Performance of cotton herbicide treatments for *Amaranthus lividus* **and** *Amaranthus hybridus***¹**

Desempenho de herbicidas utilizados no algodoeiro para o controle de *Amaranthus*

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Abstract - Some species of *Amaranthus* are widely distributed in cotton crops. In recent years, ineffective weed control after herbicide use of some species has been reported. The current study was installed in order to find effective alternatives to a proper management of these species. Two simultaneous experiments were carried out for each species in this study (*Amaranthus lividus* and *Amaranthus hybridus*), and in each of them, a different stage of weed development (2 to 4 and 4 to 6 leaves) was focused. Twenty two herbicide combinations were arranged in a completely randomized design with four replicates for each experiment. Treatments were composed by the single or combined application of pyrithiobac-sodium, ammonium-glufosinate, glyphosate and trifloxysulfuron-sodium at different doses. For pyrithiobac, the best weed control results for both *A. lividus* and *hybridus* were found with applications of doses ≥ 28 g ha⁻¹. Glufosinate and glyphosate presented excellent control of both *Amaranthus* species, despite doses or stages of application. Comparing the use of single herbicides and tank mixtures, trifloxysulfuron+pyrithiobac resulted in improved weed control only for early applications. No negative effects of *Amaranthus* species were observed for mixtures of glyphosate and pyrithiobac. Mixtures of pyrithiobac and glufosinate increased *A. lividus* and *A. hybridus* control levels.

Keywords: Livid amaranth, smooth pigweed, *Gossypium hirsutum* L., LL® cotton, RR® cotton, tank mixtures

Resumo - Algumas espécies do gênero *Amaranthus* encontram-se amplamente distribuídas em lavouras de algodão, sendo que nos últimos anos o controle químico tem sido ineficiente para o manejo dessas plantas daninhas. Com o intuito de buscar alternativas eficazes no manejo destas plantas daninhas foi instalado o presente trabalho. Foram conduzidos quatro experimentos em casa-de-vegetação sendo dois para cada espécie avaliada (*A. lividus* e *hybridus*), variando-se entre eles o estádio de aplicação (2 a 4 e 4 a 6 folhas verdadeiras). O delineamento experimental utilizado foi inteiramente casualizado com quatro repetições, avaliando-se 21 tratamentos herbicidas, além de uma testemunha sem controle químico. Os tratamentos foram compostos pela aplicação isolada e em mistura dos herbicidas pyrithiobac-sodium, amonio-glufosinate, glyphosate e trifloxysulfuron-sodium em diferentes doses. Foi avaliado a porcentagem de

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controle aos 7 e 28 dias após a aplicação dos tratamentos (DAA). O pyrithiobac em dosagens superiores a 28 g ha⁻¹ foi eficaz sobre estas espécies, em plantas de 2 a 4 folhas. A aplicação isolada de glufosinate e glyphosate apresentaram-se como boa alternativa para o controle destas plantas daninhas. Em aplicações precoces (2 a 4 folhas), a adoção da mistura entre trifloxysulfuron e pyrithiobac é benéfica. O glyphosate aplicado em mistura com pyrithiobac não teve seu desempenho comprometido. A associação entre pyrithiobac e glufosinate propicia melhoria no controle de *A. lividus* e *hybridus*.

Palavras-chave: Caruru-rasteiro, caruru-roxo, *Gossypium hirsutum* L., algodoeiro LL® , algodoeiro RR®, mistura em tanque

Introduction

Among all crops grown in Brazil, cotton the highest sensitivity level to weedimposed interference, which may lead to yield reduction up to 90%. In addition to quantitative losses, weed can cause difficult harvesting, cotton fiber depreciation, and increased herbicides costs, which contribute to reduce farmer's profitability (Salgado et al., 2002). Cotton limited capacity to compete with weeds is related to different factors such as the wide row sowing, crop slow initial development and C3 photosynthetic metabolism (Freitas et al., 2002).

In areas of cotton cropping, weeds botanically classified as *Magnoliopsida* Class are among the most important problems. This difficulty in controlling these species is related mainly to the increased frequency of occurrence and lack of selective broadleaf herbicide combinations available for postemergence applications (Freitas et al., 2006). Among the genera of greatest importance for this crop, *Amaranthus* is probably the one with the highest number of important weeds all over the world.

