Glyphosate Tolerant Soybean Response to Different Management Systems

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Abstract

The benefits of glyphosate tolerant crops technology are well-known, and its acceptance by farmers is undeniable. However, results of recent research indicate that, in some situations, glyphosate applied to herbicide-tolerant soybean crops may have phytotoxic effects affecting nutritional balance, photosynthesis and others biochemical process in plants. Despite the increasing information available on this subject, there are still scientific and technical issues that need to be clarified. Therefore, the present study aimed to assess the impact of applying different rates, management systems, and formulations of glyphosate to glyphosate-tolerant soybean trough different regions of Brazil in different environmental conditions. Two experiments were conducted over two crop seasons. A $2 \times 2 \times 5$ (formulations \times stage of application \times doses) factorial design was used in each of them, for a total of 20 treatments with four replications. The study assessed a series of variables related to agronomic performance such as total chlorophyll and yield. The results suggest some problems associated with post-emergent use of glyphosate in tolerant soybean crop as 5% total yield reduction even without phytotoxicity symptoms dependent of season. There was not found any formulation interaction with yield decrease.

Keywords: Glycine max, herbicides, phytotoxicity, selectivity, transgenic crops

1. Introduction

Transgenic plants are being increasingly used in the development of products and services that have a significant impact on the lives of rural producers and worldwide consumers. Farmers, in particular, have embraced genetically modified organisms (GMOs) because of the advantages they offer (OECD-FAO, 2015). Before the appearance and expansion of herbicide-resistant crops, the major difficulty in managing weeds that interfere with commercial crops was to find a single product that ensured the effective control of all invasive plants, i.e., that exhibited a broad spectrum of weed control and was simultaneously selective and harmless to the crop, which happens to glyphosate (Ferreira et al., 2013).

With the expansion of area occupied by soybean crops in recent years in Brazil, there has been a significant increase in transgenic soybean (with tolerant to herbicides or resistant to insects, or both), covering over 96% of the soybean-cultivated area in the 2016-17 crop year (Céleres, 2017).

Recent studies have reported that glyphosate can have phytotoxic effects on GMO soybean (Albrecht et al., 2011; Zobiole et al., 2010a). Glyphosate may affect process such secondary metabolism, mineral nutrition, photosynthesis and biomass formation and accumulation (Zobiole et al., 2010a, 2010b, 2010c; Reddy et al., 2004).

Despite the important contribution of the aforementioned studies to the understanding of pertinent issues, there are still areas that need to be investigated in the context of crop agronomic performance. Still, is not known if

these findings are a rule or if they depend on environment conditions, glyphosate formulation type or stage of plant development application. The findings of these studies will help to explain the actual impact of glyphosate on glyphosate tolerant-soybean crops under different field conditions and seasons.

2. Methods

2.1 Field Sites and Material Description

The experiments were conducted in the cities of Assis Chateaubriand (24°16'10.49"S, 53°39'40.06"W), referred hereafter as Exp. 1, and Marialva (23°22'4.01"S, 51°39'40.06"W), referred hereafter as Exp. 2, both repeated in 2011-12 and 2012-13 seasons. The region in which the experimental areas were located was Paraná, a Brazilian state with great potential for soybean production. Exp. 1 area is located at an altitude of 406 m, and the soil classified as a typical eutroferric red latosol. Exp. 2 area is located at an altitude of 612 m in a latosolic eutroferric red nitosol soil. Fertilization procedures, crop establishment, and phytosanitary measures followed the methods recommended by *Empresa Brasileira de Pesquisa Agropecuária* (EMBRAPA, 2011). The experimental areas were kept free of weed plants throughout the study via hand weeding. Data on rainfall and maximum and minimum temperatures were collected daily during the experiments.

The soybean cultivar used in both locations was NK 7059 RR (V-max RR, Syngenta Crop Protection, Santo Amaro, Brazil) the most cultivated over the last three crop years in Paraná. For Exp. 1, the dates of sowing and harvest, respectively, were September 29, 2011, and February 1, 2012, in the 2011-12 crop year and September 27, 2012, and February 3, 2013 in the 2012-13 crop year. For Exp. 2, these dates were October 20, 2011, and February 20, 2012, in the 2011-12 crop year and November 3, 2012, and March 2, 2013, in the 2012-13 crop year, respectively.

