



ORIGINAL ARTICLE

Minimized phytotoxic effects of pre-harvest desiccation with glyphosate in soybean seeds

Redução dos efeitos fitotóxicos da dessecação em pré-colheita com glyphosate em sementes de soja

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ABSTRACT: Pre-harvest desiccation of soybean plants from non-genetically modified cultivars with glyphosate have shown to be detrimental to seed physiological quality by reducing germination and increasing production of abnormal seedlings. On the other hand, many products have been applied to seeds in order to enhance quality such as growth regulators and micronutrients. This study aimed to verify the phytotoxic effects of pre-harvest desiccation with glyphosate on physiological quality of soybean seeds and if they can be overcome or minimized by the application of micronutrients and biostimulant. Seeds from soybean cultivar *Conquista* were produced with and without desiccation with glyphosate, at the dose 2.0 L ha⁻¹, applied at physiological maturity stage. Right after harvest, seeds were treated with Co + Mo + B + Zn, Co + Mo and biostimulant; a control, with no treatment, was also evaluated. Physiological quality was assessed by tests of germination and seedling development. Data was submitted to variance analysis. Means were compared by the Tukey test ($p \leq 0.05$) and analyzed as a 2 × 3 factorial. Dunnet's test ($p \leq 0.05$) was also applied. Negative effects of pre-harvest desiccation with glyphosate on germination of soybean seeds can be overcome by the application of micronutrients Co+Mo. Applying biostimulant to soybean seeds improves seedling development compared with micronutrient treatments, although it does not minimize detrimental effects of desiccation with glyphosate.

RESUMO: A dessecação em pré-colheita de plantas de soja de cultivares tradicionais com glyphosate tem-se revelado prejudicial à qualidade fisiológica das sementes, em razão da redução da germinação e do aumento no número de plântulas anormais. Por outro lado, muitos produtos, como reguladores vegetais e micronutrientes, têm sido aplicados às sementes com o objetivo de melhorar sua qualidade. Este estudo objetivou verificar se efeitos fitotóxicos da dessecação em pré-colheita com glyphosate na qualidade fisiológica de sementes de soja podem ser superados ou minimizados pela aplicação de micronutrientes e biostimulante. Sementes de soja da cultivar *Conquista* foram produzidas com e sem dessecação das plantas com glyphosate, na dose de 2,0 L ha⁻¹, aplicada na maturidade fisiológica. Logo após a colheita, as sementes foram tratadas com os complexos Co + Mo + B + Zn, Co + Mo e um biostimulante; a testemunha, sem tratamento, também foi avaliada. A qualidade fisiológica foi avaliada por testes de germinação e desenvolvimento de plântulas. Os dados foram submetidos à análise de variância. As médias foram comparadas pelo teste de Tukey ($p \leq 0,05$) e analisadas em esquema fatorial 2 × 3. O teste de Dunnet ($p \leq 0,05$) também foi aplicado. Os efeitos negativos da dessecação em pré-colheita com glyphosate na germinação das sementes de soja podem ser minimizados com a aplicação de Co + Mo. A aplicação de biostimulante às sementes de soja melhora o desenvolvimento das plântulas, quando comparada com o tratamento com micronutrientes, apesar de não minimizar os efeitos deletérios da dessecação com glyphosate.

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1 Introduction

Physiological maturity stage or R7 (FEHR; CAVINESS, 1977) is defined as a developmental stage when seeds show maximum dry matter accumulation, germination and vigor (CARVALHO; NAKAGAWA, 2012). Therefore, harvesting seeds at or close to this stage would ensure better quality at minimum deterioration levels.

Soybean (*Glycine max* (L) Merrill) seed producers have attempted to bring harvest forward by chemically desiccating plants, especially with paraquat and diquat (LACERDA et al., 2003). Nevertheless, glyphosate has also been applied as desiccant to accelerate and homogenize water loss by plants and seeds. Glyphosate is a non-selective and efficient herbicide widely applied in many Brazilian regions (VARGAS; MORAES; BERTO, 2007), although inappropriate use may cause phytotoxicity in plants, as well as grain yield and seed quality loss.

Pre-harvest desiccation of soybean plants from non-genetically modified cultivars for seed production is not recommended, because physiological quality and development of primary roots in seedlings may be harmed (EMBRAPA, 2011). Toledo, Cavariani and França Neto (2012) reported negative effects of pre-harvest desiccation with glyphosate on seedling development of two soybean cultivars, similarly to other authors (MARCANDALLI; LAZARINI; MALASPINA, 2011; DALTRO et al., 2010).

