

Foliar-Applied Glyphosate Substantially Reduced Uptake and Transport of Iron and Manganese in Sunflower (*Helianthus annuus* L.) Plants

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Evidence clearly shows that cationic micronutrients in spray solutions reduce the herbicidal effectiveness of glyphosate for weed control due to the formation of metal–glyphosate complexes. The formation of these glyphosate–metal complexes in plant tissue may also impair micronutrient nutrition of nontarget plants when exposed to glyphosate drift or glyphosate residues in soil. In the present study, the effects of simulated glyphosate drift on plant growth and uptake, translocation, and accumulation (tissue concentration) of iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu) were investigated in sunflower (*Helianthus annuus* L.) plants grown in nutrient solution under controlled environmental conditions. Glyphosate was sprayed on plant shoots at different rates between 1.25 and 6.0% of the recommended dosage (i.e., 0.39 and 1.89 mM glyphosate isopropylamine salt). Glyphosate applications significantly decreased root and shoot dry matter production and chlorophyll concentrations of young leaves and shoot tips. The basal parts of the youngest leaves and shoot tips were severely chlorotic. These effects became apparent within 48 h after the glyphosate spray. Glyphosate also caused substantial decreases in leaf concentration of Fe and Mn while the concentration of Zn and Cu was less affected. In short-term uptake experiments with radiolabeled Fe (⁵⁹Fe), Mn (⁵⁴Mn), and Zn (⁶⁵Zn), root uptake of ⁵⁹Fe and ⁵⁴Mn was significantly reduced in 12 and 24 h after application of 6% of the recommended dosage of glyphosate, respectively. Glyphosate resulted in almost complete inhibition of root-to-shoot translocation of ⁵⁹Fe within 12 h and ⁵⁴Mn within 24 h after application. These results suggest that glyphosate residues or drift may result in severe impairments in Fe and Mn nutrition of nontarget plants, possibly due to the formation of poorly soluble glyphosate–metal complexes in plant tissues and/or rhizosphere interactions.

KEYWORDS: Glyphosate; *Helianthus annuus*; iron; manganese; zinc; copper; leaf chlorosis

INTRODUCTION

Glyphosate [*N*-(phosphonomethyl)glycine] is the most widely used herbicide due to its low production costs and high effectiveness in killing a diversity of weeds. Usage of glyphosate is increasing with the widespread cultivation of transgenic plants and the adoption of no-tillage cropping systems (1). The major toxic action of glyphosate results from inhibition of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (2, 3). This enzyme is critical in the shikimate pathway, and its inhibition results in reduced biosynthesis of aromatic amino acids that subsequently impairs general metabolic processes such as protein synthesis and photosynthesis. Glyphosate also inhibits stomatal

conductance, carbon export to sink organs, and nodular metabolism (4–6). An indirect detrimental effect of glyphosate is the increased sensitivity of plants to various soil-borne pathogens. Kremer et al. (7) reported that increased susceptibility of the glyphosate-treated plants to soil-borne pathogens (e.g., *Fusarium* spp.) could be due to reduced production of phytoalexins and increased root exudation of amino acids into the rhizosphere.

In cropping systems where glyphosate is regularly applied, glyphosate spray drift and residues can cause severe damage to nontarget plants (8, 9). A significant amount of glyphosate applied to target plants reaches the soil as a result of direct contact, wash off from leaves, and exudation from roots of the treated weeds (7, 9, 10). Up to 10% of the applied glyphosate can move to nontarget plants (11, 12). The half-life of glyphosate in soil is very long and ranges from several weeks to years (10, 13, 14). Most of the glyphosate residues (up to 90%) are found

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in the top 15 cm of soil (10), and these residues represent an important threat for soil microbial activity and root uptake by nontarget plants. Very recently, Bellaloui et al. (15) showed that a simulated glyphosate drift at 12.5% of the usually applied rate impaired shoot growth and nodule activity of both nitrate reductase and nitrogenase in a nonglyphosate resistant soybean, especially during early vegetative growth.

