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Chemical Management of *Bidens pilosa* (L.) and *Euphorbia heterophylla* (L.) and Seed Germination in Genetically Modified Soybean Glyphosate Tolerant

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Abstract: The objective of this work is to propose a chemical management alternative to control *Bidens pilosa* and *Euphorbia heterophylla* in genetically modified soybean without affecting grain production and seed germination by using glyphosate applied only once and in mixture, aiming to reduce the number of applications and the amount of herbicides used. The experimental design was randomized blocks with 10 treatments and 4 replications. The soybean used was BRS-Valiosa RR. The treatments consisted of glyphosate and chlorimuron-ethyl applied once and alone (1.5; 2.0 and 80 L or g ha^{-1} pc), glyphosate in mixture with chlorimuron-ethyl (1.5+10 and 2.0+10 L or g ha^{-1} pc) and sequentially applied glyphosate (1.5/1.5; 2.0/1.5 and 2.0/1.5/1.5 L ha^{-1} pc), plus weeded (clean) and non-weeded (dirty) witnesses. The evaluations were: herbicide efficacy in *B. pilosa* and *E. heterophylla*, phytotoxicity, density, height and grain yield (kg ha^{-1}) in soybean. The production data obtained were subjected to analysis of variance and F test by the SANEST program ($P<0.05$). It was concluded that the studied species, *B. pilosa* and *E. heterophylla*, were controlled by glyphosate at levels above 90%. There was no visual injury in soybean and no significant decrease in production and germination.

Keywords: Transgenic Soybean, Biotechnology, Seeds, Herbicides

1. Introduction

For soybeans, the methods commonly used for weed control are mechanical, crop and chemical. However control is most effectively done by chemicals where the main advantages are in labor economy and speed of application. Chemical control obeys the principle that certain products are capable of killing plants and much more importantly that many of them can kill some types of plants without injuring others [15].

In conventional soybean, a preemergent product is generally applied and subsequently a selective action product in the postemergence of the crop. However this type of control may present some problems. Selective products, for example, do not control some broadleaved plants and some pre-emergent plants have a prolonged residual effect and may cause phytotoxicity in crops that are planted in succession; This drawback is even more pronounced in regions where safrinha maize is cultivated [20]. Another point to be highlighted is that the cost in this type of control is high because it is necessary to enter the field at least twice and the operation can cause greater mechanical damage to soybean plants and soil compaction.

An alternative to weed control would be the cultivation of genetically modified soybeans which would basically only involve the use of glyphosate herbicide, which is a non-selective product that controls large numbers of broadleaf and narrow-leaf plants by inhibiting EPSP synthetase an enzyme that participates in the metabolic pathway of aromatic amino acid biosynthesis (tryptophan, tyrosine and phenylalanine), which are essential for plant growth [13]. In this production system it is possible for genetically modified soybeans to develop even after glyphosate herbicide application, thus allowing chemical control to be performed when weeds reach levels of competition with soybeans, which may reduce the number and quantity of herbicides used, making production costs lower and avoiding other problems [13].

Therefore, due to the fact that, to date, there are few publications of research results related to genetically modified soybean cultivation in Brazil. This research aimed to propose an adequate weed management system in glyphosate tolerant soybean cultivars without affecting the yield production.

2. Material and Methods

The field trial was installed in an experimental area of Agrocosmos Agrícola S/A in the municipality of Engenheiro Coelho (SP), in the 2006/07 agricultural year, presenting as geographic coordinates 22°42'9" south latitude and 47°38'30" west longitude and 540 m altitude. The climate in the region is classified, according to Koeppen, as Cwa, characterized by a dry winter. The soil classified as Barrento, clayey lime subclass, pH 4.8 ($CaCl_2$); M. O.: 3.9%; P: 130 mg / dm^{-3}; K: 2.2; Ca: 29; Mg: 9; H+Al: 47; SB: 40.2; CTC: 87.2 mmolc/dm^{-3} and V: 46.1%.

The experimental design was randomized blocks with 10 treatments and 4 replications. The genetically modified soybean cultivar used was BRS-Valiosa RR, recommended for the state of São Paulo. Sowing was performed on 12/28/06 at a density of 14 to 18 linear plants per meter and spacing of 0.5 m between rows. The treatments consisted of glyphosate Roundup Ready® commercial product containing 480 g L^{-1} acid equivalent and chlorimuron-ethyl Classic® commercial product containing 80 g kg^{-1} active ingredient applied once and alone (1.5; 2,0 and 80 L or g ha^{-1} pc), glyphosate mixed with chlorimuron-ethyl (1.5+10 and 2.0+10 L or g ha^{-1} pc) and sequentially (1.5/1.5; 2.0/1.5 and 2.0/1.5/1.5 L ha^{-1} pc), in addition to weeded (clean) and non-weeded (bush) controls. For the treatment 80 g.ha^{-1} of chlorimuron-ethyl was added to 0.05% v/v mineral oil spray solution.