Many studies have brought out relevant effects of chemical desiccation such as accumulation of toxic residues in seeds and influence on germination and vigor; however, results are yet inconclusive. Nevertheless, Dick and Quinn (1995) observed that modifications in plant physiology and, consequently, seed quality occur mainly due to compounds originated from glyphosate degradation, such as the aminomethyl phosphonic acid (AMPA), rather than glyphosate itself. This substance is accumulated in seeds (DUKE et al., 2003) and may cause abnormalities to seedlings.

Elucidation of metabolic reactions involving synthesis and degradation of molecules during developmental, deteriorative and germination processes in seeds is still challenging. Cell integrity and metabolism depend on a great variety of enzymes and structural enzymes, which are specific for each species (VIEIRA; VON PINHO; SALGADO, 2006). In this context, the application of micronutrients and growth regulators in seeds has been widely discussed in literature. Most micronutrients are activators and structural components of enzymes (TAIZ; ZEIGER, 2004), improving seed germination and vigor. Growth regulators have been frequently associated with micronutrients in seed treatment, aiming to increase germination and seedling emergence in the field. According to Castro and Vieira (2001), they enhance seed performance by reducing abnormalities in seedlings. According to these authors, soybean seeds treated with biostimulants improve germination efficiently.

Efficiency of growth regulators is related to mobility within a certain organism, potential to amplify signals, and capacity to stimulate complex regulations by the interaction among biochemical and physiological processes (RODRIGUES; LEITE, 2004). Therefore, it is possible that phytotoxic effects

of pre-harvest desiccation with glyphosate, such as reduced germination and poor development of primary roots in seedlings, can be overcome or minimized by the application of micronutrients and biostimulants to seeds.

This study aimed to verify whether phytotoxic effects of pre-harvest desiccation with glyphosate on physiological quality of soybean seeds can be overcome or minimized by the application of micronutrients and biostimulant.

2 Materials and Methods

This study was conducted in the municipality of Botucatu, state of Sao Paulo, Brazil, in two stages. The first consisted of producing soybean seeds under pre-harvest desiccation with glyphosate. The experiment was conducted in area with geographical coordinates of 48° 23' W and 22° 51' S and 765 m asl. Soil in the area is a Typic Hapludox (FAO, 2006). According to Köeppen's classification, climate is Cwa, which corresponds to tropical altitude with dry winter and hot wet summer (PEEL; FINLAYSON; McMAHON, 2007).

Figure 1 shows climate data recorded in the area during production.

The experimental design in the field was the completely randomized block with four replications, analyzed as a 2 × 3 factorial (with/without desiccation × chemical treatment). Non-genetically modified soybean cultivar *Conquista* was sown mechanically over millet residues on 04 November 2008. Seeds were previously treated with carboxim+thiram fungicide (Vitavax Thiram SC® - 300 mL per 100 kg of seeds) and inoculant (Biomax Premium Turfa® - 60 g per 50 kg of seeds) and sown at 0.45-m row spacing to obtain a population of 400,000 plants ha⁻¹. Base fertilization consisted of 300 kg ha⁻¹ of the 04-20-20 NPK formulation.

Glyphosate was applied at a dose of 2.0 L ha⁻¹ (Roundup WG® - 720 g kg⁻¹ of the a.i.) at physiological maturity stage, or R7, that occurred on 24 March 2009. This growth stage was identified through pod staining, i.e. most of them yellowish and one completely mature in the main stem (FEHR; CAVINESS, 1977). The application was carried out with spraying bar with flat fan TJJ110.04 nozzles spaced 0.50-m apart from each other. Water volume and application pressure were 200 L ha⁻¹ and 50 lbf cm⁻², respectively.

Seeds were harvested mechanically on 10 April 2009 in 6 lines of 6-m length, totaling 16.2 m². Afterwards, they were dried and stored in paper bags in dry chamber (30-40% relative humidity and without temperature monitoring) during quality evaluations.

