Title

Manganese uptake and redistribution in soybeans as affected by glyphosate

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Introduction

Manganese is an important co-factor for several enzymes associated with the shikimic acid pathway, including phenolic aromatic amino acids, coumarins, lignins and flavonoids. A light Mn deficiency decreases photosynthesis and soluble carbohydrate levels in the plant, while a severe deficiency causes irreversible breakdown of chloroplast structure (Marschner, 1995). Soybean plants develop interveinal chlorosis in young leaves when subjected to Mn deficiency and leaf Mn levels between 10 and 20 mg kg^{-1} were associated with deficiency in soybean (Malavolta et al., 2000). Since the nutrient mobility in the phloem is very limited, when Mn availability is high, the highest amount of the nutrient is usually found in the roots (Rosolem, 1989).

The herbicide glyphosate is absorbed and translocated via the phloem to plant roots and rhizomes. It inhibits the activity of the enzyme enolpiruvil shikimato-3-phosphate synthase (EPSP), preventing the synthesis of aromatic amino acids which are precursors of substances such as alkaloids, flavonoids and lignin (Amarante Jr. et al., 2002). In the case of glyphosate-resistant soybean, tolerance was achieved by the insertion of a gene (Aroa) from the Agrobacterium sp genome, which encodes a variant of EPSPS (CP4 EPSPS), especially tolerant to inhibition by glyphosate (Padgett et al., 1995). Although glyphosate-resistant (GR) soybean cultivars are not severely affected, glyphosate decreases leaf and hypocotyl chlorophyll contents (Hoagland, 1980), which could be also attributed to Mn deficiency (Malavolta, 1997). Chlorophyll is highly sensitive to glyphosate because the synthesis of its precursor, the δ-aminolevulinic acid is strongly inhibited (Cole, 1985), which leads to a characteristic chlorotic growth of plants exposed to sublethal doses of the herbicide (Wong, 2002).

Glyphosate can be exuded by the target plant roots, released in small amounts in the rizosphere and be absorbed, at least in part, by non-target plants (Kremer et al., 2005). A glyphosate-resistant soybean plant may exude 1500 ng of glyphosate in 16 days (Kremer et al., 2005). Herbicide activity in the treated plant was found to be around 1.0 % in roots and 0.08 % in shoots of non-target plant as compared with the target plant (Ricordi et al., 2007). This amount transferred through the rhizosphere should not be enough to cause damage to non-target plants, however, when low rates were applied to the leaves of non-transgenic soybean plants the absorption and translocation of Mn were decreased by doses around 2% of the normally recommended (Römheld et al., 2005).

Although glyphosate is effective in controlling weeds, there are reports of various physiological side effects. Among these side effects on non-target plants a decrease in Mn absorption and translocation has been reported (Römheld et al., 2005). Since the effect was observed in plants non resistant to glyphosate, it is unclear whether this effect also occurs in resistant plants. In this work we studied Mn uptake kinetics in two near-isogenic soybean cultivars as affected by low glyphosate rates present in the nutrient solution. Furthermore, Mn uptake and translocation in glyphosate-resistant cultivars and response to Mn after application of glyphosate was also studied in soybean.

Material and Methods

Three experiments were carried out to study the kinetics of Mn absorption as affected by glyphosate applied to the nutrient solution, the comparative Mn uptake and distribution of near-isogenic soybean cultivars, and the response of soybean to Mn when treated with glyphosate on a Mn-deficient soil.

For the absorption kinetics experiment, two near-isogenic soybean cultivars, Conquista and Valiosa, were grown in nutrient solution. Twenty days after plant emergence the parameters defining Mn absorption kinetics were determined using the solution depletion technique (Claassen et al., 1974). The level of Mn in solution was 14.5 μM L^{-1} and glyphosate was applied at rates of 0, 8, 16, 32, 64 and 128 μg L^{-1} a.e. (acid equivalent). Solution samples of 5 ml were taken at 30, 45, 60, 75, 120, 210, 390, 750, 1470 minutes after applying the treatments. At the end of the depletion period, plants were collected and split in shoots and roots.

In the Mn uptake and distribution experiment, the same soybean cultivars were used and also grown in nutrient solution. The transgenic cultivar (treated or not with glyphosate) and the conventional cultivar were grown in the presence of 1.0, 2.0, 4.0, 6.0, 10.0 and 20.0 μM of Mn in the nutrient solution. Glyphosate (360 g L^{-1} of active ingredient) was sprayed at 540 g ha^{-1} (a.e.) to the respective treatments 25 days after plant emergence. Plants were set in lines 0.45 m apart (as they would be in the field) for herbicide application, so that experiment conditions would be closer to field conditions and more realistic. The experiment was harvested 40 days after plant emergence.