Two different commercial formulations of glyphosate, registered for post-emergence treatment of RR soybean crops in Brazil, were selected for the applications. One of the two glyphosate formulations applied was isopropylamine salt defined hereafter as IS (Roundup Ready[®], 480 g ai L⁻¹, Monsanto São José dos Campos, Brazil), and the other one, the potassium salt, defined hereafter as PS (Zapp QI[®], 620 g ai L⁻¹, Syngenta Crop Protection Santo Amaro, Brazil).

2.2 Experimental Design

Both experiments were conducted using a randomized block design with four replicates, being treatments combined in a $2 \times 2 \times 5$ (formulations \times stage of plant development at application \times doses) factorial arrangement, for a total of 20 treatments. Management system one defined hereafter as M1 consisted of a single application of glyphosate at the V4 soybean stage (four unfolded trifoliate leaves). Management system two, M2, consisted of glyphosate sequential applications, the first applied at the V4 soybean stage and the second between V5 and V6 soybean stages (10 days after the first application) (Fehr et al., 1971). In M2, the glyphosate dose was divided into the two applications (Table 1).

Application of glyphosate was performed with a CO_2 -pressurized backpack sprayer equipped with a bar and six flat tips (Jacto[®] 11002, Jacto São Paulo, Brazil) at a constant pressure of 29 psi and an output of 0.65 L min⁻¹. Spraying was performed at a distance of 50 cm from the target in a velocity of 1 m s⁻¹. The treated area was 50 cm wide, and the spray volume applied was 200 L ha⁻¹. All applications were performed under adequate environmental conditions.

Glyphosate rate (g ai ha ⁻¹)				
M1 (Single application)	M2 (Two sequential applications)			
0	0 + 0			
720	360 + 360			
1,440	720 + 720			
2,160	1,080 + 1,080			
2,880	1,440 + 1,440			

Table 1. Treatments performed with each glyphosate formulation (IS and PS) in 2011/12 and 2012/13 crop years

The plots consisted of six 5 m long rows with spacing of 0.45 m. A work area of 5.4 m^2 was used for assessment, which included only the four central rows and discarded the 1 m borders on each end of the rows.

2.3 Analytical Procedures

Phytotoxicity of soybean was visual evaluated at 7, 14, 21, and 28 days after application (DAA). For each experimental unit was attributed a percentage score from 0 to 100% (0% for absence of injury and 100% for plant death) (Velini et al., 1995). These data were transformed using the formula $(x + 1)^{0.5}$ for data normality, where, "x" is the phytotoxicity value.

Chlorophyll *a* and *b* and total chlorophyll contents were determined at the R2 stage (full flowering) using an electronic chlorophyll meter (ClorofiLOG[®], Falker Porto Alegre, Brazil). The Falker chlorophyll index, which correlates with laboratory measurements and takes into consideration the presence of chlorophyll *a* and *b*, was also determined using the measured light absorption values at various frequencies.

The variables related to agronomic performance evaluated included plant height, number of pods per plant, hundred-seed weight, and yield estimation. Plant height and pods number were measured in 10 randomly plants inside work area. The number of pods per plant was determined at full maturity of plants (R8 stage) by manual counting, also when plants were manually harvested. Subsequently, the pods were threshed, cleaned, and properly stored. Yield (kg ha⁻¹) was then estimated based on seed yield in the plots. Hundred-seed weight was determined by weighing eight subsamples of 100 seeds for each field replicate using analytical scales with a precision of 1 mg. Seed moisture content was adjusted to 13% on a wet basis, also used to calculate yield and hundred-seed weight.

The basic assumptions for analysis of variance were met, and all necessary tests of interaction were performed (p < 0.05). Regression analysis ($p \le 0.05$) was used to assess dose-dependent behavior, and the *F*-test was conclusive in the comparison of means of the qualitative treatments (*i.e.* management systems M1 and M2 and glyphosate formulations IS and PS).

3. Results and Discussion

3.1 Season and Environmental Conditions

Figures 1A and 2A show that crop development in both experimental locations was severely affected by climatic conditions in the 2011-12 crop year, especially due to water deficits that occurred between the R2 and R5 stages in Exp. 1 and in the V4 and R3 stages in Exp. 2.



