A Field Survey of Crop Management Practices and Distribution of ALS Resistant *Euphorbia heterophylla* in Two States in Southern Brazil[1]

Avaliação das Práticas de Manejo das Lavouras e da Distribuição Geográfica de ***Euphorbia heterophylla*** *Resistente aos Inibidores de ALS em Dois Estados do Sul do Brasil*

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ABSTRACT - Herbicide resistance was reported in Brazil almost ten years ago. One of the main weeds with herbicide resistance is wild poinsettia (*Euphorbia heterophylla*). This work evaluates the distribution of ALS-resistant *E. heterophylla* in two states in southern Brazil and determines the major contributing management causes for weed resistance selection in the area. *E. heterophylla* seeds from 148 sites located in Paraná and Rio Grande do Sul were sampled during 2001 and 2002. Farmers provided specific site data for weed control, tillage system, crop rotation and harvesting operations during previous years. ALS resistant *E. heterophylla* biotypes were found widely distributed in the survey area. Data analysis suggests seed dissemination is unlikely to explain the widespread distribution of resistance. The most probable factor for the selection of the resistant *E. heterophylla* is the persistent high use of ALS-inhibiting herbicides over time. Indirect evidence is presented demonstrating the need to educate legislators and farmers about the importance of herbicide mixtures as a strategy to prevent herbicide resistance.

Key words: imazethapyr, wild poinsettia, herbicide mixtures.

*RESUMO - A resistência aos herbicidas foi relatada no Brasil há quase uma década. Uma das principais plantas daninhas com resistência aos herbicidas é a leiteira (**Euphorbia heterophylla**). Este trabalho foi planejado para avaliar a distribuição geográfica de E. heterophylla resistente aos herbicidas nos estados do Paraná e Rio Grande do Sul e para obter dados que identificassem os principais fatores de manejo associados à seleção de biótipos resistentes na área. Sementes de **E. heterophylla** de 148 locais foram coletadas nos anos de 2001 e 2002. Em cada local, os agricultores informaram os procedimentos relativos a controle de plantas daninhas, sistema de preparo do solo, rotação de culturas e colheita. Este trabalho demonstrou que biótipos resistentes aos herbicidas inibidores da ALS estão amplamente distribuídos na região amostrada. A disseminação de sementes de plantas resistentes não é a provável causa da ampla distribuição da resistência. O principal fator responsável pela seleção de plantas resistentes na espécie **E. heterophylla** foi a elevada utilização de herbicidas inibidores de ALS nos anos que antecederam a coleta. Foram apresentadas evidências indiretas que demonstram a necessidade de educar os legisladores e agricultores com respeito à importância da mistura de herbicidas na prevenção da resistência aos herbicidas.*

Palavras-chave: imazethapyr, leiteira, mistura de herbicidas.

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INTRODUCTION

Wild poinsettia is a very common weed in the Brazilian soybean growing areas. This weed is native to South America (Kissmann & Groth, 1992) and has a severe impact on soybean yield. Each wild poinsettia plant m^{-2}, when competing during the whole season with soybean, reduces grain yield by 0.7 to 3.0% (Chemale & Fleck, 1982; Holm et al., 1997; Voll et al., 2002). Soybean must be wild poinsettia-free from 17 to 44 days after crop emergence to avoid yield losses (Meschede et al., 2002).

Soybean is planted in over 18 millions ha in Brazil. The oldest soybean growing areas are located in the southern part of the country, which account for about 50% of the grain produced. In this region, Paraná and Rio Grande do Sul states have the highest soybean acreage, with production concentrated in the western and northern regions of each state, respectively.

Since 1986, wild poinsettia control in soybeans has been achieved mainly by herbicides which inhibit the acetolactate synthase (ALS) enzyme. During this period, the adoption of the no-till system has increased in the region. Likewise, the increase in the soybean monocrop has been steadily during this decade. We hypothesize these management practices are associated to the selection of herbicide resistant biotypes.

