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Herbicide combinations to control the weed seedbank in an upland cotton field

Mezclas de herbicidas para controlar el banco de semillas de malezas en un cultivo de algodón

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Abstract. An experiment was conducted in dryland conditions of the Brazilian Northeast to determine the number of viable weed seeds (seedbank) in an upland cotton crop, and its distribution in the soil profile, before and after using various herbicide treatments. A randomized block design in a split-plot block scheme with 6 replications was used, where the main plots were constituted by a factorial (13 treatments and 2 sampling soil depths), and the subplots by 2 sampling dates. The seedbank was determined by germination of the recovered weed seeds obtained from different soil depths. The highest number of viable weed seeds in the area was found before the application of the herbicide treatments at 0 - 10 cm soil depth. The treatments metalachlor + diuron; diuron + pendimethalin and the control (no herbicide treatment, weeded weekly during the entire cotton crop cycle) were the most effective in reducing the weed seedbank in the area.

Keywords: *Gossypium hirsutum* L. r. *latifolium* H.; Agro-ecosystem; Seed viability.

Resumen. Se condujo un estudio en condiciones de secano en el nordeste brasileño a fin de determinar la distribución en el perfil del suelo del número de semillas viables de malezas (banco de semillas) en un área sembrada con algodón antes y después de aplicar varios tratamientos de herbicidas. Se utilizó un diseño factorial (parcelas principales: 13 tratamientos y 2 profundidades de muestreo en el suelo) en bloques al azar con parcelas divididas (subparcelas: tiempos de muestreo), y 6 repeticiones por tratamiento. El banco de semillas se determinó mediante la germinación, en bandejas de plástico, de las semillas de malezas que se encontraron en las muestras de suelo. El mayor número de semillas viables se encontró a 0-10 cm de profundidad en el suelo antes de la aplicación de los tratamientos de herbicidas. Los tratamientos metalachlor + diurón, diurón + pendimetalina y el control (sin herbicidas, con malezas eliminadas semanalmente durante todo el ciclo del cultivo de algodón) fueron los más eficaces en la reducción del banco de semillas de malezas en la zona.

Palabras clave: *Gossypium hirsutum* L. r. *latifolium* H.; Agroecosistema; Viabilidad de semillas.

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INTRODUCTION

The weed seedbank shows several survival features based on soil management, control over the years and the morphological and physiological characteristics of seeds (Buhler et al., 2001; Voll et al., 2001).

The characteristics related to weed seeds, such as high output, efficient dispersal, longevity and especially dormancy, produce large seedbanks in the soil, which ensures the regenerative potential of various species, even in the absence of seed production during long periods (Carmona, 1992).

Studies of samples of the soil seedbank or of the emerging weeds should allow identification and classification of the flora and subsequent characterization of the plant community (Voll et al., 1995). These primary studies can also be used in predicting the need for control, adjusting different soil, crop and rationale herbicide management to cost/benefit considerations in production. Surveys of this type are rare in Brazil, and have been restricted to the flora of established weeds in various regions and crop conditions (Carmona, 1995). The use of herbicides increases each year in cotton (*Gossypium hirsutum* L.) production, in Brazil and worldwide. This is due to a number of factors, such as a reduction in the population involved in agriculture, and increases in costs (Beltrão & Pereira, 2001). This use may also influence the species composition of the weed seedbank, and may increase it or decrease it, depending on the products used (Ball, 1992).

The use of herbicide combinations is known to be advantageous in relation to the application of a single compound (Marking, 1985, quoted by Tomital & Beyruth, 2002). The reported advantages include: (1) an increased efficiency against target organisms, (2) a better safety for non-target organisms, (3) a reduction of the quantities used without reducing the herbicide efficiencies, (4) smaller quantities of residues in the environment, and (5) reduced costs for the application of the materials. No more than three-herbicide combinations are used in cotton up to date in Brazil. Despite the considerable increase in data on the use of herbicides, there is still a lack of information on the use of herbicide combinations in upland cotton, mostly three-and four-herbicide combinations.

A field study was conducted to determine if information about the weed seedbank in the soil is essential (1) in predicting weed infestations, and (2) in the implementation of an integrated weed management program based on the rational use of herbicides in agro-ecosystems. The hypotheses tested were that a) the number of viable weed seeds vary at different soil depths, and b) herbicide treatments reduce the spatial and temporal infestation by weed seeds in the agricultural area on different ways. Thus, the study aimed to determine the number of viable seeds (seedbank) of weeds in an area planted with upland cotton, and its distribution in the soil profile, before and after control of these weeds by various herbicide treatments.

