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Geographic Distribution of Ryegrass Resistent to the Clethodim Herbicide in Rio Grande do Sul[1]

Distribuição Geográfica de Azevém Resistente ao Herbicida Clethodim no Rio Grande do Sul

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ABSTRACT - Ryegrass is a weed of annual cycle that is present in winter crops, in orchards and vineyards of the South region of Brazil. The species is normally controlled by the glyphosate herbicide, but the continuous use of this product caused the selection of resistant biotypes. The use of ACCase inhibitor herbicides is the main alternative for the control of this species, but it has not been satisfactory in some places, thus causing suspicion of resistance to this action mechanism. Thus, the objective of this paper was to evaluate the occurrence and geographic distribution of ryegrass biotypes that are resistant to the clethodim herbicide in the state of Rio Grande do Sul. For that, seeds of ryegrass plants that survived the application of clethodim were collected in crops from the north region of RS, summing up to a total of 152 samples from 72 cities. The biotypes were submitted to the application of 120 g i.a. ha^{-1} (maximum registered dose) and 60 g i.a. ha^{-1} de clethodim (half the maximum registered dose). According to the results, among the samples of ryegrass seeds collected, there were no biotypes resistant to the clethodim herbicide when the maximum registered dose was applied and in the stage of three to four leaves. However, there were biotypes with lower susceptibility that survived half the maximum registered dose.

Keywords: *Lolium multiflorum*, resistance, chemical control, acetyl coenzyme A carboxylase.

RESUMO - O azevém é uma planta daninha de ciclo anual presente em lavouras de inverno, em pomares e vinhedos da região Sul do Brasil. A espécie normalmente é controlada pelo herbicida glyphosate, porém o uso continuado desse produto selecionou biótipos resistentes. O uso de herbicidas inibidores da ACCase é a principal alternativa para o controle dessa espécie, o qual não tem sido satisfatório em alguns locais, provocando a suspeita da ocorrência de resistência a esse mecanismo de ação. Assim, o objetivo deste trabalho foi avaliar a ocorrência e distribuição geográfica de biótipos de azevém resistente ao herbicida clethodim no Estado do Rio Grande do Sul. Para isso, sementes de plantas de azevém que sobreviveram a aplicações de clethodim foram coletadas em lavouras da região norte do RS, totalizando 152 amostras de 72 municípios. Os biótipos foram submetidos à aplicação de 120 g i.a. ha^{-1} (dose máxima de registro) e 60 g i.a. ha^{-1} de clethodim (metade da máxima dose de registro). De acordo com os resultados, entre a amostras de sementes de azevém coletadas não foram encontrados biótipos resistentes ao herbicida clethodim quando aplicada a máxima dose de registro e no estádio de três a quatro folhas. No entanto, observaram-se biótipos com menor suscetibilidade, que sobreviveram à metade da máxima dose de registro.

Palavras-chave: *Lolium multiflorum*, resistência, controle químico, acetyl coenzima A carboxilase.

INTRODUCTION

In tillage system or in orchards, ryegrass control is usually done by applying non-selective herbicides, mostly glyphosate. The intensive use of glyphosate for this end is due to its high efficiency in different vegetative stages of the species, combined with its low cost when compared to the other herbicides (Christoffoleti & Lópes-Ovejero, 2003). The

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intensive use of an herbicide imposes high selection pressure on the population of plants, resulting in the selection of resistant biotypes pre-existent in the population (Powles & Yu, 2010).

Resistance is defined as the inherent and inheritable capability of a biotype, within a certain population, of surviving and reproducing after exposure to the registered dose of the herbicide for the control of the species, according to the application criteria (Grazziero et al., 2009). The resistance of weeds to herbicides is an evolution process, and the dynamics and the impact depend on several factors. The factors that are able to determine how the resistance is boosted are the genetic diversity of the population, the existence of genes resistant to a specific herbicide and operational factors (Powles & Yu, 2010).

As an alternative for the control of ryegrass populations that are resistant to glyphosate, especially in pre-sowing of maize and wheat, the herbicides used are the ones that inhibit the ACCase enzyme. The ACCase inhibitor herbicides are applied in the weed postemergence period; they control species of poaceae and are selective for magnoliopsida crops (Vidal & Merotto Jr., 2001). The repeated use of ACCase inhibitor herbicides resulted in the selection of biotypes resistant to this action mechanism in Brazil. The bioecological characteristics of the ryegrass, such as genetic variability, allogamy and elevated production of seeds, associated to inappropriate handling strategies, make the selection of resistant biotypes predictable and worrisome (Christoffoleti & López-Ovejero, 2003).

