Trends in Plant Science

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Opinion

Glyphosate-Modulated Biosynthesis Driving Plant Defense and Species Interactions

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Glyphosate has become the best-selling herbicide used in agriculture, horticulture, silviculture, and urban environments. It disrupts the shikimate metabolic pathway and thereby blocks the production of aromatic amino acids, which are the basis for several plant metabolites. Glyphosate residues are reported in soils from diverse environments, but the effects on plant physiology and consequences for species interactions are largely unknown. Here, we emphasize the complexity of these physiological processes, and argue that glyphosate residues modulate biosynthetic pathways, individually or interactively, which may affect interactions between plants and heterotrophic organisms. In this way, glyphosate residues can substantially interfere with plant resistance and the attraction of beneficial insects, both of which are essential elements in integrated pest management and healthy ecosystems.

Highlights

Residues of glyphosate, a potent herbicide, are increasingly found in diverse environments.

Glyphosate residues in soil alter plant physiological processes; however, their effects on plant defense strategies and subsequent species interactions are poorly understood.

Glyphosate inhibition of the shikimate pathway affects plant defense-related phytohormones and the phenylpropanoid pathway, the basis for many phytoalexins.

Glyphosate affects lipid peroxidation, which alters jasmonic acid levels and green leaf volatiles, in turn, important players mediating many trophic interactions and pollinator attraction.

By changing the plant biochemistry, glyphosate residues in soil have the potential to alter plant interactions with herbivores and mutualistic organisms.

Glyphosate Residues Affect Plant Defense Compound Biosynthesis

Glyphosate [N-(phosphonomethyl)-glycine], a synthesized broad-spectrum systemic herbicide, is the most widely used herbicide globally [1]. Glyphosate inhibits 5-enolpyruvylshikimate-3-phosphate synthase (**EPSPS**) (see Glossary), which is an essential enzyme in the **shikimate pathway**, catalyzing the conversion of shikimate-3-phosphate to 5-enolpyruvylshikimate-3-phosphate [2]. The shikimate pathway produces chorismate, the central precursor molecule for the production of essential aromatic amino acids in plants [3] (Figure 1, Key Figure). While aromatic amino acids are the basis of proteins, they are also precursors to many plant metabolites [4]. Their absence will lead to plant death. Although glyphosate is believed to be safe for non-target organisms, due to its mode of action and presumed rapid degradation in soils [5], an increasing number of empirical studies have shown negative effects on various non-target organisms [6–8]. Furthermore, recent studies indicate that glyphosate can persist and spread in diverse habitats, and that these residues can have a negative impact on non-target plants and other organisms [7,9,10]. Such exposure of plants to sublethal doses of glyphosate residues in the soil has an important and underappreciated consequence. Sublethal doses of glyphosates affect plant physiological processes [11], with subsequent effects on the plant's interactions with its biotic environment. This means that glyphosate can not only have direct effects on non-target organisms, but also indirect effects that are mediated by glyphosate-induced changes in the phenotype of plants, that have cascading effects on non-target organisms with which they interact (Box 1). In this article, we will focus on the effects of sublethal doses of glyphosate on defense-related processes in plants, and their potential indirect effects on the plant's biotic interactions.

The biosynthesis of several **phytohormones**, and especially plant defense-related compounds, relies on the availability of aromatic amino acids [4]. Disrupted biosynthesis of these compounds can lead to alterations in direct plant defenses against herbivorous insects, as shown for aphids and noctuid caterpillars [12–15]. However, pathogens and the plants relationship with beneficial microbes may also be affected (Box 2) [16]. Furthermore, the emission of **volatile organic**