MATERIALS AND METHODS

The experiment was conducted under dryland conditions in Northeast Brazil (Barbalha, CE State: 7° 19' S, 39 °18› W, 405 m.a.s.l.). The area has a sandy soil with the following physico-chemical characteristics: pH = 6.6; organic matter = 2.8 g/kg; Ca^{+2} = 11.0 $mmol_c/dm^3$; Mg^{+2} = 4.0 $mmol_c/dm^3$; Na^+ = 0.3 $mmol_c/dm^3$; K^+ = 6.0 $mmol_c/dm^3$; Al^{+3} = 0.5 $mmol_c/dm^3$, and P = 2.9 mg/dm^3 (Laboratory of Soil Chemistry and Physics of Embrapa Cotton, Campina Grande, PB State, Brazil). Other soil physical characteristics at 0-10 and 10-20 soil depths include, respectively, Global density (1.48 and 1.46 g/cm^3); Particle density (2.69 and 2.66 g/cm^3); Coarse sand (630 and 600 g/kg); Fine sand (300 and 310 g/kg); Silt (50 and 40 g/kg); Clay (20 and 50 g/kg), and Natural clay (10 and 10 g/kg) (Laboratory of Soil Chemistry and Physics of Federal University of Ceará – UFC, Fortaleza, CE State, Brazil).

The study area has an annual precipitation of 1150 mm, a temperature range between 24 and 26 °C, and a mean relative humidity of 63.6%. The climate is Aw´/BSh according to the Köeppen classification (Ramos et al., 2009). Total rainfall in the period of the experiment (12 March to 19 June 2004) was 318.50 mm.

The herbicides diuron (Herburon 500 BR, 500 g a.i./L, SC, MILENIA), pendimethalin (Herbadox 500 CE, 500 g a.i./L, EC, BASF), oxadiazon (Ronstar SC 480, 480 g a.i./L, SC, BAYER) and metalachlor (Dual 960 CE, 960 g a.i./L, EC, SYNGENTA) were used in combinations of 2, 3 or 4 herbicides. The evaluation of the weed seedbank at 2 soil depths (0 - 10 and 10 - 20 cm) was carried out at 2 sampling dates: before and 70 days after application of the herbicide treatments listed in Table 2.

The experimental design was a randomized block using a split-plot block scheme with six replications, where the main plots consisted of the combinations of the factor levels (13 treatments and 2 sampling depths), and the subplots, of the 2 sampling dates.

Soil tillage, done on 8 March 2004, consisted of 2 light disking. These were followed by liming (0.5 + 0.5 t of lime/ha) to correct the soil pH. The experimental area (1216 m^2) consisted of 78 experimental units, each measuring 5.0 m x 4.0 m (20 m^2). The planting of cotton, and the application of herbicide treatments, occurred on 12 March 2004. The upland cotton cultivar used was BRS 187, and it was sown at a spacing of 1.0 m between rows with 10 plants/m. Seeds were delinted and chemically treated with the insecticide Carbofuran (Furadan 350 SC, 350 g a.i./L, SC, FMC) and the fungicide Pencycuron (Trotis 25 SC, 250 g a.i./L, SC, BAYER) before planting. The soil also received NPK fertilization in the form of ammonium sulfate (150 kg/ha), superphosphate (150 kg/ha) and potassium chloride (33 kg/ha), and organic fertilization with sugarcane (*Saccharum officinarum* L.) filter cake (6 t/ ha). Pests were satisfactorily controlled.

The water used in the preparation of test herbicides was at a pH of 7.2. Treatments were all applied at pre-emergence in relation to crop and weeds, using a well-calibrated Jacto knapsack sprayer, with a 20 L-capacity, equipped with a Teejet 8004 nozzle, mesh 50, with a spray volume of approximately 300 L/ha. One of the no herbicide treatments (Treatment 12) was weeded weekly during the entire cotton crop cycle.

For each evaluation/sampling of the weed seedbank in the area (before and 70 days after application of the herbicide treatments), and at each soil sampling depth (0 - 10 and 10 - 20 cm), 4 soil subsamples of 250 g each were collected at random within each plot, using a 2.5 cm-diameter tubular auger.

Soil samples, properly identified by sampling dates, treatments and sampling depths were placed in plastic bags and subjected to a screening process using a sieve 0.5 mm mesh. This was followed by flotation of the soil samples to facilitate the separation and counting of the weed seeds present in them.