The soil experiment was conducted in 6 L pots in the greenhouse. A Cerrado soil with low original content of manganese was used. Dolomitic limestone was applied to raise soil base-saturation to 60%. The treatments consisted of application of manganese at rates of 0, 3.0, 6.0, 12 mg kg^{-1}. Three transgenic soybean cultivars (M-SOY 7908 RR, Valiosa RR and Coodetec 226 RR) were treated or not with glyphosate. Two plants were grown per pot. At the growth stage V3, glyphosate was applied to the respective treatments at 720 g ha^{-1} (a.e.), arranging the plants as in the previous experiment. Fifteen days after herbicide application, the plants were harvested and root and shoot biomass was determined as well as the Mn content in the plant tissue.

Results and Discussion

The maximum Mn influx (Vmax) was higher in Valiosa RR than in Conquista (Fig. 1). The lowest glyphosate rate (8 mg L^{-1}) increased Vmax of both cultivars, which was decreased with further increases in glyphosate rates in the nutrient solution. The affinity of the uptake system for Mn ions was increased in the presence of glyphosate in the nutrient solution, because there was a decrease of Km with glyphosate rates (Fig. 1). For Cmin (the minimum Mn concentration in the nutrient solution at which net uptake occurs), a significant decrease was observed at the higher glyphosate rates (results not shown). Thus, although there was a decrease in Mn influx with higher doses of glyphosate, the effects on Km and Cmin suggest a higher affinity of the Mn uptake system in the presence of glyphosate.

In the Mn absorption and distribution experiment in nutrient solution, soybean dry matter production was similar between cultivars and there was no response either to Mn or glyphosate applied (results not shown). Thus, it can be inferred that the introduction of the RR gene has not led to significant changes in soybean response to Mn. Particularly for the transgenic cultivar, glyphosate had no effect on plant development and dry matter production, regardless of the state of Mn nutrition. Leaf Mn concentrations were lower in Valiosa RR when grown in Mn-deficient solution. Even in the lowest Mn solution level leaf Mn concentrations in all treatments were not below 20 mg kg^{-1}, which is considered the threshold for soybean development and production (Malavolta et al., 1997).

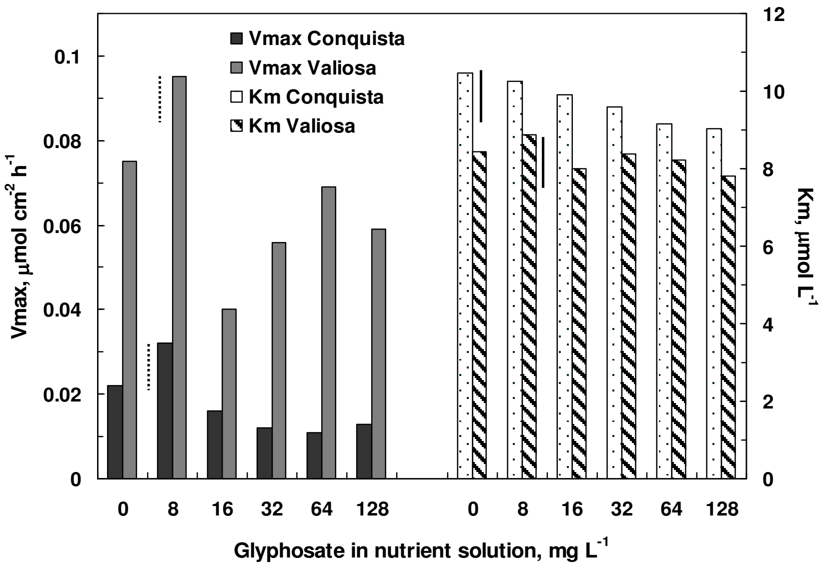

Figure 1. Vmax and Km for a transgenic and a conventional soybean cultivar as affected by glyphosate rates applied to the nutrient solution. Bars show LSD (P< 0.05), solid line – Km, dashed line – Vmax.

Within 10 to 15 days after plant emergence some plants of both cultivars showed Mn deficiency symptoms in treatments with the lowest Mn level in nutrient solution. However, new growth was normal. Furthermore, plants sprayed with glyphosate showed, a few days after application, symptoms that were similar to those of Mn deficiency (Figure 2), which also disappeared almost completely by the time the plants were harvested. This phytotoxicity of glyphosate was also observed under field conditions in the Brazilian Center-West (Broch and Ranno, 2008), with no effect on soybean Mn nutrition or yields.

Figure 2. Manganese deficiency symptoms (A) and glyphosate toxicity symptoms in the transgenic cultivar Valiosa (B).

Soybean dry matter yields were not affected by Mn rates (results not shown), but total Mn accumulation by cultivar Conquista increased linearly with Mn rates in the nutrient solution (Fig. 3), however, for Valiosa RR, its near isogenic cultivar, Mn accumulation was quadratic.

Furthermore, Mn accumulation or distribution within the plant was not affected by glyphosate in Valiosa RR. For the glyphosate non-resistant genotypes, a decrease in Mn uptake and translocation to the shoots has been reported (Römheld et al., 2005). But, from this experiment it is clear that there was no interference of glyphosate on total Mn accumulation in the genetically modified genotype.