In fact, several reports indicated the appearance of wild poinsettia biotypes resistant to ALS inhibitors in southern Brazil (Gazziero et al., 1998; Vidal & Merotto Jr., 1999). ALS resistance in wild poinsettia is a dominant trait and nuclear encoded (Vargas et al., 2001). ALS enzyme studies performed on a limited number of wild poinsettia biotypes indicate an altered target site as the cause of resistance (Vargas et al., 2001; Oliveira et al., 2002). The molecular basis for resistance to ALS inhibiting herbicides is well established in other plant species and is attributed to aminoacid substitutions in one of five regions of the ALS enzyme, which in turn occurs due to a single base-mutation on the respective regions of the ALS gene (Devine & Shukla, 2000).

There is controversy concerning the distribution of ALS resistant wild poinsettia in Brazil. Heap (2003) reports the occurrence of resistant biotypes to be limited to less than 10 places, whereas Cerqueira et al. (2002) reported the occurrence of resistant biotypes to be over 50 counties in southern Brazil. No surveys of weed resistance in Brazil are found in the literature; however several surveys document the status of weed resistance in other parts of the world (Bourgeois & Morrison, 1997; Bourgeois et al., 1997; Beckie et al.., 1999; Légere et al., 2000; Llewellyn & Powles, 2001).

The objectives of this study were to determine the distribution of ALS-resistant populations of wild poinsettia in two states in southern Brazil and to obtain data to determine the major contributing management causes for weed resistance selection in the area.

MATERIALS AND METHODS

E. heterophylla flowers between January and March, in southern Brazil, and produces mature seeds from February to April. Wild poinsettia seed is disseminated through explosive dehiscence soon after maturity almost one month before soybean harvest. For this survey, immature wild poinsettia fruits were collected in February-April 2001 and 2002 from plants occurring in soybean growing areas located in Rio Grande do Sul and Paraná states, respectively.

Fields sampled were suspected of having ALS resistance and had a minimum of 50 surviving wild poinsettia plants m^{-2}. A total of 148 seed samples were collected from soybean fields located in 24 and 42 counties in Rio Grande do Sul and Paraná, respectively (Figure 1, Table 1). The area sampled for *E. heterophylla* seeds totaled almost 13,000 ha, with 4,100 ha located in Paraná state and about 8,800 ha in Rio Grande do Sul (Table 1). In Paraná, the majority of samples were collected from small weed patches, whereas in Rio Grande do Sul, the majority of samples were collected from weed patches bigger than 50 ha (Table 1). For both states, most counties had only one or two samples collected (Table 3).

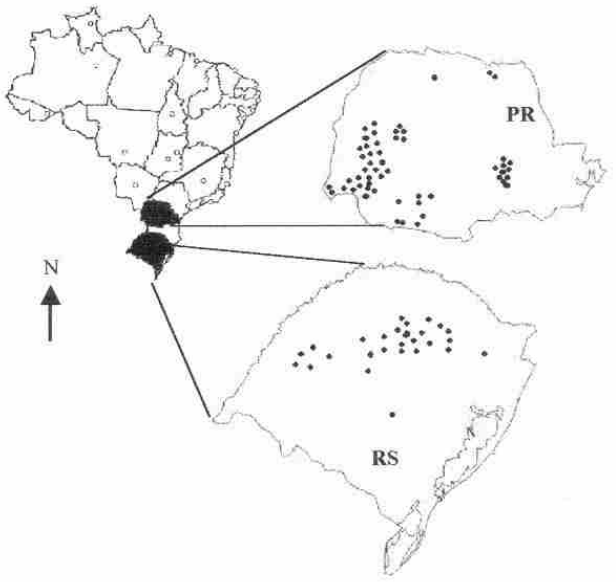

Figure 1 - Map of Brazil, with blown up maps of Paraná (PR) and Rio Grande do Sul (RS) states. Each dot on state maps represents approximate location of two sampling sites of *Euphorbia heterophylla* seeds.