In the flotation process, a saturated solution of $CaCl_2.2H_2O$ (calcium chloride, dehydrated, 75% pure) (Johnston et al., 1978) was used, which had a density of 1.40 to 1.42 g/cm^3. This density was higher than the seed density and allowed all the seeds to float after some stirring with a glass rod. Seeds, which were in specific containers for each sample, were then removed with the aid of a fine brush and left to dry in the shade.

Viable seeds at sight were counted, using a magnifying glass with a 60 mm-diameter cable and a clamp (the seeds were lightly pressed with that clamp to help identify and remove non-viable seeds). The total number of viable weed seeds in the soil was confirmed allowing their germination and subsequent seedling emergence.

Data were analysed using ANOVA. Transformations of the data were not needed to achieve normality. All analyses were performed using the Statistical Analysis Service software (SAS Institute, 2004).

RESULTS

Variance analysis of the weed seedbank in the soil of the area showed significant differences for sampling dates (D), herbicide treatments (T) and sampling depths (S), but not for the interactions tested (Table 1). There were a higher number of viable weed seeds before the application of herbicides, and at the 0 - 10 cm soil depth (Table 2).

The herbicide treatments diuron + oxadiazon, and metalachlor + pendimethalin, were less effective for controlling weed seeds in the soil of the area (seedbank), as was treatment 13 (without herbicide, not weeded during the entire cotton crop cycle). Some herbicide combinations (or one herbicide by itself) allowed more viable weed seeds in the seedbank, than other herbicide combinations (See Table 2). The treatments metalachlor + diuron (1.92 + 1.52 kg brand name/ha), diuron + pendimethalin (1.52 + 0.88 kg b.n./ha), metalachlor + diuron + oxadiazon (1.68 + 1.33 + 0.39 kg b.n./ha), diuron + pendimethalin + oxadiazon (1.33 + 0.77 + 0.39 kg b.n./ha) and pendimethalin + oxadiazon (0.88 + 0.44 kg b.n./ha) gave similar results to treatment 12 (without herbicide, weeded weekly during the entire cotton crop cycle) (Table 2).

Table 1. Summary of the analysis of variance of the number of viable weed seeds (seedbank) at different dates and soil depths in the experimental site (Barbalha, CE State, Brazil), exposed to various herbicide treatments.

Tabla 1. Resumen del análisis de varianza del número de semillas viables de malezas (banco de semillas) en distintas fechas y profundidades de muestreo en el área experimental (Barbalha, Estado CE, Brasil), expuesta a distintos tratamientos de herbicidas.

Variation source	DF	Mean square
Blocks	5	69.47**
Sampling dates (D)	1	343.24**
Error (a)	5	0.63
Treatments (T)	12	23.40**
Sampling depths (S)	1	285.05**
DxT	12	1.58 ns
DxS	1	0.08 ns
TxS	12	1.83 ns
DxTxS	12	2.10 ns
Error (b)	250	1.46
Total	311	-
Mean	-	6.20
CV (%)	-	19.40

** $p<0.01$; ns: Not significant ($p>0.05$).

In addition, before application of the herbicide treatments, the predominant weed was *Raphiodon echinus* (Nees & Mart.) Schauer (mint), and 70 days later it was *Portulaca oleracea* L. (purslane). This showed the greater presence of dicotyledon species at both sampling dates in the weed seedbank of the experimental area.

DISCUSSION

According to the analysis of variance results (Table 1), it is clear that there was variation in the number of viable weed seeds (seedbank) in the experimental area, both spatially (regarding soil depth) and temporally (sampling dates). At least one of the herbicide treatments was effective in reducing the infestation of weed seeds in the soil of the study area. These results support the hypotheses tested. The non-significance of all tested interactions indicates independence among the sources of variation considered.

Table 2. Mean values of the number of viable seeds (seedbank) of weeds at different soil depths and dates in the experimental area (Barbalha, CE State, Brazil), exposed to various herbicide treatments.
Tabla 2. Valores medios del número de semillas viables de malezas (banco de semillas) a diferentes profundidades del suelo y fechas en el área experimental (Barbalha, CE, Brasil), expuesta a distintos tratamientos de herbicidas.