Prior to the application of the treatments, weed identification and counting surveys were made within the useful area of each plot using the 0.5 m^{-2} inventory method. Glyphosate and chlorimuron-ethyl herbicides were applied once and alone and glyphosate mixed with chlorimuron-ethyl were carried out on 01/19/07, 14 days after emergence (DAE) of soybean (01/05) at the phenological stage V2, according to the classification of Fehr et al. (1971). The applications glyphosate sequences were performed with a 15 days interval between the applications being the second and third, carried out on 02/02 and 17/02 and soybean in V5 and V8 phenological stages, respectively.

The herbicides were always applied in the morning and without wind to prevent drift, with the aid of a constant pressure (CO_2) costal sprayer, set to 300 L ha^{-1} spray volume and bar equipped with fan spray tips (110°-SF-05). The control was evaluated in *Bidens pilosa* (black prick) and *Euphorbia heterophylla* (dairy), manually sown within the useful area of each plot, with an average density of 223 and 34 individuals m^{-2} of *Bidens pilosa* and *Euphorbia heterophylla*, respectively.

The experimental plots consisted of 6 lines of 5.0 m in length, with 4 repetitions. The useful area was the 4 central lines with 4.0 m in length, with 0.5 m at each end of the plot.

a) Herbicide efficacy: percentage of species control within the useful area of each plot at 7, 14, 28 and 42 days after herbicide application, obtained by the initial counting of the number of weeds and remnants. by species; b) Phytotoxicity of herbicides in soybean: evaluated by visually assigned grades always comparin with control by the EWRC (1964) scale also at 7, 14, 28 and 42 days after herbicide application; c) Plant density and height: the number of plants per linear meter was determined by counting plants sampled in two 1.0 meter rows within the useful area of each plot and the height of 10 plants randomly sampled at 42 DAT; d) Production: determined by harvesting 4.0 m long central lines of soybean plants within the useful area of each plot, with moisture corrected to 13% (wet basis); e) Germination: Soybean seeds were harvested, tracked and for the germination test three subsamples of 50 seeds were collected in each treatment totaling twelve replications. The test was performed on a Germitest paper towel roll moistened with water equivalent to 2.5 times the weight of the dried substrate and placed in plastic containers. After this procedure the plastic containers were placed in a walking germination (WG) type germinator, which were kept in a chamber at a constant temperature of 25°C. Evaluations were performed on the fifth day after the test installation, according to the criteria of Brazil (1992) and the result expressed as a percentage of normal seedlings.

Density, plant height, production and germination data were subjected to analysis of variance and F test by the SANEST program. For the significant analyzes it was performed the comparison between means by Tukey test at 5% probability.

3. Results and Discussion

As can be seen in Figure 1, the percentage of control of the studied species was above 90% when compared to the control (Figure 11), thus being considered susceptible to treatments with chlorimuron-ethyl alone at a dose of 80 g ha^{-1} (see Figure 4) glyphosate applied once and alone at doses 1.5 and 2.0 L ha^{-1} pc, glyphosate mixed with chlorimuron-ethyl at doses of 1.5+10 and 2.0+10 L or g ha^{-1} from pc and glyphosate applied sequentially doses at 1.5/1.5; 2.0/1.5 and 2.0/1.5/1.5 L ha^{-1} of p. c. See Figures 2 to 9 compared to the Figures 10 and 11.