The second stage was carried out right after harvest and aimed to assess physiological quality of seeds after treatment with Co+Mo+B+Zn (Biocriop®, 200 g ha⁻¹), Co+Mo (CoMo Plus 250®, 100 mL ha⁻¹) and a biostimulant, mixture of cytokinin + indolbutiric acid + giberelic acid (Stimulate®, 500 mL ha⁻¹). A control, with no treatment, was also evaluated.

Seed quality was evaluated by the following tests, right after harvest and treatment:

Water content: two subsamples of 20 seeds per replication were evaluated using an oven at 105 ± 3 °C for 24 h (wet basis); results were expressed as a percentage (BRASIL, 2009).

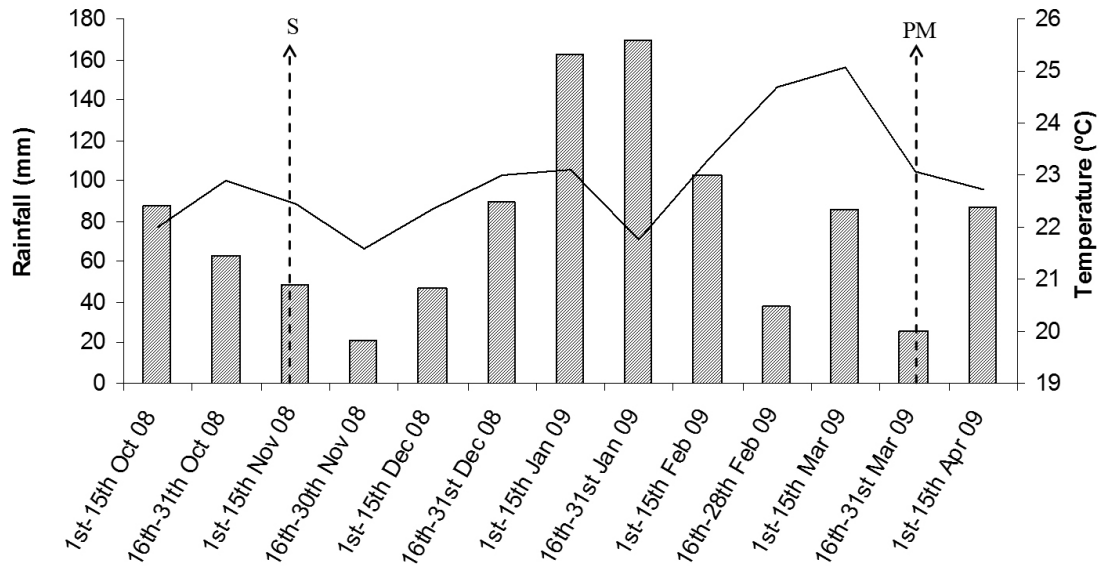


Figure 1. Rainfall (▨) and mean temperature (—) during seed production. S: sowing; PM: physiological maturity.

Germination: four subsamples of 50 seeds per replication were distributed on paper towels moistened with water equivalent to 2.5 the weight of the dry paper. Paper rolls were made and placed into plastic bags and left for germination at 25 °C. Evaluation took place eight days after sowing (BRASIL, 2009) and results were expressed as the mean percentage of normal and abnormal seedlings.

First count of germination: it was performed along with the germination test; the percentage of normal seedlings was recorded on the fifth day after sowing (BRASIL, 2009).

Seedling length: four subsamples of ten seeds per field replication were sown on a line drawn on paper towels moistened with water equivalent to 2.5 times the weight of the dry paper. Rolls were made and placed into plastic bags and left for germination in upright position at 25 °C for 5 days (NAKAGAWA, 1999). Hypocotyl, primary root and total length of seedlings were measured separately in cm. Results were obtained dividing the total sum of measurements by the number of seeds initially sown.

Seedling dry matter: normal seedlings of the seedling length test were placed into paper bags and dried using an oven at 80 °C for 24 h (NAKAGAWA, 1999). Results were obtained dividing each weight by the number of seedlings. Then, the means were obtained for each treatment, in mg.

Computer analysis: four subsamples of 25 seeds per field replication were germinated in a near vertical orientation in moistened rolled paper towels at 25 °C for five days. Image acquisition was accomplished by a custom scanning box computer system and software processing of images as described by Hoffmaster et al. (2003). The imaging platform captured multiple images of seedlings, enabling simultaneous measurements that were further processed by advanced computer methods that quantify the potential performance of the seed sample. After each seedling was processed and automatically measured, the results of each subsample were combined to produce indices of vigor and uniformity of growth as described by Hoffmaster et al. (2003). This

included the penalty term in the uniformity algorithm, to avoid the undesirable influence of dead seeds on precision of results; small adjustments to the software used for soybeans to recognize properly and mark normal seedlings. Results were expressed as mean values of seedling vigor and uniformity for each treatment.

