Glyphosate reduced seed and leaf concentrations of calcium, manganese, magnesium, and iron in non-glyphosate resistant soybean

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ABSTRACT

Greenhouse experiments were conducted to study the effects of glyphosate drift on plant growth and concentrations of mineral nutrients in leaves and seeds of non-glyphosate resistant soybean plants (*Glycine max*, L.). Glyphosate was sprayed on plant shoots at increasing rates between 0.06 and 1.2% of the recommended application rate for weed control. In an experiment with 3-week-old plants, increasing application of glyphosate on shoots significantly reduced chlorophyll concentration of the young leaves and shoots dry weight, particularly the young parts of plants. Concentration of shikimate due to increasing glyphosate rates was nearly 2-fold for older leaves and 16-fold for younger leaves compared to the control plants without glyphosate spray. Among the mineral nutrients analyzed, the leaf concentrations of potassium (K), phosphorus (P), copper (Cu) and zinc (Zn) were not affected, or even increased significantly in case of P and Cu in young leaves by glyphosate, while the concentrations of calcium (Ca), manganese (Mn) and magnesium (Mg) were reduced, particularly in young leaves. In the case of Fe, leaf concentrations showed a tendency to be reduced by glyphosate. In the second experiment harvested at the grain maturation, glyphosate application did not reduce the seed concentrations of nitrogen (N), K, P, Zn and Cu. Even, at the highest application rate of glyphosate, seed concentrations of N, K, Zn and Cu were increased by glyphosate. By contrast, the seed concentrations of Ca, Mg, Fe and Mn were significantly reduced by glyphosate. These results suggested that glyphosate may interfere with uptake and retranslocation of Ca, Mg, Fe and Mn, most probably by binding and thus immobilizing them. The decreases in seed concentration of Fe, Mn, Ca and Mg by glyphosate are very specific, and may affect seed quality.

1. Introduction

Glyphosate (N-[phosphonomethyl] glycine) is the most commonly applied herbicide in cropping systems (Duke and Powles, 2008). The major herbicidal action of glyphosate is based on the inhibition of the enzyme 5-enolpyruvylshikimate 3-phosphate synthase (EPSPS) that results in reduced biosynthesis of aromatic amino acids and alterations in protein metabolism. Glyphosate also causes adverse effects on photosynthetic carbon metabolism and sucrose translocation within plants (Geiger et al., 1999; Riberio et al., 2008). Impairments in nitrate assimilation and nitrogen fixation are reported as further detrimental effects of glyphosate in plants (King et al., 2001; De Maria et al., 2006; Bellaloui et al., 2006), especially under water stress conditions (Zablotowicz and Reddy, 2007). Even at relatively low application doses (e.g., 1.25 mM), glyphosate caused significant decreases in nitrogenase activity in nodules of lupine plants 24 h after its spray (De Maria et al., 2006).

Plant organs with high metabolic activity and growth rates such as nodules, root tips and shoot apex represent a high sink activity for glyphosate. Experimental evidence is available showing substantial accumulation of foliar-applied glyphosate in such sink tissues (Schulz et al., 1990; Hetherington et al., 1999; Feng et al., 2003). According to Feng et al. (2003) up to 80% of the glyphosate absorbed after foliar applications is translocated into shoot apex and root tips. Even at low foliar application rates, the sink tissues accumulate glyphosate at very high concentrations. Previously, it has been reported that only a single foliar application of glyphosate at a rate of 0.5 kg ha^{-1} was effective to cause an accumulation of glyphosate up to 0.3 mM in the sink organs, and this concentration could be much higher when glyphosate is applied at greater rates or repeatedly (McWhorter et al., 1980; Honegger et al., 1986, cited in King et al., 2001). In tomato and spinach plants treated with foliar glyphosate applications, it is estimated that glyphosate may account for up to 16% of the dry weight of sink tissues (Schulz et al., 1990).

