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Effects of the glyphosate-resistance gene and of herbicides applied to the soybean crop on soil microbial biomass and enzymes

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ABSTRACT

Glyphosate, a broad-spectrum herbicide used for the non-selective control of weeds, inhibits 5-enolpyruvylshikimate-3-phosphate synthase, a key enzyme in the synthesis of aromatic amino acids in the shikimic acid pathway in plants, fungi and bacteria, thus impairing the synthesis of proteins required for various life processes. Soybean genetically engineered to be glyphosate resistant (GR or Roundup Ready, RR) represents the most cultivated transgenic crop globally, including Brazil. There are concerns about the effects of RR transgenic soybean and of glyphosate on soil microbial communities and their functioning. Our study was designed to detect changes in soil microbial biomass-carbon (MB-C) and -nitrogen (MB-N) and in enzyme activities [beta-glucosidase (GLU) and acid phosphatase (PHO)] in a large set of field trials performed at six sites in Brazil for two cropping seasons. We evaluated the effects of the RR transgene, glyphosate and weed management (RR soybean + glyphosate vs. conventional soybean + conventional herbicides), with three pairs of nearly isogenic soybean cultivars evaluated per site. Soils were sampled from the 0–10 cm layer, between cropped lines, during the cropping seasons 2004/2005 and 2005/2006, at the R2 stage of soybean growth. Univariate and contrast analyses were performed in addition to multivariate analyses including all four microbial variables, and denominated as soil microbial variables (SMV). In general, microbial parameters and SMV were not affected by the transgene, type of herbicide or weed management. Differences were, rather, related to site, cropping season and cultivar.

1. Introduction

Glyphosate-resistant (GR or Roundup Ready®, RR) soybean [*Glycine max* (L.) Merr.] represents the most-cultivated transgenic crop. In Brazil, cultivation of RR genotypes continues to increase, mainly due to broad adoption of glyphosate for weed control (Pereira et al., 2008; Zobiole et al., 2010a,b,c); in the 2010/2011 growing season, RR genotypes occupied 86% of the area cultivated to soybean globally (ISAAA, 2012). In response to the widespread use of RR soybean, the quantity and frequency of glyphosate applications have escalated worldwide in recent years (Gomez et al., 2009), raising concerns regarding potential environmental impacts (Weaver et al., 2007).

Glyphosate [N-(phosphomethyl) glycine; Roundup®, Monsanto, St. Louis, MO] is a broad-spectrum herbicide used for the non-selective control of weeds. It inhibits 5-enolpyruvylshikimate-3-phosphate synthase, a key enzyme in the synthesis of aromatic amino acids in the shikimic acid pathway in plants, fungi and bacteria (Gomez et al., 2009). Without these amino acids, organisms are unable to synthesize certain proteins essential for metabolism (Ware, 2000). Transgenic soybean produces a glyphosate-insensitive 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) (Reddy et al., 2001).

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Glyphosate is often described as a simple molecule, easily degraded, exhibiting little or no activity in soil due to its rapid adsorption on inorganic and organic particles, with a half-life ranging from days to months (Forlani et al., 1999; Jonge and Jonge, 1999; Duke and Powles, 2008). Many soil microorganisms can metabolize pesticides and use xenobiotics as sources of carbon, energy and nutrients (Durkin, 2003). The rate at which glyphosate is mineralized is related to biomass and activity of microorganisms, determining its persistence in the soil (Wiren-Lehr et al., 1997).

Even though the herbicide is not applied directly to the soil, a significant amount may reach its surface during broadcast preplanting or early-season applications, with harmful effects on soil microorganisms. The quantity of herbicide available to the soil microorganisms depends on factors such as soil nutrient and pH status, temperature, and moisture content (Weber et al., 1993).

Glyphosate consumption is increasing globally, due both to the expanded area planted to RR crops, and to higher amounts applied per unit area. The increased application of glyphosate has raised concerns about changes in soil-microbial communities, demanding monitoring of microbial populations and activities (Kremer et al., 2005; Johal and Huber, 2009). Microbial biomass is correlated with essential functions in the soil, such as the decomposition of organic matter, cycling of mineral nutrients, plant-growth promotion, biological control of diseases and insects and degradation of xenobiotics. It has been proposed as a sensitive parameter to measure disturbances caused to the soil environment, particularly in relation to those resulting from agricultural management (e.g. Sylvia et al., 2005; Nogueira et al., 2006; Kaschuk et al., 2010, 2011; Lopes et al., 2013), including the monitoring of effects related to transgenic crops (Souza et al., 2008a,b, 2013). Similarly, microbial enzymatic activities are considered to be responsive to changes in the environment caused by natural or human-induced factors (Puglisi et al., 2006; Kaschuk et al., 2010; Mendes et al., 2012). The β-glucosidase enzyme plays a critical role in soils, releasing low molecular weight sugars that represent important energy sources for soil microorganisms (Bandick and Dick, 1999). The acid phosphatase (predominant in acidic soils) can provide useful information on soil biochemical activity in the tropical Brazilian soils characterized by low pH values (Balota et al., 2013). Urease is also an important enzyme; however, the experimental areas of our study have not received N-fertilizers and the soils are very poor on N and thus the activity of this enzyme was not evaluated.