Data was submitted to variance analysis. Means were compared by the Tukey test ($p \leq 0.05$). Dunnett's test ($p \leq 0.05$) was applied to compare each individual mean with the control, without any treatment.

3 Results and Discussion

Soybean seeds showed 8% moisture content before treatment and physiological quality evaluations represented uniform conditions that did not affect the results.

Germination of seeds was reduced by desiccation of plants with glyphosate before and after treatment with Co + Mo and biostimulant (Table 1). Nevertheless, negative effects of pre-harvest desiccation were not observed after the application of the complex Co + Mo + B + Zn to soybean seeds. In fact, treating seeds with this complex resulted in lower abnormal seedlings. Addition of B and Zn was beneficial to seed quality compared to other ingredients, similarly to the findings in Marschener (2002), who reported that B deficiency in seeds decreases germination and increases the number of abnormal seedlings. Those authors complemented their findings by concluding that even in seeds with low B levels seedling emergence can be overcome by B fertilization applied at sowing. Significant effects of B, Zn, or their combination on rice seed germination, compared to the control were found by Ohse et al. (2001). The authors reported high percentage of abnormal seedlings after seeds were treated with these micronutrients, although there were not any significant differences compared to the control. Boron is required for cell division and elongation; on the other hand, Zn is required for

tryptophan synthesis, which is precursor of indoleacetic acid (TAIZ; ZEIGER, 2004).

Soybean seeds treated with biostimulant showed low germination compared to the control and the micronutrient treatment and higher number of abnormal seedlings. Seed treatment with biostimulants may interfere in enzymatic activity during the germination process (SILVA et al., 2008). Low germination after treating lettuce seeds with high doses of biostimulant was reported by Diniz et al. (2009). Phytotoxic effects and accentuated number of abnormalities in maize seedlings were found even after treating seeds with giberelic acid (ARAGÃO et al., 2003).

There seems to be no relation between the effects of pre-harvest desiccation of soybean plants with glyphosate and later application of biostimulant to seeds, once it was detrimental to germination of seeds produced with no desiccation (Table 1), opposing to Castro and Vieira (2003), who found that biostimulants applied in bean seeds increased germination uniformity and favored seedling emergence.

Germination of seeds produced by desiccated plants and treated with micronutrients was significantly higher than in the control. The treatment caused a reduced number of abnormalities and increased number of normal seedlings in the first count. The application of biostimulant to seeds from non-desiccated plants was the only treatment that differed from the control, with significant decrease in germination.

Similarly to seed germination, pre-harvest desiccation of glyphosate was detrimental to seedling development (Table 2). In fact, measuring of seedling length was already found to be efficient in detecting detrimental effects of desiccation. In two growing seasons, Daltro et al. (2010) evidenced harmful effects of glyphosate on physiological quality of soybean seeds, with phytotoxic symptoms and reduced length of primary roots, similarly to the findings of Marcandalli, Lazarini and Malaspina (2011). Photosynthesis does not occur in early stages of seedling development due to an early stage in formation of photosynthetic apparatus (BERVALD et al., 2010). That way, amino acids that are essential for the synthesis of proteins and secondary metabolites are not produced by

Table 1. Variance and statistical analysis of germination percentage, abnormal seedlings and germination first count of soybean seeds affected by pre-harvest desiccation with glyphosate and subsequent chemical treatment.

Evaluation	Product	Pre-harvest desiccation ⁽¹⁾		
		+G	-G	Mean
Germination (%)	Control	63	84	-
	Co + Mo	73 aB # ⁽²⁾	83 aA	78
	Co + Mo + B + Zn	79 aA #	83 aA	81
	Bioestimulat	49 bB #	68 bA #	58
	Mean	67	78	-
	C.V. (%)		14.39	
	F product		42.555**	
	F desiccation		24.824**	
	F interaction		3.635*	
	Abnormal seedlings (%)	Control	27	8
Co + Mo		19 bB #	11 aA	15
Co + Mo + B + Zn		13 aA #	10 aA	12
Bioestimulat		32 cB	17 bA #	25
Mean		22		-
C.V. (%)			40.71	
F product			29.621**	
F desiccation			41.419**	
F interaction			6.143**	
Germination first count (%)		Control	45	79
	Co + Mo	70 aB #	82 aA	76
	Co + Mo + B + Zn	79 aA #	63 aA	81
	Bioestimulat	48 bB	67 bA #	57
	Mean	66	77	-
	C.V. (%)		15.07	
	F product		43.328**	
	F desiccation		27.017**	
	F interaction		3.633*	