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Key Figure

Plant Defense-Related Metabolic Pathways Affected by Glyphosate

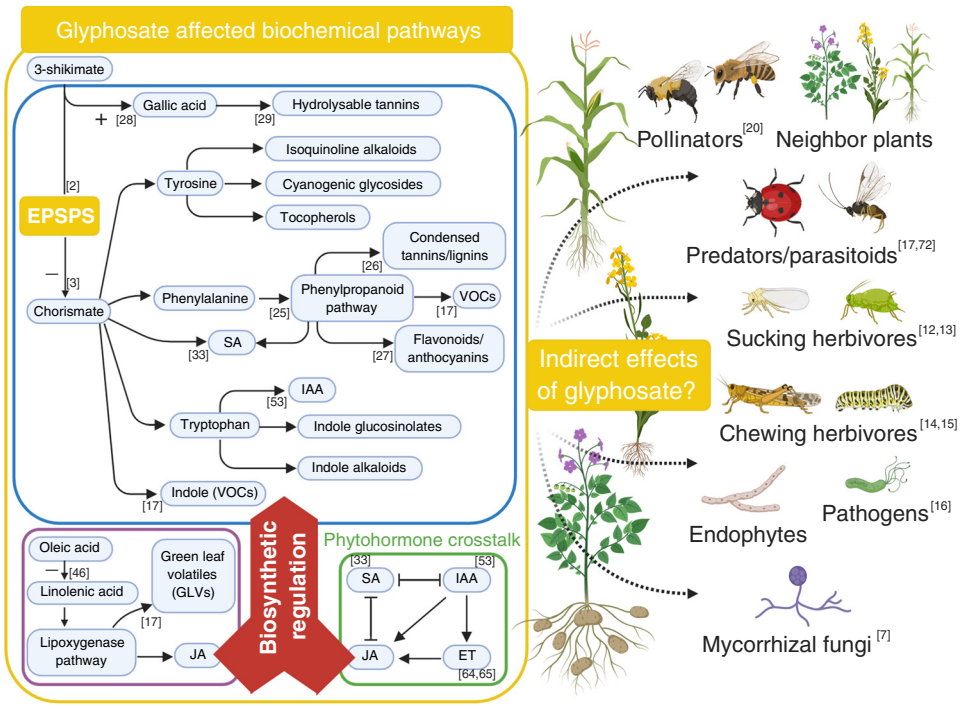

Figure 1. Glyphosate inhibits the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) enzyme, and can consequently negatively affect the biosynthesis of defense-related compounds downstream of EPSPS [indicated with minus symbol (–) in the blue box] [28], where defense-related compounds produced upstream of EPSPS can then accumulate [indicated with the plus symbol (+) in the blue box] [29]. Furthermore, glyphosate alters jasmonic acid (JA) levels, and green leaf volatiles (GLVs) (violet box). JA induces the biosynthesis of many phytoalexins (blue box) and interacts antagonistically or synergistically with other plant hormones (green box) [63,77]. Although the mechanism whereby the biosynthesis of JA and GLVs (violet box) are affected by glyphosate is mostly unknown, it may be indirectly regulated by numerous possible schemes of phytohormone crosstalk (Box 1). Alternatively, glyphosate may directly affect the lipoxygenase pathway (LOX) [46]. By affecting the biosynthesis of multiple defense-related plant compounds, glyphosate is predicted to indirectly alter plant–biotic environment interactions, with unknown consequences for ecosystems, ecosystem services, and agriculture. Numbers represent linked studies, where the results may derive from possible plant-mediated effects of glyphosate. Figures were created using the software biorender (app.biorender.com). See [2,3,7,12–17,20,25–29,33,46,53,64,65,72]. Abbreviations: ET, ethylene; IAA, indole-3-acetic acid; SA, salicylic acid; VOCs, volatile organic compounds.

compounds (VOCs) can be altered by sublethal glyphosate doses [17]. These compounds are essential signals for attracting predatory and parasitic insects, (i.e., **indirect plant defense**), but they also play a role in attracting pollinators [18,19]. For example, pollinators were shown to be decreasingly attracted by flowering plants after treatment with sublethal glyphosate doses [20]. In addition to the vast literature on the direct effects of glyphosate residues on non-target organisms, we suggest that glyphosate residues can substantially alter plant biotic interactions, exclusively by disrupting the biosynthesis of phytochemicals essential for plant defense and signaling to beneficial insects. Glyphosate residues may consequently interfere with intrinsic resistance of crops to pests and pathogens, as well as interfering with both fungal and insect biological control, which is especially relevant considering the global call for **integrated pest management** strategies [21]. We argue that the effects of glyphosate residues in the

Glossary

Endophytes: asymptomatic plant microbes inhabiting hosts locally or systemically. Some of them are well known for their plant defense-related mutualism. They interact with plant hormones for successful establishment.
EPSPS: 5-enolpyruvylshikimate-3-phosphate synthase, the enzyme and molecular target-site of the glyphosate inhibition step in the shikimate pathway.
Green leaf volatiles (GLVs): hydrocarbon chains of six carbon atoms, with different functional groups such as acetate, alcohol, and aldehyde. They are derived from enzymatic degradation of linolenic acid and are essential components of indirect plant defense responses.
Indirect plant defense: plant traits with no significant direct effect on attackers but which increase the recruitment of organisms that are harmful to plant attackers.
Integrated pest management: a holistic and sustainable approach for the control of plant pests based on the integration of natural phenomena with the goal of minimizing the use of chemical pesticides.
Lipoxygenase pathway (LOX): a biosynthesis pathway that uses polyunsaturated fatty acids from cell walls to produce plant signaling compounds, such as jasmonic acid (JA) and volatile organic compounds (VOCs).
Mycorrhizal fungi: root-associated plant symbionts known to increase plant water and nutrient uptake from the soil in exchange for photosynthesis products. Mycorrhizae interact with phytohormones, which in turn can increase the direct and indirect defense activities of a plant.
Oxylipins: enzymatically produced oxidized fatty acids; important molecules for plant signaling related to plant stress and immune signaling, such as JA.
Phenylpropanoid (PP) pathway: biosynthesis pathway present throughout the plant kingdom, which uses phenylalanine to produce diverse classes of essential compounds included in plant defense.
Phytoalexins: low-molecular-weight specialized plant secondary metabolites, rapidly synthesized following attack by pathogens or herbivores, often induced and mediated by phytohormones.
Phytohormones: low molecular weight acidic plant metabolites, with a signaling function that primarily mediates plant