Facing a scenario of elevated resistance of weeds to herbicides, it is crucial to have measures that recommend the handling of the resistance, and it is important to monitor crops for the identification of the resistance focus and the elimination of suspected plants (Lazaroto et al., 2008). After resistance is identified in the field, the adoption of practices such as crop rotation, mixture of herbicides, localized applications and association of control methods reduces the impact of the problem (Hugh, 2006).

Knowing the resistant species and the extension of the infested area provides insight on the severity of the problem, making it possible to determine the resistance handling and making the process of selection of resistant biotypes slower, helping in the reactive decision making process in the control of these populations (Owen et al., 2014). In addition, mapping the resistance enables the establishment of the resistance costs, the definition of public policies and the directed technical assistance.

In Brazil, there is no information gathering on the area infested with ACCase inhibitor herbicide-resistant ryegrass. Gathering information of ryegrass populations that are resistant to ACCase inhibitor herbicides is a tool that will enable the identification of the places where this resistance occurs and the determination of specific handling strategies for each region, according to its characteristics. With this information, the decision making process and the adoption of prevention and resistance control strategies become possible, recovering the viability of cultivation in these areas. The objective of this paper was to evaluate the geographic distribution of ryegrass biotypes that are resistant to clethodim in the state of Rio Grande do Sul.

MATERIAL AND METHODS

To perform this paper, we collected seeds of ryegrass plants that survived the application of ACCase inhibitor herbicides in crops of the north region of Rio Grande do Sul. The seeds that composed the samples came from a plant, and all the collection points were identified with geodetic coordinates, through the use of the Global Positioning System (GPS).

The harvest of seeds happened between the months of September and November 2013, in properties located in the following cities: Água Santa (3), Alto Alegre (2), Augusto Pestana (2), Barra Funda (2), Barracão (4), Boa Vista do Cadeado, Boa Vista do Incra (3), Bom Progresso, Campo Novo, Capão Bonito do Sul, Carazinho (3), Caseiros (2), Ciríaco (2), Colorado, Condor, Coqueiros do Sul (5), Coronel Bicaco, Coxilha (2), Crissiumal (4), Cruz Alta (3), David Canabarro (2), Ernestina, Erval Seco (3), Espumoso (2), Frederico Westphalen, Gentil, Horizontina (2), Humaitá (2), Ibiaçá (2), Ibiraiaras (2), Independência (2), Joia (3), Julio de Castilhos (3), Lagoa Vermelha (3), Machadinho (4), Mato Castelhano, Maximiliano de Almeida, Muitos Capões (2), Nova Candelária, Novo Barreiro, Paim Filho (2), Palmeira das Missões (2), Panambi (4), Passo Fundo, Pejuçara (3), Saldanha Marinho (2), Salto do Jacuí, Sananduva (3), Santa Rosa, Santa Bárbara do Sul (3), Santa Cecília do Sul, Santo Augusto, Santo Cristo (2), São João da Urtiga, São José do Inhacorá, São José do Ouro (2), São Miguel das Missões, Seberi (2), Selbach, Tapejara (2), Tapera, Taquaruçu do Sul (2), Tenente Portela (2), Tio Hugo (2), Três de Maio (4), Três Passos (2), Tucunduva (3), Tupanciretã (2), Tuparendi, Vacaria (2), Victor Graeff, Vila Lângaro (4) and Vista Alegre (Figure 1), summing up 152 samples of seeds harvested in 74 cities.

After the harvest, the seeds were cleaned, identified and stored until when they would be used in experiments. The experiment, carried out to confirm resistance, was done between October and December 2013, in a greenhouse, in a completely randomized design, and the treatments were arranged in a factorial scheme, in which the A factor was composed of biotypes of different places of harvest and B had two doses of the clethodim herbicide (60 and 120 g i.a. ha^{-1}), representing half the maximum registered dose and the maximum registered dose, respectively, for the control of ryegrass in pre-sowing of maize and wheat (AGROFIT, 2015). A control treatment without herbicide application was also added. The experimental units were composed of plastic vases with volumetric capacity of 500 mL, containing commercial substrate, being composed of five plants per vase.