Contrasting results have been observed by various researchers evaluating the impact of glyphosate on soil microbiota. Studies by Haney et al. (2000, 2002) indicated that glyphosate application can increase soil-microbial biomass, respiration and C and N mineralization. Evaluations on Brazilian soils indicated that glyphosate was rapidly metabolized to aminophosphonic acid, and increased respiration and fluorescein diacetate (FDA) hydrolytic activity were observed (Araujo et al., 2003). Investigation conducted under controlled conditions in the Midwestern USA found limited or no effect of glyphosate or of cropping RR soybean on soil-microbial biomass (Liphadzi et al., 2005). Similar results were observed in Brazil by Zilli et al. (2008), who found that no significant changes occurred in microbial-biomass content, soil basal respiration or metabolic quotient ($q\mathrm{CO}_2$). Pereira et al. (2008), in a field experiment, also observed that glyphosate application had no impact on soil CO_2 production or on microbial biomass. The study of Gomez et al. (2009) showed that glyphosate application promoted an initial inhibitory effect that affected microbial biomass, microbial respiration rate, $q\mathrm{CO}_2$ and dehydrogenase activity; however, the effect was transitory. In contrast, Bohm et al. (2007) showed that the application of glyphosate resulted in lower C incorporation by microbial biomass, as well as increased respiration.

One concern regarding the use of transgenic plants and their effects on soil microorganisms is the so-called "unexpected effect". Donegan et al. (1995) suggested that unexpected changes in plant traits resulting from genetic modification may impact soil microbial communities. Novel proteins have been shown to be released from transgenic plants into the soil ecosystem, and their presence can influence microbial communities by selectively stimulating the growth of specific organisms able to use these molecules (Dunfield and Germida, 2004). However, as pointed out by Dunfield and Germida (2004), genetically engineered plants differ by only one or two genes, begging the question of whether the difference is enough to influence rhizospheric microorganisms.

The great majority of the studies assessing the effects of transgenic plants and specific herbicides application is limited to greenhouse conditions, using only the transgenic treatments without comparison against the parental non-transgenic plants. When the studies are carried out under field condition, few sites are evaluated and in general for only one cropping season. The present work is part of a large set of experiments performed in six field sites of Brazil, covering several edaphoclimatic conditions, for two cropping seasons, aiming to investigate the effects of transgene resistance to glyphosate, of herbicides and of weed management strategies on soybean crop. The RR gene was introduced in the parental genotypes, in an agreement between Embrapa and Monsanto®; therefore, the comparison between transgenic and nearly isogenic parental genotypes was possible, what is rare in studies with transgenic soybean, once the material usually belongs to the private sector. The results from our study were designed to detect changes in soil microbial biomass and enzyme activities, evaluated both individually and pooled as a multivariate response.

2. Materials and methods

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

The experiments were set up during the cropping seasons of 2003/2004, 2004/2005 and 2005/2006, under no-tillage management, at six sites in Brazil (States between parentheses): Passo Fundo (Rio Grande do Sul); Ponta Grossa (Paraná) (except in 2003/2004); Londrina (Paraná); Uberaba (Minas Gerais); Planaltina (Federal District); and Luiz Eduardo Magalhães (Bahia). Details about the location, climate and soil classification at each site were given elsewhere (Hungria et al., 2014), and the locations of the sites are shown in Fig. 1. Soil chemical and physical properties were described before (Hungria et al., 2014), but are also supplied in Supplementary Table S1. Evaluation of microbial parameters started from the second year after the establishment of the field experiments.

Supplementary Table S1 related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fcr.2014.03.010.

Each trial was conducted in a completely randomized block design, with 5 treatments × 3 cultivars, with 6 replicates, in a total of 90 plots. The five treatments consisted of: (T1) RR soybean + glyphosate (2 L ha^{-1}) [Roundup Transorb®, Monsanto, applied 20–30 days after emergence]; (T2) RR soybean + conventional herbicides [0.5 L ha^{-1} of Select 240 (Clethodim, Milenia), mixed with Assist mineral oil at 0.5% of the volume (narrow-leaf weeds), and Classic (Chlorimurum-ethyl, DuPont) at 80 g ha^{-1} (broad-leaf weeds)]; (T3) conventional soybean + conventional herbicides; (T4) RR soybean + hand-weed control; (T5) conventional soybean + hand-weed control.

Three pairs of cultivars, each of which included a parental soybean genotype and its respective nearly isogenic RR genotype, were cropped at each site. Soybean cultivars tested in Ponta Grossa and Londrina were Conquista/ValiosaRR (Cultivar 1 = C1); BRS133/BRS245RR (Cultivar 2 = C2); and Embrapa 59/BRS244RR