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CROP PROTECTION

Susceptibility of *Echinochloa* populations to cyhalofop-butyl in Southern region of Brazil and impact of the weed phenology on its efficacy of control

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ABSTRACT: *Cyhalofop-butyl stands out among the herbicides in the control of imidazolinone-resistant* ***Echinochloa*** *species; but, rice farmers are not always satisfied with the control achieved with this herbicide. The objectives were to evaluate in regional scale the susceptibility of* ***Echinochloa*** *populations to cyhalofop-butyl, and quantify the effect of the weed phenology on its efficacy of control. For this, three trials were carried out under greenhouse conditions with a fully random design, using* ***Echinochloa*** *populations collected in rice fields in the southern region of Brazil. In two trials, the susceptibility level of 156 (2012/13 growth season) and 103 (2013/14 growth season) populations were evaluated with the application of cyhalofop-butyl at 360g ha^{-1}. In other trial, in which treatments were arranged in a bi-factorial design (A = 6 x B = 5), it was evaluated six cyhalofop-butyl rates and five phenological stages of* ***E. crus-galli*** *populations.* ***Echinochloa*** *populations had showed differential susceptibility to cyhalofop-butyl, especially in the 2013/14 growth season, where 20 out of the 103 populations had control lower than 90%. The efficacy of this herbicide was inversely proportional to the phenological stage, and the application timing delay contributed directly to the decrease of susceptibility to the herbicide. Cyhalofop-butyl is an effective alternative to control imidazolinone-resistant* ***Echinochloa*** *populations, as long as the application timing occurs in the early phenological stages (2 to 4 leaves).*
Key words: *barnyardgrass, development stage, imidazolinones,* ***Echinochloa crus-galli***, ***Echinochloa colona***.

Suscetibilidade de populações de Echinochloa a cyhalofop-butyl na região Sul do Brasil e impacto da fenologia da infestante sobre a eficácia do seu controle

RESUMO: *Cyhalofop-butyl destaca-se dentre os herbicidas usados no manejo de populações de capim-arroz resistente às imidazolinonas, mas nem sempre o orizicultor fica satisfeito com o resultado obtido com este herbicida. Objetivou-se avaliar a suscetibilidade de populações de capim-arroz (****Echinochloa*** *spp.) ao cyhalofop-butyl e, quantificar o efeito da fenologia da infestante sobre a eficácia do seu controle. Para isto, três experimentos foram realizados em casa de vegetação com delineamento inteiramente casualizado, utilizando-se populações de capim-arroz coletadas na região Sul do Brasil. Em dois experimentos, a suscetibilidade de 156 (safra 2012/13) e 103 (safra 2013/14) populações foram avaliadas frente à aplicação do herbicida cyhalofop-butyl na dose de 360g ha^{-1}. Em outro experimento, em que se arranjaram os tratamentos em esquema bi-fatorial, foram avaliados seis doses do herbicida e cinco estádios fenológicos de populações de* ***E. crus-galli****. Foi verificada variação na resposta das populações de capim-arroz ao cyhalofop-butyl, em especial na safra 2013/14, em que 20, das 103 populações testadas, tiveram controle menor que 90%. A eficácia do controle foi inversamente proporcional ao estádio fenológico e, o atraso no controle da infestante contribui diretamente para a diminuição da sua suscetibilidade ao herbicida. Cyhalofop-butyl é uma alternativa eficaz para controlar capim-arroz resistente às imidazolinonas, desde que a aplicação ocorra nos estádios iniciais de desenvolvimento (2 a 4 folhas).*
Palavras-chave: *capim-arroz, estádio de desenvolvimento, imidazolinonas,* ***Echinochloa crus-galli***, ***Echinochloa colona***.

INTRODUCTION

Species from the ***Echinochloa*** genus are frequently distributed over flooded rice fields in Brazil and worldwide, and historically includes a number of the most important weeds of this crop. In general, species from this genus have plants with intermediate characteristics and great morphological variability, and their field identification is very hard (DALAMAS et al., 2008). Therefore, in many situations, populations of the species ***Echinochloa crus-galli***, ***E. crus-pavonis*** and ***E. colona***, among others, are identified as ***Echinochloa*** complex (BORTOLY et al., 2015). These species present

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high adaptability level for interspecies competition, causing severe damages and yield losses even to lowly infested flooded rice fields (PANOZZO et al., 2014). Just one ***Echinochloa*** plant per m^{-2} caused enough negative interference to decrease the grain yield of flooded rice fields in Brazil ranging from 4 to 30% (GALON et al., 2007).