It has been reported that glyphosate may interfere with soybean response to Mn (Gordon, 2007). However, in this experiment the onset of Mn symptoms was not dependent on the application of the herbicide, which did not affect manganese nutrition of soybeans. Other factors, such as the activity of microorganisms responsible for Mn dynamics in the soil (Kremer et al., 2005) could influence the availability of nutrients to plants. In general, the shoots had a higher percentage of the Mn accumulated by the plant (over 50 %). However, no evidence was found that glyphosate has influenced the translocation of Mn in RR soybeans, as it had been reported for a non-resistant material (Römheld et al., 2005).

In the soil experiment, in spite of the originally low soil Mn content, liming and wet incubation must have favored organic matter mineralization increasing Mn availability. Therefore, soybean dry matter yield did not respond to Mn application. Glyphosate application had no effect on soybean growth, Mn accumulation and distribution within the plants (Fig. 4) as has been reported earlier (Gordon, 2007).

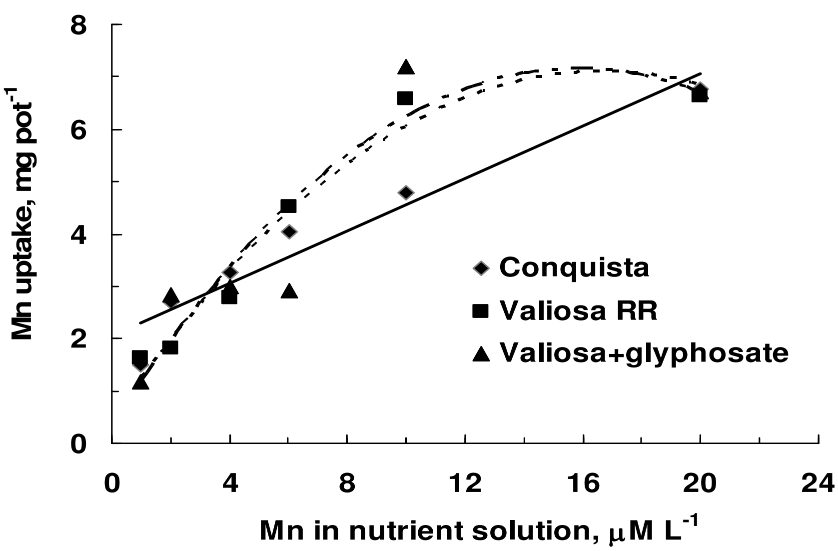

Figure 3. Mn accumulation by soybean cultivars as affected by Mn levels in the nutrient solution and glyphosate sprayed on the glyphosate-resistant cultivar.

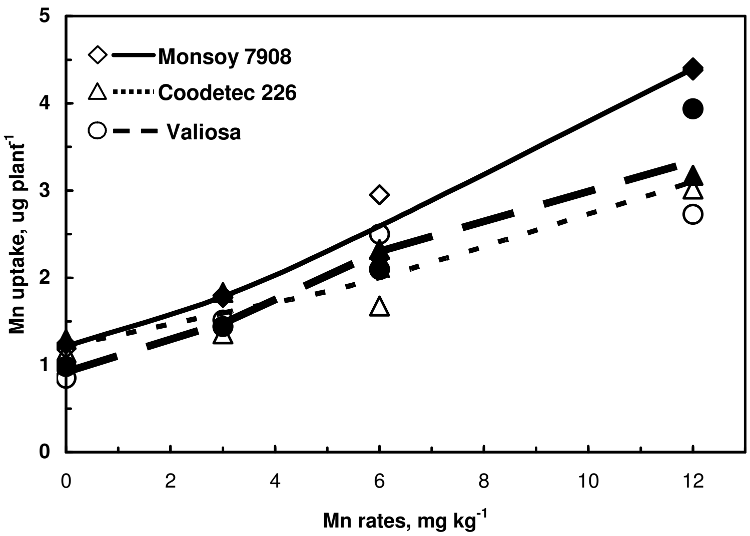

Figure 4. Manganese uptake by glyphosate-resistant cultivars of soybean as affected by Mn rates applied to a Mn-deficient soil. Open symbols: with glyphosate, filled symbols: without glyphosate. There are no significant differences between glyphosate treatments.

Microorganisms responsible for Mn dynamics in the soil could have been affected by glyphosate (Kremer et al., 2005) and eventually interfere in Mn availability and uptake by soybean, although this was not observed in the present experiment. After glyphosate application there was also some leaf yellowing for a few days, but the symptom did not persist in the new growth. Manganese uptake was increased with Mn rates for the three cultivars (Fig. 4), with no response in dry matter yields. Monsoy 7908 accumulated more Mn.

In summary, the study does not support the reported deleterious effect of glyphosate application on Mn absorption, accumulation and distribution in the plant of glyphosate-resistant soybean genotypes. However, plants sprayed with glyphosate show a temporary phytotoxicity characterized as leaf yellowing. This symptom may, under field conditions, be misinterpreted as Mn deficiency.

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