a vast number of different factors including hormones
328 such as salicylic acid (SA) and jasmonic acid (JA) (Wasternack and Strnad, 2019; Ali, 2020). Biotic
329 stress responses are carried out either by systemic acquired resistance (SAR) or induced systemic
330 resistance (ISR), depending on the site of induction and the nature of the pathogen (Vlot *et al.*, 2020).
331 SAR triggers a broad-spectrum of disease resistance in uninfected tissues in response to a local
332 infection, which plays a crucial role in balancing growth and immunity of the plant (van Butselaar and
333 Van den Ackerveken, 2020). Accessibility of carbon was shown to be critical for SA-regulated defense.
334 Previous work on a starch-free mutant of *Arabidopsis* showed delayed production of SA-regulated
335 camalexin, resulting in increased susceptibility to the hemi-biotrophic pathogen *Colletotrichum*
336 *higginsianum* (Engelsdorf *et al.* 2013).

337 In addition to SA, N-hydroxy-pipecolic acid (NHP), a lysine derivative, was shown to act as another
338 key signal for SAR in diverse plants (Chen *et al.*, 2018; Hartmann and Zeier, 2018; Homes *et al.*, 2019).

339 Chen *et al.* (2018) reported that NHP can be detected in an O-glucosylated conjugated form (NHPG)
340 upon biotic stress suggesting that its activity might be at least in part controlled by the sugar substitution.
341 Overaccumulation NHP in Arabidopsis leaves results in a dwarf phenotype that correlates with
342 constitutive defense responses in Arabidopsis. In contrast, overaccumulation of NHPG displays more
343 vigorous growth phenotype but hypersusceptibility to pathogen infection (Cai *et al.*, 2021). Recent work
344 by Cai *et al.* (2021), highlighted the importance of the NHP to NHPG ratio in the growth-defense trade-
345 off. According to their model, at a certain NHP threshold level, UGT76B1 glycosylates NHP attenuating
346 its activity and decreases SAR response, thus reestablishing normal plant growth and development
347 (Figure 3).

348 In contrast to SAR, ISR response is predominately regulated by JA (Van der Ent *et al.*, 2009). In
349 response to necrotrophic pathogens or phytophagous insects, JA biosynthesis is increased leading to
350 production of an array of defense compounds, along with a strong growth inhibition (Havko *et al.*,
351 2016). Previous work in *N. attenuata* showed the function of the JA-signaling pathway is re-allocation
352 of nitrogen from RuBisCO into nicotine and phenolamide compounds during plant-herbivore
353 interactions (Ullmann-Zeunert *et al.*, 2013). JA signaling activates the biosynthesis of several classes of
354 specialized metabolites including nicotine, artemisinin, terpenoid indole alkaloids, withanolides and
355 SGAs (Chen *et al.*, 2019; Wasternack and Strnad, 2019; Goklany *et al.*, 2013; Sharma, *et al.*, 2019, Min
356 *et al.*, 2020). In contrast, constitutively active JA signaling leads to suppressed plant growth (Campos *et al.*,
357 2016; Guo *et al.*, 2018; Major *et al.*, 2020). The coordination of chemical defense and plant growth
358 is primarily achieved through crosstalk between JA and gibberellic acid (GA) signaling pathways. It
359 involves MYC2, the JA signaling repressor JAZ and the GA response repressor DELLA (Navarro *et al.*,
360 2008) (Figure 3). Hong *et al.* (2012) reported that in Arabidopsis upregulation of DELLA sequesters the
361 JA responsive MYC2 protein and this results in transcriptional suppression of the *TPS21* and *TPS11*
362 sesquiterpene synthase genes. Loreti *et al.* (2008) found that JA signaling positively and GA metabolism
363 negatively regulate anthocyanin biosynthesis in Arabidopsis. In *N. attenuata* a pair of calcium-
364 dependent protein kinases (CDPK4 and CDPK5) were found to regulate specialized metabolism by
365 suppression of JA biosynthesis, and at the same time modulating plant growth through interaction with
366 core GA biosynthetic enzymes (GA20-OXIDASE and possibly GA13-OXIDASE) (Heinrich *et al.*,
367 2013). Additionally, Machado *et al.* (2013; 2017) demonstrated that herbivore induced nicotine
368 biosynthesis in *N. attenuata* led to strong growth suppression, while GA complementation rescued plant
369 growth. In tomato, the JA perception *jai1* mutant possesses low SGAs levels (Abdelkareem *et al.*, 2017).