Echinochloa species have herbicide resistant biotypes that were reported in various regions on Brazil and the world, and they are among the ten weed species with most number of resistance cases. In fact, the ***Echinochloa*** complex includes six species that are resistant to nine different herbicide action mechanisms, including various cases of multiple resistance to herbicides (HEAP, 2016). Over the last decade, in the southern region of Brazil, the constant use of imidazolinones on *Clearfield*® rice has selected innumerous biotypes resistant to herbicides of this chemical group. This added up to the resistance of biotypes from the same species to the auxin-mimic quinclorac, with cases of multiple resistance as well, limiting the farmers' options for herbicides on controlling ***Echinochloa*** species on this region (ANDRES et al., 2007; MATZENBACHER et al., 2013).

Cyhalofop-butyl, 2-[4-(4-cyano-2-fluorophenoxy) phenoxy] propionate, butyl ester is a systemic herbicide that controls annual and perennial grasses, and that has been used in agriculture for over two decades. As all the other aryloxyphenoxypropionate acids, it works by inhibiting the Acetyl Coenzyme A Carboxylase enzyme, which catalyzes the first synthesis route of oily acids (DÉLYE et al., 2003). It has physical-chemical characteristics that result into high selectivity over rice, due to low esterification and high metabolization of its molecules (RUIZ-SANTAELLA et al., 2006a). Conversely, it is very active on other grasses, such as the ones from the ***Echinochloa*** complex, giving high effectiveness to rates starting from 200g ha^{-1} (KIM et al., 2005). For these reasons, cyhalofop-butyl is one of the most widely used herbicides with this action mechanism for the control of grasses complex in flooded rice as well upland rice fields.

The use of alternative herbicides or their association with imidazolinones on the imidazolinone-resistant ***Echinochloa*** management has been a common practice in the Southern region of Brazil. Among the available herbicide options for this situation, cyhalofop-butyl stands out, since its action mechanism is different from imidazolinones (MATZENBACHER et al., 2015). However, in some cases, rice farmers are not satisfied with cyhalofop-butyl results, mainly when ***Echinochloa*** plants emerge at different times and are in variable growth stages at the application. In addition, cyhalofop-butyl is commonly utilized to rescue the control of imidazolinone-resistant ***Echinochloa*** which are in more advanced phenological stages of the plants, such as full tillering. This study was developed to evaluate the following two hypothesis: (i) populations of ***Echinochloa*** species has differential susceptibility to cyhalofop-butyl; (ii) the efficacy of control of this herbicide depends on the phenological stage of ***Echinochloa*** plants.

The objective was to evaluate the susceptibility of imidazolinone-resistant ***Echinochloa*** populations to cyhalofop-butyl, and quantify the effect of phenology stage of these weeds over the efficacy of control of cyhalofop-butyl.

MATERIALS AND METHODS

Vegetal material

Three trials were performed in a controlled environment greenhouse at the Experimental Station of Dow AgroSciences Ind. Ltda., in Mogi Mirim/SP, Brazil, on the 2012/13 and 2013/14 growth season. The plant material studied comes from seeds samples of ***Echinochloa crus-galli***, ***E. crus-pavonis*** and ***E. colona*** collected in flooded rice fields in Rio Grande do Sul and Santa Catarina states, which are located in the geographic area between the geographic coordinates of latitude 26°27'00''S to 33°31'08''S and longitude 48°50'39''W to 57°05'18''W. Fields were chosen based on the history usage of *Clearfield*® rice varieties, as well as imidazolinones herbicides, and ripe seeds were always collected in bulk from at least 25 plants. Plants were established using the method described by MATZENBACHER et al. (2013), and have grown over air temperature of 28±2°C and photoperiod of 12 hours (light/dark).

Susceptibility of the populations to cyhalofop-butyl

Two trials were designed as a fully random design using three or four repetitions whose one trial had 156 populations and other trial had 103 populations, respectively, on the 2012/13 and 2013/14 growth seasons. The treatment was cyhalofop-butyl at 360g ha^{-1} (Clincher®, 180g L^{-1}, EC, Dow AgroSciences) with the addition of mineral oil at 2% v. v.$^{-1}$ (Joint® Oil, 761g L^{-1}, EC, Dow AgroSciences). This rate corresponds to 133% of the maximum rate recommended for ***Echinochloa*** control and was used for a better comparison of the susceptibility among the different populations. Each treatment repetition was composed by a 5L plastic pot containing 25 ***Echinochloa*** plants, and herbicide application were always performed when plants reached 2 expanded leaves. The application was performed with a CO_2 portable spray, equipped with XR Teejet 110.015 nozzles, and spray pressure of 40psi, reaching a spray solution volume equivalent to 100L ha^{-1}.