Box 1. Phytohormone Crosstalk in Plant Defense

Plant responses to heterotrophic organisms, such as herbivores, pathogens, and beneficial microbes, are regulated by evolutionarily conserved phytohormone-mediated signaling pathways. Phytohormone pathways and their crosstalk are highly complex, but their ecological importance is becoming increasingly clear. Salicylic acid (SA) concentrations are known to increase after attack by piercing–sucking insects, and in response to biotrophic pathogen infection [61] (Table 1). Jasmonic acid (JA) and ethylene (ET) are induced by chewing herbivores and necrotrophic pathogens, where ET enhances JA induction by altering the lipoxygenase pathway (LOX) which leads to the production of JA [63] (see Figure 1 and Table 1 in the main text). As SA and JA pathways can be mutually antagonistic, increased resistance to chewing herbivores is often found to be negatively correlated with resistance to biotrophs [79] (Table 1). Ongoing research is uncovering the involvement of several other phytohormones in biotic interactions, such as abscisic acid (ABA), which is mostly known for its role in plant responses to abiotic stress, and in mediating growth and senescence processes [68]. Auxin, indole-3-acetic acid (IAA), mostly involved in plant growth regulation, can cause the suppression of SA biosynthesis and be mutually antagonistic. External SA application can attenuate auxin signaling [61] (Table 1). Furthermore, IAA can stimulate ET and JA biosynthesis, and consequently, boost JA-mediated defenses [56,65] (Figure 1 and Table 1). Plant responses to abiotic stress are commonly mediated by ABA, which has been shown to cause an increased plant susceptibility to most pests and pathogens [68]. This process is likely to be further mediated by some phytohormone crosstalk [77]. Gibberellin- (GA) mediated regulation of many developmental plant processes can cause an accumulation of SA, and consequently, antagonize JA-mediated defense responses [68].

environment have been largely understudied despite the possibility that they substantially alter plant physiology, and thereby biotic interactions, with herbivores, pathogens, and plant mutualistic organisms (Figure 2 and Box 2).

Here we analyze how sub-lethal glyphosate doses modulate plant defense-related metabolic pathways. How these modulations may subsequently affect plant interactions with the biotic environment are then explored. The aim of this article is to stimulate research on the effects of glyphosate residues on: (i) phytohormone concentrations and their regulation of biosynthetic pathways; (ii) the biosynthesis of plant defense compounds; (iii) species-specific plant defense strategies; (iv) plant-associated microbes involved in plant protection; (v) plant–herbivore dynamics; and (vi) volatile emissions and the subsequent effects on pollination and indirect plant defense responses.

Phenylpropanoid Pathway-Based Compounds

Glyphosate primarily inhibits the production of chorismate. This compound provides the basis for the biosynthesis of three essential aromatic amino acids: tyrosine, tryptophan, and phenylalanine. These amino acids act as entrance molecules to the numerous pathways responsible for the production of several plant hormones and specialized metabolites for plant defense responses, including the **phenylpropanoid (PP) pathway** [4] (Figure 1). Every plant produces PPs, which perform numerous functions in plants, particularly in relation to the plant's interactions with biotic and abiotic environmental factors [22,23]. Major groups of secondary compounds are derived from the PP pathway (e.g., lignins, flavonoids, anthocyanins, condensed tannins, and stilbenes), which are widely distributed throughout the plant kingdom [24]. However, the production of PPs is entirely dependent on chorismate supply, which is blocked by glyphosate. Several studies confirm a decreased production of PPs (e.g., condensed lignins, anthocyanins, and flavonoids) following exposure to sublethal glyphosate doses in multiple plant species [25–27]. This decrease in PPs can lead to an increased susceptibility to attackers [22].

While the biosynthesis of lignins and condensed tannins require molecules derived from the PP pathway, hydrolysable tannins require gallic acid, which is biosynthesized in branches of the shikimate pathway prior to EPSPS, and reportedly accumulates in pea roots (*Pisum sativum*) growing in glyphosate-containing substrate [28] (Figure 1). Hydrolysable tannins accumulate in leaves that have been treated with sublethal glyphosate doses, as shown in mountain birch (*Betula pubescens*) [29]. However, the effects on plant resistance against herbivores and pathogens remain unknown. It is likely that certain chemical plant defense strategies

growth, senescence, and defense responses to attackers. Most important for defense are salicylic acid (SA), jasmonates (e.g., JA, and JA-Ile), ethylene (ET), and auxins (e.g., IAA).

Secondary metabolites: compounds which are not immediately required in the plant primary metabolism, but often have a specialized role in enhancing plant performance under biotic or abiotic stress.

Shikimate pathway: a biosynthesis pathway in plants and various microbes that produces the aromatic amino acids phenylalanine (Phe), tyrosine (Tyr), and tryptophan (Trp).

Volatile organic compounds (VOCs): plant compounds released into the environment, belowground as well as aboveground, with diverse signaling functions for herbivores, their enemies, pathogens, mutualistic microbes, neighboring plants, and pollinators.

against herbivores are strengthened by sublethal glyphosate doses, due to a higher availability of precursor metabolites derived from the shikimate pathway prior to EPSPS, such as gallic acid.