Glyphosate may also affect micronutrient nutrition of plants. Field observations in Brazil and the North Central United States have reported that frequent applications of glyphosate induce Fe, Zn, and Mn deficiencies in different crop species (16–18). Application of glyphosate to field-grown soybean plants with a low supply of Fe exacerbated Fe deficiency chlorosis (16). The nature of this antagonism between micronutrients and glyphosate is not known. Possibly, it is related to the formation of insoluble glyphosate complexes with cationic micronutrients (19, 20). Iron and Mn in spray solutions are known to inhibit glyphosate herbicidal activity by limiting absorption and translocation of glyphosate in treated leaves. After absorption of glyphosate into the plant, the uptake and transport of cationic micronutrients may also be limited due to the formation of poorly soluble glyphosate–metal complexes within plant tissues. The antagonism between cationic mineral nutrients and glyphosate has been studied primarily in terms of impaired leaf absorption (penetration) and limited translocation of glyphosate to explain reduced effectiveness of glyphosate to kill target plants (19–24). In sunflower and velvetleaf plants, cationic nutrients such as Mn, Fe, and Ca bind to the glyphosate molecule via its carboxyl and phosphonate groups to form stable complexes with glyphosate (20, 22, 23). Such complexes severely reduced the absorption and translocation of glyphosate within the treated tissue and thus limited its efficacy in weed control.

Despite a significant number of studies investigating the effects of cationic nutrients on the herbicidal effectiveness of glyphosate on target plants, little is known about the effect of glyphosate on mineral nutrition of nontarget plants. In the present study, experiments were conducted to study the effect of glyphosate on shoot dry matter production, chlorophyll concentration, and the uptake, translocation, and tissue accumulation of Fe, Mn, Zn, and Cu in sunflower plants.

MATERIALS AND METHODS

Growth Conditions. Sunflower (*Helianthus annuus* L. cv. TR-6149 SA) plants were grown in nutrient solution under controlled environmental conditions (light/dark regimes of 16/8 h at 25/22 °C, relative humidity 60–70%, and a photosynthetic photon flux of 320 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at canopy height provided by fluorescent lamps). Seeds were germinated in perlite moistened with saturated CaSO_4 solution. After 5 days of germination, two seedlings were transferred to each 2.7 L plastic vessel containing continuously aerated nutrient solution [0.75 mM K_2SO_4 , 2 mM $\text{Ca}(\text{NO}_3)_2$, 1 mM MgSO_4 , 0.25 mM KH_2PO_4 , 0.1 mM KCl, 100 μM Fe-EDTA, 1 μM MnSO_4 , 1 μM ZnSO_4 , 10 μM H_3BO_3 , 0.1 μM CuSO_4 , and 0.01 μM $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$] that was changed every 3 days.

Glyphosate Applications. Glyphosate formulated as Roundup Ultra [active ingredient (ai): 480 g L^{-1} N-[phosphonomethyl]glycine isopropylamine salt, Monsanto Co.] and available in Turkish market was used in all experiments. The selected sublethal doses of 1.25, 2.5, and 6% of the recommended application rate provided on the product label for narrow or broad leaf annual weeds (i.e., 1.44 kg ha^{-1} ai glyphosate to be applied with 200 L of water per ha: 31.55 mM glyphosate as active ingredient) were equivalent to 0.39, 0.79, and 1.89 mM, respectively. In all experiments, freshly prepared glyphosate solution (or distilled water in the case of control plants) was sprayed on foliage in a volume of nearly 1.5 mL per plant. The sprayed volume of solution did not cause any runoff from leaves.

Uptake and Transport Experiments. Three different experiments were carried out. In the first and second experiments, the dosage and time of glyphosate application were tested, respectively. The third experiment investigated the effect of glyphosate on root uptake and root-to-shoot translocation of radiolabeled Fe, Zn, and Mn.

In the first experiment, glyphosate solution was applied to 20 day old sunflower plants at 0.39 or 0.79 mM. Plants were harvested 3 days after glyphosate treatment. Chlorophyll determination was based on SPAD readings (Minolta SPAD-502 chlorophyll meter) of the young leaves measured before harvest. At harvest, roots were washed initially in tap water containing 0.1 mM CaSO_4 and then in deionized water. Plants were separated into roots, old leaves, and young leaves and weighed to determine dry matter production before analysis for Fe, Mn, Zn, and Cu. The concentration of micronutrients in leaves and roots was measured after digesting dried and ground samples in a microwave in 65% (w/v) HNO_3 . After complete digestion, the concentration of micronutrients was measured by inductively coupled plasma atomic emission spectroscopy (JY-138 Ultrace, France).