Figure 1. Rainfall and temperature (minimum and maximum) at Exp. 1 location in the 2011-12 crop year (A) and 2012-13 (B)



Figure 2. Rainfall and temperature (minimum and maximum) at Exp. 2 location in the 2011-12 crop year (A) and 2012-13 (B)

Comparing Figures 1A and 2A with Figures 1B and 2B, the great difference in water resources in maturation stage between the two crop years is clear. From an ecophysiological point of view, when water stress (caused by water shortage) occurred in the initial stages of crop development (Exp. 2 during the 2011-12 crop year), growth of the aerial part of the plants are limited in favor of root development. Plants should therefore being shorter with a reduced number of nodes and pods per plant. When water stress occurred in later stages, specifically in the reproductive phase of the crop (Exp. 1 in the 2011-12 crop year), plants reproductive traits are significantly affected. Flowering abortion increased because water stress led to a reduction in the absorption of nutrients essential for flowering and fertilization. The low relative humidity also adversely affected fertilization. Moreover, during this period, to maintain growth and develop seeds, plants accumulate photoassimilates for reserves, and the source-sink relationship is altered. Therefore, grain filling (R5 stage) is considered one of the most critical phases of soybean development (Farias et al., 2007).

In Exp. 1 and Exp. 2, the adverse environmental conditions affected plant development and therefore the crop's agronomic performance. However, the climatic conditions in the second crop year in both locations were similar to historical mean conditions of the regions, and the crop developed normally attesting all possible and different environmental effects in treatments applied.

Although the conditions for crop development were more favorable in the second than the first crop year for Exp. 1, crop response to the applied formulations and management systems were not significantly different. Crop response to the applied formulations and management systems did not differ significantly between the two crop years in Exp. 2, even under different climatic conditions. The results did not support a conclusion of one product or management system being more or less harmful to the crop than another (p < 0.05), even in terms of the variables exhibiting statistical differences.

3.2 Effects on Chlorophyll Content

In the Exp. 1 location (IS glyphosate applied in M2 during 2011-12 crop year), and Exp. 2 location during 2012-13 crop year, the assessments and statistical analysis of data on chlorophyll *a* content showed some significant differences between treatments. Tests of interaction were conducted using regression analysis to identify significant effects (p < 0.05) on the variables of chlorophyll *a* content. The data's fitted a linear model with a decreasing effect on these variables with increasing glyphosate rates (Figure 3). For Exp. 1 location during 2012-13 crop year, and Exp. 2 location during 2011-12 crop year there were no differences in chlorophyll *a* content.



Figure 3. Chlorophyll a content, as a function of IS glyphosate formulation rates applied in M2 during 2011-12 crop year at Exp. 1 location (A), as a function of IS and PS glyphosate formulation rates applied in M1 during 2012-13 crop year at Exp. 2 location (B), and as a function of PS glyphosate formulation rates applied in M2 during 2012-13 crop year at Exp. 2 location (C)

For Exp. 1 and Exp. 2 locations, during 2011-12 crop year, there were also no significant differences in chlorophyll *b* content (p < 0.05) between the treatments (data not showed). For Exp. 1 location, during 2012-13 crop year, there were slight statistical differences in chlorophyll *b* content; however, there were no defined patterns for each rate. Regression analysis using the tests of interaction for the different glyphosate rates showed significant effects on chlorophyll *b* content (Figure 4A).

For Exp. 2 location, during 2012-13, tests of interaction using regression analysis were used to identify significant effects ($p \le 0.05$) on chlorophyll *b* content (Figures 4B and 4C). A linear, negative response of this variable to increasing glyphosate rates was found.

For Exp. 1 location 2011-12 crop year, first and second crop years for Exp. 2, the assessments and statistical analysis of data on, total chlorophyll content showed some significant differences between treatments.

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Figure 4. Chlorophyll b content, as a function of PS glyphosate formulation rates applied in M1 during 2012-13 crop year at Exp. 1 location (A), as a function of IS and PS glyphosate formulation rates applied in M1 during 2012-13 crop year at Exp. 2 location (B), content as a function of IS and PS glyphosate formulation rates applied in M2 during 2012-13 crop year at Exp. 2 location (C)

Regression analysis for the different rates showed significant effects on total chlorophyll content. There was a linear, decreasing response in this variable to increasing rates of glyphosate (Figure 5).



