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Short communication

Glyphosate- and imazethapyr-induced effects on yield, nodule mass and biological nitrogen fixation in field-grown glyphosate-resistant soybean

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ABSTRACT

Over half of the 21 Mha of soybean planted in Brazil is now transgenic glyphosate-resistant (GM_{RR}). A field experiment was carried out to investigate whether the application of glyphosate or imazethapyr to the GM_{RR} variety reduced the input of N_2 fixation (BNF). No effects on yield, total N accumulation, nodulation and BNF ($\delta^{15}N$) could be assigned to the genetic modification of the plant. Imazethapyr reduced soybean yield but had no significant effect on BNF. Even though yields were not affected by glyphosate, the significant reduction of nodule mass and BNF to the GM_{RR} suggests that the use of this herbicide could lead to an increased dependence on soil N and consequently an eventual decrease of SOM reserves.

Transgenic soybean resistant to the herbicide glyphosate (GM_{RR}) already occupies over 10 Mha in Brazil (as of 2007) and it is still in doubt whether the great dependence of the crop on BNF is affected. Malty et al. (2006) reported that glyphosate reduced growth of the *Bradyrhizobium japonicum* in culture medium whilst other studies pointed out to negative effects on nodulation and leghaemoglobin content, as well as on nitrogenase activity and N content in the plants (Reddy et al., 2000; King et al., 2001; Reddy and Zablotowicz, 2003). However, the results of Zablotowicz and Reddy (2007) showed little sign of inhibition of nodulation or nitrogenase activity by glyphosate application, even when the plants were treated with twice the recommended rate of herbicide on two occasions during growth. The objective of this study was to compare the BNF contribution to GM_{RR} crops and the respective non-GM parent variety and the effect of the addition of glyphosate to the former. An experimental treatment structure based upon these varieties and herbicides (Table 1) was established in a field experiment. Imazethapyr was chosen as an alternative herbicide as both the GM_{RR} and the "parent" non-GM soybean variety are known to be tolerant to this herbicide.

The experiment was installed at the Federal University of Pelotas, RS (27° 57′ 21″ S, 51° 48′ 32″ W, altitude 643 m) on a Solodic Planosol (FAO classification), with 16% clay. Plot size was 4 × 5 m and each treatment was replicated four times in a completely randomised design. The experiment was planted on December 2006 with seeds inoculated with a commercial peat-based inoculant (>10^9 viable cells g^{-1}) of *Bradyrhizobium japonicum* (SEMIA 5079) and *B. elkanii* (SEMIA 587) at a rate of 250 g inoculant per 20 kg seeds. N-free fertilizer was applied at a rate of 22 kg P ha^{-1} and 42 kg K ha^{-1}.

At the R6 stage of growth, ten 90 DAP plants were harvested from each plot for evaluation of the number and dry weight of the nodules, and the dry matter, N content and ^{15}N natural abundance of the shoot tissue. Samples of the aerial tissue of spontaneous weeds taken from the plots not treated with herbicide were also harvested on the same day from each plot to serve as reference plants for the use of the ^{15}N natural abundance technique to quantify the BNF contribution to the soybean plants. All samples were analysed for total N and ^{15}N natural abundance as described by Ramos et al. (2001). At physiological maturity (150 DAP) the grain was harvested from an area of 4 m^2 per plot to evaluate the grain yield of each treatment. In order to evaluate the possible effect of glyphosate application on soil organic matter (SOM) mineralization, four soil samples of 100 g were taken from each

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Table 1
The treatment structure adopted to examine the effect of two herbicides on transgenic glyphosate-resistant and non-transgenic soybeans.

Treatment	Soybean variety	Herbicide applied	Addition rate (g active ingredient ha^{-1})	Time of application (days after planting)
T1	GM_{RR} BRS 244RR	None[a]		
T2	BRS 154 (non-transgenic)	None		
T3	GM_{RR} BRS 244RR	Glyphosate	960	28
T4	GM_{RR} BRS 244RR	Glyphosate	960	28 and 56
T5	GM_{RR} BRS 244RR	Imazethapyr	100	28
T6	BRS 154 RR	Imazethapyr	100	28

[a] No herbicide addition implies that plots were hand weeded.

plot at 90 DAP of the treatments T1, T3, T4 and T5, to quantify CO_2 evolution as described by Anderson and Domsch (1990). The proportion of N in the soybean plants derived from BNF (%Ndfa) was calculated using the equation of Shearer and Kohl (1986) adapted for this application, using the value of −1.3‰ (Bergersen et al., 1988) as the '*B*' value – the ^{15}N abundance of soybean plants grown entirely on N_2 fixation. Statistical comparisons of all parameters (dry matter, N content, %Ndfa) were performed using the conventional ANOVA procedure and the Tukey's honestly significant difference test for means comparisons.