Plant Hormones

Salicylic Acid

Besides being the precursor for the biosynthesis of aromatic amino acids, chorismate is needed to produce the phytohormone salicylic acid (SA). SA mediates plant defense responses to biotrophic pathogens and piercing-sucking insect herbivores, but other guilds of herbivores can also be affected [30] (Table 1). The signaling role of SA in symbioses with beneficial microbes has been observed numerous times (Box 2). To date, three pathways leading to SA biosynthesis have been described, all derived from the shikimate pathway, either branching directly from chorismate, or via phenylalanine [31,32] (Figure 1). The effect of glyphosate residues in soil on SA biosynthesis, however, has rarely been studied. One study reported an increase in SA concomitantly with a decrease in cinnamic and coumaric acid, following spraying of low doses of glyphosate onto yellow nutsedge leaves (*Cyperus esculentus*) [33]. The authors suggest that a glyphosate-mediated increase in SA may be possible via an alternative SA biosynthesis pathway using dehydroshikimic acid, which is a metabolite in the shikimate pathway**,** upstream of EPSPS [33]. However, all three SA biosynthesis pathways discovered to date require chorismate which is produced downstream of EPSPS [34]. It is more likely that the increased SA, reported by Cañal *et al*. [33], is derived from SA glycoside, which serves as a storage form of SA, and may be readily deglycosylated in response to stressors such as glyphosate [35].

Jasmonic Acid and Green Leaf Volatiles

Linolenic acid is one of the major polyunsaturated fatty acids in plant cell-membrane lipids [36]. Enzymatic metabolization, together with oxygenation of linolenic acid via the **lipoxygenase pathway (LOX)**, creates several **oxylipins** that have important functions in the physiology of the plant and its interactions with the environment [37,38]. Damage to cell walls causes the enzymatic degradation of cell wall-bound linolenic acid into compounds that are essential in plant direct and indirect defense processes [37,38]. One product is the plant hormone jasmonic acid (JA) which largely mediates the defense response to chewing herbivores and necrotrophic pathogens [39], but also plays a role in the mutualistic relationship between cool-season grasses and fungal *Epichloë* **endophytes** [40] (Box 2). JA is responsible for inducing many defense responses in plants through the rapid production of effective compounds, such as alkaloids, terpenoids, antioxidants, and PPs [41] (Figure 1). Reduced levels of JA have often been correlated with increased plant susceptibility to herbivorous insects [42]. Glycoalkaloid concentrations in potato (*Solanum tuberosum*) plants decrease when grown in soil containing glyphosate residues [43]; yet it is unknown whether this decrease derives from a direct glyphosate effect on glycoalkaloid biosynthesis, or whether it is indirectly mediated via disrupted JA signaling. JA is further known to interfere with SA-mediated plant defenses and vice versa [44] (Box 1). Certain herbivores adapt their feeding mode to target the induction of SA, which in turn reduces JA-mediated induction of **secondary metabolites** [45]. However, herbivore-elicited manipulations of hormone crosstalk may be disrupted when glyphosate-residues interfere with JA biosynthesis. In glyphosate resistant soybean seeds (*Glycine max*), glyphosate mediates a decrease in linolenic acid concentrations [46], although the concentration of its precursor (oleic acid) increases [47]. These results indicate that glyphosate represses the FAD_2 enzyme responsible for the conversion of oleic acid into linolenic acid [48] (Figure 1). Ultimately, this glyphosate-mediated decrease in linolenic acid concentrations indicates that there is also a decrease in

products based on the enzymatic metabolization of polyunsaturated cell-wall fatty acids, such as JA.

Plant emitted volatile blends can affect the behavior of many heterotrophic insects, including pollinators, herbivores, and their natural enemies. Peroxidation of linolenic acid also leads to the production of **green leaf volatiles (GLVs)**, a type of plant VOC produced following plant damage as a 'cry for help' message, to attract the natural enemies of herbivores [19] (Figure 1 and Table 1). VOCs are essential signaling components of indirect plant defense responses, often attracting predatory or parasitic insects (Figure 1 and Table 1). A recent study on maize (*Zea mays*) showed a decreased concentration in the GLV Z-3-hexenyl acetate following exposure to sublethal glyphosate doses [17]. Furthermore, in the same study, glyphosate decreased the production of phenolic-based VOCs, such as phenethyl acetate and indole [17]. Volatile benzenoids and PPs are the second largest group of volatiles after terpenoids [18], and similar to non-volatile PPs, their concentrations can be greatly altered by glyphosate (Figure 1).

Indolic Compounds

Indole derives from the aromatic amino acid tryptophan and concentrations can greatly decrease following exposure to sublethal glyphosate doses [17]. Indole plays a central role in herbivore-induced volatile emission and indirect plant defense responses [19,49], and its emission is further induced by JA [19].

Additional to its role in attracting parasitic wasps as an indirect plant defense agent, indole has an essential function in airborne communication between neighboring maize (*Z. mays*) plants, as a priming signal for incoming herbivory [49] (Figure 1). By affecting indole-dependent volatile biosynthesis, glyphosate may indirectly affect myriads of insects as well as the responses of surrounding plants, despite having virtually no physical contact with them [50] (Figure 2).