370 Schubert *et al.*, (2019) showed that the tomato *jail* mutant has lower JA, but higher GA levels as
371 compared to wild-type plant.

372 Arabidopsis *della* mutants exhibited elevated GA response, but reduced JA level (Cheng *et al.*,
373 2009). Pauwels and Goossens (2011) suggested DELLA-mediated modulation of JA responses in
374 Arabidopsis following competition with MYC2 for JAZ binding. In Arabidopsis, low GA levels allows
375 DELLA to bind with JAZ consequently enabling MYC2 mediated transcription of JA response genes
376 (Hou *et al.*, 2010). JAZ proteins inhibit MYC2 activity not only by recruiting corepressors but also by
377 blocking the binding of MYC2 to target promoters (Pauwels and Goossens, 2011). In Arabidopsis,
378 MYC2 was shown to transcriptionally regulate the expression of one of the *DELLA* gene (*RGL3*) to
379 modulate plant growth (Wild *et al.*, 2012) (Figure 3). Additionally, Yang *et al.*, (2012) reported that
380 active JA signaling interfere with GA-mediated DELLA degradation in Arabidopsis. Wang *et al.* (2020)
381 identified diverse DELLA proteins throughout the plant kingdom that are possibly responsible for
382 restricting plant development. JA-mediated growth suppression was likely oversimplified as most of the
383 focus was on DELLA-dependent growth inhibition (Yang *et al.*, 2012; Davie`re and Achard, 2016; Jang
384 *et al.*, 2020). However, recently Major *et al.* (2020) demonstrated that in Arabidopsis, JAZ mutations
385 inhibit plant growth likely independent of DELLA. Thus, the molecular mechanism of JAZ-MYC2-
386 DELLA-mediated JA-GA crosstalk and regulation of the growth vs. defense tradeoff remains
387 controversial.

388 Apart from MYC2, other TFs such as MYB21, MYB24 and PHYTOCHROME INTERACTING
389 FACTORS (PIFs) were shown to interact with DELLAs, and might play a role in the crosstalk between
390 the JA and GA signaling pathways (De Lucas *et al.*, 2008; Feng *et al.*, 2008; Hong *et al.*, 2012; Li *et al.*,
391 2016; Pham *et al.*, 2018; Huang *et al.*, 2020). In Arabidopsis, PIFs were found to regulate the JA-GA
392 crosstalk either by reducing JA-Ile biosynthesis or by competing with DELLA for JAZ binding,
393 consequently modulating MYC2 activity (Li *et al.*, 2016; Pierik and Ballaré, 2020; Fernández-Milmanda
394 *et al.*, 2020) (Figure 3). In a recent study, isoprene (i.e. 2-methyl-1,3-butadiene) was found to pair GA-
395 mediated growth regulation and JA-mediated defense responses in *Eucalyptus globulus* by the
396 modulation of JAZ, MYC2, DELLA and PIFs (Zuo *et al.*, 2019). In transgenic poplar, heterologous
397 overexpression of Arabidopsis *DELLAs* lead to compromised plant growth, due to reduced carbon flux
398 through lignin metabolism (Busov *et al.*, 2006; Ribeiro *et al.*, 2012).

