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Effects of the glyphosate-resistance gene and herbicides on soybean: Field trials monitoring biological nitrogen fixation and yield

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ABSTRACT

The commercial use of glyphosate-resistant (also known as Roundup Ready®, GR or RR) soybean was initiated in 1996 in the United States. This genetically engineered crop now occupies 75.4 million ha worldwide, 20.6 million of which are in Brazil where it occupies 86% of the total area cropped with the legume. Biological nitrogen fixation (BNF) is critical for economic sustainability of soybean in Brazil; therefore, to investigate the effects of the RR gene—using pairs of nearly isogenic cultivars—and herbicides on BNF, we conducted an extensive series of field experiments for three cropping seasons. The experiments were set up at six sites with five treatments, three pairs of nearly isogenic cultivars, and six replicates. The treatments consisted of: (T1) RR soybean + glyphosate; (T2) RR soybean + conventional herbicides; (T3) conventional parental soybean + conventional herbicides; (T4) RR soybean + hand weed control; and (T5) conventional parental soybean + hand weed control. Parameters of nodulation, plant biomass production, total N and ureide-N were evaluated at the V4 and R2 stages of growth, and grain yield and total N in grains were evaluated at crop maturity. Data were analyzed by ANOVA, analyses of contrasts, and multivariate analyses considering a pool of six variables, denominated as symbiotic efficiency (SyEf). The comparison of the pairs of non-transgenic and RR soybean cultivars showed that the transgenic trait negatively affected some BNF variables, but over a three-year period these effects had no significant impact on soybean grain yield. No consistent differences between glyphosate and conventional herbicide application were observed on BNF-associated parameters. When compared to conventional soybean and conventional herbicides, weed-management strategy with RR soybean and glyphosate did not affect symbiotic efficiency. In addition, at three sites, grain yields increased in the treatments with glyphosate and RR soybean over the three cropping seasons. The results from the multivariate analyses indicate that BNF and yield parameters were more affected by location, cropping season and cultivar than by the transgene, herbicides, or weed-management strategy. Despite the lack of effects of the transgene on yield in the three-year period, longer-term effects on BNF and N accumulation should be monitored.

1. Introduction

The commercial use of glyphosate-resistant soybean [*Glycine max* (L.) Merr.], also called Roundup Ready®—RR started in 1996 in the United States; and today this crop occupies about 75.4 million ha worldwide (ISAAA, 2012). The release of commercial RR soybean cultivars opened new opportunities for weed control by enabling: (i) reduction and replacement of pre-emergence herbicides; (ii) early seeding and no-tillage cropping; and (iii) less crop injury (Carpenter and Gianessi, 2001). In Brazil, RR soybean was released in 2003 and rapidly adopted by farmers. In the 2010/2011 growing season it was grown on 20.6 million ha, representing 86%

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of the total area cultivated with this crop in Brazil (ISAAA, 2012); in other major producing countries, such as the United States and Argentina, the area planted with RR soybean is close to 100%.

Glyphosate [N-(phosphomethyl) glycine, marketed as Roundup® by Monsanto Company, St. Louis, MO] is a broad-spectrum (controls both broad-leaf and grass weeds), non-selective herbicide, with a short mean active life in the soil environment (Franz et al., 1997). Its mechanism of action is based on the inhibition of the synthesis of aromatic amino acids (phenylalanine, tyrosine and tryptophan), by blocking the shikimic acid pathway present in plants and microorganisms, but absent from animals; hence, it shows low mammalian toxicity (Jaworski, 1972; Fisher et al., 1986). The basis of resistance to glyphosate in soybean is the insertion of a 5-enolpiruvylshikimic acid-3-phosphate synthase (EPSPS) gene derived from *Agrobacterium* strain CP4, which is insensitive to this herbicide, allowing the functional expression of the shikimic acid pathway in its presence (Padgette et al., 1995; Zablotowicz and Reddy, 2004).

The release of genetically engineered soybean cultivars has raised the question of whether the symbiotic nitrogen fixation process would be affected or not. The use of herbicides may affect biological nitrogen fixation (BNF) directly through effects on the bacteria (Jaworski, 1972), indirectly through effects on the legume host (Johnson, 1971), and also in the symbiosis (Gonzalez et al., 1996). Therefore, it is important that the biotechnological advances that have enabled the generation of genetically modified soybeans are also accompanied by rigorous studies of environmental safety. This is necessary not only to ensure the occurrence of minimal impacts on the environment, but also that gains already obtained from research, such as the contribution of high rates of nitrogen fixation, are not negatively affected. The importance of BNF in Brazil is highlighted when we consider that, in 2013, it was estimated that the application of inoculants containing N_2-fixing bacteria, to the 27 million ha cropped with soybean, produces an annual savings of approximately US$ 10 billion due to the non-use of nitrogen fertilizers, without taking into account the benefits on the environment (Hungria et al., 2013).