When the plants reached a vegetative state of three to four leaves, the herbicide was applied, using the backpack sprayer, pressured at CO_2, calibrated to provide application volume of 120 L ha^{-1} of herbicide spray, equipped with spray nozzles in the form of a fan 110.015. The Lanzar® adjuvant was also used in the spray in the dose of 0,5% v/v.

The variable evaluated was visual control at 28 days after the application of treatments (DAT), and the biotypes were identified, according to the response to the herbicides, as susceptible or resistant, adopting a binary scale in which zero (0) represented the death of the plants and one (1) represented survival. The data obtained was analyzed by descriptive statistics, seeking to establish relations between the distributions of cases of ryegrass suspected to be resistant to the ACCase enzyme inhibitors.

Source: Adapted from Geolivre - Mapas temáticos RS, 2011.

Figure 1 - Geographic location of the cities where there was harvest of seeds of ryegrass plants suspected of being resistant to the clethodim herbicide in the state of Rio Grande do Sul.

RESULTS AND DISCUSSION

The data analysis revealed that all the 152 harvested biotypes were controlled with a doses of 120 g i.a. ha^{-1} clethodim, proving the non-occurrence of resistance to this herbicide (Table 1). However, 14 biotypes survived the application of half the maximum registered dose (60 g i.a. ha^{-1}) for the control of ryegrass, showing sensitivity difference. Similar results were observed in biotypes sensitivity studies of *Euphorbia heterophylla* and *Eleusine* spp. with suspicion of resistance to the glyphosate herbicide, originated from soy crops RR from Rio Grande do Sul – RS (Ulguim, 2012; Vargas et al., 2013).

The use of the maximum registered dose of the herbicide is due to the fact that a plant can only be classified as resistant to the herbicide if it survives and reproduces after exposure to the registered dose of the product for the control of the species, according to the

Table 1 - Localization and response (0 = death, 1 = survival) of biotypes of *Lolium multiflorum* in function of the application of 60 and 120 g ha^{-1} of clethodim visually evaluated at 28 days after the treatment (DAT)

