Alteration of plant physiology by glyphosate and its by-product aminomethylphosphonic acid: an overview

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Abstract

It is generally claimed that glyphosate kills undesired plants by affecting the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) enzyme, disturbing the shikimate pathway. However, the mechanisms leading to plant death may also be related to secondary or indirect effects of glyphosate on plant physiology. Moreover, some plants can metabolize glyphosate to aminomethylphosphonic acid (AMPA) or be exposed to AMPA from different environmental matrices. AMPA is a recognized phytotoxin, and its co-occurrence with glyphosate could modify the effects of glyphosate on plant physiology. The present review provides an overall picture of alterations of plant physiology caused by environmental exposure to glyphosate and its metabolite AMPA, and summarizes their effects on several physiological processes. It particularly focuses on photosynthesis, from photochemical events to C assimilation and translocation, as well as oxidative stress. The effects of glyphosate and AMPA on several plant physiological processes have been linked, with the aim of better understanding their phytotoxicity and glyphosate herbicidal effects.

Key words: Aminomethylphosphonic acid, AMPA, glyphosate, herbicide exposure, oxidative stress, photosynthesis, phytotoxicity, plant nutrition.

Introduction

Glyphosate [N-(phosphonomethyl)glycine] is currently the most widely used herbicide in the world and its use in agriculture has increased tremendously since the introduction of glyphosate-resistant (GR) plants (Duke and Powles, 2008). The glyphosate molecule was first synthesized by Henri Martin from Cilag, a small Swiss pharmaceutical company, but was only tested as a herbicide in 1970 by John E. Franz of Monsanto Co. (Franz et al., 1997). In 1974, after being patented for herbicide use, glyphosate (as an isopropylamine salt of glyphosate) reached the market as a post-emergence, non-selective herbicide (Duke and Powles, 2008), and has been used for total control of vegetation (Cobb, 1992) since it is a broad-spectrum, non-selective systemic herbicide.

In agricultural fields, glyphosate is sprayed on plant foliage; however, a portion of the chemical can be deposited directly to the soil surface or blown by the wind to neighbouring soils and plants, leading to exposure of non-target plants. Moreover, waterway contamination is a source of glyphosate...
transfer to adjacent agricultural fields, especially in fields irrigated through pumping into surface water bodies. Another important source of glyphosate exposure is exudation from roots of sprayed plants (Coupland and Caseley, 1979) and its release from dead plants (Neumann et al., 2006). Recent studies suggested a risk of glyphosate toxicity to non-target plants due to rhizosphere transfer of glyphosate (Tesfamariam et al., 2009). Once in soil, glyphosate may be adsorbed onto soil particles, degraded by microbes, or transferred to deeper soil horizons, migrating via soil pores or root canals. However, some agricultural practices, such as phosphorous amendment, may re-solubilize glyphosate in soils, making it available for leaching (Borggaard and Gimsing, 2008) and to the rhizosphere of non-target plants.

Recently, a number of studies have focused on the environmental presence of the major glyphosate metabolite amino-methylphosphonic acid (AMPA). Having a short half-life, glyphosate is quickly degraded to AMPA in soils by microorganisms (Franz et al., 1997; Van Eerd et al., 2003). A similar mechanism of glyphosate degradation has been proposed in plants (Reddy et al., 2004); therefore the co-occurrence of glyphosate and AMPA is expected in plant tissues due to glyphosate degradation and/or AMPA uptake from environmental matrices. Upon penetrating the plant tissues, glyphosate will reach active metabolic sites, such as root and shoot meristems, after being translocated through vascular tissues (Satchivi et al., 2000), following the same pathway as photoassimilates (Monquero et al., 2004). Similarly, AMPA can also be translocated to diverse plant tissues (Reddy et al., 2004). Therefore, plant organs such as nodules, root tips, and shoot apices, which show high rates of metabolism and growth, represent important sinks for glyphosate/AMPA (Hetherington et al., 1999; Feng et al., 2003).

The herbicidal effects of glyphosate are due to the inhibition of the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), an enzyme from the shikimate pathway, which leads to prevention of the biosynthesis of the amino acids phenylalanine, tyrosine, and tryptophan (Siehl, 1997). Even though the changes in physiological plant processes induced by glyphosate and AMPA have not been considered as primary effects, they could contribute to its herbicidal effects. In addition, physiological processes of GR plants may also be affected by glyphosate. Although at a lower extent compared to glyphosate-sensitive (GS) plants, glyphosate effects on photosynthesis, mineral nutrition, and oxidative events in GR plants have been reported (Zobiole et al., 2009, 2010c, 2012). In addition, AMPA is a recognized phytotoxin (Reddy et al., 2004) whose exact mechanism of action has not yet been completely elucidated. Once present in plant tissues AMPA can contribute to the effects of glyphosate on physiological processes. Moreover, Reddy et al. (2004) attested that glyphosate-induced injuries in GR plants are due to AMPA formed from glyphosate degradation. The glyphosate-sensitivity of GR plants is widely variable depending on species and cultivar. So, it is intrinsically important to elucidate the glyphosate and AMPA effects on physiological processes related to plant growth and production in order to better understand the glyphosate-herbicidal mechanism and its possible effects on non-target plants.

Glyphosate has been shown to affect plant physiological mechanisms such as photosynthesis, C metabolism, mineral nutrition, and oxidative events, and to disturb plant–microorganism interactions (Kremer and Means, 2009; Kielak et al., 2011; Zobiole et al., 2012). Despite the fact that AMPA has been less studied, it was shown to affect chlorophyll biosynthesis and to cause plant growth reduction (Reddy et al., 2004; Serra et al., 2013). In this review, we provide an overall picture of the effects of glyphosate and AMPA on plant primary physiological processes, with a particular focus on photosynthesis, from photochemical events to C assimilation and translocation, and oxidative stress in plants.

Uptake and translocation of glyphosate and AMPA in plants

In leaves, the uptake of glyphosate is a biphasic process which involves a rapid initial penetration through the cuticle, followed by a slow uptake through the symplast (Monquero et al., 2004). Entrance into the symplast could be either through a passive diffusion mechanism that is not affected by pH (Gougler and Geiger, 1981), or by an endogenous transport system, possibly a phosphate carrier within the cell membrane (Burton and Balke, 2012). The duration of this process depends on several factors such as species and plant age, environmental conditions, and herbicide concentration (Monquero et al., 2004). Environmental factors are also known to affect glyphosate absorption. For example, glyphosate absorption is modulated by any factors that alter the water potential of plants (such as soil moisture and relative humidity) ( Sharma and Singh, 2001), the production of cuticular wax (such as low light intensity) (Franz et al., 1997), or its hydration and the mineral absorption through influencing transpiration rate (such as temperature) (Sharma and Singh, 2001).