Adverse effects of glyphosate on *Bradyrhizobium japonicum* have been related to the accumulation of shikimic acid and hydroxibenzoic acids, causing growth inhibition, and sensitivity varies among strains (Moorman et al., 1992; Hernandez et al., 1999; Drouin et al., 2010). At high concentration of glyphosate *Bradyrhizobium* death can occur (Fisher et al., 1986). In evaluations of the symbiosis soybean-*Bradyrhizobium*, although some deleterious effects of glyphosate on the nodulation and/or N_2 fixation processes have been reported (Reddy et al., 2000; King et al., 2001; Reddy and Zablotowicz, 2003; Zablotowicz and Reddy, 2004; Dvoranen et al., 2008; Bohm et al., 2009; Kremer and Means, 2009), grain-yield reductions have not been demonstrated. The magnitude of the effects on the symbiosis, including nodulation and plant-related parameters vary with glyphosate dose, salt, time of application, soybean cultivar, geographical location and environmental conditions, and are accentuated under water stress and in sandy soils (Zablotowicz and Reddy, 2004).

It is possible that, in the long term, the reductions of nodule mass and BNF rates by RR soybeans can lead to increased scavenging of mineral N. The uptake of more N from the soil will result in depletion of organic matter reserves and lower soil fertility. The situation is especially critical in sandy soils with limited N availability (Zablotowicz and Reddy, 2004; Bohm et al., 2009).

Considering the potential for reduced BNF in the RR soybean under field conditions, in this work we report the results of an extensive series of field experiments carried out between 2003 and 2006 in the most prominent soybean-producing regions of Brazil. Our objective was to evaluate the effects of the RR transgene, glyphosate and weed-management strategy on BNF and yield parameters, by using pairs of nearly isogenic soybean cultivars.

2. Materials and methods

2.1. Geographic location, general description of the field sites, treatments and logistics

The experiments were set up in the 2003/2004, 2004/2005 and 2005/2006 growing seasons at six sites in Brazil: Passo Fundo (Rio Grande do Sul State); Ponta Grossa (Paraná State; except in 2003/2004); Londrina (Paraná State); Uberaba (Minas Gerais State); Planaltina (Federal District); and Luiz Eduardo Magalhães (Bahia State). Details on location, climate and soil classification are given in Table 1. At each site soil samples were collected before the establishment of the experiment at a depth of 0–20 cm in five different points to form a composite sample. At each point 10 sub-samples were taken. These sub-samples were homogenized in large plastic bags, transported to the laboratory and sieved through a 4-mm sieve. Soil analyses were performed as described before (Hungria et al., 2006b) and the results are shown in Table 1. Table 1 also shows data on soil organic carbon (SOC), which under tropical conditions gives a good reference in terms of the soil capacity to supply N.

In each region, the trials were conducted in a completely randomized block design, with 5 treatments × 3 pairs of cultivars, with 6 replicates, in a total of 90 plots. The five treatments consisted of: (T1) RR soybean + glyphosate; (T2) RR soybean + conventional herbicides; (T3) conventional parental soybean + conventional herbicides; (T4) RR soybean + hand weeding; (T5) conventional parental soybean + hand weeding, as the control.

For T1, Roundup Transorb® (glyphosate, Monsanto), was applied at a dose of 2 L ha^{-1} in 200 L of water, 20–30 days after emergence, whereas 0.5 L ha^{-1} of Select® 240 (Clethodim®, Milenia, Londrina), mixed with mineral oil at 0.5% of the volume (to kill narrow-leaf weeds), and Classic® (chlorimuron-ethyl, DuPont, Wilmington, DE) at 80 g ha^{-1} (to kill broad-leaf weeds) were used for T2 and T3. Three pairs of cultivars, each consisting of the parental non-transgenic cultivar (from Embrapa Soja's breeding program) and its respective nearly transgenic counterpart (from a Embrapa Soja-Monsanto partnership) were cropped at each site, in a total of 15 treatments per site. In the three growing seasons, the pairs of soybean cultivars tested in Ponta Grossa and Londrina were Conquista/ValiosaRR (Cultivar 1 = C1); BRS133/BRS245RR (Cultivar 2 = C2); and Embrapa 59/BRS244RR (Cultivar 3 = C3). These same cutivars were tested in Passo Fundo, except that in 2004/2005 and 2005/2006, when Conquista/ValiosaRR were replaced by Embrapa58/BRS242RR. In 2003/2004, the soybean cultivars tested in areas of the central region (Uberaba, Planaltina, Luiz Eduardo Magalhães) were Conquista/ValiosaRR (Cultivar 1 = C1); BRS133/BRS245RR (Cultivar 2 = C2); and Jataí/SilvâniaRR (Cultivar 3 = C3). In 2004/2005 and 2005/2006, the same cultivars were tested, except for BRS133/BRS245RR, which were replaced by Celeste/BalizaRR. Genealogy and maturity groups of parental conventional types were as follows: Conquista MG/BR 46 (Lo76-4484 × Numbaíra, G.8.1); BRS 133 (FT Abyara × BR 83-146, G.7.3); Embrapa 59 (FT Abyara × BR83-147, G.7.3); Embrapa 58 (Paraná × BR83-143, G.7.4); BRS/GO Jataí [Embrapa 313 (Anhanguera) × BR92-31910 (Cristalina CARDF-30*3 × FT Estrela), G.8.9]; BRS Celeste (Bossier × BR 1T, G.8.1); they all have determinate type of growth. The replacements were made when cultivars showed relatively poor performance, being replaced by better adapted, newly released genotypes.

All areas were managed under a no-tillage system and cropped with soybean in the summer. In the winter, the areas were sown