Biotype	City	Coordinates		Responses to Clethodim	
		Latitude	Longitude	60 g	120 g
AGS 01	Água Santa	28º17’ 38’’	51º58’ 34”	0	0
AGS 02	Água Santa	28º15’ 11’’	51º58’ 50”	1	0
AGS 03	Água Santa	28º 14’ 38’’	51º 58’ 57’’	1	0
ALA 01	Alto Alegre	28º 46’ 34’’	52º 56’ 15’’	0	0
ALA 02	Alto Alegre	28º 46’ 45’’	52º 59’ 06’’	0	0
APE 01	Augusto Pestana	28º 31’ 43’’	54º 01’ 11’’	0	0
APE 02	Augusto Pestana	28º 33’ 04’’	54º 03’ 51’’	0	0
BAF 01	Barra Funda	28º 55’ 55’’	53º 03’ 27’’	0	0
BAF 02	Barra Funda	27º 55’ 59’’	53º 00’ 30’’	0	0
BAR 01	Barracão	27º 48’ 60’’	51º 26’ 42’’	0	0
BAR 02	Barracão	27º 39’ 51’’	51º 32’’ 55’’	0	0
BAR 03	Barracão	27º 35’ 55’’	51º 38’ 25’’	0	0
BAR 04	Barracão	27º 41’ 04’’	51º 30’ 53’’	0	0
BVC 01	Boa Vista do Cadeado	28º 32’ 45’’	53º 47’ 40’’	0	0
BVI 01	Boa Vista do Incra	28º 56’ 40’’	53º 24’ 59’’	0	0
BVI 02	Boa Vista do Incra	28º 56’ 45’’	53º 24’ 49’’	0	0
BVI 03	Boa Vista do Incra	28º 52’ 38’’	53º 27’ 51’’	0	0
BOP	Bom Progresso	27º 34’ 25’’	53º 51’ 58’’	0	0
CAN	Campo Novo	27º 40’ 48’’	53º 50’ 31’’	0	0
CBS	Capão Bonito do Sul	28º 18’ 60’’	51º 21’ 09’’	0	0
CAR 01	Carazinho	28º 20’ 09’’	52º 55’ 34’’	0	0
CAR 02	Carazinho	28º 20’ 36’’	52º 51’ 19’’	0	0
CAR 03	Carazinho	28º 20’ 34’’	52º 54’ 39’’	0	0
CAS 01	Caseiros	28º 13’ 30’’	51º 38’ 27’’	0	0
CAS 02	Caseiros	28º 16’ 44’’	51º 40’’ 44’’	0	0
CIR 01	Ciríaco	28º 18’ 26’’	51º 57’ 11’’	0	0
CIR 02	Ciríaco	28º 19’ 06’’	51º 54’ 34’’	0	0
COL 01	Colorado	28º 22’ 18’’	52º 17’ 21’’	0	0
CON 01	Condor	28º 09’ 49’’	53º 28’ 05’’	0	0
COQ 1	Coqueiros do Sul	28º 11’ 20’’	52º 44’ 33’’	0	0
COQ 2	Coqueiros do Sul	28º 11’ 09’’	52º 44’ 19’’	1	0
COQ 3	Coqueiros do Sul	28º 11’ 23’’	52º 44’ 38’’	0	0
COQ 4	Coqueiros do Sul	28º 11’ 37’’	52º 44’ 23’’	0	0
COQ 5	Coqueiros do Sul	28º 11’ 33’’	52º 44’ 17’’	1	0
COB	Coronel Bicaco	27º 46’ 13’’	53º 34’ 37’’	0	0
COX 01	Coxilha	28º 07’ 01’’	52º 15’ 51’’	0	0
COX 02	Coxilha	28º 10’ 06’’	52º 18’ 51’’	0	0
CRI 01	Crissiumal	27º 30‘ 55’’	54º 08’ 13’’	0	0
CRI 02	Crissiumal	27º 32’ 37’’	54º 01’ 18’’	0	0
CRI 03	Crissiumal	27º 33’ 06’’	54º 15’ 34’’	0	0
CRI 04	Crissiumal	27º 30’ 47’’	54º 12’ 12’’	0	0
CRA 01	Cruz Alta	28º 51’ 52’’	53º 36’ 44’’	0	0
CRA 02	Cruz Alta	28º 47’ 26’’	53º 36’ 16’’	0	0
CRA 03	Cruz Alta	28º 42’ 47’’	53º 35’ 07’’	0	0
DAC 01	David Canabarro	28º 23’ 53’’	51º 48’ 02’’	0	0
DAC 02	David Canabarro	28º 22’ 57’’	51º 51’ 11’’	1	0
ERN 01	Ernestina	27º 33’ 19’’	52º 30’ 35’’	0	0
ERS 01	Erval Seco	27º 31’ 16’’	53º 28’ 31’’	1	0
ERS 02	Erval Seco	27º 31’ 24’’	53º 28’ 17’’	0	0
ERS 03	Erval Seco	27º 31’ 57’’	53º 28’ 39’’	0	0
ESP 01	Espumoso	28º 49’ 39’’	52º 54’ 08’’	0	0
ESP 02	Espumoso	28º 52’ 18’’	52º 43’ 19’’	1	0

To be continued ...

Table 1, cont.