Table 1 - Number of sites sampled for *Euphorbia heterophylla* seeds, counties, and total area with suspected ALS resistance, organized by state

	State		Total
	Paraná	Rio Grande do Sul	
Number of samples	103	45	148
Number of counties	42	24	66
Area sampled (ha)	4.100	8.760	12.860

Table 2 - Sites (%) sampled for *Euphorbia heterophylla* seeds from plants suspected of resistance to herbicides, organized by size distribution of the area, and by state

Size distribution (ha)	State		Average
	Paraná	Rio Grande do Sul	
< 10	32	20	26
11 – 20	21	9	15
21 – 30	9	4	6
31 – 40	7	4	5
41 – 50	9	7	8
51 – 100	9	17	13
101 - 200	9	14	12
> 200	4	24	14

Table 3 - Number of counties, by state, according to sampling frequency per county assessed for *Euphorbia heterophylla* seeds from plants suspected of resistance to herbicides.

Sites per county	State		Average
	Paraná	Rio Grande do Sul	
1	16	10	13
2	13	10	11
3	6	2	4
4	1	1	1
5 or +	6	1	3

Fruit were sampled from at least 20 plants along a W shaped path. At least 1000 fruits were collected on surviving wild poinsettia plants located in each soybean field. Wild poinsettia fruits were stored at room temperature inside paper bags, and when completely ripe, the seeds were stored at room temperature. After fruit/seed collection, farmers were asked a series of management questions, presented on Table 4. Questionnaire answers were converted to percentage in relation to the total of respondents per state, and averaged for both states.

In August of each year, about 15 wild poinsettia seeds were planted in 300 cm^{-3}pots. Seeds from a known susceptible population were planted as a control. Each biotype was planted with two replicates. The potting mix consisted of 33% soil, 33% sand and 33% peat. When most plants were at the four-leaf stage, imazethapyr was sprayed at 200 g ha^{-1}, with a nonil-phenol adjuvant (0.2% v/v) using a hand-held sprayer delivering a total (two passes) of 220 L ha^{-1} at 200 kPa. This herbicide application rate was twice the maximum labeled rate. Only populations with plants surviving both replicates were documented as resistant.

RESULTS AND DISCUSSION

Weed control information. Seed collected from all sites demonstrated resistance to the ALS inhibitor tested, with very few susceptible plants in each biotype. As the sampled region was randomly distributed around the soybean growing areas in Paraná and Rio Grande do Sul state, respectively (Figure 1), these results

suggests *E. heterophylla* ALS-resistant biotypes are distributed throughout the soybean area of southern Brazil.

For both states, the majority of the samples (71%) were collected in areas where the farmer suspected weed resistance for two or more years (Table 5). This is not surprising because ALS-inhibiting herbicides have been used in the region for at least 15 years and a rapid increase in the magnitude of herbicide-resistance problems is expected in areas where few alternative herbicide mode of action are available (Heap & Lebaron, 2001).

The predominant tillage system in the sampled area was the no-till. Overall for both states, 68% of the farms were using no-tillage for 5 or more years (Table 6). A no-tillage system makes farmers more dependent on herbicides and less likely to adopt other weed control strategies, thus increasing the selection pressure of the chemicals used for the vegetation management (Owen, 2001).

Table 6 - Sites (5), by state, according to tillage system in areas sampled for *Euphorbia heterophylla* resistance to herbicides, and number of years in the no-tillage system

Tillage system	State		Average
	Paraná	Rio Grande do Sul	
No-tillage	100	91	95
Conventional	0	9	4
Years in no-till			
< 4 years	24	10	17
5 – 8 years	55	48	51
> 8 years	21	42	31

Table 4 - Questionnaire assessed for each *Euphorbia heterophylla* population sampled