399 Apart from the JA-GA crosstalk, JA interrelates with the biosynthesis of growth-promoting
400 brassinosteroids (BRs; Ren *et al.*, 2009). Kim *et al.* (2013) reported that JA treatment can represses

401 transcript accumulation of DWARF4, a key BRs biosynthesis enzyme, in a CORONATINE
402 INSENSITIVE 1 (COI1)-dependent manner. Previous reports demonstrated that two BRs responsive
403 transcription factors BRASSINAZOLE-RESISTANT 1 (BZR1) and BRI1-EMS-SUPPRESSOR 1
404 (BES1) can interact with DELLA, PIF and MYB proteins, hence modulating Arabidopsis glucosinolate
405 metabolism (Guo *et al.*, 2013; Bruyne *et al.*, 2014; Liao *et al.*, 2020). Taken together, –hormone
406 signaling cascade coordinately modulates biosynthesis of plant specialized metabolites and consequently
407 suppresses plant growth (Figure 3).

408 **The role of specialized metabolites in the regulation of the plant defense vs. growth tradeoff**

409 Hormone-mediated regulation of the defense vs. growth tradeoff has been studied extensively (Huot *et al.*,
410 2014; Guo *et al.*, 2018). Yet, specialized metabolites and/or their breakdown products were also
411 found to participate in the regulation of plant development and might take a direct part in balancing such
412 tradeoff. Malinovsky *et al.* (2017) showed that 3-hydroxypropylglucosinolate fine-tunes the target of
413 rapamycin pathway in Arabidopsis to suppress root growth. Although the exact target of 3-
414 hydroxypropylglucosinolate is still unknown, indole-3-carbinol, a breakdown product of glucosinolate
415 was reported to bind the auxin receptor TRANSPORT INHIBITOR RESPONSE 1, and regulate auxin-
416 mediated root growth. Furthermore, the Arabidopsis auxin repressors (auxin-responsive protein; IAA5,
417 IAA6, and IAA19) were found to regulate 4-(methylsulfinyl)butylglucosinolate (4-MSO; glucoraphanin)
418 level and consequently control stomatal aperture (Salehin *et al.*, 2019). Khokon *et al.*, (2011)
419 demonstrated the role of the glucosinolate breakdown product allyl isothiocyanate in stomatal closure
420 through ROS production. In addition to stomatal closer, 4-MSO was found to regulate the circadian
421 clock and flowering time in Arabidopsis by transcriptional regulation of circadian genes (Kerwin *et al.*,
422 2011).

423 Apart from glucosinolates, flavonoids are also known to regulate plant growth by modulating auxin
424 transport. The Arabidopsis *transparent testa 4* mutant possesses elevated auxin transport and increased
425 ROS levels in the guard cells, which can be recovered by the addition of naringenin, a flavonoid
426 precursor (Murphy *et al.*, 2000; Brown *et al.*, 2001). Several studies demonstrated the mechanistic link
427 between flavonoid biosynthesis, auxin transport, ROS accumulation and plant growth (Peer and
428 Murphy, 2007; Santelia *et al.*, 2008; Hernández *et al.*, 2009; Muhlemann *et al.*, 2018). According to Erb
429 and Kliebenstein (2020), flavonoids could be the possible signaling molecules as they are reported to
430 change the oxidative state of cells by ROS activation which can modify the disulfide bridge formation

431 between partner proteins of cell signaling. In addition to flavonoids, β -carotene derived β -cyclocitral is
432 known to regulate root stem cell behavior in diverse plant species (Dickinson *et al.*, 2019). Recently
433 Mitra *et al.* (2021) found that herbivore interaction in *Arabidopsis* induces β -cyclocitral-mediated volatile
434 signal which elevates plant resistance but inhibits the MEP pathway that is significant for plant growth.
435 The direct involvement of specialized metabolism in growth suppression and the growth-defense
436 tradeoff is not yet clear and needs to be critically examined.