Biotype	City	Coordinates		Responses to Clethodim	
		Latitude	Longitude	60 g	120 g
FRW 01	Frederico Westphalen	27° 24' 12''	53° 24' 41''	0	0
GEN 01	Gentil	28° 18' 57''	51° 02' 06''	0	0
HOR 01	Horizontina	27° 36' 23''	54° 21' 30''	0	0
HOR 02	Horizontina	27° 35' 09''	54° 20' 57''	0	0
HUM 01	Humaitá	27° 33' 12''	53° 55' 39''	0	0
HUM 02	Humaitá	27° 34' 19''	53° 55' 54''	0	0
IBI 01	Ibiaçá	28° 03' 37''	51° 49' 44''	0	0
IBI 02	Ibiaçá	28° 03' 47''	51° 50' 43''	0	0
IBR 01	Ibiraiaras	28° 20' 47''	51° 38' 49''	1	0
IBR 02	Ibiraiaras	28° 20' 34''	51° 38' 45''	0	0
IND 01	Independência	27° 52' 54''	54° 11' 49''	0	0
IND 02	Independência	27° 52' 28''	54° 11' 52''	0	0
JOI 01	Joia	28° 44' 18''	54° 10' 07''	0	0
JOI 02	Joia	28° 43' 33''	54° 09' 56''	0	0
JOI 03	Joia	28° 44' 14''	54° 10' 06''	0	0
JCA 01	Julio de Castilhos	29° 16' 01''	53° 39' 19''	0	0
JCA 02	Julio de Castilhos	29° 06' 56''	53° 39' 57''	0	0
JCA 03	Julio de Castilhos	29° 11' 02''	53° 40' 40''	0	0
LAV 01	Lagoa Vermelha	28° 04' 23''	51° 27' 58''	0	0
LAV 02	Lagoa Vermelha	28° 17' 52''	51° 27' 01''	0	0
LAV 03	Lagoa Vermelha	28° 17' 31''	51° 23' 48''	0	0
MAC 01	Machadinho	27° 35' 55''	51° 38' 25''	0	0
MAC 02	Machadinho	27° 39' 37''	51° 33' 53''	0	0
MAC 03	Machadinho	27° 34' 18''	51° 40' 38''	0	0
MAC 04	Machadinho	27° 36' 46''	51° 42' 18''	0	0
MCA 01	Mato Castelhano	28° 18' 53''	51° 08' 35''	0	0
MAX 01	Maximiliano de Almeida	27° 36' 58''	51° 47' 35''	0	0
MUC 01	Muitos Capões	28° 19' 32''	51° 15' 04''	0	0
MUC 02	Muitos Capões	28° 19' 34''	51° 14' 10''	0	0
NCA 01	Nova Candelária	27° 38' 06''	54° 08' 17''	0	0
NBA 01	Novo Barreiro	27° 55' 07''	53° 05' 11''	0	0
PAF 01	Paim Filho	27° 43' 30''	51° 44' 27''	0	0
PAF 02	Paim Filho	27° 41' 48''	51° 47' 53''	0	0
PAM 01	Palmeira das Missões	27° 53' 53''	53° 15' 45''	0	0
PAM 02	Palmeira das Missões	27° 45' 51''	53° 30' 06''	0	0
PAN 01	Panambi	28° 20' 31''	53° 33 60''	1	0
PAN 02	Panambi	28° 15' 36''	53° 28' 18''	0	0
PAN 03	Panambi	28° 21' 50''	53° 27' 39''	0	0
PAN 04	Panambi	28° 21' 03''	53° 27' 39''	0	0
PAS 01	Passo Fundo	28° 14' 13''	52° 34' 15''	0	0
PEJ 01	Pejuçara	28° 19' 30''	53° 39' 52''	0	0
PEJ 02	Pejuçara	28° 19' 27''	53° 39' 48''	0	0
PEJ 03	Pejuçara	28° 25' 53''	53° 33' 24''	1	0
SAM 01	Saldanha Marinho	28° 25' 59''	53° 08' 11''	0	0
SAM 02	Saldanha Marinho	28° 23' 40''	53° 03' 15''	0	0
SAJ 01	Salto do Jacuí	29° 03' 43''	53° 09 32''	0	0
SAN 01	Sananduva	27° 58' 20''	51° 47' 35''	0	0
SAN 02	Sananduva	27° 53' 37''	51° 49' 15''	0	0
SAN 03	Sananduva	27° 58' 20''	51° 47' 34''	0	0
SAR 01	Santa Rosa	27° 50' 59''	54° 32 59''	0	0
SAB 01	Santa Bárbara do Sul	28° 24' 27''	53° 13' 53''	0	0
SAB 02	Santa Bárbara do Sul	28° 22' 48''	53° 24' 13''	0	0
SAB 03	Santa Bárbara do Sul	28° 23' 25''	53° 24' 13''	0	0

To be continued ...

Table 1, cont.