Figure 5. Total chlorophyll content, as a function of IS glyphosate formulation rates applied in M2 during 2011-12 crop year at Exp. 1 location (A), and Exp. 2 location (B). Total chlorophyll content, as a function of IS and PS glyphosate formulation rates applied in M1 (C) and M2 (D) during 2012-13 crop year at Exp. 2 location

In the second crop year for Exp. 2, tests of interaction using regression analysis were used to identify significant effects (p < 0.05) on total chlorophyll content. However, for other experiments, there was no clear pattern suggesting that one formulation or management system was more or less harmful to the crop than another for each rate. For Exp. 1 location, second season, there were no differences in total chlorophyll content (data not showed). The reductions in chlorophyll content net photosynthesis, probably affected and accumulation of biomass of agronomic interest, which, in turn, affected hundred-seed weight and yield (Zobiole et al., 2010c).

3.3 Plant Phytotoxicity of Glyphosate Formulations

In Exp. 1, for booth crop years, and in Exp. 2, for first crop year, there were no significant effects on phytotoxicity in the crop. However, for Exp. 2, during 2012-13 crop year, phytotoxicity (Figures 6-8) exhibited a linear, positive response as a function of increasing rates. With the evolution of DAA, symptoms of phytotoxicity decreased.

The environmental conditions in this location and crop year did produce visually detectable phytotoxicity symptoms that had, until that point, remained latent. This observation was not associated with a reduction in yield, but rather, with a negative effect on other traits, such as plant height and photosynthetic systems. This finding indicates that a positively relation between traits and final yield are not always present, and the same, for initial phytotoxicity symptoms.

The inverse relationship between the reduction in chlorophyll content and increased phytotoxicity (yellow flashing) observed in the crop is noteworthy. This unwanted symptom in the RR soybean crop may be associated with the accumulation of aminomethylphosphonic acid, the main phytotoxic metabolite of glyphosate (Reddy et al., 2004).







Figure 7. Visual plant phytotoxicity 7 DAA as a function of IS and PS glyphosate formulation rates applied in M1 during 2012-13 crop year at Exp. 2 location (A). Visual plant phytotoxicity 7 DAA as a function of IS and PS glyphosate formulation rates applied in M2 during 2012-13 crop year at Exp. 2 location (B)



Figure 8. Visual plant phytotoxicity 21 DAA as a function of IS and PS glyphosate formulation rates applied in M1 during 2012-13 crop year at Exp. 2 location (A). Visual plant phytotoxicity 21 DAA as a function of IS and PS glyphosate formulation rates applied in M2 during 2012-13 crop year at Exp. 2 location (B)

3.4 Effects on Plant Height

In Exp. 1 location, for booth crop years, there were also no significant differences in plant height, ($p \le 0.05$) between the treatments. In the first crop year for Exp. 2, plant height differed between treatments; however, there was no clear pattern suggesting that one formulation or management system was more or less harmful to the crop than another for each rate (data not showed). During 2012-13 crop year, tests of interaction using regression analysis were used to identify significant effects (p < 0.05) on plant height (Figure 9). A linear, negative response of these variables to increasing glyphosate rates was found.



Figure 9. Plant height as a function of IS and PS glyphosate formulation rates applied in M1 during 2012-13 crop year at Exp. 2 location

Plant height affected by glyphosate rates probably as a result of water and xenobiotic stress (from the glyphosate or aminomethylphosphonic acid). Although plant height was not compromised, the photosynthetic apparatus was probably affected.

3.4 Effects on Hundred-Seed Weight and Number of Pods per Plant

For Exp. 1 location, during 2012-13 crop year, the assessments and statistical analysis of data on and hundred-seed weight showed some significant differences between treatments. The data's fitted a linear model with a decreasing effect on these variables with increasing glyphosate rates (Figure 10).



Figure 10. Hundred-seed weight as a function of IS glyphosate formulation rates applied in M1 during 2012-13 crop year Exp. 1 location

For first crop year statistical analysis of data showed some significant differences between treatments (data not showed). However, for booth crop years, there was not a single pattern suggesting the superiority of one formulation or management system over another.