The aromatic amino acid tryptophan provides the basis for auxins, which promote plant growth and are known to be involved in plant defense against pathogens [51]. Indole-3-acetic acid (IAA) is the most abundant auxin and functionally essential for plant growth and development [52]. Sublethal glyphosate doses have been shown to decrease plant growth, which in turn was correlated with lower levels of free IAA and an increase in the metabolism rate of IAA, leading to faster IAA breakdown or binding [53,54]. Tryptophan is further biosynthesized into the plant hormone camalexin, and the secondary metabolites indole glucosinolates, both of which are involved in the defense responses of Brassicaceae species [4] (Figure 1). IAA shares a biosynthetic pathway with indole alkaloids and indole glucosinolates, and has a diverse hormone crosstalk with ethylene (ET), SA, and JA [55–57] (Box 1 and Table 1). When attacked, plants can direct resources into the biosynthetic pathway branches for glucosinolate production instead of IAA biosynthesis [56]. Allocating resources for secondary metabolite biosynthesis instead of growth promoting hormones, follows the concept of a growth-defense trade-off [56,58]. However, in and of itself, glyphosate blocks the biosynthesis of tryptophan, which interferes with any pathway that requires tryptophan for the production of IAA or indole glucosinolates (Figure 1). Besides indole glucosinolates, many Brassicaceae plants can also produce aliphatic glucosinolates, which are created independent of the shikimate pathway and are, theoretically, not affected by glyphosate residues [59]. It is unknown whether these glyphosate-mediated effects on growth-related hormones can result in a decrease in both types of glucosinolates and, in turn, how this may impact insect–plant interactions and, consequently, insect communities.

Plant-induced IAA production can induce JA signaling, and a rapid production of defense compounds against necrotrophic pathogens [56,60]. IAA is mutually antagonistic to SA and, in turn, SA is mutually antagonistic to JA [56]. Thus, while suppressing SA biosynthesis, IAA indirectly promotes JA signaling, and consequently increases resistance to necrotrophic pathogens [61] (Box 1 and Figure 1). Several biotrophic pathogens have been shown to synthesize IAA in an attempt to decrease SA-mediated plant defense responses, and to enhance plant vulnerability to their attack [57]. Low glyphosate-mediated IAA concentrations may decrease JA-induced defense responses, while also increasing SA-mediated defense responses.

Ethylene

The plant hormone ET is produced from the amino acid methionine, and is known for its roles in plant growth, development, and senescence [62]. However, ET can also interact with defense related hormones, and affect plant responses to herbivores up to the third trophic level [63]. Glyphosate has been shown to increase ET levels in common bean (*Phaseolus vulgaris*) [64]. In tobacco (*Nicotiana tobacum*) calluses, the presence of IAA can cause an increase in ET concentrations, previously diminished following the addition of glyphosate [65] (Box 1). ET is one of the first hormones to be synthesized following chewing–biting herbivory [44]. Furthermore, ET has been shown to positively interact with JA in the regulation of herbivore-induced volatile emissions in maize (*Z. mays*) [66] (Table 1).

In summary, ET plays an important role in fine tuning direct and indirect plant defense responses. It often complements the JA-mediated defense response, but ET concentrations can be affected by IAA (Box 1 and Table 1). Thus, by altering ET biosynthesis, glyphosate may disrupt specific plant responses to herbivores, including the successful signaling of indirect plant defense mechanisms. The question of how, specifically, glyphosate affects ET biosynthesis, release, and signaling warrants detailed further study.

Abscisic Acid

The phytohormone abscisic acid (ABA) is involved in mediating abiotic stress, dormancy, and seed development. Sublethal doses of glyphosate have been found to increase concentrations of ABA in soybean (*G. max*) [67]. While ABA has seldom directly been linked to plant defense processes, it has been correlated with increased susceptibility to any kind of plant attack [68]. In particular, ABA can act synergistically with JA, but it also has been linked to decreased JA–ET mediated plant defenses following exogenous application and shown to interfere with SA-mediated pathogen defenses in soybean (*G. max*) [68]. In case of co-occurring abiotic stress (such as drought or salt stress) with biotic stress, plant evolutionary processes may favor the allocation of resources to cope with abiotic stress, which is potentially more lethal (Box 1). Glyphosate may be perceived as a form of abiotic stress due to the inhibition of essential pathways, which may explain the observed increase in ABA concentrations mediated by glyphosate [67]. It is not clear if glyphosate residues in soil continually increase endogenous ABA concentrations, and thereby, cause a decrease in plant responses to biotic stress. To understand how glyphosate affects phytohormone biosynthesis and signaling, evidence from mechanistic studies that reveal clear causalities between glyphosate application, gene expression, and phytohormone concentrations in plant tissues of different plant species, is urgently needed. Future research needs to unravel the degree to which glyphosate residues in soil disrupt phytochemical processes, and the consequences for biotic interactions and hormone crosstalk (Figure 1).