Biotype	City	Coordinates		Responses to Clethodim	
		Latitude	Longitude	60 g	120 g
SAC 01	Santa Cecília do Sul	28° 15’ 30’’	51° 59’ 47’’	0	0
SAG 01	Santo Augusto	27° 43’ 08’’	53° 50’ 58’’	0	0
SCR 01	Santo Cristo	27° 50’ 30’’	54° 34’ 44’’	0	0
SCR 02	Santo Cristo	27° 50’ 30’’	54° 35’ 23’’	1	0
SJU 01	São João da Urtiga	27° 51’ 15’’	51° 49’ 26’’	0	0
SJI 01	São José do Inhacorá	27 41’ 37’’	54° 09’ 12’’	0	0
SJO 01	São José do Ouro	27° 46’ 55’’	51° 38’ 29’’	0	0
SJO 02	São José do Ouro	27° 43’ 12’’	51° 32’ 15’’	0	0
SMM 01	S. Miguel das Missões	28° 59’ 31’’	54° 22’ 35’’	0	0
SEB 01	Seberi	27° 25’ 44’’	53° 25’ 17’’	0	0
SEB 02	Seberi	27° 25’ 36’’	53° 25’ 24’’	0	0
SEL 01	Selbach	28° 38’ 02’’	52° 56’ 07’’	0	0
TAP 01	Tapejara	28° 03’ 44’’	51° 54’ 58’’	0	0
TAP 02	Tapejara	28° 04’ 11’’	51° 58’ 21’’	0	0
TPE 01	Tapera	28° 38’ 44’’	52° 51’ 14’’	0	0
TAQ 01	Taquaruçu do Sul	27° 23’ 56’’	53° 28’ 45’’	1	0
TAQ 02	Taquaruçu do Sul	27° 24’ 01’’	53° 27’ 54’’	0	0
TPO 01	Tenente Portela	27° 23’ 32’’	53° 49’ 59’’	1	0
TPO 02	Tenente Portela	27° 23’ 34’’	53° 47’ 54’’	0	0
TIH 01	Tio Hugo	28° 35’ 28’’	52° 38’ 02’’	0	0
TIH 02	Tio Hugo	28° 33’ 28’’	52° 34’ 18’’	0	0
TRM 01	Três de Maio	27° 44’ 40’’	54° 12’ 53’’	0	0
TRM 02	Três de Maio	27° 48’ 52’’	54° 15’ 16’’	0	0
TRM 03	Três de Maio	27° 49’ 00’’	54° 15’ 05’’	0	0
TRM 04	Três de Maio	27° 47’ 10’’	54° 14’ 59’’	0	0
TRP 01	Três Passos	27° 25’ 33’’	53° 55’ 22’’	0	0
TRP 02	Três Passos	27° 29’ 47’’	53° 53’ 36’’	0	0
TUC 01	Tucunduva	27° 36’ 39’’	54° 28’ 49’’	0	0
TUC 02	Tucunduva	27° 39’ 26’’	54° 27’ 10’’	0	0
TUC 03	Tucunduva	27° 36’ 39’’	54° 28’ 58’’	0	0
TUP 01	Tupanciretã	29° 02’ 59’’	53° 40’ 55’’	0	0
TUP 02	Tupanciretã	29° 02’ 43’’	53° 40’ 18’’	0	0
TPA 01	Tuparendi	27° 44’ 22’’	54° 24’ 23’’	0	0
VAC 01	Vacaria	28° 25’ 25’’	51° 01’ 19’’	0	0
VAC 02	Vacaria	28° 30’ 39’’	50° 51’ 49’’	0	0
VGR 01	Victor Graeff	28° 37’ 52’’	52° 45’ 32’’	0	0
VLA 01	Vila Lângaro	28° 08’ 28’’	52° 12’ 28’’	1	0
VLA 02	Vila Lângaro	28° 08’ 03’’	52° 09’ 09’’	0	0
VLA 03	Vila Lângaro	28° 08’ 34’’	52° 09’ 08’’	0	0
VLA 04	Vila Lângaro	28° 08’ 19’’	52° 09’ 18’’	0	0
VIA 01	Vista Alegre	27° 22’ 35’’	53° 29’ 34’’	0	0

application criteria (indicated vegetative stage, climate conditions and others) (Gazziero et al., 2009). The use of half the maximum registered dose is justified by the fact that the process of resistance selection may be influenced by the use of sub doses of herbicides and because there is differential susceptibility of biotypes to the application of the herbicide (Neve & Powles, 2005; Yu et al., 2013; Yu & Powles, 2014).

The 14 biotypes that survived the application of half the registered dose presented susceptibility difference to the clethodim herbicide, being concentrated in 12 cities of the northwest, northeast and Midwest regions, those being: Água Santa (2), Coqueiros do Sul (2), David Canabarro, Erval Seco, Espumoso, Ibiraiaras, Panambi, Pejuçara, Santo Cristo, Taquaruçu do Sul, Tenente Portela and Vila Lângaro (Figure 2).