Factors	Doses (kg brand name/ha)	Viable seeds (number)
Herbicide treatments		
1. metolachlor + pendimethalin	1.92 + 0.88	7.48 a
2. metolachlor + diuron	1.92 + 1.52	5.04 d
3. metolachlor + oxadiazon	1.92 + 0.44	7.11 ab
4. diuron + pendimethalin	1.52 + 0.88	5.14 d
5. diuron + oxadiazon	1.52 + 0.44	7.54 a
6. pendimethalin + oxadiazon	0.88 + 0.44	5.53 cd
7. metolachlor + diuron + pendimethalin	1.68 + 1.33 + 0.77	6.48 abc
8. metolachlor + diuron + oxadiazon	1.68 + 1.33 + 0.39	5.28 d
9. metolachlor + pendimethalin + oxadiazon	1.68 + 0.77 + 0.39	7.05 ab
10. diuron + pendimethalin + oxadiazon	1.33 + 0.77 + 0.39	5.31 d
11. metolachlor + diuron + pendimethalin + oxadiazon	1.44 + 1.14 + 0.66 + 0.33	7.24 ab
12. Weeded weekly during the entire cotton crop cycle cycle	No herbicides	5.21 d
13. Not weeded during the entire cotton crop cycle	No herbicides	6.14 bcd
MSD	-	1.16
Sampling depths (cm)		
0 – 10	-	7.15 a
10 – 20	-	5.24 b
Sampling dates		
Before the application of herbicides treatments	-	7.25 a
70 days after the application of herbicides treatments	-	5.15 b

For herbicide treatments, means followed by the same letter in the column do not differ by the Tukey's Studentized Range (HSD) test at 0.05 probability; for sampling depths and dates, means followed by the same letter in the column do not differ by the F test at 0.01 probability.
Para los tratamientos de herbicidas, los promedios seguidos de la misma letra en la columna no difieren por la prueba de Tukey a una probabilidad de 0,05; para las fechas y profundidades de muestreo, los promedios seguidos de la misma letra en la columna no difieren por la prueba de F a una probabilidad de 0,01.

The results listed in Table 2 corroborate the findings of Voll et al. (1996). They reported that the application of herbicides can lead to exhaustion of the seedbank. Also, Carmona (1992), in studying the influence of land preparation on the seedbank, found that the application of certain chemicals contributed to an accelerated decay rate of seeds in the soil.

Regarding the efficacy of the study herbicides in reducing the weed seedbank, combinations of two and three herbicides appeared especially promising, where diuron was present in 80% of them (Table 2). The use of two, three and four herbicide combinations have been recommended because they are less costly in weed control, since doses are reduced by 10, 20 and 30%, respectively, in comparison to those using single herbicides. These combinations thus also reduce the risk of damage to the crop, and the residual effects of the herbicide active ingredients on the environment.

Diuron is slightly soluble in water, and it is not carried into deep soil layers. It remains in the soil surface layers due to its adsorption to soil particles and organic matter. Due to its systemic action, it is carried by the movement of sap in the plant to parts where photosynthesis occurs. The plant loses moisture, yellows and dies. The product usually remains active for four months (Hertwig, 1983).

Nobrega et al. (1998) tested herbicide doses, alone and in combinations, on weed control of rainfed upland cotton. The combinations diuron + alachlor and diuron + pendimethalin in doses of 1.5 + 1.5 kg/ha, respectively, were some of the most efficient in controlling weeds for up to 60 days after emergence.

The observation that the majority of viable seeds were found at 0 - 10 cm soil depth possibly resulted from the little previous disturbance of the ground, since the field was fallow for two years and prepared before planting with superficial tillage. O'Donovan & Mcandrew (2001) tested four tillage systems, ranging from intensive to zero, and observed that the majority of seeds were near the soil surface with the zero system. Lacerda (2003), Lacerda et al. (2005) and Steenwerth et al. (2010) found that conventional tillage provided some homogenization of the seedbank along the vertical soil profile.

Vasileiadis et al. (2007) evaluated the effects of tillage regime (mouldboard plough, chisel plough and rotary tiller), cropping sequence [continuous cotton (*Gossypium* spp. L.), cotton–sugar beet (*Beta vulgaris L. altissima*) rotation and continuous tobacco (*Necotiana tabacum* L.)] and herbicide treatment on weed seedbank dynamics. These authors concluded that in light-textured soils, conventional tillage with herbicide use gradually reduced seed density of small seeded weed species in the top 15 cm soil depth over several years.

Rahman (2001) reported that the emergence of seedlings from weed seeds was higher near the soil surface than deeper in the soil. Thus, this work emphasizes the importance of surveying the weed seedbank in agricultural areas for predicting weed species infestations and the implementation of well programmed weed controls.

It can be concluded that the highest number of viable weed seeds in the study area was found before the application of the herbicide treatments at 0 - 10 cm soil depth, and that the treatments metalachlor + diuron, diuron + pendimethalin and treatment 12 (no herbicide treatment, weeded weekly during the entire cotton crop cycle) were the most effective in reducing the weed seedbank in the area.

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