Property information			
Property name:	Size of farm:		Phone number:
Farmer name:	County:		State:
Soil Preparation system (no-till/conventional):		Number of years in this system:	
Weed control information			
Number of years suspected weed resistance to herbicides:		Size of problem area:	
Last herbicide used:		Rate:	Surfactants:
Other herbicides used in the season:		Rate:	Surfactants:
Herbicides used last winter:		Herbicides used last summer:	
Herbicides used 2 winters ago:		Herbicides used 2 summers ago:	
Herbicides used 3 winters ago:		Herbicides used 3 summers ago:	
Crop rotation information			
Crop planted last winter:		Crop planted last summer:	
Crop planted 1 winter ago:		Crop planted 2 summer ago:	
Crop planted 2 winters ago:		Crop planted 3 summers ago:	
Harvesting information			
Owns equipment (yes/no):	Harvest for neighbors (yes/no):	Neighbors have weed resistance (yes/no):	

Table 5 - Sites (%), by state, according to the time of suspecting resistance of *Euphorbia heterophylla* to ALS herbicides.

Time	State		Average
	Paraná	Rio Grande do Sul	
The year of sampling	15	11	13
One year before sampling	13	17	15
Two years before sampling	33	26	29
Three years before sampling	39	46	42

The mechanism of action of the herbicides used on the seed sampling year was mainly (68%) ALS inhibitors. For both years before the time of *E. heterophylla* sampling, ALS inhibitors were also the predominant mode of action used (Table 7). Several factors modify the rate of evolution of herbicide resistance in a region; however the most important factors controlling this phenomenon are the intensity of herbicide use and the initial frequency of the herbicide resistant gene (Maxwell & Mortlmer, 1994; Winkler et al., 2002). The initial frequency of ALS-resistant cells in *Nicotiana tabacum* cell culture was 10^{-8} (Harms & Dimaio, 1991) and in *Gossipium hirsutum* cell culture was 10^{-7} (Rajasekaran et al., 1996), implying that in a few generations of the constant use of an ALS-inhibiting resistant biotypes would dominate a weed community (Maxwell & Mortlmer, 1994). However, recent research indicates that in *Lolium rigidum* populations never sprayed with ALS-inhibiting herbicides, the initial frequency of individuals resistant to this group of herbicides can be as high as 10^{-4} (Preston & Powles, 2002), explaining the appearance of resistant biotypes with only 3 years of constant use of herbicides from this group (Powles et al., 1997).

There was a trend of increased use of PROTOX inhibitors with time. In fact, only 5% of sites used herbicides with this mode of action two years before seed sampling and 17% at the time of sampling. Likewise, for both states, the use of mixtures of herbicides *for E. heterophylla* control followed an increasing trend from 5% of sites using compounds with several mechanisms of action two years before seed sampling to 13% at the time of sampling (Table 7).

In the state of Rio Grande do Sul, there was a trend of higher use of herbicide mixture from different mechanisms of action, when compared to Paraná state. This may be, in part, a consequence of severe law enforcement in Paraná. In Brazil, against all biological and agronomical logics, the pesticide law forbids the mixture of herbicides and the state of Paraná is known to strongly enforce this law. We speculate the lack of mixture of herbicide from different mechanisms of action may have severe negative consequences for the selection of herbicide resistant biotypes. In fact, Trezzi et al. (2004) documented the first case of multiple herbicide resistance in Brazil, in Paraná state, in the species *Euphorbia heterophylla*.

The trend of increased use of herbicides from alternative mechanism of action, or increased use of herbicide mixture, for *E. heterophylla* control is in agreement with the results from Table 5, that demonstrated the farmers suspected ALS resistant biotypes to be present on the area for a couple of years before sampling and probably were advised to adopt alternative herbicides to control the ALS-resistant biotypes.