The genus *Amaranthus* comprises approximately 60 species, many of them commonly found in the main crops grown around the world. Among those species, *A. hybridus*, *A. lividus*, *A. retroflexus*, *A. viridis* and *A. spinosus* are important weeds in Brazil (Carvalho et al., 2006; Raimondi et al., 2010). These weeds are characterized by being very competitive and aggressive in cotton areas, and

their effects include damages to the quality of the fiber produced.

In recent years, there have been several reports in Brazil on the difficulty to manage *Amaranthus* areas, mainly aroused from cotton producers. Apparently, the lack of control of these species in cotton areas could be related to the lack of proper knowledge on positioning the registered broadleaf herbicide combinations. For instance, applications performed either beyond the ideal stage of weed control or in inappropriate rates are usually related to some reports. A second issue that has been raised recently is the probability of selection of herbicide-resistant *Amaranthus* populations. Biotypes of two species of *Amaranthus* collected around the most important cotton areas in Brazil were recently reported a resistant to both to ALS inhibitors and FS II inhibitors (Francischini et al., 2012).

Currently, the two main herbicide options for broadleaf post-emergent weed control in cotton are pyrithiobac and trifloxysulfuron. Although effective control of some species like *A. viridis* have been reported (Oliveira Jr. et al., 2001), both options have limited efficiency on species like *A. lividus and A. hybridus*. With the recent availability of cotton LL (glufosinate tolerant) and RR (glyphosate tolerant) varieties, it is expected that mixtures of these herbicides will be used to provide more effective weed control. In this context, the objective of this study was to assess the effect of herbicides registered for conventional cotton (pyrithiobac and

trifloxysulfuron) and those used for genetically modified varieties (glufosinate and glyphosate) in relation to *Amaranthus lividus* and *Amaranthus hybridus* control.

Material and Methods

Experiments were conducted in greenhouse at Irrigation Training Center (CTI) of Universidade Estadual de Maringá (UEM) (23º24'12''S; 51º56'24''W and altitude of 560 m). Experimental units were composed by 3 dm³ pots filled with soil (pH H_2 0=6.3; 7.9 g dm⁻³ OC; 51% sand; 47% clay). After moistening the soil in the pots, the same number of *Amaranthus lividus* and *Amaranthus hybridus* seeds were distributed per experimental unit (3-cm deep). After seedling emergence, the number of plants per pot was reduced to ten. Eventual emerged seedlings after herbicide application were eliminated from each pot, to prevent conflicts in weed control evaluations.

For each species, two simultaneous experiments were carried out, one for each stage of development. In all experiments, treatments were composed by twenty two postemergent herbicide treatments, including a nosprayed check for. Herbicides and respective doses (g active ingredient ha^{-1} or g acid equivalent ha^{-1}) were as follows: pyrithiobac (16.8; 28; 56; 84), glufosinate (300; 400; 500), glyphosate (648; 972), glufosinate+pyrithiobac (300+16.8; 300+28; 300+56; 400+16.8; 400+28; 400+56), glyphosate+pirythiobac (648+16.8; 648+28; 648+56), trifloxysulfuron (3) and trifloxysulfuron+pirythiobac (2.25+16.8; 2.25+42). Treatments with glufosinate were applied with Aureo[®] (0.2% v v^{-1}) and remaining treatments were applied with Iharol[®] (0.5% v v⁻¹), except those containing glyphosate, which received no adjuvant. Herbicide treatments were applied at two different stages of weed development: 2 to 4-leafed plants (S1) and 4-6 leafed-plants (S2).

Applications of glyphosate were performed with a $CO₂$ –pressurized backpack sprayer equipped with flat-fan nozzles calibrated to deliver 200 L ha⁻¹ at 241 kPa and 3.6 km h^{-1} . Applications were always Applications were always performed considering a distance of 0.5 m between tips and target plants. Environmental conditions at applications (S1 and S2, respectively) were: Temperature = 27 and 19^oC; Relative Humidity = 60 and 70%; Wind Speed = 1.7 and 1.5 km h^{-1} .

Evaluations of weed control were performed at 7 and 28 days after application (DAA), using a visual scale where 0% corresponded to no injury and 100% to plant death (SBCPD, 1995).