In the second experiment, 20 day old plants were treated with 0.79 mM glyphosate as described above. Plants were harvested at 24, 48, and 72 h after glyphosate application and analyzed for dry matter, chlorophyll value, and micronutrient concentrations in roots and shoots as described above.

The third experiment was conducted to study the effect of glyphosate on root uptake and root-to-shoot translocation of radiolabeled Fe (^{59}Fe), Mn (^{54}Mn), and Zn (^{65}Zn) in separate uptake experiments. On the day of the uptake experiments, plants were 16 days old for the ^{59}Fe and 24 days old for ^{54}Mn and ^{65}Zn uptake studies. Glyphosate (1.89 mM) was applied 12 h prior to starting the ^{59}Fe and 24 h before the ^{54}Mn and ^{65}Zn uptake experiments. Plants were then transferred into a fresh nutrient solution without the corresponding micronutrient. To initiate the uptake experiment, the specific labeled micronutrient was added into nutrient solution and plants were grown under light for 4 h. The ^{59}Fe nutrient solution contained 100 μM Fe as ^{59}Fe -labeled Fe(III)–EDTA with a specific activity of 74 GBq mol^{-1} Fe. At the end of the 4 h uptake period, roots were washed in 500 mL of Fe-free nutrient solution and treated with bipyridyl and sodium dithionite to remove apoplasmic root Fe (25). The plants were then separated into shoot and roots, oven dried at 70 °C, weighed, and ashed at 550 °C. The ash was dissolved in 5 mL of 1% HCl (w/v), added to 5 mL of scintillation cocktail, and assayed for ^{59}Fe in a liquid scintillation counter (1414 WinSpectra, Wallac, Germany). In separate uptake experiments with ^{54}Mn and ^{65}Zn , the uptake solutions contained 1 μM Mn as ^{54}Mn -labeled MnSO_4 and 1 μM Zn as ^{65}Zn -labeled ZnSO_4 with a specific activity of 79 GBq mol^{-1} Mn and 83 GBq mol^{-1} Zn, respectively. At the end of the uptake period, roots were washed for 15 min in 1 mM CaSO_4 and then 1 mM Na–EDTA to remove extracellular Zn and Fe from roots.

Statistics. With the exception of the experiment conducted with radiolabeled micronutrients, in all experiments, each treatment consisted of four independent replications, and each replication (pot) had two plants. The experiment with radiolabeled nutrients was conducted with three independent replications. Least significant difference (LSD) calculations were performed according to Student's *t*-test using MSTAT-C software.

RESULTS

Effects of Increasing Glyphosate Doses. Three days after glyphosate application, a severe chlorosis developed on young leaves and roots became dark-colored (Figure 1). Leaf chlorosis was pronounced on the shoot tips and basal (expanding) parts of young leaves. Older leaves did not show chlorosis. The amount of chlorophyll (SPAD values) in younger leaves significantly declined as the concentration of glyphosate increased (Table 1). Similarly, dry matter production of young leaves and roots was significantly decreased by glyphosate (Figure 1 and Table 1). Glyphosate had no effect on dry matter of older leaves.

Following 3 days of glyphosate treatment, there was a very sharp decrease in Fe concentration in both young and old leaves,