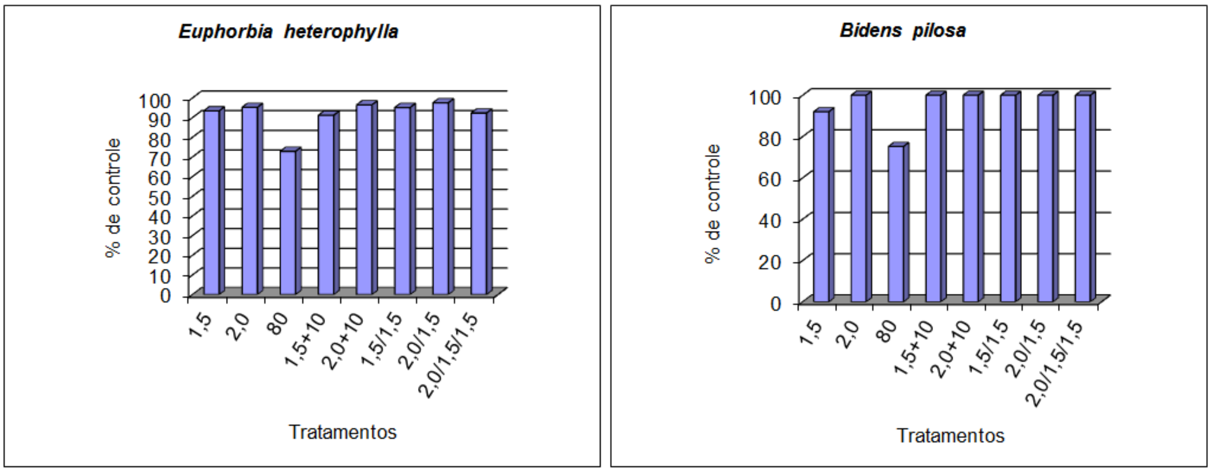

Figure 1. *Control percentage of Euphorbia heterophylla and Bidens pilosa as a function of glyphosate and chlorimuron-ethyl application at 7 DAT.*

Search performed were treatments was constituted of glyphosate applications in the following doses: 0; 720; 960; 1200; 1440; 1680 e 1920 g ha^{-1} i.a., in the species *Bidens pilosa* L., *Commelina benghalensis* L., *Digitaria insularis* L. (Fedde), *Ipomoea grandiofolia* L. and *Tridax procumbens* L. which were sowed and transplanted in vases of polyethylene, containing three plants per vase. It was concluded that the herbicide glyphosate controlled *B. pilosa* and *D. insularis* occurring control of 100% of these species in 14 DAA in the rate of 720 g ha^{-1} i.a. *C. benghalensis* was considered specie of difficult control. The necessary rate to reach control indexes above 91% was of 1680 g ha^{-1} i.a. for *C. benghalensis*, 960 g ha^{-1} i.a. in the species *T. procumbens* and 1440 g ha^{-1} i.a. of glyphosate for *I. grandifolia* in 21 DAA [14].

Figure 2. *Results of single application of glyphosate (1.5 L.ha^{-1}).*

Figure 3. *Results of single application of glyphosate (2.0 L ha^{-1}).*

Figure 4. *Results of chlorimuron-ethyl application (80 g ha^{-1}).*

Figure 5. *Results of glyphosate + chlorimuron-ethyl mixture (1.5+10 L or g ha^{-1}).*

Figure 6. *Results of glyphosate + chlorimuron-ethyl mixture (2.0+10 L or g ha^{-1}).*

Figure 7. *Results of sequential glyphosate application (1.5/1.5 L ha^{-1}).*

Figure 8. *Results of sequential glyphosate application (2.0/1.5 L.ha^{-1}).*

Figure 9. *Results of sequential glyphosate application (2.0/1.5/1.5 L.ha^{-1}).*

Figure 10. *Control (weeding).*

Figure 11. *Control (no weeding).*

Thus in conventionally cultivated soybean areas where there are failures of resistance control of *Euphorbia heterophylla* and *Bidens pilosa* biotypes to ALS inhibitor herbicides, found by researchers [6, 18, 19, 21, 23, 24, 25] the genetically modified soybean production system, allows glyphosate to be a new alternative chemical management by rotation herbicide with another mechanism of action (EPSPs).

In the treatment with chlorimuron-ethyl at a dose of 80 g ha^{-1} of p. c., the herbicide was not interesting because it did not control over 75% for weeds. Perhaps noting the difficulty of controlling the herbicide to these species according to research already cited [6, 18, 19, 21, 23, 24, 25].

Table 1 shows the variance analysis data and average values of density, plant height and soybean yield. Density is within the limits recommended by [8]. Herbicides did not significantly affect plant height and yield. The highest yield of treatments (1.956 kg ha^{-1}) was found to be the mixture of glyphosate with chlorimuron-ethyl (2.0+10) as this has a residual effect, giving conditions for the culture to close without the presence of consequently expressing their maximum yield potential by reducing weed competition and reinfestation in the critical period. Mixing glyphosate with chlorimuron-ethyl, ALS inhibitor, has also been shown to be effective in desiccating *Euphorbia heterophylla* and *Bidens pilosa* in a planting system soybean crop [4, 5, 26].