After penetrating the leaves, glyphosate will reach active metabolic sites, such as root and shoot meristems, after being translocated to vascular tissues (Satchivi et al., 2000). Glyphosate’s movement through phloem follows the same pathway as other photoassimilates, which are produced in photosynthetically active tissues and migrate towards growth or storage tissues such as roots, tubers, rhizomes, young leaves, and meristematic zones (Monquero et al., 2004). Plant organs with high rates of metabolism and growth, such as nodules, root tips, and shoot apices, represent important sinks for glyphosate (Cakmak et al., 2009). As for the photoassimilates, glyphosate translocation to these tissues changes during the plant life cycle (Monquero et al., 2004). Using $^{14}$C-glyphosate, Feng et al. (2003) demonstrated that in Abutilon theo- phrasti, 45 and 34% of the glyphosate absorbed after foliar application was translocated to roots and meristems, respectively. Low inhibition thresholds (0.23 and 0.21 ppm glyphosate, respectively) were measured in these tissues, indicating glyphosate phytotoxicity. By contrast, stem tissue showed a much higher inhibition threshold (8.4 ppm). Moreover, the authors observed a linear relationship between glyphosate dose and tissue concentration, but the tissue distribution
pattern was independent of the dose used. Even at low foliar application rates, the sink tissues accumulate glyphosate at very high concentrations, and a single foliar application of glyphosate at a rate of 0.5 kg ha⁻¹ can lead to glyphosate accumulation up to 0.05 ppm in the sink organs (King et al., 2001).

A new topic of interest in glyphosate translocation in plants concerns the exudation from roots to soil which has been observed in some plant species (Neumann et al., 2006; Laitinen et al., 2007). It was shown that following exudation, glyphosate can inhibit growth of adjacent plants and seedlings (Kremer et al., 2005). For example, a GR soybean (Glycine max) may exude 1500 ng of glyphosate in the rhizosphere in 16 days (Kremer et al., 2005). Using ¹⁴C-glyphosate, Ricoldi et al. (2007) reported the translocation of glyphosate from target to non-target plants. However, the non-target plants showed little growth inhibition in relation to the decrease observed in the target plant, and so the growth of non-target plants was considered not to be affected by the amount of glyphosate exuded through the rhizosphere of nearby plants (Ricoldi et al., 2007). Laitinen et al. (2007) observed after 8 days, 12% of the glyphosate applied on leaves of Chenopodium quinoa was detected in roots and about 4% in the surrounding soil. Two weeks later, 8 to 12% of the applied glyphosate was detected in soil samples. According to these authors, the same results were found in field studies, where the plant roots contribute to the glyphosate amounts detected in soil residues. Glyphosate exudation by plant roots can also be considered as an environmental source of glyphosate exposure for non-target plants and should be included in risk assessment models.

The results of Ricoldi et al. (2007) presented above also confirm glyphosate uptake through a root pathway. However, in soil, glyphosate has been proposed to be quickly degraded to AMPA (Franz et al., 1997; Van Eerd et al., 2003). Due to their chemical similarities, both glyphosate and AMPA are expected to be taken up from soil solution. Once in root tissues, glyphosate and AMPA can reach active metabolic sites such as the shoot meristems through xylem conduction. Despite the possibility that root uptake of glyphosate and AMPA could constitute an important source of these molecules to plants, information regarding glyphosate root uptake are scarce, and no studies have been performed with AMPA. As AMPA phytotoxic effects were found even in GR plants (Reddy et al., 2004), these studies are urgently needed.

Alteration of plant physiology by glyphosate and AMPA

Glyphosate has been shown to affect several plant physiological processes which could also be linked to glyphosate-herbicidal effects. Reinforcing this statement, some studies have contested the glyphosate effects being strictly due to EPSPS inhibition, as depletion of aromatic amino acids was not verified in glyphosate-treated plants (Wang, 2001; Serra et al., 2013). As a metal chelator, glyphosate could deprive plants of important nutrients which have major roles as enzymatic co-factors and biomolecular constituents. Additionally, AMPA is known itself to be a phytotoxin, which could, in addition to its own effects, amplify indirect effects of glyphosate on physiological processes. On the other hand, due its chemical similarity, AMPA can compete with glycine in biological sites and pathways, affecting among others chlorophyll biosynthesis and, thus, the photosynthetic process.

Photosynthesis

Photosynthesis is the major biochemical process occurring in photoautotrophic organisms and is known to be affected by various anthropogenic factors. Some herbicides were found to directly interrupt photosynthetic electron transport. For example, 3-(3,4-dichlorophenyl)-1,1-dimethyleurea (DCMU) is known to block the electron flow between QA and QB by competing for QB binding sites (Töth et al., 2005). Other herbicides, such as glyphosate, will affect photosynthesis indirectly by inhibiting the biosynthesis of carotenoids, chlorophylls, fatty acids, or amino acids (Fedtke and Duke, 2005). As an EPSPS competitive inhibitor, glyphosate blocks the shikimate pathway, inhibiting the biosynthesis of secondary metabolites in plants, including compounds related to photosynthesis, such as quinones (Dewick, 1998). However, it is unclear how glyphosate leads to plant death, and hypotheses such as depletion of protein stocks and drainage of C from other vital pathways have been put forward (Duke and Powles, 2008). A closer look at glyphosate’s effects on photosynthetic processes may shed light on this hypothesis. Indeed, numerous field and greenhouse studies have indicated a decreased photosynthetic rate in plants following glyphosate exposure (Mateos-Naranjo et al., 2009; Yanniccari et al., 2012; Zobiole et al., 2012).