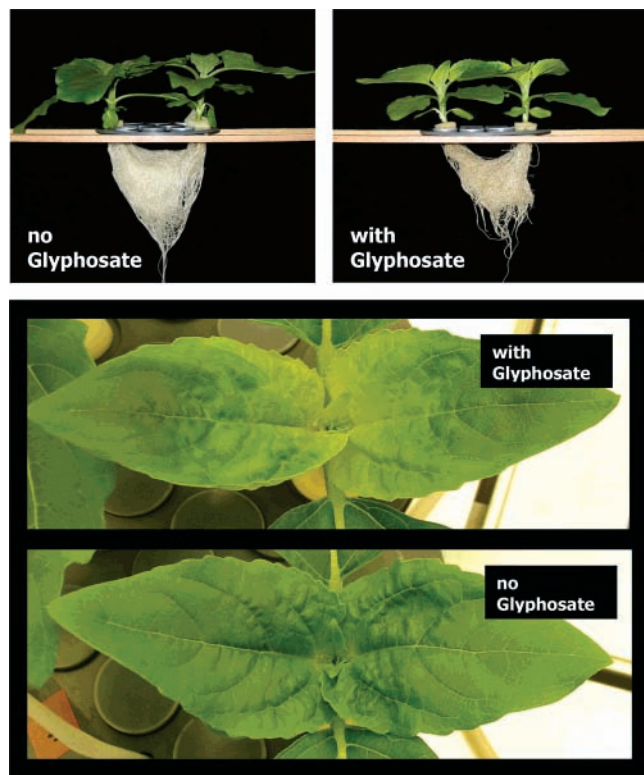


Figure 1. Effect of 0.79 mM glyphosate (equivalent to 2.5% of the recommended dosage for weed control in the field) on growth of 20 day old sunflower plants (upper picture) and leaf chlorosis in young leaves (lower picture). The picture was taken 3 days after glyphosate treatment.

Table 1. Effect of Increasing Glyphosate Rates on SPAD Values and Dry Matter Production of Leaves and Roots in 23 Day Old Sunflower Plants^a

glyphosate application ^b (mM)	SPAD values	dry matter production (mg DW plant ⁻¹)		
	young leaves	young leaves	old leaves	root
0	46.3	1429	1066	1078
0.39	27.5	1099	1114	839
0.79	17.7	790	1176	781
LSD _{0.05}	5.2	241	ns	140

^a Glyphosate was sprayed onto shoots of 20 day old plants that were harvested 3 days after the glyphosate treatment. SPAD values representing chlorophyll levels were measured on the young leaves. For each treatment, data represent means of four independent replications with two plants in each replicate. ^b Roundup Ultra was applied to sunflower foliage at a concentration of 1.25% (0.39 mM glyphosate) and 2.5% (0.79 mM glyphosate) of the recommended application rate for weed control.

while the root concentration of Fe was not affected (**Table 2**). This result indicates that glyphosate application resulted in severe decreases in root-to-shoot translocation of Fe during 3 days of treatment (see below), while uptake and translocation of Fe continued normally in the plants without glyphosate application, leading to nearly 3-fold difference in leaf Fe concentrations between treated and nontreated plants. Applying 0.39 mM glyphosate (1.25% of the recommended dose) was sufficient to cause a strong decrease in leaf Fe. Increasing the glyphosate 2-fold did not result in a further decrease in Fe concentration (**Table 2**). Increasing rates of glyphosate gradually decreased Mn concentrations in leaves and roots about 50% (**Table 2**). Leaf and root concentrations of Zn and Cu were also

Table 2. Effect of Increasing Rates of Glyphosate on the Concentration of Fe, Mn, Zn, and Cu in Leaves and Roots of 23 Day Old Sunflower Plants^a

glyphosate application ^b (mM)	mg kg ⁻¹ DW		
	young leaves	old leaves	root
	iron		
0	244	202	727
0.39	73	64	744
0.79	78	69	668
LSD _{0.05}	18	39	ns
	manganese		
0	89	139	33
0.39	60	101	22
0.79	53	88	15
LSD _{0.05}	10	8	10
	zinc		
0	72	89	38
0.39	56	68	35
0.79	68	75	43
LSD _{0.05}	8	9	5
	copper		
0	13.7	13.7	11.9
0.39	9.8	9.5	10.8
0.79	10.0	9.7	9.6
LSD _{0.05}	1.8	1.7	1.6

^a Glyphosate was sprayed onto the shoot of 20 day old plants that were harvested 3 days after the glyphosate treatment. For each treatment, data represent means of four independent replications with two plants in each replicate. ^b Roundup Ultra was applied to sunflower foliage at a concentration of 1.25% (0.39 mM glyphosate) and 2.5% (0.79 mM glyphosate) of the recommended application rate for weed control.

significantly decreased by glyphosate (**Table 2**); however, the magnitude of these decreases was lower than the changes observed with leaf Fe and Mn.

Micronutrient Concentrations over 72 h after Glyphosate Treatment. In the second experiment, the effect of glyphosate on micronutrient concentrations was studied over a 72 h period after application of 0.79 mM (2.5% of the recommended dose) glyphosate (**Table 3**). The SPAD values (chlorophyll concentrations) of nontreated control plants slightly increased during this period while there was a gradual decline in the glyphosate-treated plants. Following glyphosate treatment, the dry matter production of glyphosate-treated plants was significantly reduced as compared to plants without glyphosate (**Table 3**). Decreases in dry matter production were especially pronounced in young leaves and roots, with young leaves more sensitive to glyphosate in terms of the reduction in dry matter production.