Table 7 - Herbicide use (%), organized by mechanism of action and state, in relation to the time of sampling for seeds for *Euphorbia heterophylla* resistance to ALS herbicides

Time of seed sampling	MoA*	State		Average
		Paraná	Rio Grande do Sul	
Year of seed sampling	ALS	78	58	68
	PROTOX	15	20	17
	MIX	5	22	13
One year before	ALS	61	65	63
	PROTOX	5	18	11
	MIX	8	10	9
Two years before	ALS	88	82	85
	PROTOX	5	6	5
	MIX	4	6	5

* MoA = mechanism of action; ALS = herbicides inhibitors of the acetolactate synthase enzyme; PROTOX = inhibitors of protoporphyrinogen oxidase enzyme; MIX = mixture of herbicides from these two MoA.

Another supporting evidence that the farmers encountered herbicide resistance prior the sampling date is the trend of increased need of a second herbicide for *E. heterophylla* control, starting from 59% of the sites two years before the sampling up to 71% of the sites at the sampling year (Table 8). Unfortunately, overall for both states, 48 to 62% of the second herbicide chosen to complement the weed control was ALS inhibitors (Table 8). This result suggests the need to improve farmer education on herbicide mode of action and its rotation as strategy to minimize the selection of herbicide resistant plants.

Crop rotation and equipment information. On average, the majority of the sampled sites were cultivated with soybean crop on the assessment year or on the years preceding it. There was a trend of increased use of crop rotation in Rio Grande do Sul than in Paraná (Table 9). The weed communities that have developed in soybean monocrop tend to have a large seedbank of some species due to limited options of alternative herbicides available for their control (Owen, 2001). With a large weed seed bank, higher weed densities are expected in these areas, which predispose these sites to an increased probability of selecting for a resistant biotype (Jasieniuk et al., 1996; Vidal & Fleck, 1997).

For both states, the majority of the farmers (81%) where the samples were collected own the combine used to harvest the crop (Table 10). Of these, only 34% harvest for neighbors (Table 10). On average, 63% of the

Table 8 - Use (%) of a second herbicide in the season, by state, in relation to the time of sampling for seeds for *Euphorbia heterophylla* resistance to ALS herbicides

Time of seed sampling	State		Average
	Paraná	Rio Grande do Sul	
Year of seed sampling	82	60	71
(ALS)*	(63)	(34)	(48)
One year before	67	65	66
(ALS)	(73)	(52)	(62)
Two years before	63	55	59
(ALS)	(62)	(36)	(49)

* Number in parenthesis represents percentage of use of herbicides inhibitors of the acetolactate synthase enzyme, within the result above it.

Table 9 - Sites (%) with soybean crop, by state, according to the time of sampling for seeds of *Euphorbia heterophylla* ALS resistant

Time	State		Average
	Paraná	Rio Grande do Sul	
The year of sampling	91	64	77
One year before sampling	89	77	83
Two years before sampling	89	74	81

Table 10 - Sites (%), by state, where farmers own combine or harvest for neighbors

Combine ownership	State		Average
	Paraná	Rio Grande do Sul	
Owns combine: Yes	72	91	81
No	28	9	19
Combine use			
Harvest for neighbors: Yes	40	27	34
No	60	73	66

neighbors from farmers that harvest for them have resistant *E. heterophylla,* however this result is not significantly different from the ones without resistance (Table 11). This result suggests the use of shared combine is not responsible for the spread of weed resistance. In fact, by the time of soybean harvest, seeds on *E. heterophylla* plants have dehisced (Kissmann & Groth, 1992) and none are expected to be harvested by the combine.

This study demonstrates that ALS resistant *E. heterophylla* biotypes are widely distributed in southern Brazil. The analysis of questionnaire data suggests the dissemination of seeds from resistant plants is unlikely to be the explanation for the widespread distribution of resistance. The primary factor contributing to the selection of the ALS resistant biotype probably was the high use of ALS-inhibiting herbicides in years preceding our sampling. The results suggest the need to educate farmers on the importance of rotation of herbicides of different mode of action and the importance of crop rotation to prevent selecting resistant biotypes. Indirect evidence is presented demonstrating the need to educate legislators about the importance of herbicide mixtures as a strategy to prevent herbicide resistance. More studies should be done on integrated weed management in the no-tillage systems to minimize its limitation on weed control methods.

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