Chlorophyll biosynthesis

The first step in photosynthesis occurs in the thylakoid membranes of chloroplasts by light excitation of photosynthetic pigments, including chlorophylls. This initiates electron flow, where electrons are transferred from a special pair of chlorophyll a molecules to plastoquinone, the primary electron acceptor (Rohacek et al., 2008). Some studies reported a decreased chlorophyll content in plants after glyphosate application due to the degradation or inhibition of chlorophyll biosynthesis (Mateos-Naranjo et al., 2009; Zobiole et al., 2011b; Huang et al., 2012). Glyphosate may prevent chlorophyll synthesis indirectly by decreasing the Mg content in leaves, as shown by Cakmak et al. (2009), which leads to a decreased chlorophyll content and photosynthetic rate (Zobiole et al., 2012). Indeed, the incorporation of Mg by Mg chelatase in the porphyrin structure is a necessary step leading to the synthesis of chlorophyll molecules (Tanaka and Tanaka, 2007). Cakmak et al. (2009) demonstrated that foliar application of glyphosate will decrease concentrations of cations in shoots and seeds of GS soybeans. Similarly, by inducing Fe deficiency, glyphosate may prevent the biosynthesis of δ-aminolevulinic acid (ALA), a component of the chlorophyll biosynthetic pathway (Marsh et al., 1963). Catalase (CAT) and peroxidase, both enzymes implicated in ALA biosynthesis, are highly
sensitive to Fe deprivation (Marsh et al., 1963). Glyphosate is a strong cation chelator, due to its carboxyl and phosphonate groups, forming complexes with nutrients in plant tissues, thus making them unavailable for biological processes, including photosynthesis (Cakmak et al., 2009). In addition, glyphosate has been proposed to interfere with ALA biosynthesis by controlling the conversion of alpha-ketoglutarate to ALA and/or the condensation of glycine with succinyl-CoA to form ALA and CO₂ (Kitchen, 1980).

Interestingly, Reddy et al. (2004) reported that glyphosate’s deleterious effects on chlorophyll biosynthesis are primarily dependent on the degradation rate of glyphosate into AMPA. In other words, AMPA (not glyphosate) was proposed to be responsible for the hazardous effects on chlorophyll biosynthesis, but by an unknown mechanism. Recently, Serra et al. (2013) showed decreased amounts of glycine, serine, and glutamate in plants treated with AMPA. The lack of glycine/glutamate in AMPA-treated plants could thus reduce ALA and chlorophyll contents, since these amino acids are required during ALA biosynthesis through ALA-synthetase and γ,δ-dioxivalerate (DOVA) cycles, respectively (Fig. 1). Furthermore, we suggest, based on the ALA metabolic pathway, that due to its similarity with glycine, AMPA can affect ALA production by competing (i) with glycine in the photosynthesis process (leading to deprivation of glutamate content) and/or (ii) with the substrate of the ALA synthetase active site. However, further studies are needed to provide evidence as to how exactly AMPA may alter chlorophyll biosynthesis.

Similarly, in the case of plants without contact with AMPA and devoid of glyphosate oxidoreductase (GOX) (the enzyme responsible for glyphosate degradation into AMPA), glyphosate can induce a decrease in the chlorophyll content by depriving N assimilation (Zobiole et al., 2011b), which will lead to reduced glutamate production, affecting ALA and chlorophyll biosynthesis as shown previously by Kitchen (1980).

Photochemical reactions
After light excitation of chlorophyll molecules in the light-harvesting complexes, the energy is transferred to the reaction centres of photosystems I (PSI) and II (PSII) (Rohacek et al., 2008). The PSII reaction centre is formed by two monomers, the D1 and D2 proteins, with the chlorophyll a complex (P680), pheophytin a (Pheo), and plastoquinone (QA) as cofactors (Kern and Renger, 2007). The D1/D2 heterodimer possesses two redox tyrosine subunits Tyr₉ (Y₂) and Tyr¹₉ (Y₉) (Kern and Renger, 2007). Vivancos et al. (2011) have shown that glyphosate affects the abundance of proteins associated with PSII by disrupting aromatic amino acid biosynthesis, including tyrosine, in GS soybeans. Tyr₉ is actively implicated in the electron transport chain as an electron donor from the Mn cluster to the oxidized P680 complex. The Mn cluster is the enzymatic complex involved in the water photoysis process. It oxidizes two water molecules, releasing O₂, four protons (H⁺) and four electrons entering the photosynthetic electron transport chain (Zouni et al., 2001). The released protons contribute to the proton gradient in the thylakoid membrane, leading to ATP synthesis (Kern and Renger, 2007). Limited capacity of electron transport after glyphosate exposure was shown by Vivancos et al. (2011), but implication of the amino acid tyrosine was unclear. Another factor that has to be considered is the Mn-cluster structure. This cluster contains four Mn ions, one Ca ion, five oxygen atoms, and water molecules (Zouni et al., 2001). There is no evidence that glyphosate will disturb the formation of the Mn cluster associated with PSII, however one could reasonably expect an effect since glyphosate is known to be a strong metal chelator, forming stable complexes with Mn and Ca in plants, which become unavailable for biological processes (Cakmak et al., 2009). Reducing the availability of PSII-associated metals and amino acids could reduce its capacity to transfer light energy into the electron transport chain, and may explain the reduced photosynthetic activity observed when plants are exposed to glyphosate.

It has been demonstrated that chlorophyll a fluorescence measurements can be used to evaluate plant photosynthetic performance, and therefore it is useful to study direct and indirect effects of herbicides on photosynthesis (Juneau et al., 2007). The effects of glyphosate on photosynthesis (evaluated by chlorophyll fluorescence kinetics) have been investigated by some authors, using in vitro and in vivo studies (Table 1). In these studies, glyphosate was shown to inhibit PSII activity, electron transport rate, and non-photochemical energy dissipation processes. In addition to these effects, glyphosate can also alter the activity of PSI (Muñoz-Rueda et al., 1986) and decrease NADH and NADPH pools (Vivancos et al., 2011). On the other hand,
Table 1. Effects of glyphosate on plants, measured by chlorophyll a fluorescence

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<th>Effect</th>
<th>Parameters</th>
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<tr>
<td>↓</td>
<td>$F_0$</td>
<td>Minimal fluorescence level</td>
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<td>$F_m$</td>
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<td>↓</td>
<td>$qP$</td>
<td>Photochemical quenching</td>
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<td>↓</td>
<td>$F/F_m$</td>
<td>Maximum quantum efficiency of PSII</td>
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<td>NPO or qN</td>
<td>Non-photochemical quenching</td>
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<tr>
<td>↓</td>
<td>ETR</td>
<td>Electron transport rate</td>
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<tr>
<td>↓</td>
<td>$\Phi_{PSII}$</td>
<td>Quantum efficiency of PSII</td>
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<tr>
<td>↓</td>
<td>$F_{v}/F_{m}'$</td>
<td>Intrinsic efficiency of PSII</td>
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$^a$ Upward- and downward-pointing arrows indicate an increase or decrease of the parameter, respectively.

some studies revealed no effects of glyphosate on photosynthetic activity using chlorophyll a fluorescence measurements (Cañero et al., 2011). Differences observed among these studies may be due to methodological differences. For example, Cañero et al. (2011) treated young olive trees (Olea europaea) by adding glyphosate to their soil medium, while other fluorescence studies on superior plants (Vivancos et al., 2011; Zobiole et al., 2012) used foliar applications. Ralph (2000), meanwhile, did his experiment using an immersed seagrass, Halophila ovalis, planted in sandy loam sediments in media with liquid IPA. Glyphosate salts diluted in filtered seawater, using a recirculating flow-through system, with chlorophyll a measurements being done underwater. This methodology is quite different and this may explain the differences among the effects of glyphosate on the measured photosynthetic parameters.