Phenology effect on cyhalofop-butyl effectiveness

One trial was designed as a fully random design with bi-factor arrangement design (A=6 x B=5) and four repetitions; each repetition consisted of a 5L plastic pot containing five ***Echinochloa*** plants. For this trial, the vegetal material consisted of five barnyardgrass (***E. crus-galli***) populations of the 2012/13 growth season from several rice regions across Rio Grande do Sul State. The A factor consisted of six rates of cyhalofop-butyl, as following: 0, 45, 90, 180, 360 and 720g ha^{-1} with the addition mineral oil at 2% v. v.$^{-1}$, as described in the previous section. The B factor consisted of five phenological stages, as following (BBCH scale): 2 leaves (12), 4 leaves (14), 2 tillers (22), 4 tillers (24) and full flowering (55) (HESS et al., 1997). The stages were defined when 50% +1 of the plants reached the determined characteristic. Herbicide treatments applications were always performed as described in the previous section.

Evaluations and statistic analysis

The efficacy of control of ***Echinochloa*** populations was always evaluated 30 days after herbicide treatment application (DAT), using conventional scale of visual control, with values from 0 to 100%. In the susceptibility trials, histograms of efficacy of control ranges were created to classify the ***Echinochloa*** populations according to their susceptibility to cyhalofop-butyl. In the phenology effect trial, the data was submitted to analysis of variance by F test ($P \leq 0.05$) and, subsequently, to non-linear regression analysis by three parameters logistic model. With the obtained functions, it was used the inverse equation principle to estimate GR_{50}, that is, the herbicide rate that gives 50% of efficacy of control over ***Echinochloa*** plants. Finally, GR_{50} values of the populations were adjusted to the second order polynomial model, to estimate the relation between phenological stage and the response to cyhalofop-butyl herbicide.

RESULTS

Susceptibility of the populations to cyhalofop-butyl

The ***Echinochloa*** populations were satisfactorily controlled by cyhalofop-butyl in most situations (>80% control); but, they showed differential susceptibility to the treatment in both growth seasons (Figure 1). During the 2012/13 growth season, 360g ha^{-1} of cyhalofop-butyl presented high control over the 156 evaluated populations; in all situations, the minimum efficacy of control was 91%. During the 2013/14 growth season, there has been higher variability in the response of populations to the same treatment, since 20 out of 103 populations presented lower than 90% response. Moreover, 3 out of 103 populations were not controlled in a satisfactory way (<80% control) and would possibly survive in a situation of flooded rice commercial field. This results pointed out the existence of some ***Echinochloa*** populations less sensitive to cyhalofop-butyl and not properly controlled, even with the use of higher rates than the maximum label one.

Phenology effect on cyhalofop-butyl effectiveness

Efficacy of control varied based on the significant interaction between rate and phenological stage; this allowed the interaction decomposition with dose-response curves for each phenological stage. On table 1, the parameters of the logistic model equation for the five barnyardgrass are shown, in five phenological plant stages, obtaining high adjustment to the non-linear logistic model. It is possible to verify a clear decrease in the efficacy of control based on the progress of the barnyardgrass development, especially once the plants started the tillering growth stages. For example, when the plants were in the initial phenological stages (2 to 4 leaves), an effective control was obtained in most cases, using the label rates (from 180 to 315g ha^{-1}); however, when the plants were in advanced phenological stages such as 2 to 4 tillers, barnyardgrass was only effectively controlled with the application of twice the rate on the label.

As plants developed, there was a decrease in control levels, since the obtained value for GR_{50} was proportional to the phenological weed stage, on the average of the five barnyardgrass populations (Figure 2). Actually, barnyardgrass was very sensitive when treated during the 2 and 4 leaf stages, since the average GR_{50} was obtained with lower than 100g ha^{-1} cyhalofop-butyl rates. Conversely, in control situations during the most advanced phenological stages, the magnitude of GR_{50} grew exponentially,