437

438 **CONCLUDING REMARKS**

439 The concept of secondary metabolism was first described by Albrecht Kossel, Nobel Prize winner for
440 physiology and medicine in 1910 (Jones, 1953). From that time, the advancement of analytical, genetic and
441 molecular tools facilitated understanding and manipulation of specialized metabolite production in a large
442 number of plant species. A large body of information was collected with respect to biosynthesis, transport and
443 regulation of different classes of specialized metabolites. There are a few reports from Baldwin and his
444 colleagues (Zavala and Baldwin, 2004; Zavala *et al.*, 2004) that assessed the fitness costs and benefits in
445 tobacco under control as well as during herbivory event. Baldwin and colleagues showed that the plants
446 lacking trypsin protease inhibitors (TPIs) were more vigorous compared to the TPI-producing genotypes,
447 indicating the high costs of TPI production to the plant (Zavala and Baldwin, 2004; Zavala *et al.*, 2004).
448 However, during herbivory the benefits of TPI biosynthesis are becoming higher than the costs (Zavala *et al.*,
449 2004). However, additional studies are necessary to investigate the role of TPIs in the control of primary
450 metabolism and plant fitness. For instance, it was shown that PIF factors regulate specialized metabolism in
451 addition to light-regulated development, but do specialized metabolites have any effect on modulation of the
452 plant photosynthetic capacity? Additionally, numerous reports describe the “growth-defense tradeoff”,
453 predicting the plant ‘budget’ for specialized metabolites. Yet, it is difficult and currently impossible to
454 integrate the ‘cost’ of multiple stresses imposed constantly on plants in an everchanging environment.

455 A promising approach to induced resistance in agriculture is achieved by administering 4-
456 fluorophenoxyacetic acid, a synthetic plant strengthener, that induces formation of flavonoid polymers in plant
457 cell walls suppressing the sap-sucking insect population and therefore increasing the yield without
458 compromising plant growth (Wang *et al.*, 2020). However, from a breeder’s point of view, the ultimate goal of
459 research into the “growth-defense tradeoff” and budget allocation, is to develop cultivars that can be
460 simultaneously high yielding and resistant to a broad spectrum of plant’s natural enemies. Nevertheless, the

461 current research points to mutual exclusiveness of growth and defense, thus, it seems difficult to identify a
462 “soft spot” and obtain plants that would be optimized for sustainable agriculture.
463

464 **DATA AVAILABILITY**

465 No experimental data is associated with this review.

466

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475

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478

479 **LITERATURE**

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966 **Figure legends**

967 **Figure 1. Mechanisms to reduce costs of specialized metabolites production by controlling biosynthesis**
968 **and avoiding self-toxicity of intermediates and final products. A,** Metabolon formation facilitates rapid
969 channeling of reaction products between catalytic sites of enzymes, allowing their quick turnover to more
970 stable and less toxic metabolites without damaging cells. Schematic representation of the *Sorghum bicolor*
971 metabolon involved in dhurrin biosynthesis (Laursen *et al.*, 2016). It is comprised of two cytochrome P450
972 isoforms (CYP79A1 and CYP71E1), NADPH-dependent cytochrome P450 oxidoreductase (POR) and a
973 soluble glycosyltransferase (UGT85B1). **B,** In *N. tabacum*, nicotine biosynthesis enzymes are localized in
974 roots, and nicotine is transported via the xylem to leaves and acts as an insecticide. **C,** Sequestration to the
975 vacuole was reported for several groups of specialized metabolites. Known exporter and importer proteins and
976 their target molecules are shown. *Cj*, *Coptis japonica*, *Cr*, *Catharanthus roseus*, *Nt*, *Nicotiana tabacum*, *Sb*,
977 *Sorghum bicolor*, *Sl*, *Solanum lycopersicum*. Additionally, ER-associated vesicular transport to the vacuole is
978 depicted for the alkaloid sanguinarine produced by *Papaver somniferum*. **D,** Spatial separation of potentially
979 self-toxic metabolites and the enzymes that catalyze metabolite breakdown to toxic products. Upon wounding
980 and herbivore attack damaged cells containing enzymes and substrates join and release toxic defense
981 compounds. The glucosinolate–myrosinase defense system is found in plants of the *Brassicaceae* family.
982 Glucosinolates and myrosinases, their hydrolytic enzymes (specific class of β -thioglucosidases), are stored in
983 separate cells in intact tissues. Upon tissue disruption, myrosinases are encounter and act on glucosinolate
984 substrates, forming an unstable aglycone, that, depending on pH and other conditions is converted to nitriles
985 and potent products. ESP, epithio specifier protein. For additional information, see Rask *et al.*, (2000). **E,** In *C.*
986 *sativa* toxic cannabinoids are produced in the storage cavity of glandular trichomes.