A completely randomized design with four replicates was utilized for all experiments. Data of both trials were submitted to joint analysis in order to find out if there was any effect of the stage in which the herbicides were applied to *Amaranthus* plants. Variance analysis was subsequently performed by F test, and when a significant effect was observed for any variable, means were compared by Scott-Knott (*p*<0.05).

Results and Discussion

Amaranthus lividus:

Levels of *A. lividus* control after application of herbicides in two stages of weed development are summarized in Table 1. For applications performed at S1 (2 to 4 leaves), increment in pyrithiobac dose aiming at the *A. lividus* control had little influence on the initial control (7 DAA), but provided reasonable increments on final control (28 DAA). Based on 28 DAA results, doses \geq 56 g ha⁻¹ of pyrithiobac provided satisfactory control of livid amaranth.

When pyrithiobac was applied in later stages (4 to 6 leaves), the same trend of increment in weed control with dose increment was found. However, control final control

levels were much lower than those found for applications in earlier stage. These results indicate that lack of adequate control reported by farmers due to pyrithiobac applications could be related to the stage of weed development at spraying, since application to younger plants provided effective control of *A. lividus*.

Due to its contact effect, *A. lividus* plants submitted to glufosinate manifested sharp symptoms of herbicide action right after application. Initial control action of *A. lividus* was \geq 85% at 7 DAA – regardless of the stage

in which the herbicide was applied. This rapid control imposed by this herbicide is related to its mechanism of action, which causes the inhibition of glutamine synthetase, triggering an accelerated accumulation of intracellular NH_4^+ . The association of NH_4^+ accumulation with chloroplast structure rupture leads to photosynthesis inhibition and subsequent plant cell death (Fleck et al., 2001). Due to high control levels observed at 28 DAA, glufosinate proved to be a good option to control *A. lividus*, particularly for LL® varieties.

Table 1. *A. lividus* control (%) after application of different post-emergent herbicide treatments. Maringá - PR, 2010.

	Stages of weed development									
Treatments $(g ha-1)$	S1(2 to 4 leaves)					S2(4 to 6 leaves)				
	7 DAA		28 DAA			7 DAA		28 DAA		
01. PYR (16.8)	71.3	\mathbf{c}	67.5	d		37.5	d	48.8	e	
02. PYR (28)	76.0	\mathbf{c}	85.8	$\mathbf b$		42.5	d	53.8	e	
03. PYR (56)	67.0	\mathbf{c}	87.5	$\mathbf b$		43.8	d	51.3	e	
04. PYR (84)	52.5	d	95.3	a		57.5	\mathbf{C}	65.0	d	
05. AG (300)	85.3	$\mathbf b$	84.5	$\mathbf b$		91.3	a	84.0	$\mathbf b$	
06. AG (400)	92.0	a	90.3	$\mathbf b$		95.0	a	100.0	a	
$07. \text{AG } (500)$	87.0	$\mathbf b$	95.0	a		95.0	a	100.0	a	
08. GLY (648)	91.3	a	100.0	a		90.0	a	100.0	a	
09. GLY (972)	82.0	b	100.0	a		94.5	a	100.0	a	
10. AG + PYR $(300 + 16.8)$	84.0	$\mathbf b$	87.8	$\mathbf b$		95.0	a	100.0	a	
11. AG + PYR $(300 + 28)$	84.3	b	86.5	$\mathbf b$		95.0	a	100.0	a	
12. AG + PYR $(300 + 56)$	83.3	$\mathbf b$	97.3	a		93.8	a	100.0	a	
13. AG + PYR $(400 + 16.8)$	91.8	a	90.3	$\mathbf b$		95.0	a	100.0	a	
14. AG + PYR $(400 + 28)$	89.5	a	93.8	a		92.5	a	100.0	a	
15. AG + PYR $(400 + 56)$	90.3	a	87.5	$\mathbf b$		93.8	a	100.0	a	
16. GLY + PYR $(648 + 16.8)$	92.3	a	99.8	a		91.3	a	97.5	a	
17. $GLY + PYR (648 + 28)$	92.3	a	98.8	a		90.0	a	100.0	a	
18. GLY + PYR $(648 + 56)$	93.3	a	100.0	a		92.5	a	100.0	a	
19. TRI (3)	51.3	d	67.5	d		78.8	$\mathbf b$	73.8	\mathbf{C}	
20. TRI + PYR $(2.25 + 16.8)$	53.8	d	78.8	\mathbf{C}		57.5	\mathbf{C}	55.0	e	
21. TRI + PYR $(2.25 + 42)$	46.3	d	89.5	$\mathbf b$		58.8	\mathbf{c}	66.3	d	
22. Check without herbicide	0.0	e	0.0	e		0.0	e	0.0	$\mathbf f$	
CV(%)	6.82		8.99			7.44		5.79		