Biotic Interactions

Herbivorous Insects

Most studies conducted to test the effects of glyphosate on insect performance have assessed the effects of direct exposure to glyphosate or glyphosate-based herbicides. These studies have shown negative effects on insect survival, growth, and reproduction [69]. By contrast, this opinion piece is focused on the indirect effects of this chemical on biotic interactions, through changes in the plant biochemical processes involved in plant defense responses. A closer look into the effects of glyphosate on hormone crosstalk, and the resulting effects on defense compound composition, is an essential step towards a better understanding of the role of this herbicide in plant–herbivore interactions (Figure 1). Therefore, thorough studies on the indirect glyphosate-induced effects on plant defense responses are urgently required. To date, studies using aphids as model organisms for testing plant responses to piercing–sucking insects, following treatment with sublethal doses of glyphosate, have produced mixed results. In a field study, Dewar *et al*. [12] found high aphid populations on glyphosate-treated, but glyphosate-tolerant sugar beet (*Beta vulgaris*) plants, whereas Lipok [13] observed a suppressed development in *Aphis fabae* aphids feeding on *Vicia faba* L. treated with glyphosate, as well as a feeding preference for untreated control plants in an olfactometer assay, which indicates a herbicide-mediated change in VOC emission, driving aphid preference for untreated plants. It is unknown whether a glyphosate-mediated effect on SA biosynthesis contributed to the reported effects on aphid performance. A decrease in glycoalkaloid concentrations in potato (*S. tuberosum*) leaves mediated by glyphosate residues in the soil did not affect the performance of herbivorous *Leptinotarsa decemlineata* [43]. However, Campbell *et al*. [14] observed a decrease in weight-gain in *L. decemlineata* larvae following treatment of their host plant with sublethal doses of glyphosate. Similarly, Abo El-Ghar [15] observed decreases in larval growth rate, pupation, and adult emergence of *Spodoptera littoralis* following consumption of *Ricinus communis* leaves sprayed with glyphosate. In cases such as these, understanding the role of glyphosate-mediated changes in JA concentrations, may elucidate the mechanisms underlying the reduced performances observed in chewing insects. Studies are needed to disentangle the effect of glyphosate-altered plant quality (e.g., due to reduced availability of amino acids), and the effects of glyphosate-altered plant defense mechanisms on herbivorous insects. Both types of changes in plant chemistry may determine herbivore behavior and performance.

Box 2. Indirect Effects of Glyphosate on Plant-Associated Microbes

Glyphosate may be able to interact in two ways with plant-associated microbes: (i) by directly affecting their metabolism, especially those sharing the shikimate pathway, such as several fungal and bacterial guilds [80,81]; or (ii) indirectly, by inducing a substantial change in the plant metabolome, which is essential to the status of plant–fungi symbioses [40,82] (Figure 2). Signaling between plants and associated microbes can depend on plant metabolites derived from the shikimate pathway, such as flavonoids, which are essential for successful nodulation between legumes and nitrogen-fixing rhizobacteria [83]. In recent years, plant associated microorganisms have been increasingly shown to be essential for plant defense against biotic threats, and the same plant signaling pathways can regulate plant–microbe and plant–insect interactions [84]. Interestingly, **mycorrhizal fungi** may improve plant defenses by inducing changes in plant hormone levels, or by improving volatile emissions for indirect plant defense [82]. Other symbiotic microorganisms strongly interact with plant immune responses, such as the systemic fungal *Epichloë* endophytes of cool-season grasses [85]. Glyphosate effects on the plant metabolome may disrupt mutualisms between plants and microbes. Furthermore, glyphosate may disrupt the fine-tuned signaling between plant and microbes on which mutualistic interactions rely. Microorganisms interact with the plant immune system by actively lowering salicylic acid (SA) concentrations in order to establish symbioses [86] (Table 1). However, jasmonic acid (JA) can affect the symbiotic relationships between mutualistic fungi and their host plants [40,78]. This indicates that JA concentrations determine the fate of the plant–fungus mutualism, and that alterations as a result of glyphosate applications may disturb the growth of plant-associated fungi [78]. Since most defense-related hormones are affected by glyphosate, it is likely that plant–fungus symbiotic interactions are disrupted by glyphosate residues in the soil (see Figure 1 in the main text).