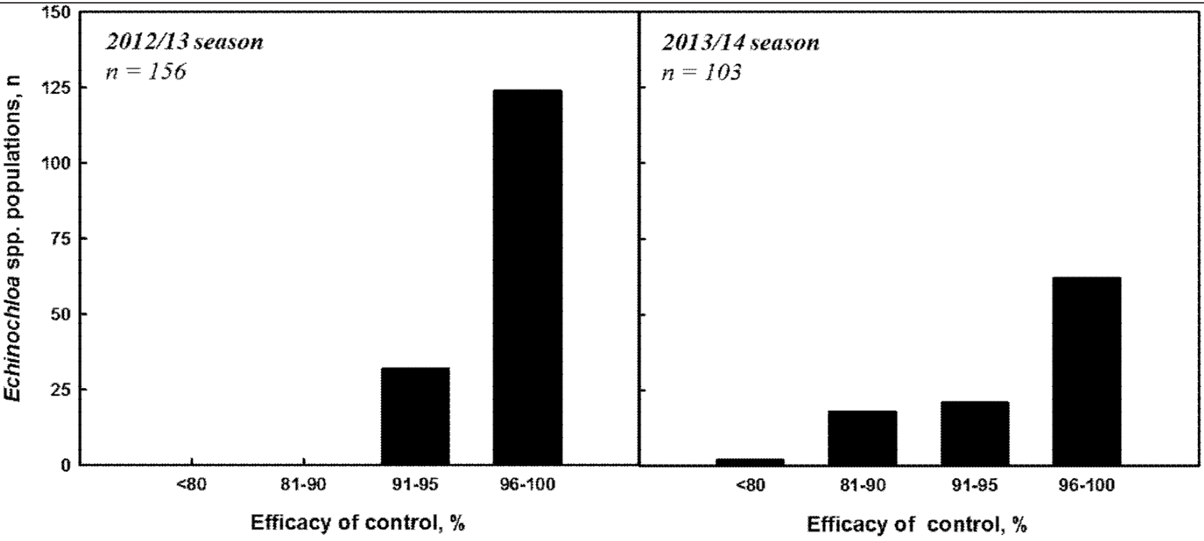

Figure 1 - Histogram for efficacy of control ranges of different ***Echinochloa*** populations with cyhalofop-butyl herbicide (360g ha^{-1}), evaluated 30 days after application. Mogi Mirim/SP, Brazil, 2012/13 and 2013/14 growth seasons.

exceeding the herbicide rate of 400g ha^{-1} of cyhalofop-butyl. In fact, it was observed that the progress in each unit of the BBCH scale requires an increase of 3.3g ha^{-1} of cyhalofop-butyl herbicide, in order to obtain efficacy of control of 50%. However, with barnyardgrass plants in advanced stages, as example full flowering, the increase in the rate only compensated for the decrease of weed control and not provided satisfactory control.

DISCUSSION

The ***Echinochloa*** complex increased its importance in rice farming during the last decade in Brazil and worldwide, due to the appearance of herbicide resistant biotypes in six species of this genus (HEAP, 2016). In Brazil, imidazolinones and/or quinclorac resistant ***Echinochloa*** populations are widespread over flooded rice fields in the southern region of the country (MATZENBACHER et al., 2015). This problem requires a changing attitude from rice farmers and technicians, not only on alternative herbicide usage, but also on attention towards good practices for the use of these products. Thus, for the cyhalofop-butyl herbicide, this study addresses two topics that have great relevance for its efficacy of control over imidazolinone-resistant ***Echinochloa*** populations: (i) there is differential susceptibility among populations to cyhalofop-butyl herbicide (Figure 1); (ii) the performance of this herbicide depends on the phenological stage of the plants (Figure 2; Table 1).

In other studies with ***Echinochloa*** species, differential susceptibility to cyhalofop-butyl applied from 150 to 300g ha^{-1} rates occurred as well (VIDOTTO et al., 2007; DALAMAS et al., 2008). In these cases, higher sensibility was noticed in the ***E. crus-galli*** species compared to ***E. erecta***, ***E. phyllopogon*** and ***E. oryzoides***, even though variations took place within each species. In the present research, the identification of species was not performed in susceptibility trials, since its objective was only to widely evaluate the response of populations in regional scale. In the phenology trial, the five populations evaluated were from the specie ***E. crus-galli*** and differences were observed among them in the response to increased herbicide rates (Table 1). This may be related to populations' adaptations in metabolizing cyhalofop-butyl at higher level and/or speed velocity (RUIZ-SANTAELLA et al., 2006b; MATZENBACHER et al., 2015).