The failures in the control of ryegrass observed in areas with the application of clethodim, in wheat and maize crops in RS, and the result of this study show the control of the biotypes suspected to be resistant, indicating that these failures of control can be a result of other factors. Handling practices such as the use of sub doses of clethodim, intensive use of the herbicide and absence of crop rotation were suggested as causes of failure on the control of *Euphorbia heterophylla* by glyphosate in RS (Vargas et al., 213), and it can also be the reason for this observation in ryegrass with clethodim herbicide.

The resistance of weeds to herbicides is an evolutionary process and it pre-exists in nature, influenced by the biology of the species and by genetic factors related to the herbicide in question (Maxwell & Mortimer, 1994) among which the pressure made by the rate of herbicides application is an important factor in the selection of resistant biotypes (Powles & Yu, 2010). However, recent studies have shown that recurrent selections with reduced doses (smaller than the registered dose) of ACCase inhibitor herbicides result in a rapid evolution of the resistance to herbicides in only three generations (Neve & Powles, 2005; Manalil et al., 2011). The susceptibility difference found in the 14 biotypes that survived the application of half the herbicide dose may be a result of the use of low doses, such as 72 at 84 g i.a. ha^{-1} of clethodim by the producers.

Source: Adapted from Geolivre - Mapas temáticos RS, 2011.

Figure 2 - Geographic location of the cities where there were biotypes with lower susceptibility to the clethodim herbicide in the state of Rio Grande do Sul.

In general, in most cases of resistance to ACCase inhibitor herbicides, the resistance mechanism is related to an alteration in the place of action of the herbicide due to a mutation in the gene that encodes the expression of the ACCase enzyme. However, the metabolization that enables a plant to detoxify the herbicide to non-toxic compounds is also an important mechanism that gives resistance and, in many cases, this mechanism gives crossed or multiple resistance to different herbicides of the chemical group and different action mechanisms (Délye, 2005; Powles & Yu, 2010). The primary action mode of ACCase inhibitor herbicides consists in the inhibition of the lipid synthesis, because it inhibits the enzyme (Roman, 2007). The ACCase present in poaceae is sensitive to inhibition by these herbicides (Rodrigues, 1994). However, some conditions are recommended so that the herbicides can reach a higher efficiency, such as the application when the plants present an elevated metabolic activity (Pereira et al., 2011).

Thus, according to the results obtained, resistance of *Lolium multiforum* to clethodim is rejected, but susceptibility difference cannot be discarded. In the field, there are several reports on the failure of ryegrass control with the application of clethodim, which suggests resistance –, a fact that is not confirmed by this paper. The survival of plants in the field occurs without a logical explanation, once it is applied by tractors and with precision sprayer with up to twice the registered dose. The change of environment and the time of growth different from the time when there is chemical control in the field, caused by harvest in the field and sowing in greenhouse, can result in a smaller interference of environmental factors, perhaps interfering in the metabolism of the plants. That way, there is a scientific explanation for the survival of these plants.

On the other hand, one cannot ignore the fact that there are control failures in the field, and that the remaining plants are not controlled with doses above the indicated ones in the clethodim label (120 g i.a. ha^{-1}). Considering that the characteristic is not hereditarily transmitted, the options are reduced to epigenetic factors. Epigenetic resistance happens when a gene is activated and results in the over expression of the target enzyme of the herbicide, or even in the expression of enzymes of the metabolism or kidnapping in the vacuole or intercellular spaces of the herbicide molecule (Gressel, 2009). The epigenetic effects may or may not be transferred to the following generation through meiosis, opposing to the theory of resistance heritability (Bruce et al., 2007).

Considering the results obtained in this paper, it is evident to see the need for an evaluation of epigenetic resistance in ryegrass. In addition, it is worth highlighting the importance of ryegrass handling, especially with the use of integrated handling of weeds, based on the implementation of crop rotation, rotation and/or association of herbicides with different action mechanisms, in order to delay the emergence and evolution of biotypes resistant to herbicides.

Therefore, the biotypes AGS 02, AGS 03, COQ 02, COQ 05, DAC 02, ERS 01, ESP 01, IBR 01, PAN 01, PEJ 03, SCR 01, TAQ 01, TPO 01 and VLA 01 present smaller susceptibility, not being controlled with the application of the maximum registered dose of clethodim for the control of ryegrass.

There is no difference between the sensitive biotypes and resistance suspected that could justify survival, in the field, with the application of 120 g i.a. ha^{-1} of clethodim.

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