Iron concentrations in leaves and roots were slightly decreased after 24 h in both the glyphosate-treated and the nontreated plants (**Table 4**); however, at 48 and 72 h after glyphosate treatment, Fe in leaves increased in plants without glyphosate, while it distinctly decreased in the glyphosate-treated plants. Root Fe concentrations did not significantly change in the glyphosate-treated plants but declined gradually in nontreated plants (**Table 4**). The concentration of Mn in old and young leaves of nontreated plants was similar over the 72 h study period, while root Mn significantly declined irrespective of glyphosate application over the 72 h of study period but was especially reduced in glyphosate-treated plants (**Table 4**). The concentration of Zn in leaves of the nontreated plants remained similar over the 72 h while it tended to decline with time after glyphosate treatment (**Table 4**). As found with Mn, the concentration of Zn in roots declined over the 72 h period in the treated and nontreated plants (**Table 4**). Irrespective of

Table 3. Changes in SPAD Values and Dry Matter Production of Leaves and Roots of 23 Day Old Sunflower Plants 72 h after Glyphosate Application^a

time (h)	SPAD values		dry matter production (mg DW plant ⁻¹)					
	young leaves		young leaves		old leaves		roots	
	-glyphosate	+glyphosate ^b	-glyphosate	+glyphosate	-glyphosate	+glyphosate	-glyphosate	+glyphosate
0	42.7	42.7	113	113	494	494	215	215
24	46.3	38.1	299	232	659	614	325	285
48	52.3	25.5	459	231	851	751	453	317
72	50.6	19.9	717	382	832	881	610	401
LSD _{0.05} (int)	2.9		154		ns		88	

^a For each treatment, data represent means of four independent replications with two plants in each replicate. ^b Roundup Ultra was applied to sunflower foliage at a concentration of 2.5% (0.79 mM glyphosate) of the recommended application rate for weed control.

Table 4. Changes in Fe, Mn, Zn, and Cu in Leaves and Roots of 23 Day Old Sunflower Plants 72 h after Glyphosate Application^a

time (h)	mg kg ⁻¹ DW					
	young leaves		old leaves		roots	
	-glyphosate	+glyphosate ^b	-glyphosate	+glyphosate	-glyphosate	+glyphosate
			iron			
0	113	113	98	98	1490	1490
24	92	110	89	91	1452	1490
48	150	82	126	72	1314	1385
72	155	85	137	72	997	1525
LSD _{0.05} (int)	21		19		ns	
			manganese			
0	76	76	131	131	66	66
24	72	68	127	120	48	41
48	79	56	130	101	41	25
72	75	54	133	95	29	16
LSD _{0.05} (int)	8		15		ns	
			zinc			
0	70	70	82	82	63	63
24	70	78	81	87	49	58
48	60	71	74	72	44	52
72	64	67	81	67	46	46
LSD _{0.05} (int)	ns		7		4	
			copper			
0	15.7	15.7	14.3	14.3	18.6	18.6
24	12.5	13.4	12.1	12.7	17.5	17.3
48	11.2	12.5	11.8	11.1	15.6	14.4
72	11.6	11.6	11.8	10.4	16.2	14.2
LSD _{0.05} (int)	ns		ns		ns	

^a For each treatment, data represent means of four independent replications with two plants in each replicate. ^b Roundup Ultra was applied to sunflower foliage at a concentration of 2.5% (0.79 mM glyphosate) of the recommended application rate for weed control.

glyphosate treatment, Cu concentrations of leaves and roots decreased similarly, and glyphosate treatment did not affect the Cu concentration in the plants (Table 4).

Glyphosate Effects on Uptake and Translocation of Radiolabeled Micronutrients. In order to study the effect of glyphosate on root uptake and shoot translocation of micronutrients, glyphosate was applied 12 h prior to starting the ⁵⁹Fe and 24 h before the ⁵⁴Mn and ⁶⁵Zn uptake experiments. Root uptake of Fe and, especially, Mn were significantly reduced in short-term uptake experiments using radiolabeled Fe (⁵⁹Fe), Mn (⁵⁴Mn), and Zn (⁶⁵Zn) (Figure 2). In the case of Zn, there was a trend indicating decreased uptake, but this trend was not statistically significant. The effect of glyphosate on the root-to-shoot translocation of Fe, Mn, and Zn was pronounced. Glyphosate substantially decreased shoot translocation of Fe and Mn. The shoot translocation rate of Zn was decreased by glyphosate, but this decrease was not statistically significant (Figure 2).