For Exp. 2 location, during booth crop years, there were also no significant differences in this variable ($p \le 0.05$) between the treatments (data not showed). There were also no significant differences in number of pods per plant, between the treatments (data not showed), for all experiments.

3.5 Effects on Crop Yield

In the Exp. 1 location, during crop year 2011-12, there were also no significant differences in yield ($p \le 0.05$) between the treatments (data not showed). In soybean, yield reduction can be correlated with injury to other characteristics of the plants, such as chlorophyll content, plant height, and number of pods per plant (Sediyama et al. 1993). Instead, the general environmental conditions observed for this season and soybean characteristics affected by glyphosate in this experiment (such as chlorophyll and seed weight) were not sufficient for soybean yield interference.

In the Exp. 1 location, during crop year 2012-13, there were slight statistical differences in yield between treatments; however, there were no defined patterns for each rate (Table 2).

Table 2. Soybean yield after the application of different rates of two glyphosate formulations (IS and PS) under two management systems (M1 and M2) during 2012-13 crop year in Exp. 1 location.

Rates (g ai ha ⁻¹)	Yield (kg ha ⁻¹)				
	IS f	IS formulation		PS formulation	
	M1	M2	M1	M2	
0	3,257.42 Aa	3,252.99 Aa	3,140.65 Aa	3,049.37 Aa	
720	3,206.80 Aa	3,247.41 Aa	3,142.18 Aa	2,767.01 Bb	
1,440	3,097.87 Aa	3,181.35 Aa	2,853.20 Aa	3,087.19 Aa	
2,160	2,957.62 Aa	2,978.42 Aa	3,142.59 Aa	2,839.01 Aa	
2,880	3,283.63 Aa	2,955.30 Aa	3,038.61 Aa	2,832.95 Aa	
Means	3.160,67	3.123,09	3.063,45	2.915,11	
CV (%)	8.58				

Note. M1: Management system 1, with a single glyphosate application, M2: Management system 2, with glyphosate sequential application. CV: Coeficient of variation. Same uppercase letter in in rows indicates no significant difference (p < 0.05) between formulations (IS and PS) within each management system and rate with the *F*-test. Same lowercase letter in rows indicates no significant difference (p < 0.05) between management systems (M1 and M2) within each formulation and rate with the *F*-test.

Regression analysis using the tests of interaction for the different glyphosate rates showed significant effects on yield (Figure 11). The data fitted a linear model with a negative effect on all variables from increasing glyphosate rates.



Figure 11. Soybean yield as a function of IS glyphosate formulation rates applied in M2 during 2012-13 crop year at Exp. 1 location

The mean yield for this year was similar to the historical regional mean (around 3,000 kg ha⁻¹) and different to that obtained in the first year (1,843 kg ha⁻¹). There was a clear reduction in yield with increasing rates of glyphosate. Figure 11 shows a regression-curve fitting for formulation IS in M2 displaying a linear decreasing response, with a 0.120 kg reduction in yield grain for every glyphosate g ia ha⁻¹ increase. In this experiment, the number of pods was not affected. Therefore, the variable that correlated with decreasing yield was seed weight.

With the results observed in the two seasons, for Exp. 1 location, it noticeable that the stress caused by environmental conditions can be more harmful to soybean productivity than glyphosate (Carvalho et al., 2002). The yield reduction in these location reached 40% caused by water stress, while glyphosate had no effect. In addition, when there exists favorable conditions for plants development, glyphosate can cause yield losses even without symptoms on soybean, in this case, more pronounceable with potassium salt in a sequential application. The differences between formulations were already observed (Santos et al., 2007a), which observed differences in the translocation of ¹⁴C-glyphosate, for application of IS and PS products, especially in the nodules of soybean plants.

In Exp. 2 location, during crop year 2011-12, there were no differences in yield. The results obtained from both locations in this crop year were similar; some previous considerations are valid for this experiment. Even not statistical significant, there was a reduction in yield with increasing glyphosate PS rates in management M1. There was a 0.0397 kg reduction in grain yield for every g ia ha^{-1} increase in glyphosate and, therefore, a reduction in seed weight.