Accumulation of glyphosate in shoot apex or root tips at high amounts may induce impairments in cellular utilization of cationic mineral nutrients via reducing the free activity of these nutrients by chelation. Glyphosate has high ability to complex several divalent

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cationic nutrients such as calcium (Ca), magnesium (Mg), manganese (Mn) and iron (Fe) (Lundager-Madsen et al., 1978; Motekaitis and Martell, 1985; Barja et al., 2001). These cationic nutrients easily bind to the glyphosate molecule via the carboxyl and phosphonate groups forming poorly soluble or very stable complexes. Formation of such insoluble glyphosate complexes with cations in spray solution reduces effectiveness of glyphosate to kill weed plants. In velvetleaf plants, Bernards et al. (2005) showed that the presence of Mn in spray solution caused formation of stable Mn–glyphosate complexes which in turn resulted in reduced penetration and translocation of glyphosate from the treated leaves. Similar antagonistic reactions with glyphosate have been also shown for Ca and Mg (Nalewaja and Matysiak, 1991; Thelen et al., 1995). The presence of monovalent cations in glyphosate spray solutions did not cause any change in the glyphosate efficacy (Stahlman and Phillips, 1979). Calcium has been shown to precipitate glyphosate by forming a 1:1 complex in aqueous solutions (Gauvrit et al., 2001; Schoenherr and Schreiber, 2004). Therefore, it is often recommended by the manufacturer companies not to use hard water in preparing glyphosate spray solutions.

These results and observations indicate that divalent cations may reduce the efficacy of glyphosate in plant and soil systems. When complexed by glyphosate, the activity of divalent cations at the physiological level might also be reduced. This can be considered as a significant side-effect for non-target plants exposed to glyphosate spray drift. Glyphosate spray drift is currently an increasing concern in cropping systems where glyphosate is being repeatedly applied (Burke et al., 2005; Bellaloui et al., 2006; Buehring et al., 2007; Rolder et al., 2007). Up to 10% of the foliarly applied glyphosate may move to non-target plants (Al-Khatib and Peterson, 1999; Snipes et al., 1991) and this spray drift may be as high as 37% of the applied glyphosate rate depending on the speed of wind and accuracy of the glyphosate application method (Nordby and Skuterud, 1975).

There are, however, limited data on the effects of glyphosate drift on mineral nutritional status of the non-target plants. Applying glyphosate up to 12.5% of the recommended rate adversely affected nitrate assimilation and nitrogen fixation of soybean plants (Bellaloui et al., 2006). In both glyphosate resistant and glyphosate non-resistant crops, it has been shown that shoot concentrations of micronutrients, especially Mn and Fe, show a decrease upon glyphosate applications (Eker et al., 2006; Bott et al., 2008). The results of Bott et al. (2008) clearly indicate higher demand for Mn of the glyphosate-tolerant crops when treated with glyphosate. Because of high affinity of glyphosate to chelate and immobilize divalent cations glyphosate reduces shoot Mn concentration irrespective of whether crops are glyphosate tolerant or not. Interestingly, Gordon (2007) showed that leaf concentrations of Mn in glyphosate-tolerant soybean are lower than the glyphosate-sensitive soybean plants and therefore glyphosate-tolerant soybean cultivars require adequate Mn application to achieve highest yield. The fact that glyphosate is a very strong chelator for divalent cations indicates that glyphosate can physiologically immobilize these nutrients in the tissues. The 'yellow flashing' commonly observed in RR crops after glyphosate applications is attributable to immobilization of divalent cations especially Fe and Mn (Franzen et al., 2003; Hansen et al., 2004; Jolley et al., 2004; Eker et al., 2006). Most probably, the length of this 'flashing' is dependent on the ability of the plants to recover by adequate root uptake of the concerned elements which are immobilized by the glyphosate in plant tissues, assuming no foliar fertilization of these nutrients have been made.

In the present study, we tested the effects of foliar application of glyphosate on mineral nutrient concentrations of non-glyphosate resistant soybean plants at both early growth stage and at the grain maturation. To our knowledge, the effects of increasing glyphosate rates on concentration of mineral nutrients in soybean grain have not been described before. In the case of the experiment conducted until grain maturation, glyphosate has been sprayed at V4, V6 and R1 stages. These growth stages are widely considered application times for glyphosate, and suggested to be critical stages for an effective weed control (Halford et al., 2001; Krausz and Young, 2001; Bellaloui et al., 2008; Miller et al., 2008).