DISCUSSION

The present study shows that subherbicidal rates of glyphosate effectively reduce the uptake and transport of Fe and Mn in plants. The effects of glyphosate on the concentration of Fe and Mn in roots and leaves became very distinct after 48 h of glyphosate treatment. Glyphosate had less effect on Zn and Cu in plants (Tables 2 and 4). In contrast to leaves, root concentrations of Fe were only slightly affected by glyphosate, while there was a large decline in root Mn concentration (Tables 2 and 4). Uptake experiments with ⁵⁹Fe, ⁵⁴Mn, and ⁶⁵Zn corroborated the rapid and significant decreases in root uptake and root-to-shoot translocation of Fe and Mn after glyphosate treatment (Figure 2). Such rapid and significant decreases in root uptake of Fe and Mn by glyphosate application suggest a strong interference of glyphosate with the root uptake process of micronutrients. The nature of this inhibitory effect of glyphosate on uptake and accumulation of micronutrients is not known. It seems that such a marked decline in transport and accumulation of Fe and Mn by glyphosate is possibly related

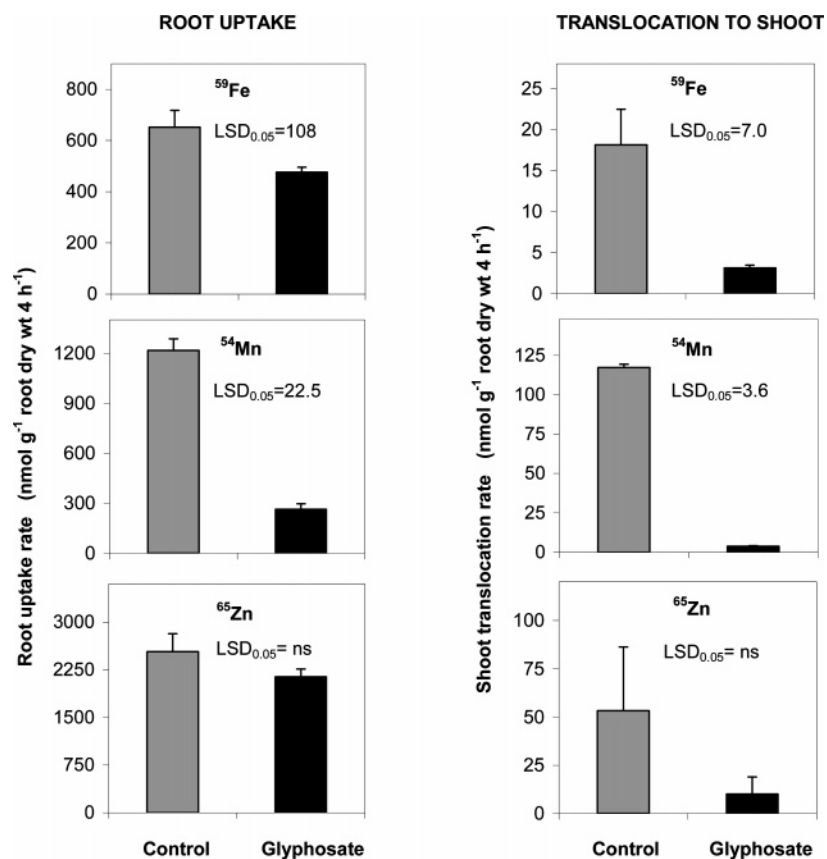


Figure 2. Effect of 1.89 mM glyphosate (equivalent to 6% of the recommended dosage for weed control in the field) on root uptake and shoot translocation of ⁵⁹Fe, ⁵⁴Mn, and ⁶⁵Zn in sunflower plants. On the day of the uptake experiments, plants were 16 days old for the ⁵⁹Fe and 24 days old for ⁵⁴Mn and ⁶⁵Zn uptake studies. Glyphosate was applied 12 h prior to starting the ⁵⁹Fe and 24 h before the ⁵⁴Mn and ⁶⁵Zn uptake experiments. Data are the means of three independent replications (\pm SD).