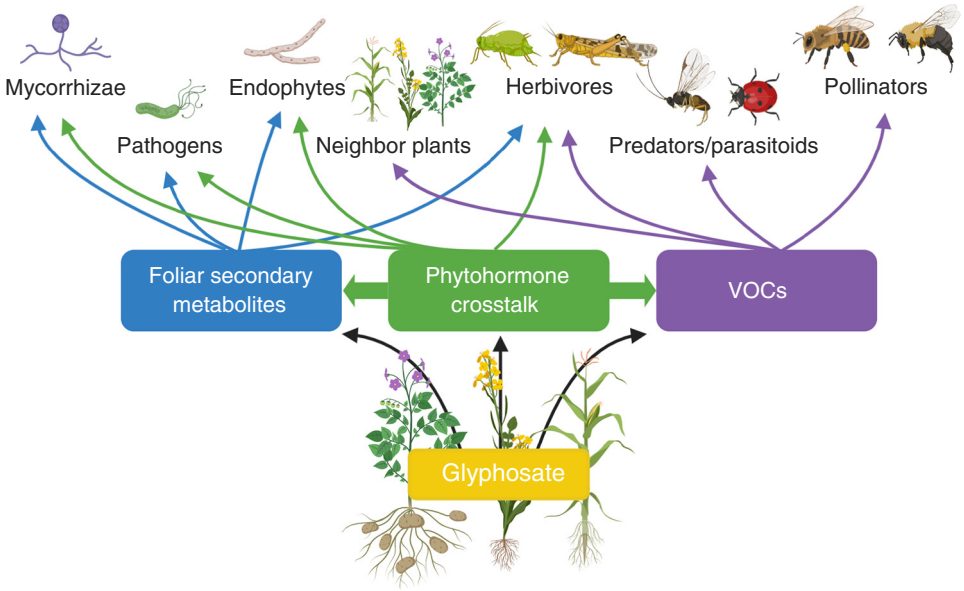

Figure 2. Glyphosate Mediated Effects on Plant Metabolites and Species Interactions. By affecting plant physiological processes, glyphosate alters the biosynthetic pathways which are involved in a plant's interactions with its biotic environment. Here highlighted, are interactions which may be affected by: (i) foliar secondary metabolites; (ii) phytohormones and crosstalk effects; and (iii) volatile organic compounds (VOCs). Besides directly affecting the interactions of species, phytohormones have indirect effects on these interactions by regulating the biosynthesis of foliar secondary metabolites and VOCs (Figure 1). Figures were created using the software biorender (app.biorender.com).

Pathogens

Various microorganisms interact with the plant immune system. Pathogens commonly decrease plant performance in contrast to mutualistic microbes which often benefit plant performance by increasing an individual's defenses against attack (Box 2). Defense against biotrophic pathogens is commonly mediated by the phytohormone SA and defense against necrotrophic pathogens is commonly mediated by JA. However, pathogens have been found to produce or induce auxin production as a strategy to lower SA-mediated plant defenses [57,61]. This makes it difficult to predict the outcome of plant–pathogen interactions when glyphosate residues interact with a plant's biochemistry. In a literature review by Johal and Huber [16], it was clearly demonstrated that there is an enhanced susceptibility of plants to pathogenic infections following treatment with sub-lethal glyphosate doses, both above and belowground. Some plant defense strategies involving the PP pathway, such as lignification, are repressed by glyphosate, while the **phytoalexin-**mediated defense is temporarily maintained [16]. Whereas lignification is a so called 'quantitative plant defense trait' that requires many lignan components, many phytoalexins are 'qualitative plant defense compounds' effective in small amounts [70]. Plants store many phytoalexins as glycosylated compounds, that can be quickly activated upon detection of an immediate threat. This is done until induced biosynthesis delivers more phytoalexins in sufficient amounts [71]. By contrast, quantitative defense mechanisms require a constant supply of biosynthetic resources. Stored glycosylated phytoalexins may be one explanation for the observed, temporarily stable phytoalexin supply. Lignin biosynthesis requires large amounts of lignan components, and stored molecules may be quickly depleted.

Plant Beneficial Insects

The effects of glyphosate on defense-related compounds may not only affect the antagonists of plants but also potential mutualists. Predators of herbivores are commonly used in pest control in

Table 1. Glyphosate Effects on Some Well-Studied Plant Defense-Related Hormones and Selected Defense-Related Compound Classes[a,b]

Compound + target of glyphosate	Function	Plant responses to		Compound interactions	Type of interaction	Refs
SA	Hormone/signaling	Sucking herbivores	[30]	SA–JA ⇨	Antagonism	[44]
EPSPS		Biotrophic pathogens	[30]	SA–VOCs ⇨	Induction/shared pathway	[19]
		Beneficial microbes	[84]	SA–PPs ⇨	Shared pathway	[4]
				SA–IAA ⇨	Antagonism	[61]
JA	Hormone/signaling	Chewing herbivores	[30]	JA–SA ⇨	Antagonism	[43]
LOX pathway/ glyphosate target unknown		Necrotrophic pathogens	[30]	JA–ET ⇨	Synergism	[66]
		Beneficial microbes	[84]	JA–IAA ⇨	Synergism	[56]
				JA–PPs ⇨	Induction	[39]
				JA–VOCs ⇨	Induction/shared pathway	[19]
IAA	Hormone/signaling	Bacterial pathogens	[52]	IAA–JA ⇨	Synergism	[56]
EPSPS				IAA–ET ⇨	Synergism	[56]
				IAA–SA ⇨	Antagonism	[61]
ET	Hormone/signaling	Herbivores	[63]	ET–JA ⇨	Synergism	[66]
Indirectly regulated via IAA				ET–IAA ⇨	Synergism	[56]
				ET–VOCs ⇨	Induction	[63]
PPs	Phytoalexins	Herbivores	[4]	PPs–SA ⇨	Shared pathway	[4]
EPSPS	Antioxidants	Pathogens	[16]	PPs–JA ⇨	Induction	[39]
		Beneficial microbes	[83]	PPs–VOCs ⇨	Shared pathway	[4]
VOCs	Intra/interspecific signaling	Herbivores	[18]	VOCs–SA ⇨	Induction/shared pathway	[19]
LOX pathway/EPSPS		Pollinators	[18]	VOCs-–JA ⇨	Induction/shared pathway	[19]
		Third trophic level	[19]	VOCs–ET ⇨	Induction	[63]
		Neighbor plants	[49]	VOCs–PPs ⇨	Shared pathway	[4]

