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Tolerance of *Bradyrhizobium* strains to glyphosate formulations

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Abstract

Application of glyphosate to soybean plants, resistant to this herbicide, may be harmful to *Bradyrhizobium*, the symbiotic bacterium. This study compared the effect of two glyphosate commercial formulations, ZappQi® and Roundup Transorb® with the standard *N*-(phosphonomethyl) glycine on the growth of four *Bradyrhizobium* strains under laboratory conditions. Commercial formulations were applied at a concentration of 43.2 $\mu g\,L^{-1}$ of a.e. and the strains were inoculated in yeast extract mannitol (YEM) broth used with or without a mixture of aromatics amino acids (phenylalanine, tyrosine, tryptophan). Herbicide effect on the growth of the *Bradyrhizobium* strains was assessed by optical density reading in a spectrophotometer. Roundup Transorb® had the biggest effects, probably, because of the presence of ethylamine as a surfactant. Pure glyphosate had minimal effects on *Bradyrhizobium* strains when aromatics amino acids have been added to YEM broth. Glyphosate application as Roundup Transorb® in the initial stage of soybean development may affect the nodulation process by *Bradyrhizobium*.

Keywords: Adjuvant; Aromatic amino acids; Rhizobium; Laboratory bioassay

1. Introduction

Recent biotechnological developments have resulted in the development of plants resistant to non-selective herbicides. Among the crops, soybean resistant to glyphosate stands out and has been cultivated on a large scale in several countries.

In Brazil soybean occupies more than 13.6 million hectare, representing the largest crop area in the country, with projections of about 54 million tonnes of beans for 2003/2004. This crop is the largest herbicide consumer, and almost 100% of the planted area in Brazil is treated with those products. The advantages of herbicide use are the high efficiency in weed control, the presence of selective products to soybean at the smallest cost, when compared to the other available weed control methods (Silva et al., 2003). In order to fully understand indirect effects related to the use of genetically modified soybean crop (transgenic soybean) in Brazilian agriculture, research work to examine possible problems generated from this technology are necessary.

Herbicide application can bring undesirable consequences to the soil micro-organisms. This depends on the active compound, commercial formulation, dose (Royuela et al., 1998), climatic conditions and soil type (Silva et al., 2003). In this respect, the search for herbicide molecules and commercial formulations less aggressive to the environment is a shared objective for all those who want to use the technology.

Glyphosate is a non-selective herbicide that inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase (Jaworski, 1972). However, introduction into

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Brazil of transgenic soybean may result in higher utilization of glyphosate in post-emergence cultivation of soybean. In spite of the satisfactory results in weed control, there are questions about glyphosate effects on the N_2 fixation process, since the Brazilian soybean crop is dependent on symbiosis with *Bradyrhizobium* (Bollich et al., 1988; Marenco et al., 1993; Zawoznik et al., 1995; Novo et al., 1996).

Research in other countries (Mallik and Tesfai, 1985; Schuls et al., 1985; Eberbach and Douglas, 1989; Moorman et al., 1992) showed that glyphosate had effects on rhizobia strains or it harmed nodulation in other legumes. According to González et al. (1996), the risk of herbicide toxicity to micro-organisms may be higher, since the products of metabolism can inhibit biochemical processes related to symbiosis between plants and micro-organisms.

The acute toxicity of glyphosate has been considered low. According to Monsanto the manufacturer of glyphosate the LD_{50} is 5600 mg kg^{-1} (Amarante Jr. et al., 2002). However, according to the World Health Organization (WHO, 1994), it is 4320 mg kg^{-1}. When glyphosate is injected on rats' abdomen, there is decrease in the activity of some enzymes. activity (Amarante Jr. et al., 2002). The product half-life in soil may surpass 40 days (Richardson and Gangolli, 1994), which is enough time to harm the nodulation process in soybeans.

Glyphosate inhibits the synthesis of EPSP synthase resulting in the inhibition of aromatic amino acid synthesis (phenylalanine, tyrosine and tryptophan). The lack of synthesis of these amino acids may be the reason for the toxic action of this herbicide on micro-organisms (Eberbach and Douglas, 1989). However, Moorman (1986) added aromatic amino acids to the culture medium and verified that growth of *Rhizobium japonicum* strains was not influenced by glyphosate. In contrast other work showed that the toxic effect was derived from the adjuvants present in the commercial herbicide compositions, including glyphosate, which affected or impeded the development of many soil micro-organisms (Katan and Eshel, 1973; Johal and Rahe, 1984; Sawada et al., 1988; Berner et al., 1991).

Some surfactants, a special class of adjuvants, may increase herbicide effects, by decreasing or eliminating the selectivity of some herbicide to crops by removing the wax layer (Kissmann, 1997). Furthermore, by the reduction of surface tension, the penetration is facilitated and the bacteria can become more sensitive to herbicide action (Malkones, 2000).

The objective of this work was to evaluate the effect of glyphosate, as pure material or in two commercial formulations, added to yeast extract broth (YEM), with or without aromatic amino acids, on the growth of four *Bradyrhizobium* strains.

2. Materials and methods

Four commercial *Bradyrhizobium* strains were examined: two *Bradyrhizobium elkanii* (SEMIA 5019 and SEMIA 587) and two of *B. japonicum* (SEMIA 5079 and SEMIA 5080). Each strain was inoculated in basal yeast extract and mannitol (YEM) broth with 43.2 μg L^{-1} of pure glyphosate either as the active ingredient glyphosate or as formulated products Zapp Qi® or Roundup Transorb®. The untreated control (without herbicide), was used either with or without 30 mg L^{-1} of a mixture of each aromatic amino acid, L-phenylalanine, L-tyrosine and L-tryptophan. The experimental design was a 4 × 2 factorial, with six replicates. Each strain was analyzed individually.

Rhizobia strains were activated in YEM broth, at 25 °C on a shaker with 150 rpm.The glyphosate solutions were diluted in distilled and de-ionized water and filtered using 0.45 μm pore filters (Millipore®). Microplates 96 cells with 300 μL capacity were used. In each cell 180 μL of YEM broth (1.33 × concentrated) were added, supplemented or not with amino acids at 30 mg L^{-1}, and 15 μL of active bacterial culture and 60 μL of herbicide stock-solution. The plates were incubated at 25 °C, in darkness. The bacterial growth was evaluated by optical density (OD) spectrophotometer at 560 nm, during 132 h. The OD values were transformed into colony forming units (CFU), using previously determined regression curves. Growth curves were determined for each strain under different treatment and growth data after 132 h were analyzed by analysis of variance. Means were compared using Tukey 0.05%, after ANOVA.

3. Results and discussion

Amino acids supplementation decreases rhizobial growth in the absence of the herbicide (Fig. 1 and Table 1). This can be attributed to an unbalanced YEM broth when the amino acids are supplemented, as observed by Moorman et al. (1992). When the medium was enriched with 0.45 mg L^{-1}, using one of the three aromatic amino acids, it reduced *B. japonicum* USDA 138 growth by 23%.

In the media with glyphosate, irrespective of the herbicide formulation, the growth of all strains tested was reduced, but presented some differential results over the incubation period (Fig. 1 and Table 1). In the medium containing Zapp Qi® a reduction of 50% was observed on average, of the number of total cells produced by SEMIA 5019, SEMIA 5080 and SEMIA 587 in the final period of incubation, compared to the control. The growth of these strains was not altered by the supplementation of aromatic amino acids (Table 1). However, for the strain SEMIA 5079, the