987
988 **Figure 2. Biological activation, detoxification and recycling of the cyanogenic glycoside dhurrin in**
989 ***Sorghum bicolor*. Bioactivation.** In response to wounding or herbivore attack, dhurrin is hydrolyzed by a
990 specific β -glucosidase, dhurrinase (DHR), forming an unstable cyanohydrin, which releases toxic HCN, either
991 spontaneously or mediated by the enzyme hydroxynitrile lyase (HNL). **Detoxification.** HCN is detoxified by
992 formation of β -cyanoalanine (by β -Cyanoalanine synthase; CAS) that can be converted to Asn, Asp and
993 ammonia by heteromers of nitrilases A and B (NIT4A/B). **Recycling.** In Dhurrin, either spontaneously or
994 catalyzed by an unknown glutathione transferase (GST), glucose is replaced by a glutathione moiety. This
995 results in formation of a glutathione conjugate of *p*-hydroxyphenyl acetonitrile (GS-pOHPACN). It is next
996 cleaved by GSTs converting reduced GSH to its oxidized form (GSSG) and releasing GS-pOHPACN. NIT4

997 heteromers catalyze hydrolysis of *p*-hydroxyphenyl acetonitrile to *p*-hydroxyphenylacetic acid and free
998 ammonia that can be reincorporated into the primary metabolite pool. For additional information, see
999 Bjarnholt *et al.*, (2018).

1000

1001 **Figure 3. A schematic representation of the crosstalk between hormone signaling pathways and their**
1002 **roles in chemical defense vs plant growth strategies.** Transcriptional activation is highlighted in green,
1003 while inhibition in red. Broken lines indicate indirect manipulation of processes or unknown mechanisms.
1004 [GA, gibberellic acid; JA-Ile, jasmonate-isoleucine; SA, salicylic acid; BR, brassinosteroid; COI1,
1005 CORONATINE INSENSITIVE 1 receptor protein; JAZ, JASMONATE ZIM DOMAIN proteins; GID1,
1006 GIBBERELLIN INSENSITIVE DWARF1 receptor protein, SLY1, SLEEPY1; PIFs, PHYTOCHROME
1007 INTERACTING FACTOR proteins; BZR1, RASSINAZOLE-RESISTANT 1 protein; BES1,
1008 BRASSINAZOLE-RESISTANT 2/BRI1-EMS-SUPPRESSOR 1 protein; HY5, LONG HYPOCOTYL 5;
1009 PAP1, PRODUCTION OF ANTHOCYANIN PIGMENT1; NPR1, NONEXPRESSOR OF
1010 PATHOGENESIS-RELATED GENES 1; NHP, N-hydroxy-pipecolic acid; NHPG, O-glucosylated N-
1011 hydroxy-pipecolic acid]. The GLYCOALKALOID METABOLISM 9 (GAME9) TF is known to work
1012 synergistically with MYC2, to transcriptionally activate SGA biosynthetic genes (Cárdenas *et al.*, 2016).
1013 Similarly, anthocyanin pigment 1 (PAP1)/MYB75 from Arabidopsis is known to transcriptionally activate
1014 anthocyanin biosynthesis genes (Shin *et al.*, 2015).

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