It was not possible to fit a model of yield response to this Exp. 2 location during 2012-13 crop year (Table 3). However, the environmental conditions in this location and crop year did produce visually detectable phytotoxicity symptoms that had, until that point, remained latent. This observation was not associated with a reduction in yield, but rather, with a negative effect on other traits, such as plant height and photosynthetic systems. This finding indicates that a positively relation between traits and final yield are not always present, and the same, for initial phytotoxicity symptoms.

	Yield (kg ha ⁻¹)			
Rates (g ai ha ⁻¹)	IS formulation		PS formulation	
	M1	M2	M1	M2
0	3,843.51 Aa	3,634.80 Aa	3,745.07 Aa	3,833.50 Aa
720	3,583.66 Ab	3,996.49 Aa	3,859.52 Aa	3,793.84 Aa
1440	3,971.09 Aa	3,795.36 Aa	4,025.80 Aa	3,982.16 Aa
2160	3,692.32 Aa	4,004.31 Aa	3,502.91 Ab	3,937.71 Aa
2880	3,879.27 Aa	3,864.28 Aa	3,956.64 Aa	3,683.97 Aa
Mean	3,793.97	3,859.05	3,817.99	3,846.24
CV (%)	7.12			

Table 3. Soybean yield after the application of different rates of two glyphosate formulations (IS and PS) under two management systems (M1 and M2) during 2012-13 crop year in Exp. 2 location

Note. M1: Management system 1, with a single glyphosate application, M2: Management system 2, with glyphosate sequential application. CV: Coeficient of variation. Same uppercase letter in in rows indicates no significant difference (p < 0.05) between formulations (IS and PS) within each management system and rate with the *F*-test. Same lowercase letter in rows indicates no significant difference (p < 0.05) between management systems (M1 and M2) within each formulation and rate with the *F*-test.

The results suggests that the differences in the effects on the study variables between a single application and sequential applications of glyphosate (management systems 1 and 2) and between the two formulations used (IS and PS) were not significant for each rate. Significant effects, however, were evident within the analyzed rate levels (either simple or interactive effects).

Although single and sequential-application management systems are recommended for post-emergent use in crops, information on their effect on crops with regard to factors such as infestation and species present in the area is scarce in the relevant literature (Alonso et al., 2013). Although the two formulations contained different salts, which, together with the surfactants, facilitated differential penetration, absorption, and translocation, their final effects on the plants and agronomic performance were similar (Santos et al., 2007b).

Xenobiotic stress caused by glyphosate or its degradation products on RR soybean plants is often unnoticed by the producer and even by expert technicians. This is because, in most cases, there is no visual phytotoxic effect on the plants, even at the highest doses, as was demonstrated here in Exp. 1, second season. Even without symptoms, there is a yield reduction on crop that can reach almost 5% of total production. It is, however, possible to infer from the data and the tests of interaction that increasing glyphosate rates caused damage to the plants and, specifically, the crop's yield components and that this is not related to visual crop observations. This can be related to the development of new generation tolerant-soybeans.

The lack of significant visual effects on plants from the application of glyphosate is an important phenomenon that can prevent a producer from understanding the actual damage caused by a high rate of glyphosate to the crop. The widespread notion that RR soybean plants are totally resistant to glyphosate is an obstacle to the awareness of this phenomenon associated with the secondary effects of glyphosate (Zobiole et al., 2010b).

The putative deleterious effects of glyphosate on crop characteristics of agronomic interest have been directly discussed by several authors. These impacts are probably associated with a result of injury from the adverse action of glyphosate and its metabolites (Zobiole et al., 2010b, 2010d; Albrecht et al., 2012). Changes to other physiological mechanisms, such as photosynthesis and other biosynthetic processes leading to biomass accumulation result from glyphosate use and directly affect soybean yield components (Zobiole et al., 2010d, 2010e). The presence of any symptom is clear related to the glyphosate application, as GMOs proved to have the same compositional variability as conventional soybean in different regions in Brazil (Zhou et al., 2011).

4. Conclusion

The results obtained in the present study demonstrate that, in general, any of the investigated formulations and management systems of glyphosate can be used. However, the glyphosate rate is the main limiting factor because it significantly affects crop performance and, therefore, crop yield depending on location or environmental conditions and this often occurs in the absence of visual phytotoxic symptoms. Despite the large number of experiments and assessments within the study, these results are not final. Further research is needed, especially because Brazil has numerous soybean cultivars adapted to different environmental conditions and a large land extension.

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