2. Materials and methods

2.1. Growth conditions

Non-glyphosate resistant soybean plants (*Glycine max* [L.] Merr. Cultivar: Nova) were grown under greenhouse conditions equipped with an evaporative cooling system (24–28; 21–24 °C day/night) under natural daylight during the summer season. Soybean seeds were sown in a 3.3-l plastic pots containing 2.8 kg loamy clay soil with pH 7.6, organic matter 1.5%, $CaCO_3$ 17.6%. The soil used in the experiments was transported from the Eskisehir region in Central Anatolia in Turkey where soil Zn deficiency has been well documented (Cakmak et al., 1999). Top 40 cm of the soil profile has been taken and dried at the ambient temperature before the transportation. The concentrations of DTPA-extractable Zn, Fe, Mn and Cu were 0.10, 5.6, 7.2 and 1.7, respectively, measured according to the method described by Lindsay and Norvell, 1978). Before potting soil was homogenously mixed with a basal treatment of 200 mg N kg^{-1} soil as $Ca(NO_3)_2$, 100 mg P kg^{-1} soil and 126 mg K as KH_2PO_4, 20 mg S kg^{-1} soil as $CaSO_4$, 5 mg Fe kg^{-1} soil as FeEDDHA and 2 mg Zn kg^{-1} soil as $ZnSO_4{\cdot}7H_2O$. Soil was not inoculated with N-fixing bacteria. Eight seeds were sown in each pot, and after emergence the seedlings were thinned to four per pot. Plants were watered daily by using deionized water.

Two separate pot experiments were conducted in a randomized block design with 4 (in the first experiment) or 6 (in the second experiment) replications. In the first experiment, plants were grown for 3 weeks under greenhouse conditions, and foliar glyphosate applications were made at 13 days after planting. At harvest, the shoot apex together with the youngest expanded leaves was harvested to represent the young plant parts and the remaining leaves as old plant part. The leaf samples were analyzed for mineral nutrient concentrations as described below. In the second experiment, plants were grown until seed maturation in the plastic pots containing 2.8 kg loamy clay soil as mentioned above. In addition to the basal fertilizer treatment, 100 mg N as $Ca(NO_3)_2$ and 50 mg P and 63 mg K as KH_2PO_4 per kg soil was applied into soil at the R1 growth stage. Glyphosate applications during the second experiment were made at the V4, V6 and early R1 stages as described below. Plants were harvested at the grain maturation stage, and the grains harvested were used for determination of grain yield and for analysis of mineral nutrient concentrations.

2.2. Glyphosate applications

In all foliar applications, the isopropylamine salt of glyphosate (Roundup Ultra Herbicide, Monsanto Ltd., Adana, Turkey) with an active ingredient (ai) 480 g L^{-1} was used. In the first experiment, when plants were 13 days old, foliar applications of glyphosate was initiated and realized three times with two days intervals. The doses applied for simulating foliar glyphosate drift were 0.06, 0.2, and 0.6% of the recommended application rate (i.e. 1.44 kg ha^{-1} ai glyphosate applied with 200 L of water ha^{-1}: 31.55 mM glyphosate as active ingredient), which are equivalent to 19, 63 and 189 μM glyphosate in the spray solution, respectively. These concentrations have been selected based on the preliminary tests to determine the different sublethal doses of glyphosate, and the selected doses were similar to those used previously by De Maria et al. (2006) and Eker et al. (2006).

In the experiment done until seed maturation, the simulated drift rates of the glyphosate applied at the V4, V6 and early R1 stages and were 0, 0.3, 0.6, 0.9 and 1.2% of the recommended application rate which are equivalent to 0, 95, 189, 284 and 379 μM glyphosate in the spray solution, respectively. Glyphosate solutions were freshly prepared before the foliar treatments. Applications were made onto the leaves using a plastic hand-sprayer until most of the leaves became wet but not allowing any surface run-off.

2.3. Chlorophyll and mineral nutrients

Changes in levels of chlorophyll (SPAD value) were measured on the same area of the youngest expanded leaves before harvest using a chlorophyll meter (Minolta SPAD-502, Japan). For the measurement of mineral nutrient concentrations, the dried and ground leaf and seed samples were digested in 2 ml 35% H_2O_2 and 4 ml 65% HNO_3 by using a microwave digestion system (MarsExpress; CEM Corp., Matthews, NC, USA). The analysis of P, K, Mg, Ca, Mn, Fe, Zn and Cu was performed by using inductively coupled plasma optical emission spectrometry (ICP-OES) (Vista-Pro Axial; Varian Pty Ltd., Mulgrave, Australia). Total nitrogen concentrations of the samples were measured by an automated N analyzer (TruSpec CN, LECO Corp., Michigan, USA).