*DAA: Days after application; PYR (pyrithiobac-sodium); AG (ammonium-glufosinate); GLY (glyphosate); TRI (trifloxysulfuron-sodium). Means followed by the different letters in the same column differ significantly by Scott-Knott test ($p \leq 0.05$).

Among all single herbicide treatments, glyphosate was the one that stood out as the best alternative for *A. lividus* control, since even the lowest dose (648 g ha^{-1}) was enough to provide death of all plants.

For mixtures, an increment on *A. lividus* control (2 to 4 leaves) was observed when glufosinate (300 g ha^{-1}) was associated to pyrithiobac. For pyrithiobac mixtures with the highest dose of glufosinate, control levels were not altered, indicating that no antagonistic effect for these mixtures. Glufosinate shows reduced or no residual activity in soil due to its rapid microbial degradation and low adsorption in soil colloids (Mahan et al., 2006). The absence of antagonism for the mixture pyrithiobac+glufosinate may be adopted with the benefit of soil residual weed control imposed by pyrithiobac. Effective soil residual control of *Ipomoea lacunosa, Sida spinosa, Senna obtusifolia* and *Amaranthus palmeri* after applications of pyrithiobac have been found in previous works (Branson et al., 2005).

No evidences of antagonism were found for glyphosate when mixed with pyrithiobac, despite the application stage. Considering that glyphosate alone controlled all plants of this species, the utilization of this herbicide mixed with others would not bring any additional benefits and could implicate in additional costs for farmers. However, in general, weed communities present great heterogeneity in species composition and sensitivity to herbicide treatments. Thus, the addition of pyrithiobac to glyphosate could be an option in areas where weed composition is composed by more tolerant species, and, therefore, a wider weed control spectrum is demanded (Constantin & Oliveira Jr., 2009) with the additional benefit to provide residual control due to pyrithiobac activity in soil.

For pyrithiobac+trifloxysulfuron mixtures, it was observed that there was some synergism in early applications to control *A. lividus*. However, when applied in later stages,

weed control was inferior to that obtained with trifloxysulfuron alone. Benefits for the utilization of these post-emergent herbicides mixture were already reported in literature, and are related mainly to wider spectrum of weeds controlled due to this association (Richardson et al., 2006).

By assessing the results of this current study, it has been observed that *A. lividus* presents greater sensitivity to pyrithiobac alone in earlier applications, and an increment of this herbicide dose provides increased control. Glufosinate alone has stood out as a good alternative to this species management, regardless of weed development stage. Among the herbicides applied alone, the best performance has been observed by glyphosate. The lowest dose of this product was able to control all the plants present in both application stages.

The association between pyrithiobac and glufosinate improved *A. lividus* control in most treatments evaluated and no negative effect in any stages was observed for this mixture. The combined application of pyrithiobac and glyphosate was considered additive because the levels of control exerted by the mixture were similar to that of glyphosate alone. Use of these treatments may be beneficial due to the amplification of weed control spectrum and to residual control imposed by pyrithiobac. Associations of pyrithiobac and trifloxysulfuron was only considered as beneficial for the first stage of application (2 to 4 leaves), once when applied to later stages, reductions in control levels were observed.

Amaranthus hybridus:

A. hybridus control after the application of different post-emergent herbicide treatments is shown in Table 2. Comparing both stages of herbicide application, pyrithiobac provided more effective initial (7 DAA) weed control when applied to older plants (S2). However,

better final control (28 DAA) was achieved with applications to younger plants (S1). Previous reports have shown more effective control of *A. hybridus* than those found here by the application of pyrithiobac (56 g ha⁻¹) at 5-6 leaves (Carvalho et al., 2006), what could reflect the selection of more tolerant biotypes due to the continuous use of ALS inhibitors in cotton. The occurrence of differential susceptibility of weeds to herbicides in distinct locations is deeply related to the history of herbicide use.