Carbon metabolism
Glyphosate and AMPA also affect photosynthesis by modifying C metabolism in plants. It was reported that net C exchange and stomatal conductance was decreased after foliar application of glyphosate and AMPA (Mateos-Naranjo et al., 2009; Ding et al., 2011; Zobiole et al., 2011b). In these conditions, CO₂ assimilation capacity is reduced, leading to an increased intracellular concentration of CO₂ (Mateos-Naranjo et al., 2009; Ding et al., 2011).

In addition to these effects on gas exchange, the levels of ribulose-1,5-bisphosphate (RuBP) and 3-phosphoglyceric acid (PGA) are reduced after glyphosate exposure (Servaites et al., 1987; Siehl, 1997). It seems that glyphosate may also reduce ribulose 1,5-bisphosphate carboxylase oxygenase activity (Rubisco) in sugar beet (Servaites et al., 1987). This result was also reported by de Maria et al. (2006) in Lupinus albus leaves, where a 26% reduction of Rubisco activity occurred after five days of exposure to 10 mM glyphosate. All these effects have an impact on the plant’s efficiency in fixing atmospheric C and reduce it into sugars. Another hypothesis that was advanced to explain glyphosate’s perturbation of C metabolism is related to C flow into the shikimic pathway caused by a lack of regulation. Indeed, glyphosate’s inhibition of the shikimate pathway leads to an accumulation of shikimate-3-phosphate in the chloroplasts, which becomes a C sink (Siehl, 1997; Duke and Powles, 2008). Arogenate, a by-product of chorismate, was shown to inhibit the enzyme 3-deoxy-D-arabinoheptulosonic acid-7-phosphate (DAHP) synthase, the first enzyme involved in the shikimic pathway catalysing the condensation of phosphoenolpyruvate (PEP) with D-erythrose-4-phosphate (Siehl, 1997). Siehl (1997) suggested that the inhibition of DAHP synthase by arogenate is the key regulation process for the shikimate pathway. In the case of glyphosate exposure, this important regulatory pathway cannot occur since chorismate and all its by-products are not synthesized, resulting in C flow towards this pathway and the accumulation of shikimate-3-phosphate.

Glyphosate can also impair C metabolism by interfering with sugar metabolism and translocation. Studying the effects of glyphosate on Pisum sativum plants, Orcaray et al. (2012) found carbohydrate accumulation in both leaves and roots of glyphosate-treated plants. As growth was stopped, carbohydrate accumulation in roots was attributed to a lack of utilization of available sugars, which also caused soluble carbohydrate accumulation in leaves. Servaites et al. (1987) reported that glyphosate had no effect on sucrose synthesis and transport, but did reduce starch synthesis 4 h after foliar application on sugar beet leaves. They also found that glucose-6-phosphate levels increased during day-time, but there was no difference observed in the levels of fructose-6-phosphate or triose-phosphate.

Although only very few studies have been conducted on the effect of AMPA on C metabolism, it was shown that soluble sugar content was decreased in AMPA-treated plants (Serra et al., 2013). However, we found no study demonstrating direct AMPA effects on photochemical and C assimilatory pathways, as reported for glyphosate. AMPA effects on photosynthesis were obtained in plants a few days after exposure to AMPA and were related to the effects on chlorophyll contents (Reddy et al., 2004; Ding et al., 2011). GR- and GS-soybeans had decreased
photosynthetic activity in the presence of AMPA up to 10 days after exposure, and the recovery of photosynthesis was slowed down and occurred 28 days after exposure (Ding et al., 2011). Nitrogen nutrition and the activity of nitrate reductase in these plants were unaffected. Thus, in contrast to glyphosate, AMPA does not appear to affect N assimilation in plants. Reduced photosynthesis could thus be linked to AMPA-induced alterations in glutamate, glycine, and serine contents (Serra et al., 2013), which could lead to a decreased chlorophyll content by inhibition of ALA biosynthesis (Fig. 1). As plants are not deprived of N, the production of amino acids can be recovered leading to normalization of chlorophyll contents and photosynthesis, as demonstrated by Ding et al. (2011).

Nitrogen metabolism

The effects of glyphosate on N metabolism have mostly been studied in soybean (Leguminosae), in which symbiotic N fixation represents about 40–70% of the plant’s total N requirement (Zablotowicz and Reddy, 2007). Maintaining this significant N input is important for profitable soybean yields, and for sustaining long-term soil productivity, especially in soils with low concentrations of available N where cultural rotations are done with high N-consuming crops such as maize (Zea mays) (Zablotowicz and Reddy, 2007). Glyphosate can influence N metabolism through direct effects on the rhizobial symbiont or indirectly by affecting the physiology of the host plants (Zablotowicz et al., 2010b). Aside from plants, microorganisms also possess EPSPS enzymes and are therefore susceptible to glyphosate (Fischer et al., 1986). For example, the soybean N-fixing symbiont Bradyrhizobium japonicum possesses a GS EPSPS and accumulates shikimate and hydroxybenzoic acids, such as protocatechuic and/or gallic acids, upon exposure to glyphosate. This leads to growth inhibition and induces death at high glyphosate concentrations (de Maria et al., 2006). The accumulation of protocatechuic acid in soybean nodules of glyphosate-treated plants suggested possible translocation of the herbicide to the nodules. This hypothesis was reinforced by the reduced nitorgenase activity shown in B. japonicum bacteroids (Hernandez et al., 1999). Moreover, glyphosate residues were also found in nodules of GR soybean from plants under routine herbicide application in field conditions (Reddy and Zablotowicz, 2003). The toxic effects of glyphosate in prokaryote constituents of bacteroids may be attributed to: (i) its negative effect on the synthesis of aromatic amino acids; (ii) the accumulation of potential toxic intermediates of the shikimate pathway; or (iii) the excess chemical energy (ATP and PEP) spent in the shikimate pathway (Fischer et al., 1986).