The effect of plant stage over herbicide control is widely known and has been shown in literature for ***Echinochloa*** species; it has been demonstrated for various herbicides (PINTO et al., 2008; PANOZZO et al., 2014). However, the present study is innovating in quantifying this effect

Table 1 - Logistic type regression equations used to quantify the effect of the weed phenological stage and of cyhalofop-butyl rates over efficacy of control of barnyardgrass (***E. crus-galli***) populations, being evaluated 30 days after application. Mogi Mirim/SP, Brazil, 2012/13 growth season.

Population	Stage (BBCH)	-----------------Parameters[a]-----------------			R^{2c}	F[d]
		a	*b*	*c*[b]		
48URUG	2 leaves (BBCH 12)	99.6	3.0	34.4	0.99	4,646.4*
69STMA	2 leaves (BBCH 12)	99.8	3.4	36.0	0.99	676.2*
75SGAB	2 leaves (BBCH 12)	98.8	3.5	34.5	0.99	5,346.7*
108ARGR	2 leaves (BBCH 12)	96.9	2.4	30.7	0.99	1,160.7*
136PALM	2 leaves (BBCH 12)	98.2	2.4	32.4	0.99	1,232.3*
48URUG	4 leaves (BBCH 14)	96.1	19.8	62.0	0.98	476.8*
69STMA	4 leaves (BBCH 14)	96.4	24.1	65.6	0.98	473.4*
75SGAB	4 leaves (BBCH 14)	93.9	12.4	76.7	0.98	298.1*
108ARGR	4 leaves (BBCH 14)	100.2	59.0	127.3	0.97	291.0*
136PALM	4 leaves (BBCH 14)	91.5	61.4	115.3	0.95	134.9*
48URUG	2 tillers (BBCH 22)	92.9	34.1	100.3	0.99	639.0*
69STMA	2 tillers (BBCH 22)	99.4	40.4	110.6	0.99	888.4*
75SGAB	2 tillers (BBCH 22)	92.7	58.2	190.3	0.98	350.6*
108ARGR	2 tillers (BBCH 22)	81.4	54.7	119.7	0.94	53.7*
136PALM	2 tillers (BBCH 22)	93.1	109.5	245.9	0.94	118.9*
48URUG	4 tillers (BBCH 24)	93.0	19.4	52.8	0.98	416.7*
69STMA	4 tillers (BBCH 24)	88.6	29.5	58.9	0.92	88.5*
75SGAB	4 tillers (BBCH 24)	82.8	12.5	36.6	0.91	77.9*
108ARGR	4 tillers (BBCH 24)	89.5	85.3	153.5	0.94	120.4*
136PALM	4 tillers (BBCH 24)	90.5	87.1	143.2	0.93	101.5*
48URUG	Flowering (BBCH 55)	69.9	53.9	138.0	0.93	98.8*
69STMA	Flowering (BBCH 55)	88.5	134.7	304.5	0.97	234.6*
75SGAB	Flowering (BBCH 55)	53.9	39.1	82.4	0.94	118.5*
108ARGR	Flowering (BBCH 55)	30.7	158.6	346.0	0.89	61.0*
136PALM	Flowering (BBCH 55)	32.2	95.4	197.1	0.88	55.9*

[a]Logistic type equation $[y = a / (1 + ((x / c)^b))]$, where *a, b* and *c* are the parameters and *x* and *y* are the variables. [b]Parameter that indicates the cyhalofop-buty rate (g ha^{-1}) that gives 50% of reduction of the parameter *a* of the equation. [c]Determination coefficient value of the equation. [d]F value significant at 1% of probability of experimental error.

for cyhalofop-butyl and in demonstrating that it may be even higher in cases of populations that are less sensitive to it. Thus, when the presence of imidazolinone-resistant ***Echinochloa*** populations is suspected, rice farmers must proceed with the application of cyhalofop-butyl as soon as possible. These pieces of information have great practical applicability, especially for the timing of control, justifying its implementation at early development stages of ***Echinochloa*** and, consequently, giving the chance to reduce cyhalofop-butyl rates, its application cost and its selection pressure over other weed species that can infest flooded rice as well as upland rice fields.

CONCLUSION

Imidazolinone-resistant ***Echinochloa*** populations shown differential susceptibility to cyhalofop-butyl herbicide, with cases in which they survived the exposure to the maximum rate on the herbicide label. The efficacy of this herbicide was inversely proportional to the phenological stage, and the application timing delay contributes directly to the decrease of susceptibility to the herbicide. Cyhalofop-butyl is an effective alternative to control imidazolinone-resistant ***Echinochloa*** populations, as long as the application timing occurs in the early phenological stages (2 to 4 leaves).

