Effects of glyphosate-resistant gene and herbicides on biological nitrogen fixation symbiotic efficiency and grain productivity of soybean in Brazil.

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ABSTRACT
We reported the effects of transgenic glyphosate resistant soybean cultivars (GRC), glyphosate and weed management strategies (glyphosate + GRC vs. conventional herbicides + non-GRC) on biological nitrogen fixation (BNF), symbiotic efficiency (SyEf) and grain yield (SGY) in soybean. Six variables related to BNF were pooled and analyzed as SyEf. Data were obtained in field trials in six sites in Brazil and three growing seasons. Small effects of GRC and glyphosate where observed on SyEf in some sites. SyEf did not differ between the two weed management strategies. SGY was higher in the glyphosate + GRC management in three out of the six sites.

INTRODUCTION
Glyphosate-resistant soybean cultivars (GRC) were released in Brazil in 2003 and, in 2010, have already accounted for 86% of the cultivated area in the country. In 2010, it was estimated that the inoculation of soybean with N_2-fixing bacteria led to savings of US$ 7 billion/year due to the non-use of N fertilizers. The widespread use of glyphosate and GRC has raised concerns whether the biological N fixation (BNF) would be affected. Results on the effects of glyphosate or GRC on BNF are inconsistent (Bohm *et al*., 2009; Kremer and Means, 2009; Zablotowicz and Reddy, 2004). When deleterious effects of glyphosate are observed on the BNF variables, they have not been accompanied by decreases in soybean grain yields (Bohm *et al*., 2009). In this work we reported the results of experiments carried out between 2003 and 2006 in six major producing areas of Brazil. Our objective was to evaluate the effects of GRC, glyphosate and weed management strategies on the BNF symbiotic efficiency and grain productivity in soybean.

MATERIAL AND METHODS
The experiments were set up under no-till in the 2003/2004; 2004/2005, and 2005/2006 growing seasons at six sites in Brazil: Passo Fundo (RS); Ponta Grossa (PR); Londrina (PR); Uberaba (MG); Planaltina (DF); and Luiz E. Magalhães (BA). Trials were conducted in a RBD, with 5 treatments x 3 cultivars, with 6 replicates. Treatments were 1) GRC + glyphosate; 2) GRC + conventional herbicides; 3) non-GRC + conventional herbicides; 4) GRC + hand weed control; 5) non-GRC + hand weed control. Three pairs of cultivars, each including the non-GRC and its nearly-isogenic GRC, were cropped in each site. Soybean was inoculated with *Bradyrhizobium elkanii* SEMIA 587 and *B. diazoefficiens* SEMIA 5080. At the V4 stage, plants were evaluated for nodule dry weight (NDW), and at R2, for NDW, shoot total N and N concentration, total N-ureide and percentage of N-ureide. SyEf was described by these six variables pooled and analyzed by multivariate techniques. SGY was determined at harvest. Means contrasts were used to evaluate the effects of transgenic trait, type of herbicide in GRC, and weed management strategies on SGY and SyEf. Multiresponse permutation procedures (MRPP, Mielke and Berry, 2000) were used to test for differences in SyEf between treatments.

RESULTS E DISCUSSION
Biological Nitrogen Fixation Symbiotic Efficiency (SyEf).
SyEf was affected by the type of cultivar (Contrast 1) in four out of six areas, and by the type of herbicide (Contrast 2) only in Passo Fundo (Table 1). In all these cases, effects were very small, as observed by the chance-corrected within-group agreements (*A*

values) (see footnotes in Table 1). SyEf did not differ between the two weed management strategies (Contrast 3, Table 1).

Table 1. Statistical significance (p) and effect size (A value)[1] of means contrasts[2] comparing transgenic GR trait, type of herbicide and weed management strategy on BNF symbiotic efficiency in six areas in Brazil.

	p values (*A* values)		
Site	Contrast 1	Contrast 2	Contrast 3
Passo Fundo	0.040 (<0.01)	0.005 (0.023)	0.069 (<0.01)
Ponta Grossa	0.100 (<0.01)	0.251 (<0.01)	0.418 (<0.01)
Londrina	0.003 (0.011)	0.073 (<0.01)	0.611(<0.01)
Uberaba	0.716 (<0.01)	0.470 (<0.01)	0.473 (<0.01)
Planaltina	0.009 (0.013)	0.484 (<0.01)	0.408 (<0.01)
L. E. Magalhães	0.047 (<0.01)	0.179 (<0.01)	0.554 (<0.01)

[1] *A* value represents the chance-corrected within-group agreement. When *A* equals zero, in a scale from 0 to 1, the within-group heterogeneity of the samples equals that observed by chance.
[2] Contrast 1 compares GR and non-GR cultivars; Contrast 2 compares glyphosate and conventional herbicide; Contrast 3 compares GR-cultivars + glyphosate and non-GR cultivars + conventional herbicide.

Grain Yield.

GRC reduced SGY only at Passo Fundo (-21 %). In GRC, the use of glyphosate resulted in SGY increases (+ 8 to 33%) in four out of the six areas evaluated. SGY was higher in the glyphosate + GRC-based weed management in three of the six regions (+10 to 12%) (Table 2).

Table 2. Soybean grain yield as affected by gliphosate resistant cultivar (GRC) (Contrast 1, C1), herbicide type (Contrast 2, C2) and weed management strategy (Contrast 3, C3) in six major producing areas in Brazil

		Passo Fundo	Ponta Grossa	Londrina	Uberaba	Planaltina	Luis E. Magalhães
C1	GRC	859	2545	2230	2957	3378	2550
	Non-GRC	1094	2512	2249	3023	3442	2572
	p	<0.01	ns	ns	ns	ns	ns
C2	GRC with glyphosate	1089	2747	2389	2939	3572	2766
	GRC with conventional herbicide	815	2474	2200	2931	3405	2448
	p	<0.01	<0.05	<0.05	ns	ns	<0.05
C3	GRC + glyphosate	1089	2747	2389	2939	3572	2766
	Non-GRC + conventional herbicide	1067	2466	2178	2984	3387	2462
	p	ns	<0.05	<0.01	ns	ns	<0.05

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