All treatments were performed in four and six independent replications in the first and second experiment, respectively. Differences among the means and treatments were compared by the least significant difference (LSD) test at $P = 0.05$ probability level.

2.4. Shikimic acid

Shikimic acid accumulation was determined in the youngest leaves at the growing points and oldest trifoliate leaves using a modified method of Cromartie and Polge (2002). About 500 mg (±25 mg) of fresh leaf tissues was extracted in 5 ml of 0.25 N HCl in an ice cold mortar and centrifuged at 15,000 × *g* for 15 min at +4 °C. The resulting supernatant was diluted with 0.25 N HCl at a ratio of 1:10 (v:v) and directly used in the colorimetric assay. Aliquots of 200 μL of 1:10 diluted samples were mixed with 400 μL of reaction solution consisting of 0.25% periodic acid and 0.25% Na meta-periodate. All samples were incubated at room temperature for 1 h to oxidize the shikimic acid. Finally 400 μL of quenching solution consisting of 0.6 M NaOH and 0.22 M Na_2SO_3 was added to yield a yellow color chromophore that correlates directly with the amount of shikimic acid in the samples. Concentration of shikimic acid was determined by measuring the absorbance at 380 nm using a standard curve of 0–200 μM shikimic acid (S5375; Sigma, St. Louis, MO, USA) and presented on a fresh weight basis.

3. Results

The effect of foliar-applied glyphosate on the shoot growth and leaf concentrations of mineral nutrients was first studied in 21-day-old plants by analyzing young and old leaves separately. The first reaction of plants to increasing glyphosate application up to 0.6% of the recommended dose was the development of chlorosis on the youngest leaves. As expected, chlorophyll concentrations (SPAD values) showed a clear decrease by glyphosate (Table 1). Increasing application of glyphosate significantly reduced dry weight of young leaves, but did not affect the dry weight of the old leaves (Table 1). The dry weight ratio of older parts to younger parts of plants was nearly 2 at the nil glyphosate treatment and increased to about 10 at the highest glyphosate treatment (Table 1). Increasing rates of glyphosate caused marked increases in shikimate concentrations of young parts of plants; but, remained less effective on the shikimate concentrations in the old parts of plants (Table 1).

Among the mineral nutrients analyzed, the leaf concentrations of K, P, Cu and Zn were not affected, or even increased significantly in case of P and Cu in young leaves by glyphosate (Table 2). Phosphorus concentrations of young leaves showed significant increases by increasing glyphosate rate that might be related to accumulation of P-containing glyphosate in young parts of plants (Table 2). In the case of Fe concentrations of leaves, there were inconsistent changes by glyphosate applications. But, at the highest glyphosate rate, leaf concentrations of Fe showed a clear declining tendency when compared to other glyphosate treatments (Table 2). In contrast to other mineral nutrients analyzed, the leaf concentrations of Ca, Mn and Mg were reduced greatly by glyphosate, particularly Ca and Mn in young leaves (Table 2). For example, in young leaves, the concentration of Ca was reduced from 14.9 to 9.0 mg kg^{-1} and Mn was reduced from 182 to 129 mg kg^{-1}.

In the second experiment, plants were grown until grain maturation, and glyphosate was sprayed three times at the V4, V6 and early R1 stages. Distinct decreases in shoot biomass production and seed yield by glyphosate were found at the higher glyphosate rates (e.g., 0.9 and 1.2% of the recommended dose) which also resulted in development of chlorosis on young leaves (Fig. 1; Table 3). When compared to the shoot dry weight, seed yield was more clearly declined by glyphosate. At the 1.2% glyphosate application, seed yield was declined by nearly 5-fold when compared to the plants without glyphosate application; but, this decrease was only 2.8-fold for shoot dry weight, indicating higher sensitivity of generative organs to glyphosate.