[a]We highlight their interactions with plant beneficial and detrimental species interaction types, but further the multifaceted chemical crosstalk among those compounds.
[b]Abbreviations: EPSPS, 5-enolpyruvylshikimate-3-phosphate synthase; ET, ethylene; IAA, indole-3-Acetic Acid; JA, jasmonic Acid; LOX, lipoxygenase pathway; PPs, phenylpropanoids; SA, salicylic acid; VOCs, volatile organic compounds.

many agricultural systems, and play a major role in sustainable alternatives to pesticides in integrated pest management [21]. However, declines in carabid beetles and spiders have been observed as a delayed response to the spraying of field margins with glyphosate [28], which may be attributed to a change in foliage cover, although further cascading negative effects throughout the food chain cannot be excluded. In a field study, Pereira *et al*. [72] recorded an increase in leaf beetles, which they attributed to a reduction in their predators. It was assumed that this reduction was due to glyphosate-mediated behavioral changes in predators of the leaf beetle. Disrupted plant indirect defense signaling via changes in VOC emissions are likely to have contributed to these behavioral changes (Figure 1). Parasitoids of the genus *Cotesia*, did not change their behavior, as the third trophic level in response, to herbivore-induced volatile emissions from maize (*Z. mays*) treated with sublethal glyphosate doses [17]. However, many specialized parasitoids rely on distinct herbivore-induced volatile blends to locate their prey [73]. Due to the substantial alteration of plant volatile emissions caused by glyphosate, we

argue that glyphosate-mediated changes in volatile biosynthesis may strongly interfere with indirect plant defense mechanisms, the effects of which may vary greatly between specialist and generalist insect species (Figure 2 and Table 1).

The effect of glyphosate on VOCs may not only affect defense-related species interactions, but further the interactions with pollinators. Pollinators are essential for the reproduction of most flowering plant species [74]. Agronomically, pollinating insects provide the most valuable ecosystem service of all insect species [75]. Besides leaf-derived volatiles, plants release floral volatiles, which contribute to pollination success [75]. While it is unknown whether terpenoid biosynthesis is affected by glyphosate, many floral volatile blends consist of a mixture of volatiles, among which, PP-derived VOCs play a vital role in floral volatile blends, and are important signals for pollinators to find and identify forage plants [76] (Figure 2). A recent study provides the first evidence that the proportion of flower visitors to a mixture of perennial flowering plants, decreases following treatment of the soil and foliage with sublethal glyphosate doses [20]. The authors attribute the observed changes to alterations in the phenotypical appearance of the flowering plants, but possible changes in VOC emission may contribute to those results [18]. As approximately 75% of global crop production depends on insect pollination [74], a thorough understanding of glyphosate-mediated effects on floral volatiles and their effects on pollinator behavior is urgently needed.

Concluding Remarks

Sublethal doses of glyphosate, in the form of persistent glyphosate residues in soils, can alter many physiological plant processes, including the regulation of plant defense responses by plant hormones. Although much more research is required to understand glyphosate-mediated effects on plant defense responses, the literature to date indicates that this herbicide substantially changes how plants interact with their biotic environment. Glyphosate may disrupt trophic interactions while affecting plant hormone biosynthesis and action. The interconnectivity and crosstalk between plant signals, microorganisms, herbivores, and their natural enemies, are foundational elements within healthy ecosystems. These connections have significant agro-ecological value in terms of natural pest control (i.e., integrated pest management) and pollination services. Unfortunately, plant and insect diversities across the globe are declining at an alarming rate. Consequently, a holistic understanding of how chemical herbicides are impacting plant–insect interactions across all ecosystems is urgently needed. It is our responsibility to uncover the consequences of adding overwhelming amounts of potentially destructive agrochemicals into ecosystems. Perhaps now is the time to use novel technologies to estimate the consequences of agrochemical damage to the natural processes driving ecosystem dynamics (see Outstanding Questions).

Outstanding Questions

How do glyphosate residues in soil affect phytohormone crosstalk and plant defense responses against different types of plant herbivores and pathogens?

Do glyphosate residues differentially affect SA-mediated and JA-mediated plant defense responses?

How do glyphosate residues in soil affect defense responses that are biosynthesized independent of the shikimate pathway?

What mechanism accounts for glyphosate-mediated repression of the lipoxygenase pathway?

To what extent do glyphosate residues in soil affect the relationship between plants and symbiotic microorganisms?

How do glyphosate residues in soil affect plant volatile-mediated indirect plant defense responses and pollination?

How do glyphosate residues in soil affect multitrophic interactions important for integrated pest management?

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