to the ability of glyphosate to form immobile stable complexes with Fe and Mn. Glyphosate contains several active groups (phosphonate and carboxylate groups) with high affinity to bind with metals (26, 27). There are an increasing number of studies showing that metals in spray solutions impair effectiveness of glyphosate as an herbicide due to formation of insoluble metal–glyphosate complexes, particularly with Mn and Fe (20, 23, 24, 28, 29). The main toxic action of glyphosate in plant cells appears to result from the aminomethylphosphonic acid (AMPA) metabolite of glyphosate (30). Barja et al. (27) demonstrated that AMPA complexes with Fe via its phosphonate groups. Glyphosate is known to be very rapidly translocated within plants and accumulates predominantly in meristematic areas, e.g., actively growing parts of roots and young shoots (1, 31, 32). According to Feng et al. (33), nearly 80% of the absorbed foliar-applied glyphosate is transported to the roots and youngest parts of shoots. Accumulation of glyphosate in roots may result in the formation of immobile Fe and Mn complexes and consequently limit root-to-shoot transport of essential metal nutrients. Glyphosate-treated plants can accumulate up to 0.3 mM glyphosate in root tissues (34, cited by 7) that can immobilize micronutrients in roots as found in spray solutions or leaf tissues (20, 23, 24). The complexing action of glyphosate in roots is probably the main reason for the marked depression in root-to-shoot transport of Mn and Fe by glyphosate (Figure 2). De Ruiter et al. (35) showed that glyphosate reacts with Mn, Fe, and other cations present in the xylem sap of quack grass plants. Recently, Kremer et al. (7) showed that glyphosate translocated from treated leaves to roots was then released into the rhizosphere at high amounts (up to 1 μ g per plant). The

leakage of glyphosate from roots into the soil solution may impair root uptake of metals due to formation of stable metal–glyphosate complexes. Glyphosate may also interfere with the Fe reductase process on the root cell membranes, which is an essential step for root Fe uptake by dicotyledonous plants (36).

Development of chlorosis upon glyphosate treatment is confined to the expanding basal parts of young leaves and shoot tips (Figure 1). These shoot parts are also the parts where glyphosate specifically accumulates (33). Glyphosate could reduce chlorophyll by preventing its biosynthesis or stimulating its degradation under high light (5, 30, 37). An additional reason for the glyphosate-induced leaf chlorosis could be related to impairment of the physiological availability of micronutrients. As discussed above, glyphosate forms very stable and poorly soluble salts with Fe and Mn. Formation of such insoluble compounds in the youngest parts of shoots where glyphosate is particularly accumulated may be responsible for physiological inactivation of Fe and Mn. The occurrence of chlorosis on younger leaves and shoot tips caused by glyphosate may be a reflection of the physiological inactivation of Fe and Mn in these glyphosate-accumulating leaf tissues. The occurrence of such physiological inactivation of Fe and Mn in the sink organs could be possible because cytosolic glyphosate concentrations in shoot sink organs are very high and reach up to 1 mM (38). Additional studies should clarify the glyphosate-induced physiological deficiency of Fe and/or Mn in the chlorotic tissue by measuring enzyme activity that is Fe- and Mn-dependent such as Fe-dependent nitrate reductase and nitrogenase (36). Field observations in Minnesota support this speculation where glyphosate treatment of soybean plants growing in soils with low Fe

availability promoted leaf chlorosis resulting in further reductions in yield (16, 17). Recent reports show that glyphosate causes marked decreases in nitrate reductase and nitrogenase activity in nodules of glyphosate-treated soybean (15) and lupine plants (6). One possible reason for the reduced activity of these Fe-dependent enzymes in the glyphosate-accumulating nodules might be related to the physiological immobilization of Fe by glyphosate.

In conclusion, this study clearly shows that glyphosate is antagonistic to the uptake, transport, and accumulation (tissue concentration) of Fe and Mn in sunflower plants. The formation of poorly soluble glyphosate–metal complexes is possibly the main factor responsible for the antagonism between glyphosate and cationic micronutrients. In agricultural systems with intensive glyphosate application, contamination of crop plants with glyphosate commonly occurs as a result of glyphosate spray drift and root uptake of glyphosate residues from soils (9). In view of the fact that cultivated soils globally have very low levels of plant available Fe, Zn, and Mn (36, 39), special attention should be paid to the micronutrient nutritional status of plants in cropping systems with widespread usage of glyphosate.

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