Increases in glyphosate application resulted in significant changes in seed concentrations of all measured elements with exception of P (Table 4). At the highest level of the glyphosate application, the seed concentrations of N, K, Zn and Cu were significantly increased when compared to the control plants without glyphosate treatment. By contrast, the seed concentrations of Ca, Mn, Fe and Mg were significantly reduced by the highest rates of glyphosate, particularly in the case of Ca and Mn (Table 4). To verify

Table 1
Effect of increasing glyphosate rates on SPAD values (chlorophyll) and dry matter production of young and old leaves of 21-day-old soybean plants grown under greenhouse conditions. The values represent means of four independent replications.

Glyphosate rate (% of recommended)[a]	SPAD value	Dry matter production		Shikimate	
		Young leaves (mg $plant^{-1}$)	Old leaves (mg $plant^{-1}$)	Young leaves (μmol g^{-1} FW)	Old leaves (μmol g^{-1} FW)
0	34.4	183	346	1.6	2.4
0.06	31.9	103	353	1.4	2.2
0.2	23.0	120	373	9.3	2.2
0.6	12.0	40	440	34.6	6.3
$LSD_{0.05}$	1.6	33	NS	3.2	1.7

[a] Glyphosate was applied to foliage at the concentrations of 0.06% (19 μM glyphosate), 0.2% (63 μM glyphosate) and 0.6% (189 μM glyphosate) of the recommended application rate for weed control.

Table 2
Effect of increasing glyphosate rates on the concentrations of mineral nutrients in young and old leaves of 21-day-old soybean plants grown under greenhouse conditions. The values represent means of four independent replications.

Glyphosate rate (% of recommended)[a]	P (g kg^{-1} DW)	K (g kg^{-1} DW)	Ca (g kg^{-1} DW)	Mg (g kg^{-1} DW)	Fe (mg kg^{-1} DW)	Mn (mg kg^{-1} DW)	Zn (mg kg^{-1} DW)	Cu (mg kg^{-1} DW)
Young leaves								
0	4.5	33.7	14.9	6.4	45	182	81	5.5
0.06	5.0	28.6	12.5	6.1	59	152	78	5.8
0.2	5.2	30.9	11.2	5.6	53	148	77	6.1
0.6	6.0	28.3	9.0	4.6	42	129	89	7.2
$LSD_{0.05}$	0.9	NS	1.0	0.5	14	25	NS	0.8
Old leaves								
0	2.3	26.3	22.3	8.8	49	232	93	5.5
0.06	2.2	24.0	18.4	7.2	54	160	78	4.8
0.2	2.3	25.6	19.2	7.6	51	190	84	4.9
0.6	2.3	25.2	15.6	5.8	40	121	65	4.1
$LSD_{0.05}$	NS	NS	NS	2.7	NS	75	NS	NS

[a] Glyphosate was applied to foliage at the concentrations of 0.06% (19 μM glyphosate), 0.2% (63 μM glyphosate) and 0.6% (189 μM glyphosate) of the recommended application rate for weed control.

Table 3
Effect of increasing glyphosate rates on grain and shoot dry matter production of soybean plants grown until grain maturation stage under greenhouse conditions. The values represent means of six independent replications.

Glyphosate rate (% of recommended)[a]	Seed yield (g plant^{-1})	Shoot yield (g plant^{-1})
0	12.5	19.3
0.3	13.4	18.7
0.6	12.1	20.0
0.9	9.2	14.6
1.2	2.3	6.8
$LSD_{0.05}$	1.9	2.6

[a] Glyphosate was applied to foliage at the concentrations of 0.3% (95 μM glyphosate), 0.6% (189 μM glyphosate), 0.9% (284 μM glyphosate) and 1.2% (379 μM glyphosate) of the recommended application rate for weed control.