Glufosinate represented a good alternative for *Amaranthus hybridus*

management, once for both application stages, rates ≥ 400 g ha⁻¹ provided at least 86% of final weed control. Similar to that observed for *A. viridis*, glyphosate also emerged as the most stable herbicide option to control *A. hybridus*. Final (28 DAA) control levels were >97% for both stages, indicating high sensitivity of this weed to glyphosate. *A. hybridus* sensitivity to glyphosate was already reported in other studies, and even older stages were successfully controlled by this herbicide (Monquero et al., 2001; Werlang & Silva, 2002).

Table 2. *A. hybridus* control (%) after application of different post-emergent herbicide treatments. Maringá - PR, 2010.

	Stages of weed development								
Treatments $(g ha-1)$		S1(2 to 4 leaves)	S2(4 to 6 leaves)						
	$7\,\mathrm{DAA}^*$	28 DAA	7 DAA	28 DAA					
01. PYR (16.8)	10.0	81.5	67.5	52.5					
	f	d	$\mathbf b$	e					
02. PYR (28)	15.0	89.8	47.5	56.3					
	f	\mathbf{C}	\mathbf{c}	e					
03. PYR (56)	10.0	92.5	55.0	61.8					
	f	b	\mathbf{c}	d					
04. PYR (84)	61.3	87.8	70.0	72.0					
	\mathbf{c}	\mathbf{c}	b	\mathbf{c}					
05. AG (300)	63.8	81.0	72.0	79.0					
	\mathbf{c}	d	b	\mathbf{c}					
06. AG (400)	51.3	86.0	83.8	98.0					
	d	\mathbf{C}	a	a					
07. AG (500)	77.0	97.8	91.3	100.0					
	b	a	a	a					
08. GLY (648)	88.5	98.0	81.3	100.0					
	a	a	a	a					
09. GLY (972)	84.3	95.0	90.8	100.0					
	a	b	a	a					
10. AG + PYR $(300 + 16.8)$	51.3	97.8	72.5	87.5					
	d	a	$\mathbf b$	$\mathbf b$					
11. AG + PYR $(300 + 28)$	38.8	98.5	82.5	98.0					
	e	a	a	a					
12. AG + PYR $(300 + 56)$	57.5	99.3	78.8	96.8					
	\mathbf{C}	a	a	a					
13. AG + PYR $(400 + 16.8)$	51.3	99.8	82.5	100.0					
	d	a	a	a					
14. AG + PYR $(400 + 28)$	48.8	99.5	82.5	100.0					
	d	a	a	a					
15. AG + PYR $(400 + 56)$	43.8	99.5	87.5	100.0					
	e	a	a	a					
16. GLY + PYR $(648 + 16.8)$	90.3	100.0	71.3	100.0					
	a	a	b	a					
17. $GLY + PYR (648 + 28)$	85.8	100.0	72.0	100.0					
	a	a	$\mathbf b$	a					
18. GLY + PYR $(648 + 56)$	87.3	100.0	78.3	100.0					
	a	a	a	a					
19. TRI (3)	75.8	87.3	63.8	75.3					
	b	\mathbf{C}	b	\mathbf{c}					
20. TRI + PYR $(2.25 + 16.8)$	74.0	95.3	43.8	61.3					
	b	$\mathbf b$	\mathbf{c}	d					
21. TRI + PYR $(2.25 + 42)$	42.5	93.5	60.0	76.5					
	e	b	b	\mathbf{c}					
22. Check without herbicide	0.0	0.0	0.0	0.0					
	g	e	d	$\mathbf f$					
CV(%)	9.51	4.11	11.27	6.30					

*DAA: Days after application; PYR (pyrithiobac-sodium); AG (ammonium-glufosinate); GLY (glyphosate); TRI (trifloxysulfuron-sodium). Means followed by the different letters in the same column differ significantly by Scott-Knott test ($p \leq 0.05$).