The introduction of GR plants may have unforeseen consequences for symbiotic microorganisms associated with soybeans (Zablotowicz et al., 2011a) since glyphosate can be transferred to root nodules (Reddy and Zablotowicz, 2003) and excreted into the rhizosphere (Kremer et al., 2005). Several studies report decreased N nutrition in plants exposed to glyphosate, which has been related to glyphosate effects on N fixation/assimilation (Zablotowicz and Reddy, 2007; Bellaloui et al., 2008; Zablotowicz et al., 2012). Zablotowicz and Reddy (2004) demonstrated direct effects of glyphosate on soybean symbiotic bacteria B. japonicum by monitoring nodule parameters and acetylene reduction activity (ARA). However, until now, results mentioned in the literature are still inconsistent regarding the effects of glyphosate on the nodulation and N metabolism of GR plants. King et al. (2001) reported decreased nodulation and N fixation activity (by evaluating ARA) in 21-day-old soybeans plants. Similarly, Zablotowicz et al. (2012) reported decreased nodulation in GR plants at different growth stages. ARA inhibition has been associated with sensitivity of B. japonicum strains to glyphosate (Hernandez et al., 1999). In a three-year field study, Zablotowicz and Reddy (2007) reported only a slight effect on N fixation and/or assimilation in GR soybean at recommended application rates of glyphosate, but a consistent reduction for higher concentrations. On the other hand, Bellaloui et al. (2008) have not observed effects of glyphosate on N fixation in a 2-year field study. However, these authors reported decreased nitrate assimilation, probably due to glyphosate-induced effects on C metabolism. Although decreased nodulation may vary among soybean cultivars and plant growth stages, it was previously shown that glyphosate may affect the nodulation of approximately 45% of GR soybean cultivated in Brazil (Zablotowicz et al., 2010b). The loss of energy and fixed N2 provided by B. japonicum may be significant factors responsible for reduced growth and production observed in some GR-soybean fields (Hernandez et al., 1999).

It was reported that the effect of glyphosate on symbiotic N2 fixation was due to inhibition of photosynthesis and C substrate availability (Zablotowicz and Reddy, 2007). Kremer and Means (2009) reported that glyphosate can affect rhizospheric interactions between plants and microorganisms, for example interfering in the balance of plant indole-3-acetic acid (IAA), which leads to lower root nodulation. Moreover, the reduction of nutrient accumulation in plants exposed to glyphosate can also affect symbiotic N2 fixation (Zablotowicz et al., 2012). Zablotowicz et al. (2010b) reported that Ni was immobilized by glyphosate and could thereby compromise symbiotic N2 fixation in GR soybean since Ni is required by N2-fixing microorganisms for the hydrogenase that processes hydrogen gas generated during N2 fixation. In addition, glyphosate may interfere with the availability of other minerals which are metal cofactors required for the activity of many enzymes involved in the N2 fixation process (Zablotowicz et al., 2012).

The effects of AMPA on nodulation, N fixation, and N assimilation need further investigation. However, AMPA was proposed not to interfere with N nutrition (Ding et al., 2011). Indeed, studying the AMPA effect on GR- and GS-sensitive soybean plants, Ding et al. (2011) did not observe any effect on nodulation parameters (nodule number and dry weight) or on N fixation through acetylene reduction. These results suggest that even if AMPA is being translocated to nodules, it does not appear toxic to the symbionts involved in fixation.

Glyphosate and plant mineral nutrition

The effect of glyphosate on plant mineral nutrition has not yet been extensively studied (Zablotowicz et al., 2010b). Moreover,
results published concerning GR crops are contradictory. Some studies did not report any effect (Bailey et al., 2002; Rosolem et al., 2010), while others have shown that glyphosate disturbed plant nutritional status (Cakmak et al., 2009; Senem Su et al., 2009; Zobiole et al., 2010b, 2011b, 2012).

Studying the effects of one or two glyphosate applications (at the recommended rate of 0.86 kg ha⁻¹) on the mineral content in leaves and seeds of GR soybean grown in greenhouse and field, Duke et al. (2012b) found no change in the concentrations of Ca, Mg, Mn, Zn, Fe, Cu, Sr, Ba, Al, Cd, Cr, Co, or Ni. Hence, glyphosate did not appear to influence mineral nutrition of GR soybean while being used for weed management in the field. Accordingly, Rosolem et al. (2010) did not find any effect of glyphosate on soybean Mn nutrition, and concluded that the effect of glyphosate on soybean nutrition depends on the cultivar, the stage of plant development, the physiological conditions, and finally on the growth conditions. However, the glyphosate molecule was first patented as a metal chelator (Bromilow et al., 1993), and this primarily reported mode of action could support the hypothesis that its application can exert some effects on plant nutrition, and therefore affect growth, even in resistant plants (Zobiole et al., 2011b).

According to Cakmak et al. (2009), glyphosate may induce nutritional disturbances by immobilizing certain nutrients in plants and/or interfering with their uptake and translocation. Moreover, the effects of glyphosate on the mineral content of GR crops were associated with the greater susceptibility of these crops to plant diseases (Johal and Huber, 2009; Kremer and Means, 2009). The accumulation of glyphosate in plant tissues may, in association with its chelating property, reduce the free activity of cationic mineral nutrient, leading to their deficiency in cells (Cakmak et al., 2009). Glyphosate is easily bound to divalent cations by its carboxyl and phosphonate groups, forming insoluble or very stable complexes, leading to immobilization of several divalent cations in plant tissues (Bellaloufi et al., 2009; Cakmak et al., 2009; Senem Su et al., 2009; Zobiole et al., 2011b). Significant decreases in all macronutrients and micronutrient contents have been observed in GR-soybean plants exposed to glyphosate (Zobiole et al., 2011a, 2012). According to the aforementioned authors, these plants were rendered less efficient in nutrient uptake and translocation and suffered from potential chelating effects of glyphosate. The decreased mineral concentrations in glyphosate-exposed soybean leaves (Zobiole et al., 2012) is in agreement with the possible inhibition of uptake and/or transport of nutrients due to the formation of glyphosate–metal complexes within plant tissues (Eker et al., 2006). Moreover, reduced nutrient uptake and accumulation may be related to glyphosate reducing root growth (Zobiole et al., 2012). According to Zobiole et al. (2011b), glyphosate doses affect plant macronutrient accumulation in the following order, whether glyphosate was applied as a single dose or as multiple doses: Cu > Mg > N > S > K > P. Meanwhile, micronutrient accumulation is affected in the following order, for single and sequential glyphosate applications, respectively: Fe > Mn > Co > Zn > Cu > B > Mo; and Fe > Co > Zn > Mn > Cu > Mo > B. Moreover, the negative effects of glyphosate on plant mineral nutrition varied depending on their growth stages, with younger plants being more sensitive than plants receiving glyphosate at a later growth stage (Zobiole et al., 2011b). Nutritional disturbances in GR plants may be related to the frequently observed plant injury known as ‘yellow flashing’ following glyphosate application (Zablotowicz and Reddy, 2007). This yellow flashing, occurring shortly after glyphosate application, was also proposed to be attributed to AMPA toxic effects and not to mineral deficiencies (Duke et al., 2012b).