The treatments showing the highest grain yield were the glyphosate treated GM_{RR} soybean, being significantly ($P < 0.05$) higher than the treatments with imazethapyr (Table 2). There was a similar trend for shoot dry matter and N accumulation (Table 3) although not statistically significant. The highest grain yields were for those treatments (GM_{RR} soybean treated with glyphosate) for which nodule weight and BNF parameters were the lowest (Table 3). This indicates that while the application of glyphosate inhibited nodule development and BNF, it stimulated the plants to accumulate soil N. In contrast to glyphosate, imazethapyr reduced soybean yield of both varieties but had no significant effect on BNF (Table 3).

The total CO_2 evolution from the soil was found to be significantly ($P < 0.05$) higher in the samples taken from plots treated with herbicide, 22.1, 18.0 and 21.8 mg CO_2-C 100 g $soil^{-1}$, for the treatments with one or two applications of glyphosate and one application of imazethapyr, respectively, than for the soil where herbicide was not applied (14.4 CO_2-C 100 g $soil^{-1}$).

Yields of soybean crops have been reported to be unaffected by glyphosate application (e.g. Delannay et al., 1995; Elmore et al., 2001; Norsworthy, 2004). Even though our results suggest the same, the herbicide may inhibit nodulation and BNF. Thus, the N accumulation by the crop was maintained by increased uptake of N from the soil. The greatest SOM decomposition (CO_2 data) from herbicide treated soil supports this hypothesis.

Maintaining SOM and N reserves is important for the long-term sustainability of the cropping system. Alves et al. (2003) showed that under zero tillage (which now dominates soybean production in Brazil) the high export of N in soybean grain (approximately 80% of total shoot N) was approximately compensated for by the high proportion of N derived from BNF. The possibility that the proportion of N derived from BNF by GM_{RR} soybean is in general lower than that for the non-GM varieties would mean that the N

Table 2
Shoot dry matter, nodule number and mass, and grain yield of genetically modified (cv. GM_{RR}, BRS 244RR) and non-GM parental variety (cv. BRS 154) soybean treated with the herbicides glyphosate, imazetapyr or hand weeded. Summer 2005/2006.

Treatments	Shoot dry matter ($g\,m^{-2}$)	Nodules (no./plant)	Nodule dry weight (g/plant)	Grain yield ($g\,m^{-2}$)
T1-244RR hand weeded	842	31.8	0.447^{ab}	207^{bc}
T2-154 hand weeded	1038	37.3	0.399^{abc}	222^{abc}
T3-244RR 1 × glyphosate	1143	17.5	0.229^{c}	242^{a}
T4-244RR 2 × glyphosate	1239	33.8	0.293^{bc}	225^{ab}
T5-244RR 1 × imazethapyr	777	43.0	0.413^{ab}	177^{d}
T6-154 1 × imazethapyr	919	43.5	0.525^{a}	200^{cd}
Mean	993	34.5	0.385	212
Coefficient of variation (%)	29.2	42.4	31.4	7.5

Means in the same column followed by the same letter are not significantly different at $P < 0.05$ (Tukey's honestly significant difference test).

Table 3
Total N accumulation, ^{15}N natural abundance and estimates of N derived from N_2 fixation of genetically modified (cv. GM_{RR}, BRS 244RR) and non-GM parental variety (cv. BRS 154) soybean treated with the herbicides glyphosate, imazetapyr or hand weeded. Summer 2005/2006.

	Shoot total N accumulation (g/m^2)	Shoot ^{15}N abundance (‰)	Estimated %Ndfa	Total N derived from N_2 fixation (g/m^2)	Total N derived from soil (g/m^2)
T1-244RR hand weeded	17.2	0.02^{c}	80.2^{a}	13.8	3.4^{b}
T2-154 hand weeded	24.8	0.48^{bc}	73.3^{ab}	18.2	6.6^{ab}
T3-244RR 1 × glyphosate	26.8	1.61^{a}	56.5^{c}	15.0	11.8^{a}
T4-244RR 2 × glyphosate	30.5	1.01^{b}	65.5^{bc}	20.1	$10.4^{a,b}$
T5-244RR 1 × imazethapyr	20.8	$0.48^{b,c}$	73.3^{ab}	15.5	5.3^{ab}
T6-154 1 × imazethapyr	26.3	$0.53^{b,c}$	72.6^{ab}	19.1	7.1^{ab}
Bidens pilosa	nd	5.49	–	–	–
Echinochloa spp.	nd	5.36	–	–	–
Mean	24.4	0.69	70.2	16.9	7.5
CV (%)	33.0	56.6	8.3	33.4	42.1

Means in the same column followed by the same letter are not significantly different at $P < 0.05$ (Tukey's honestly significant difference test).
nd, not determined.

balance for this crop would become negative. This would result in the loss of SOM over time, increasing CO_2 emissions, and prejudicing the system sustainability.

It is apparent from this study and others that glyphosate application on GM_{RR} soybean does not usually reduce grain yield, but still leaves open the question of whether this high protein crop is obtaining virtually all of its N from symbiotic N_2 fixation, or these GM varieties are stimulating the mining of soil N and SOM reserves.

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