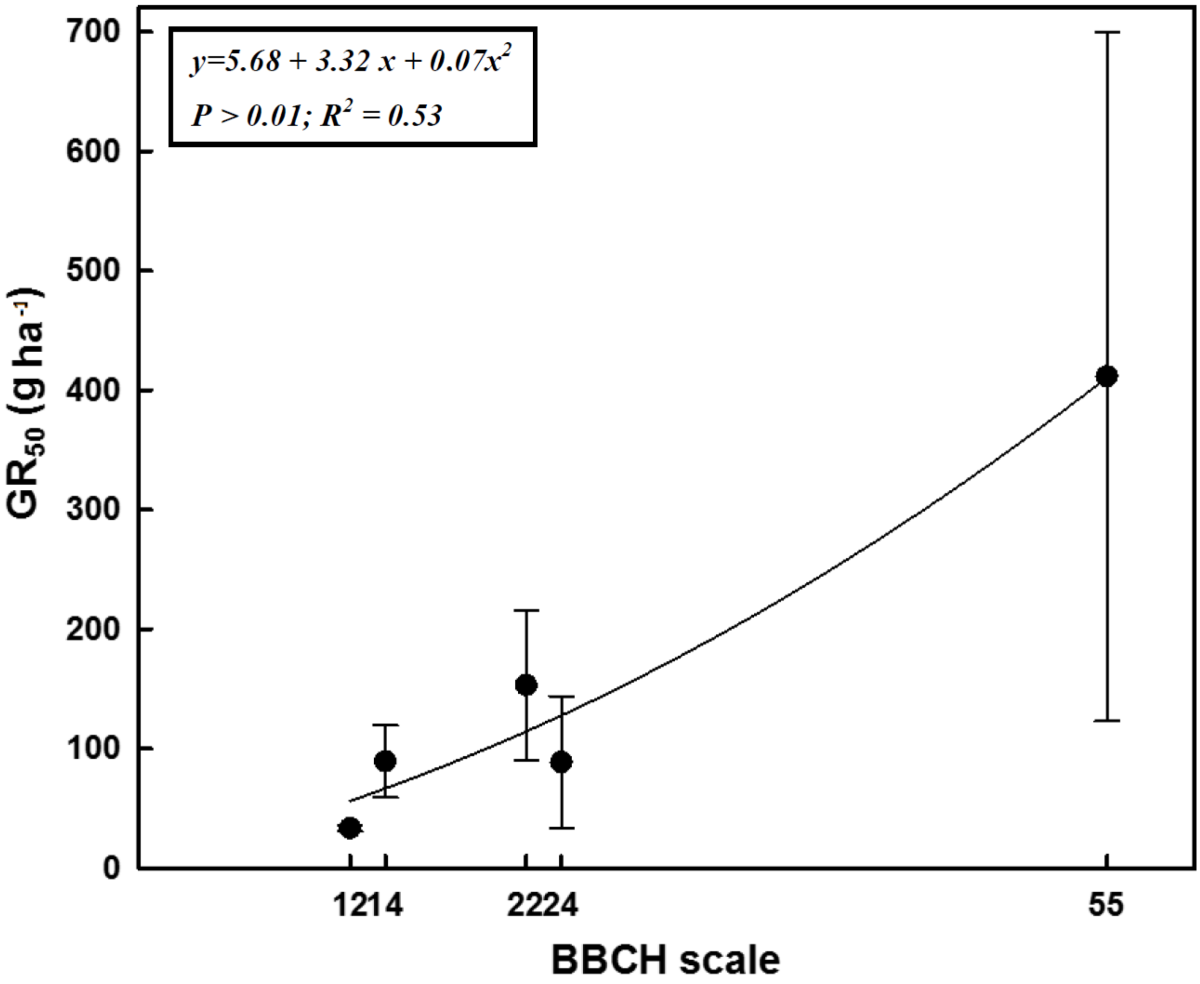

Figure 2 - Necessary cyhalofop-butyl rate for 50% efficacy of control (GR_{50}) on barnyardgrass (***E. crus-galli***) populations, adjusted to the phenological stages[a] of plants, evaluated 30 days after the application. Mogi Mirim/SP, Brazil, 2012/13 growth season. [a]Second order polynomial equation ($y = a + bx + cx^2$), where *a, b* and *c* are the parameters and *x* and *y* are the variables. [b]BBCH 12 (2 leaves), BBCH 14 (4 leaves), BBCH 22 (2 tillers), BBCH 24 (4 tillers) and BBCH 55 (full flowering).

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