In a short-term uptake study using radiolabelled elements, Eker et al. (2006) reported that root-to-shoot translocation of micronutrients was severely inhibited in sunflower plants in the order Mn > Fe > Zn. Studying the mineral nutrition of some turf-grass species, Senem Su et al. (2009) observed that glyphosate applications reduced shoot concentration of mineral nutrients, especially Ca, Mg, Fe, and Mn. Although research in this field is still limited, it is important to know that glyphosate interactions with plant mineral nutrition could amplify the toxicity of glyphosate for GS plants and lead to some glyphosate toxicity in GR plants by interfering with mineral nutrient availability (Senem Su et al., 2009).

**Oxidative stress**

Along with the inhibition of specific target sites, glyphosate action also leads to oxidative stress in plants, which is most probably a secondary effect of the blocked shikimate pathway (Ahsan et al., 2008). Plants have developed mechanisms to cope with oxidative stress induced by reactive oxygen species (ROS) accumulation by synthesizing enzymatic and non-enzymatic antioxidants (Gunes et al., 2007). Among enzymatic systems, activities of ROS-scavenging enzymes and content of malondialdehyde (MDA), a product of membrane lipid peroxidation, are frequently used as indicators of oxidative stress in plants (Gunes et al., 2007). Although changes in these oxidative stress markers were reported under various stress conditions, little information is currently available concerning the effects of glyphosate on oxidative stress.

Maize leaves exposed to glyphosate showed an increased level of lipid peroxidation, glutathione (GSH), free proline content, and ion flux (Serenk et al., 2006). In a gene expression analysis, Ahsan et al. (2008) found that glyphosate application generates hydrogen peroxide (H₂O₂), resulting in peroxidation and destruction of lipids in rice (Oryza sativa) leaves. Moreover, these authors also observed a decrease of the Rubisco large subunit content and an increase in the accumulation of antioxidant enzymes, including ascorbate peroxidase (APX), glutathione-S-transferase (GST), thioredoxin h-type, nucleoside diphosphate kinase 1 (NDPK1), peroxiredoxin, and the chloroplast precursor of superoxide dismutase [Cu-Zn] (SOD) within leaves treated with glyphosate.

Testing GR and susceptible (GS) soybean plants exposed to glyphosate, Moldes et al. (2008) did not observe a significant impact of glyphosate on lipid peroxidation. However, there was a significant increase in the levels of soluble amino acids in roots and leaves, which was greater in GS- than in GR-soybean cultivars. Soluble amino acids have antioxidant action (Samaranayaka and Li-Chan, 2011), which may
prevent lipid peroxidation. Furthermore, the oxidative stress generated by glyphosate in these plants was noted by the modulation in CAT and guaiacol peroxidase (GPX) activities. Studying the phytotoxicity of glyphosate in duckweed (Lemna minor), Kielak et al. (2011) observed that glyphosate causes damage similar to abiotic and biotic stressors, leading to an over-accumulation of putrescine, spermidine, and total polyamines, which were connected to the oxidative burst induced by glyphosate. Duckweed tissues treated with glyphosate also showed higher CAT and APX activities, demonstrating that oxidative stress can be induced by glyphosate.

Oxidative stress in P. sativum, wheat (Triticum aestivum) and maize was observed in plants exposed to glyphosate. Indeed, increased MDA (lipid peroxidation) and H$_2$O$_2$ contents and activation of antioxidant enzymes (SOD, CAT, and GPX) were found (Sergiev et al., 2006; Miteva et al., 2010). Moreover, glyphosate application on both leaves and roots of pea plants resulted in the activation of GSH reductase and enhancement of the GST activities which, in addition to the increase in both total and oxidized GSH contents, highlight the oxidative stress induced by glyphosate in these plant tissues (Miteva et al., 2010).

Studying herbicidal effects on Arabidopsis thaliana plants, Serra et al. (2013) observed increased accumulation of inositol, ascorbate, and serine. Inositol and ascorbate are oxidative-stress markers and their increased content is related to increased oxidative stress in plants (Foyer and Noctor, 2011). In addition, serine (as a cysteine precursor) is involved in glutathione metabolism (Foyer and Noctor, 2011), and its accumulation could also be an indicator of oxidative stress. Interestingly, A. thaliana treated with AMPA did not show an increase in these oxidative-stress markers (Serra et al., 2013). While ascorbate and inositol contents did not statistically differ from control plants, the serine content was decreased, due to decreased glycine content (as these two amino acid are intrinsically linked to metabolic pathways) (Serra et al., 2013). Thus, in contrast to glyphosate, AMPA was not suggested to induce oxidative stress in A. thaliana plants (Serra et al., 2013).

The oxidative damage due to glyphosate exposure could be associated with glyphosate effects on plant nutrition. For instance, metal deficiency could increase oxidative stress in plants because redox-active metals such as Cu are known to perform antioxidative protection in plant cells (Cuypers et al., 2001). Moreover, metal deficiency such as Zn and Fe could impair activities of antioxidant enzyme systems (Fig. 2). On the other hand, AMPA may not induce oxidative damage since this glyphosate by-product has not been proven to induce nutritional disturbance (as glyphosate does). However, no study has confirmed this hypothesis. Apart from acting as hazardous molecules in the oxidative burst, ROS can also act in plant signalling and are involved in several plant physiological processes. Specifically, ROS are intrinsically related to plant hormone action, thus it is important to study glyphosate-induced oxidative events as they can directly interfere with plant growth and development.

