RESEARCH ARTICLE

Journal of Research in Weed Science 1 (2) (2018) 110-122

Journal of Research in Weed Science

Journal Homepage: www.jrweedsci.com

The occurrence of resistance to ALS and ACCase-inhibiting herbicides in ryegrass (*Lolium rigidum* Gaudin) in Bizerte region

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Accepted: 17 November 2018

Abstract

Ryegrass (*Lolium rigidum* Gaudin) is a most prevalent weed in Bizerte region and caused losses in yield. Farmers used herbicides to control ryegrass in cereal crops. Thirteen herbicides (ALS inhibitors, ACCase inhibitors and PSII inhibitors) were used in the field experiment to evaluate their efficacy and their effect on yields of wheat and of ryegrass. The trial was installed in Randomized Complete Block Design (RCBD) in Bizerte. Seeds of ryegrass collected from Bizerte during surveys were the subject of the resistance detection to the ALS inhibitor herbicides [AmilcarWG® (mesosulfuron+iodosulfuron)] and to the ACCase inhibitor herbicides [Topik® (clodinafop-propagyl)] by a pot experiment method. The results of the field experiment showed that only Tolurex® [Chlortoluron (PSII inhibitor)] has the best efficacy (83.6%), but all other herbicides (ALS and ACCase inhibitors) have insufficient efficacy on ryegrass. Then, over 60% of ryegrass populations were escaped to treatments ACCase and ALS-inhibiting herbicides that are not statistically different. The low efficacy of herbicides (ALS and ACCase inhibitors) was not explained by a high density of ryegrass, but by the presence of herbicide resistance in ryegrass populations in Bizerte. These results were confirmed by the pot experiment method. The count of the surviving plants of ryegrass (30 DAT) in pot experiment method revealed that 70% of ryegrass populations are resistant to both herbicides inhibitors (ACCase and ALS). Similarly, the percentage of reduction in fresh weight of ryegrass, compared to untreated control, showed over 80% of the ryegrass populations was resistant to ACCase inhibitor herbicides and to ALS inhibitor herbicides.

Keywords: Herbicides, Weed management, Rice

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1. Introduction

Since the fifties, chemical weed control in crops has been widely used and agriculture has become highly dependent on the herbicides. However, environmental pressures against the systematic use of herbicides may encourage farmers to choose a different approach to control weeds (Liebman and Davis, 2000). In addition, the systematic use of herbicides (Hole and Powles 1997), sometimes at lower rates (Gasquez, 2005; Neve and Powles, 2005), leads to the selection of weed populations resistant to herbicides, 254 species weeds have developed resistance to one or more herbicides (Heap, 2018). Ryegrass (*Lolium* spp.) is one of the weeds has developed resistance against several grass herbicides products in different countries of the world including France, England, Spain, the USA, Australia and Tunisia. The number of populations of ryegrass keeps growing and poses a major problem for the control of this weed in cereal systems. Today ryegrass throughout the world presented a resistance to all graminicides and also glyphosate (Heap, 2009). However, ryegrass presented resistance to herbicides of different chemical families. For example, in Australia, the ACCase inhibitors herbicides (Heap and Knight, 1986), the ALS inhibitors herbicides (Burnet et al. 1994) and the glycine inhibitor herbicides (Powles et al. 1998). In Tunisia, the first case of resistance to the ACCase inhibitors herbicides in populations of ryegrass (*Lolium* sp.) was reported in 1996. Other studies conducted subsequently was also reported the presence of ryegrass resistant to ACCase inhibitor herbicides (Gasquez, 2000; Souissi et al. 2004). The control of resistant weed was based essentially on understanding the population dynamics. Similarly, different approaches have been suggested to reduce the use of herbicides. However, the most promising practical alternative to herbicides is the integrated weed management (Buhler, 2002). Many studies had been focused on the effects of cultural practices on the dynamics and management of weed species (Wilson, 1981; Gill and Holmes, 1997; Widderick et al. 2002). In the case of ryegrass (*Lolium rigidum*), an important annual grass weeds especially cereals, choice of control strategies were based on information on the biology of weeds and the effects of cultural practices to limit the development of resistance in this annual weed. This study aims to i) evaluate the efficacy of herbicides and their effects on the yield of wheat and the yield of ryegrass and ii) detect the resistance in ryegrass to ALS and ACCase-inhibiting herbicides by the pot experiment method.