Trifloxysulfuron applied alone exerted satisfactory control levels of *A. hybridus* only when applied in early stages. From the first to the second stage of herbicide application, a decrease of herbicide efficiency around 12% was observed. Although the magnitude of difference in control was small, such differences may have an important impact when the seed production ability of this species is considered.

Most of these species are usually referred as pigweeds by farmers and, in most cases, are considered as one single target in weed control. Evaluations of comparative tolerance of *Amaranthus* species to different herbicides have demonstrated inconsistent results. For instance, Raimondi et al. (2010) compared *A. hybridus*, *A. lividus*, *A. spinosus* and *A. viridis* and found similar susceptibility to pre-emergence applications of alachlor, diuron, trifluralin, clomazone and prometeryn. In contrast, species of *Amaranthus* evaluated by Carvalho et al. (2006) presented differences of susceptibility to postemergence application of ALS herbicides (trifloxysulfuron and chlorimuron). *A. deflexus* was the least susceptible species, followed by *A. spinosus, A. viridis, A. hybridus* and *A. retroflexus*. In the present work, both *Amaranthus* species had similar behavior when submitted to postemergence applications of trifloxysulfuron. Differences in herbicide tolerance within this species could be related to the particular species response, to the history of herbicide use in area or to the combined effect of both.

The combined use of glufosinate and pyrithiobac demonstrated synergism to *A. hybridus* control in earlier (S1) stages. Comparing the application of glufosinate alone or in tank mixture with pyrithiobac, similar weed control was achieved either by the highest dose of glufosinate (500 g ha⁻¹) or by tank mixtures of lower (300 or 400 g ha^{-1}) doses of glufosinate combined with any dose of pyrithiobac. These results demonstrate the potential use of these mixtures in cotton. Main benefits of mixtures would be the reduced selection pressure by the use of an additional herbicide mode of action and the soil residual control by pyrithiobac, due to its considerable persistence in soil (Guerra et al., 2011).

In later (S2) applications, when *A. hybridus* plants had 4 to 6 leaves, the synergism pyrithiobac+glufosinate was less pronounced and only found for the lowest dose of glufosinate (300 g ha^{-1}). By other side, by increasing glufosinate dose, no negative effect in weed control was found by addition of a range of pyrithiobac doses (16.8 to 56 g ha⁻¹), and therefore, no antagonistic effects were found.

Similar to the previous results found for *Amaranthus deflexus*, glyphosate and pyrithiobac tank mixture did not present any *A. hybridus* control reduction when compared to that of glyphosate alone for both stages of application. Synergistic effect was also observed in S1 applications of pyrithiobac+trifloxysulfuron. However, the improved weed control by this mixture was not found for S2 stage, where final mixture control was less than that of trifloxysulfuron alone.

As well as for *A. deflexus,* results of *A. hybridus* control demonstrate that this weed presents greater sensitivity to pyrithiobac in early development stages. In addition, dose increment of this herbicide provides improved weed control. Both glufosinate and glyphosate were excellent alternatives to *A. hybridus* effective control. Trifloxysulfuron provided a satisfactory control of this weed only when applied in S1 stage.

Associations of pyrithiobac and glufosinate provided improved *A. hybridus* control for most combinations assessed, and no antagonistic effect for this herbicide mixture has been observed in any of application stages. Pyrithiobac and glyphosate mixtures were considered additive and weed control levels were similar to the use of glyphosate alone. An

additional benefit of pyrithiobac and glufosinate mixtures would be the soil residual control imposed by pyrithiobac and the addition of a second mode of action. The association between pyrithiobac and trifloxysulfuron demonstrated increment in *A. hybridus* control for the S1 stage, whereas no increment was observed for S2 stage.

Conclusions

For pyrithiobac, the best weed control results for both *A. lividus* and *hybridus* were found with applications of doses ≥ 28 g ha⁻¹. Glufosinate and glyphosate presented excellent control of both *Amaranthus* species, despite doses or stages of application. Comparing the use of single herbicides and tank mixtures, trifloxysulfuron +pyrithiobac resulted in improved weed control only for early applications. No negative effects of *Amaranthus* species were observed for mixtures of glyphosate and pyrithiobac. Mixtures of pyrithiobac and glufosinate increased *A. lividus* and *A. hybridus* control levels.

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