Table 1. *Analysis of variance data and average values of density, plant height and production[1].*

Treatments (Doses L ou g ha^{-1})	Density (pl/m)	Plant height (cm)	Production (kg ha^{-1})
1 - glyphosate (1.5)	18 a	76.8 a	1796 a
2 - glyphosate (2.0)	15 a	68.9 a	1893 a
3 - chlorimuron-ethyl (80)	17 a	72.5 a	1875 a
4 - glyphosate +chlorimuron-ethyl (1.5+10)	15 a	74.9 a	1814 a
5 – glyphosate +chlorimuron-ethyl (2.0+10)	18 a	71.0 a	1956 a
6 - glyphosate /glyphosate (1.5/1.5)	14 a	73.0 a	1846 a
7 - glyphosate /glyphosate (2.0/1.5)	14 a	69.0 a	1853 a
8 - glyphosate /glyphosate /glyphosate (2.0/1.5/1.5)	17 a	72.3 a	1924 a
9 - Control (weeding)	17 a	71.6 a	2175 a
10 – Control (no weeding)	16 a	69.4 a	760 b
Teste F	0.29543^{ns}	0.6371^{ns}	8.39 **
DMS Tukey (5%)	7	15.50	633.4
C. V. (%)	17.2	8.9	14.6

[1]Means followed by the same letter in the column do not differ statistically from each other by Tukey test at 5% probability.

Genetically modified soybean plants well supported the sequential glyphosate applications as demonstrated by the average yield of 1.924 kg ha^{-1} in the plots where 3 herbicide applications were made at doses of 2.0/1.5/1.5. L ha^{-1} (treatment 8). The lowest yield (760 kg ha^{-1}) was obtained in non-weeding control (dirty), demonstrating a relative loss of 72.3% of grain loss compared to weeded control (2.175 kg ha^{-1}) and from 57.7; 59.8; 59.4; 58.1; 61.1; 58.8; 58.9; 60.4% for treatments 1, 2, 3, 4, 5, 6, 7 and 8, respectively, proving the aggressiveness of *Bidens pilosa* and *Euphorbia heterophylla* in soybean yield losses and herbicide efficacy in this system agricultural production.

The obtained 74% in average of germination. As can be seen in Table 2, herbicides did not significantly influence germination, resulting in: 79.7; 79.5; 76.5; 74.5; 72.8; 72.5; 72.0; 70.5; 70.5% respectively for: control, chlorimuron ethyl (80 g ha^{-1}), sequentially applied glyphosate (2.0/1.5/1.5; 2.0/1.5 and 1.5/1.5 L ha^{-1} pc), glyphosate applied once and alone (1.5 and 2.0 g ha^{-1} pc), glyphosate mixed with chlorimuron-ethyl (1.5+10 and 2.0+10 L or g ha^{-1} pc), see Figures 12 to 20.

Table 2. *Herbicide treatments and germination average values.*

Treatments (Doses L ou g ha^{-1})	Germination (%)
1 - glyphosate (1.5)	72.5 a
2 - glyphosate (2.0)	72.0 a
3 - chlorimuron-ethyl (80)	79.5 a
4 - glyphosate +chlorimuron-ethyl (1.5+10)	70.5 a
5 - glyphosate +chlorimuron-ethyl (2.0+10)	70.5 a

Treatments (Doses L ou g ha^{-1})	Germination (%)
6 - glyphosate /glyphosate (1.5/1.5)	72.8 a
7 - glyphosate /glyphosate (2.0/1.5)	74.5 a
8 - glyphosate /glyphosate /glyphosate (2.0/1.5/1.5)	76.5 a
9 – Control	79.7 a
Teste F	12.1
DMS Duncan (5%)	0.0051
C. V. (%)	8.9

[1]Means followed by the same letter in the column do not differ statistically from each other by the Duncan Test at the 5% probability level.

However, genetically modified soybean seeds did not have sufficient germination for commercialization, as they were considered to be of low physiological quality, since the germination capacity of a seed lot under laboratory conditions must be higher than 80% to obtain a good field stand [16, 27, 29].

Figure 12. *Glyphosate (1.5 L.ha^{-1}).*

Figure 13. *Glyphosate (2.0 L.ha^{-1}).*

Figure 14. *Chlorimuron-ethyl (80 g. ha^{-1}).*

Figure 15. *Glyphosate +chlorimuron-ethyl (1.5+10 L ou g. ha^{-1}).*

Figure 16. *Glyphosate +chlorimuron-ethyl (2.0+10 L ou g.ha^{-1}).*

Figure 17. *Sequential glyphosate application (1.5/1.5 L.ha^{-1}).*

Figure 18. *Sequential glyphosate application (2.0/1.5 L.ha^{-1}).*

Figure 19. *Glyphosate (2.0/1.5/1.5 L.ha^{-1}).*

Figure 20. *Control.*

4. Conclusions

There were no visual injuries in soybean crop and no significant reduction in grain yield and seed germination due to the treatments tested. However germination was sufficient to be classified as seeds. All glyphosate treatments had control over 90% for *Bidens pilosa* and *Euphorbia heterophylla*. The single and sequential applications of glyphosate were the most effective, but the most cost-effective dose was 1.5/1.5 L ha^{-1}.

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