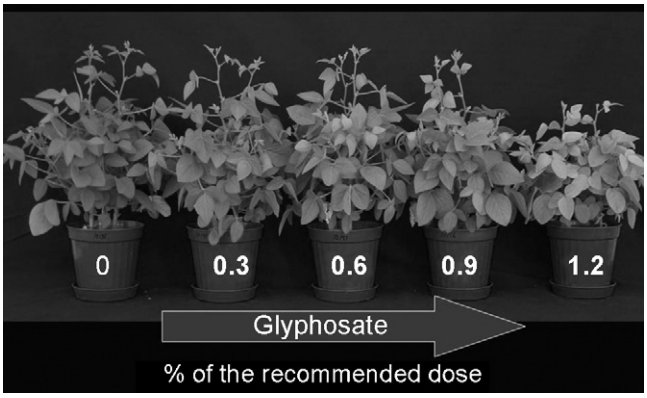

Fig. 1. Effect of increasing glyphosate rates on growth of soybean plants under greenhouse conditions. The values on the pots represent % of the recommended application rate for weed control corresponding to 0, 95, 189, 284 and 379 μM glyphosate.

such clear effects of glyphosate on seed concentrations of Mn and Ca and partly on Fe and Mg, this experiment was repeated by using 0, 0.3 and 1.2% glyphosate doses, and the results obtained confirmed the results given in Table 4 (no data shown).

4. Discussion

Reproductive organs are known to be more sensitive to glyphosate than the vegetative tissues. As shown in cotton, tobacco and soybean plants (Pline et al., 2003; Walker et al., 2006; Yasuor et al., 2007), glyphosate application results in greater damage to generative organs than the vegetative parts of plants. The reason for higher sensitivity of reproductive tissue to glyphosate might be related to higher accumulation of glyphosate in the reproductive tissues. Actively growing parts of plants (e.g., reproductive organs, root tips and shoot tips) represent an important sink for glyphosate accumulation and thus cell damage (Schulz et al., 1990; Feng et al., 2003; Hetherington et al., 1999). Accumulation of glyphosate in the youngest part of plants has been also shown in the present study (Table 1). The concentration of shikimate, a good indicator for glyphosate accumulation in plants, was increased only by 2.3-fold in older parts of plants by increasing glyphosate applications, while this increase was 16-fold for the young parts of the plants when compared to the control plants without glyphosate treatment (Table 1). In the present study, decreases in seed production by glyphosate were much greater than the decreases found in shoot

Table 4
Effect of increasing glyphosate rates on the concentrations of mineral nutrients in mature grains of soybean plants grown under greenhouse conditions. The values represent means of six independent replications.

Glyphosate rate (% of recommended)[a]	N (g kg^{-1} DW)	P (g kg^{-1} DW)	K (g kg^{-1} DW)	Ca (g kg^{-1} DW)	Mg (g kg^{-1} DW)	Fe (mg kg^{-1} DW)	Mn (mg kg^{-1} DW)	Zn (mg kg^{-1} DW)	Cu (mg kg^{-1} DW)
0	46	5.9	14.1	3.9	2.4	71	56	44	11
0.3	46	6.0	14.0	4.0	2.4	65	46	43	10
0.6	46	6.2	13.8	3.7	2.4	76	43	43	9
0.9	50	6.1	14.0	3.1	2.3	61	33	48	12
1.2	62	6.1	14.7	2.9	2.1	36	31	58	13
$LSD_{0.05}$	3	NS	0.5	0.6	0.2	13	8	5	2

[a] Glyphosate was applied to foliage at the concentrations of 0.3% (95 μM glyphosate), 0.6% (189 μM glyphosate), 0.9% (284 μM glyphosate) and 1.2% (379 μM glyphosate) of the recommended application rate (31.55 mM) for weed control.

biomass production (Table 3). Increasing glyphosate application from 0 to 1.2% of the recommended dose reduced shoot biomass production by nearly 3-fold, while this decrease was 8.8-fold in the case of seed production (Table 3). Another reason for higher sensitivity of sink organs to glyphosate might be related to reduced carbohydrate transport from source into sink organs (Geiger et al., 1999).