**Glyphosate and lignin**

Lignin represents a significant proportion of all the C fixed by plants, accounting for approximately 30% of the organic C in the biosphere (Gosselink et al., 2004). The lignification of cellulose microfibrils has been described as an adaptive mechanism that helps to maintain plant stability and tolerance to biotic and abiotic stresses (Gomes et al., 2011). Lignin production is controlled by phenylalanine, a key product of the shikimate pathway (Zobiole et al., 2010a), and thus can be highly affected by glyphosate. Indeed, even GR-soybean plants treated with glyphosate have been shown to produce less lignin when compared to non-treated plants (Zobiole et al., 2010b). According to Marchiosi et al. (2009), glyphosate may reduce lignin synthesis due to the inhibition of EPSPS followed by the reduced supply of cinnamate precursors. Reduction in lignin content is an important physiological aspect of glyphosate’s herbicidal effect since the lignin content is associated with the morphological and functional quality of plant organs (Gaspar and Coumans, 1987). Depriving plants of suitable lignin content may make them vulnerable to diseases as well as to nutrition and water-balance disturbances. The role of lignin in plant–pathogen defence has been widely discussed and the lignin deposition in root cortical cell walls is recognized to help maintain root turgescence (Gomes et al., 2011), leading to adequate water and mineral uptake. Despite its importance, however, studies of glyphosate affecting lignin content in plants remain scarce.
Glyphosate, AMPA and plant hormones

Glyphosate and AMPA accumulation in active metabolic tissues (i.e. growing and metabolically active sites, shoot and root apical meristems) (Cakmak et al., 2009) are identical to the main production sites of plant hormones, and thus glyphosate/AMPA detrimental effects in plants can induce hormonal disturbance. Thus, glyphosate-exposed plants could have both growth and development affected.

Auxin is a critical hormone related to plant growth and developmental processes. Indole-3-acetic acid (IAA), the key auxin, is synthesized from tryptophan and indole tryptophan precursor (products from the shikimate acid pathway). Therefore, by inhibiting the shikimate pathway, glyphosate may prevent auxin biosynthesis. Evaluating the gene expression of the apical bud in soybean, Jiang et al. (2013) observed different expression of genes involved in auxin metabolic pathways (genes related to auxin response factor, genes encoding proteins in the auxin-responsive family, and IAA genes). The results indicated that the effects of glyphosate on these genes may perturb cell enlargement and plant growth (Jiang et al., 2013). Moreover, by interfering in the balance of IAA, glyphosate can affect rhizospheric interactions between plants and microorganisms, leading, for example, to lower root nodulation (Kremer and Means, 2009).

Sublethal doses of glyphosate were also shown to reduce the velocity of indolaecetic acid (IAA) basipetal transport in cotton (Gossypium hirsutum) seedlings (Baur, 1979). Application of 1.44 kg acid equivalent ha⁻¹ at the beginning of flower bud appearance (eight-leaf stage) in GR cotton resulted in an increase in IAA level in anthers, resulting in inhibition of anther dehiscence (Yasuor et al., 2006). This IAA accumulation was related to glyphosate inhibition of auxin transport (Yasuor et al., 2006). Pretreatment of tobacco (Nicotiana tabacum) callus with glyphosate and AMPA perturbed IAA metabolism by increasing both conjugation and oxidation, and consequently lowered the level of free IAA leading to growth inhibition (Lee et al., 1983). However, at identical concentrations, AMPA was less active than glyphosate (Lee et al., 1983).

Sergeiv et al. (2006) observed alleviation of the detrimental effects of glyphosate in maize plants following treatment with the phenylurea cytokinin 4PU-30. Three- and four-year-old Picea pungens treated with a mixture of glyphosate and haxazinone showed decreased cytokinin (Cyt) content (Matschke and Macháčková, 2002). The strongest decrease was observed in the upper and middle part of the root (Matschke and Macháčková, 2002), which coincides with the Cyt-producing and glyphosate-accumulating sites. These results indicate that glyphosate can also affect Cyt metabolism in plants. Similarly, application of gibberellic acid (GA₃) to GR cotton has some remedial effects on pollen viability, which can also indicate glyphosate effects in GA metabolism (Pligne et al., 2003). In higher plants, in addition to participating in the biosynthesis of lignins, fatty acids, flavonoids, etc., the cytochrome P450 monoxygenases also participate in GA, brassinosteroid, and jasmonic acid biosynthetic pathways (Bolweel et al., 1994). Glyphosate inhibition of P450 activity was seen in yeast (Xiang et al., 2005), which could also be expected in plants, corroborating the glyphosate effects on plant hormones. Glyphosate was also shown to interfere with other hormones such as ethylene (Lee and Dumas, 1983) and abscisic acid (ABA) (Jiang et al., 2013). Although extremely important, the effects of glyphosate and AMPA in plant hormone metabolism are still unclear, and little attention has been given to the effects of AMPA in plant hormone metabolism and biosynthetic pathways.

Glyphosate and plant diseases

Herbicides are known to increase specific plant diseases (Hornby et al., 1988). Due to its interaction with rhizosphere microorganisms and plant physiological features, glyphosate is also expected to modulate diseases in plants. Glyphosate effects on plants diseases were extensively reviewed (Johl and Huber, 2009; Duke et al., 2012a). Due to the induction of mineral nutrient disturbances, glyphosate can greatly affect plant growth and resistance to diseases and pests (Johl and Huber, 2009). Moreover, increase in root infection may be due to the shutdown of plant protection compound production, such as phytoalexins (Kremer et al., 2005), which are synthesized by plants via the shikimate pathway (Sharon et al., 1992).

Studying GR-soybean plants, Kremer (2003) reported that fungal colonization of their roots increased significantly after application of glyphosate but not when conventional post-emergence herbicides were applied. Gressel (2002) argued that transgenic EPSPS of GR soybean could be less efficient than the wild-type genotype and might produce insufficient phytoalexins to prevent fungal infection. However, in the sole study on phytoalexin production in GR soybeans, Duke et al. (2003) showed no effect on phytoalexin content in plants following glyphosate exposure. Thus, the susceptibility of GR plants to pathogenic infection should not be associated with decreased phytoalexin content (due to ineffective EPSPS) but to overall changes in plant physiology which could reduce plant resilience.

Descalzo et al. (1998) observed an increased population of the fungus-like pathogen Pythium sp. in the rhizosphere and in soils amended with heat-killed roots from glyphosate-treated beans. This population increase was not observed in control treatments (without glyphosate). The authors concluded that herbicide treatment of plants increased the population's potential for damping off the soil. Similarly, Smiley (1992) observed a relationship between glyphosate application and plant diseases in a Rhizoctonia-infected soil. As the interval between glyphosate application and planting spring barley was shortened, severity of Rhizoctonia root rot increased. Thus, glyphosate treatment can induce a massive release of organic compounds from dying roots of target plants which can induce proliferation of a wide range of soil-pathogenic fungi.