* and ** significant at a probability level of 5 and 1%, respectively. ⁽¹⁾ +G: pre-harvest desiccation with glyphosate; -G: without pre-harvest desiccation with glyphosate. ⁽²⁾ Means followed by the same small letter in the column and capital letter in the row do not differ significantly by the Tukey test ($p \leq 0.05$). #Means followed by this symbol differ from the control by the Dunnet test ($p \leq 0.05$).

Table 2. Variance and statistical analysis of growth and dry matter of soybean seedlings from seeds affected by pre-harvest desiccation with glyphosate and subsequent chemical treatment.

Evaluation	Product	Pre-harvest desiccation ⁽¹⁾		
		+G	-G	Mean
Primary root length (cm)	Control	3.70	13,34	-
	Co + Mo	2.56 bB # ⁽²⁾	6,22 bA #	4,39
	Co + Mo + B + Zn	3.11 abA	3,81 cA #	3,46
	Bioestimulat	3.59 aB	7,86 aA #	5,73
	Mean	3.09	5,96	-
	C.V. (%)		24.86	
	F product		32.893**	
	F desiccation		156.278**	
	F interaction		23.101**	
	Hypocotyl length (cm)	Control	3.81	5,38
Co + Mo		2.94 aB #	3,95 aA #	3,44
Co + Mo + B + Zn		3.29 aA	3,22 bA #	3,25
Bioestimulat		3.53 aB	4,56 aA #	4,05
Mean		3.25	3,91	-
C.V. (%)			21.15	
F product			9.611**	
F desiccation			17.866**	
F interaction			5.476**	
Seedling total length (cm)		Control	7.52	18,71
	Co + Mo	5.50 bB #	10,16 bA #	7,83
	Co + Mo + B + Zn	6.40 abA	7,02 cA #	6,71
	Bioestimulat	7.13 aB	12,42 aA #	9,77
	Mean	6.34	9,87	-
	C.V. (%)		20.99	
	F product		26.570**	
	F desiccation		102.958**	
	F interaction		17.734**	
	Seedling dry matter (mg)	Control	28.38	36,45
Co + Mo		17.67 aA #	20,85 bA #	19,26
Co + Mo + B + Zn		19.52 aA #	19,35 bA #	19,44
Bioestimulat		19.53 aB #	30,87 aA	25,20
Mean		18.91	23,69	-
C.V. (%)			36.01	
F product			6.213**	
F desiccation			9.324**	
F interaction			4.769*	

* and ** significant at a probability level of 5 and 1%, respectively. ⁽¹⁾+G: pre-harvest desiccation with glyphosate; -G: without pre-harvest desiccation with glyphosate. ⁽²⁾ Means followed by the same small letter in the column and capital letter in the row do not differ significantly by the Tukey test ($p \leq 0.05$). #Means followed by this symbol differ from the control by the Dunnet test ($p \leq 0.05$).

seedlings, especially aromatic amino acids whose synthesis is blocked by glyphosate. In consequence, it may decrease levels of soluble proteins in plant tissues and harm germination and other early processes.

Nevertheless, the subsequent treatment of seeds produced by desiccated plants with Co + Mo and Co + Mo + B + Zn was not efficient in overcoming phytotoxic effects and negative influence on root length, reflected in the production of shorter seedlings. In truth, these micronutrients caused reduction of seedling development compared with the other products.

Harmful effects of Mo + Co + B treatment at the highest dose (4 mL kg⁻¹ of seeds) were reported in literature whenever applied along with fungicide and polymer (BAYS et al., 2007).

Seedlings originated from seeds produced conventionally and treated with Co + Mo + B + Zn showed reduced length, compared to other products. There were no significant differences in seedling length for seeds treated with Co + Mo + B + Zn produced with and without pre-harvest desiccation. Regardless, seedling development was impaired in relation to the control. Similar results with maize were

Table 3. Variance and statistical analysis of vigor and growth uniformity of soybean seedlings from seeds affected by pre-harvest desiccation with glyphosate and subsequent chemical treatment.