A likely inactivation of Ca in actively growing parts of plants by glyphosate might be a further reason for the high sensitivity of reproductive organs to glyphosate. It is well documented that herbicidal activity of glyphosate is very sensitive to the presence of Ca in spray solution or plant tissues. High Ca concentrations in spray solutions limit the efficacy of glyphosate to kill weeds (Thelen et al., 1995; Schoenherr and Schreiber, 2004; Gauvrit et al., 2001). The reason of this Ca antagonism with glyphosate activity is related to formation of insoluble or poorly soluble Ca–glyphosate complexes or precipitations (Sundaram and Sundaram, 1997; Schoenherr and Schreiber, 2004). High tolerance of velvetleaf plants to glyphosate has been ascribed to high Ca concentrations in leaf tissue (Nalewaja et al., 1992). Calcium is a critical mineral nutrient affecting cell wall stabilization and cell elongation such as in shoot meristematic tissues (Marschner, 1995). Due to their low transpiration capacity, shoot tips are highly sensitive to small changes in Ca concentrations. Accumulation of glyphosate in such sink organs with low Ca concentration would induce physiological Ca deficiency by complexing Ca. Glyphosate may also hinder Ca transport into reproductive organs (e.g., seeds) by immobilizing Ca in root or vegetative tissues. In this study, increasing glyphosate application severely reduced Ca concentration both in leaves and also seeds (Tables 2 and 4), most probably by interfering with Ca transport from roots into both shoot and seed. Previously, Duke et al. (1983) also showed that glyphosate very significantly reduced root-to-shoot transport in soybean plants, and this inhibitory effect of glyphosate on Ca translocation was not related to any changes in transpiration capacity of plants.

Magnesium has also high affinity to bind glyphosate and form insoluble Mg–glyphosate complexes, leading to both its inactivation and inactivation of glyphosate in plant tissues or spray solutions (Subramaniam and Hoggard, 1988; Thelen et al., 1995; Mueller et al., 2006). The decrease in Mg concentrations of seeds and partly in leaves by glyphosate can be, therefore, attributed to restricted transport of Mg within plant tissue. Nalewaja and Matysiak (1991) showed that glyphosate damage to wheat was reduced by high Mg concentrations in spray solution. Higher tolerance of some broadleaved weeds, such as *Abutilon theophrasti,* to glyphosate was related to high concentrations of both Mg and Ca on the leaf surface or within plant (Hall et al., 2000). In contrast to Ca and Mg, the concentrations of P, K and S were not affected by glyphosate indicating that the decline in tissue concentrations of Mg and Ca by glyphosate is a specific phenomenon, and cannot be generalized to all macronutrients.

In the case of micronutrients, only the concentrations of Fe and Mn in the seed and Mn in the leaves were declined by glyphosate (Tables 2 and 4). Recent studies indicate that Fe and Mn uptake systems are susceptible to glyphosate. In short-term experiments, root uptake and shoot translocation of Mn and Fe were severely depressed by glyphosate applications (Roemheld et al., 2005; Neumann et al., 2006; Eker et al., 2006), possibly due to formation of poorly soluble glyphosate complexes. To our knowledge, the present study shows for the first time that seed Mn and Fe concentrations decline upon foliar application of drift rates of glyphosate. Very recently, it has been shown that foliar-applied glyphosate severely impaired activity of root ferric reductase (Ozturk et al., 2008), an obligatory enzyme required for uptake of Fe by dicot and non-grass species (Chaney et al., 1972; Marschner and Roemheld, 1994). In addition to its inhibitory effects on root uptake and root-to-shoot transport of Fe and Mn, glyphosate may also interfere with phloem loading and phloem transport of Fe and Mn into sink organs (e.g., seed tissue). This is an important topic and needs to be investigated in future studies.

The reported decreases in seed concentration of Fe, Mn, Ca and Mg by glyphosate (Table 4) raise concerns in terms of seed quality. Seed reserves of mineral nutrients play an important role in seed viability and seedling vigor and establishment, particularly under adverse soil conditions (Welch, 1999). It is of great importance to examine the concentrations of mineral nutrients in grains harvested in cropping systems with frequent glyphosate applications. Preharvest application of glyphosate to stimulate grain maturation is a common practice in many cropping systems. In most cases, this practice is, however, associated with poor seed germination and reduced seedling vigor (Bennett and Shaw, 2000; Baig et al., 2003). Such adverse effects of preharvest-applied glyphosate on seed germination and seedling growth might also be related to the reduction or physiological inactivation of mineral nutrients in seeds, such as Ca and Mn. For better understanding and characterization of the adverse effects of glyphosate on plant growth, future studies should also focus on differentiation between bound and unbound divalent cations in the glyphoste-treated plants. Currently, experiments are on-going to test the role of glyphosate on physiological availability of divalent cations by measuring the activity of enzymes which are dependent on those cations.

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