GR soybeans can also release glyphosate into the rhizosphere which could influence the microbial community, enhancing communities of microorganisms pathogenic to GR soybean and causing a buildup of detrimental species that may affect subsequent crops (Kremer et al., 2005). Indeed,
glyphosate can be used as a nutrient source by specific fungal species. Moreover, Kremer et al. (2005) demonstrated that glyphosate exudes from soybean root tips and that glyphosate application also increases carbohydrate and amino acid contents in root exudates. Together, both glyphosate and high levels of soluble carbohydrates and amino acids associated with glyphosate treatment of the soybean plants may provide a selective C and N source that stimulates growth of selected rhizospheric fungi.

Glyphosate effects on photosynthesis and oxidative stress may also be linked to glyphosate increasing plant susceptibility to diseases. For example, ROS have crucial roles in pathogenesis, and are reported to be involved in processes such as the hypersensitive response of plant–pathogen incompatibility interactions; limiting pathogen infection by reinforcing plant cell walls and/or killing pathogens directly; programmed cell death (PCD); and signalling acquired resistance (Shetty et al., 2008). Modulations of the system coordinating ROS content may lead to loss of ROS-beneficial functions, thereby causing oxidative stress and subsequent effects, and favouring pathogenic infections.

**Glyphosate resistance in weeds**

Until 1993, the natural development of weed tolerance or resistance to glyphosate was a topic virtually absent from the literature (Holt et al., 1993). However, glyphosate-tolerant weed populations have now increased substantially, as reported by the International Survey of Herbicide Resistant Weeds (www.weedscience.org). The morphological or physiological reasons for such wide genetic variation in resistance to glyphosate are not well understood (Senem Su et al., 2009). Glyphosate resistance in crops and weeds develops via two primary mechanisms referred to as ‘non-target site’ resistance (reduced absorption and/or translocation) and ‘target site’ resistance (mutation and gene amplification) (Cruz-Hipolito et al., 2011). Sequestration of glyphosate into vacuoles (Ge et al., 2010), EPSPS gene amplification (Gaines et al., 2010), EPSPS mutation (Preston et al., 2009; Powles and Preston, 2010), reduced translocation of glyphosate (Powles and Preston, 2010), and poor penetration and translocation of the herbicide to apical growing points (Cruz-Hipolito et al., 2011) have been described to confer plant glyphosate resistance. Mechanisms involving plant metabolism of glyphosate have not been found to contribute to resistance of any weeds (Feng et al., 2004). However, Duke (2011) recently suggested that some plants possess a GOX enzyme involved in degradation of glyphosate into AMPA. This enzyme may be related in part to the observed natural tolerance of some species. However, as AMPA could also be toxic, these plants might be able to degrade AMPA by C-to-phosphorus (C-P) lyases, enzymes responsible for direct cleavage of organophosphonate C-P, avoiding AMPA accumulation (Van Eerd et al., 2003). Studying the weed species *Digitaria insularis*, de Carvalho et al. (2012) observed that more than 90% of glyphosate was degraded into AMPA, glyoxylic acid, and sarcosine in resistant biotypes, whereas only a small amount of herbicide (up to 11%) was degraded by the susceptible biotype.

In resistant biotypes, the substitution of a proline with threonine and of a tyrosine with a cysteine at positions 182 and 310 of the EPSPS, respectively, were observed. Therefore, the authors concluded that absorption, translocation, metabolism, and gene mutation play an important role in *D. insul-aris* glyphosate resistance. Studying GR *Amaranthus palmeri* populations, Gaines et al. (2010) verified that the EPSPS gene amplification was heritable in pseudo-F2 populations and conferred glyphosate resistance. Both resistant and susceptible *A. palmeri* plants had EPSPS activity inhibited by glyphosate; however, resistant plants contained 5- to 160-fold more copies of the *EPSPS* gene in their genome.

Mineral nutrition could also lead to glyphosate resistance, as plants showing high concentrations of some cationic nutrients may significantly reduce glyphosate phytotoxic effects through formation of poorly soluble glyphosate complexes (Senem Su et al., 2009). Moreover, the horizontal transfer of GOX or C-P lyase genes from soil microbes to plants may also be possible (Duke, 2011). Glyphosate-tolerant weed populations have increased vertiginously, as reported by the International Survey of Herbicide Resistant Weeds (www.weedscience.org). Consequently, further studies in relation to glyphosate resistance are needed as the population of glyphosate-tolerant weeds has considerably increased.

**Conclusion**

It is generally claimed that glyphosate kills undesired plants by affecting the EPSPS synthase enzyme, disturbing the synthesis of aromatic amino acids. However, glyphosate has several secondary or indirect effects on plant physiology which may also explain its herbicidal effects. The toxicity of glyphosate could be related to its effects on other physiological processes such as mineral nutrition and photosynthesis, and to the plant’s hormone and oxidative status. The alteration of these cellular processes could be directly linked to the deleterious effects of glyphosate observed on plant growth and production. As a metal chelator, glyphosate could deprive plants from important nutrients which have important roles as enzymatic co-factors, biomolecular constituents, and antioxidative systems. Oxidative stress, more specifically lipid peroxidation, induced by glyphosate, is known to severely damage the cell integrity which may lead to cell death. Moreover, increased ROS production can negatively interfere with photosynthetic processes, for example by decreasing the chlorophyll content, photochemical efficiency, and C metabolism, leading to reduction in plant growth.

It is also important to note that some plants can metabolize glyphosate to AMPA or be exposed to AMPA via different environmental matrices, which could amplify glyphosate effects on plant physiology. AMPA by itself has been considered phytotoxic, although not to the same extent as glyphosate. AMPA has been proposed to be responsible for injuries in GR plants exposed to glyphosate (Reddy et al., 2004). In fact, AMPA could interfere in chlorophyll biosynthesis by inhibiting ALA formation. However, some studies showed rapid recovery of plant chlorophyll content and photosynthetic activity after AMPA exposure (Ding et al., 2011).
Therefore, further studies of AMPA effects on plant physiology are clearly needed. These studies will provide evidence for the development of more-efficient GR plants. Since some GR plants are able to degrade glyphosate to AMPA, it is important to study whether this metabolite is responsible for the observed deleterious effects found in some plants treated with glyphosate or if these symptoms are related to the indirect effects of glyphosate on plant physiology.

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