Evaluation	Product	Pre-harvest desiccation ⁽¹⁾		
		+G	-G	Mean
Vigor index	Control	352.44	351,31	-
	Co + Mo	335.50 # ⁽²⁾	358,38	346,94 ab
	Co + Mo + B + Zn	352.75	362,50	357,63 a
	Bioestimulat	337.44 #	334,56	336,00 b
	Mean	341.90 B	351,81 A	-
	C.V. (%)		6.72	
	Fproduct		6.882**	
	Fdesiccation		4.341*	
	Finteraction		2.439ns	
	Growth uniformity	Control	849.63	817,50
Co + Mo		817.06 aA #	844,94 aA	831,00
Co + Mo + B + Zn		834.75 aA	836,50 aA	835,63
Bioestimulat		832.56 aA	798,50 bB	815,53
Mean		828.13	826,65	-
C.V. (%)			4.83	
Fproduct			2.217ns	
Fdesiccation			0.033ns	
Finteraction			4.838*	

* and ** significant at a probability level of 5 and 1%, respectively; ns: not significant. ⁽¹⁾+G: pre harvest desiccation with glyphosate; -G: without pre harvest desiccation with glyphosate. ⁽²⁾ Means followed by the same small letter in the column and capital letter in the row do not differ significantly by the Tukey test ($p \leq 0.05$). # Means followed by this symbol differ from the control by the Dunnett test ($p \leq 0.05$).

described by Silva et al. (2008), where applying a complex with Zn, Mo and giberelic acid to seeds caused reduced seedling development and dry matter accumulation.

Application of biostimulant to soybean seeds improved root development and total length as a consequence. However, applying biostimulant to seeds produced conventionally, with no desiccation with glyphosate, seems to improve seedling growth only compared to the other combination of treatments, and seeds from the control still showed significantly better quality.

Although many authors have reported positive influence of biostimulants on seed quality (CASTRO; VIEIRA, 2003; VIEIRA; CASTRO, 2001), this study showed that even ingredients, applied to improve germination and vigor, have their effects limited by toxic residues of glyphosate desiccation. In fact, it is the accumulation of AMPA in seeds (DUKE et al., 2003) that needs to be thoroughly studied. Toxic potential of AMPA is known in growing plants and still not elucidated completely, especially regarding its influence on acting in seed germination processes and seedling development (ALBRECHT; ÁVILA, 2010).

Vigor of soybean seedlings was affected by pre-harvest desiccation and seed treatment singly (Table 3). Regardless of any combinations, the use of glyphosate as desiccant reduced vigor of seedlings as well as the application of biostimulant to seeds, which are consonant with results previously discussed in Table 2. On the other hand, growth uniformity was significantly influenced by the interaction between seed treatment and desiccation conditions. Application of biostimulant caused

the main decreases of assayed parameters in seeds produced conventionally.

Many studies have been conducted to detect metabolic reactions involving synthesis and degradation of molecules during seed development, germination and deterioration. That way, applying micronutrients and biostimulants to soybean to increase physiological quality may not be efficient because toxic residues of glyphosate from desiccation are accumulated in seeds. In this case, it is extremely important to consider that negative effects may still be found if those residues remain after harvest. Once degradation and further effects of AMPA, accumulated from glyphosate metabolization, are still not clear (REDDY; RIMANDO; DUKE, 2004), it is possible that metabolites may be degraded during storage, as a result of deterioration processes.

As previously detailed, the application of Co + Mo improved germination of soybean seeds produced by desiccated plants. However, seed germination was not equal to percentage values from the control due to a high number of abnormal seedlings. Seed treatment with micronutrients and biostimulant was not efficient in overcoming phytotoxic effects of glyphosate-desiccation on seedling growth. In truth, the application of those substances decreased seedling length and dry matter compared to the control.

4 Conclusions

Negative effects of pre-harvest desiccation with glyphosate on germination of soybean seeds can be minimized by the application of micronutrients Co+Mo. Also, the application of

biostimulant to soybean seeds improves seedling development compared with micronutrient treatments, although it does not minimize detrimental effects of pre-harvest desiccation with glyphosate.

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