2. Materials and Methods

2.1. Evaluation of the efficacy of herbicides on populations of ryegrass

Field trial experiments were carried out during the cropping season 2011-12 in the farm of Fritissa at the region of Bizerte, in a field naturally infested with rigid ryegrass. The soil was a clay-loam with a pH of 8.5 and 2.3% of organic matter. The seedbed was moldboard plowed and land leveled prior to crop sowing. Durum wheat cv. Karim was sown at December 12th, 2011 and fertilized with DAP (Di-Ammonium-Phosphate) at the rate of

100 kg/ha two weeks before planting and ammonium nitrate was fractioned at the 2-3 leaf stage (100 kg/ha), end of tillering (150 kg/ha) and stem extension (50 kg/ha). The experiment was performed according to a randomized complete block design (RCBD) with thirteen herbicide treatments and four replications. The experimental unit was 40 m² (4 m × 10 m). Herbicides have different sites of action (5 herbicides are ALS inhibitors, 7 herbicides are ACCase inhibitors and 1 herbicide is PSII inhibitor) (Table 1). Herbicides were applied at their recommended rates at the 3-leaf stage of ryegrass, with a backpack sprayer calibrated to deliver 200 l/ha at 3 KPa. The Efficacy of tested herbicides was assessed by the percentage of damaged plants ryegrass compared to an untreated control after 30 days after the date of the chemical treatments (DAT).

2.2. Resistance in ryegrass

2.2.1. Seed Collection

A total of 31 ryegrass populations were collected during three successive growing seasons (2012, 2013 and 2014) in wheat fields across Bizerte region (Figure 1). Cereal fields were targeted randomly and ryegrass seed samples were collected at maturity (end of May to end of June) by following a w-shaped path. A global positioning system unit was used to record latitude and longitude for each site. Ninety percent of the surveyed fields were cropped to durum wheat (*Triticum durum* L.) the remaining with barley (*Hordeum vulgare* L.) and small faba bean (*Vicia faba* v. minor). Ryegrass seed collections were subsequently stored at room temperature in the laboratory until use.

2.2.2. Detection of Resistance using pot experiment method

Ryegrass seeds (31 samples) were seeded in pots in open area (Table 2). Ryegrass seeds were sown in pots during the optimum period of sowing of wheat (Mid-November) at 15 seeds/pot. The seeds were sown in pots of 9 L (23 cm diameter) with a substrate composed of peat and soil (1: 2 by volume) and irrigated as needed. The pots were arranged in Fischer block and placed in the open air at natural light and at the ambient temperature. Each dose of the herbicide treatment was replicated twice (two pots) with an untreated control (2 pots) and with a sensitive control (P872) and a resistant control (P2461). After the emergence of ryegrass, manual thinning of plants were executed to have the same number of plants per pot (10 plants/pot). At stage 2-3 leaves of ryegrass, as scale Zadoks et al. (1974), tow herbicides [Topik® (ACCase inhibitors) and Amilcar WG®(ALS inhibitors)] were applied at the registered doses (0.5 liters/ha and 330 g/ha) with a sprayer with flat fan nozzle tips delivering 200 L/ha at 2 kPa. Thirty days after the application of herbicides (DAT), surviving plants (not damaged by herbicide) was counted resistance classification was carried out according to the scale adopted by Llewellyn and Powels (2001) [Sensitive (S) : = 0%, Development of Resistance (DR) : 1-20% et Resistance (R) : > 20%]. Then, all plants of each treatment were cut after 30 DAT at the soil surface and were weighed to determine the fresh weight (biomass) of the each population. The percent of the reduction in the fresh weight of ryegrass was calculated, relative to untreated control, for each population and the classification of resistance "R" has been described by Moss et al. (1999) [fresh weight <40%, Resistance confirmed (RRR), fresh weight between 40% and 80%, Resistance confirmed (RR), fresh weight between 80% and 90%, Development of Resistance (DR), fresh weight > 90% sensitive (S)].

2.3. Statistical Analysis

Experimental data were subjected to analysis of variance (ANOVA) using the statistical package SAS® (SAS, 1985). When F-values were significant at the P = 0.05 level, the means were compared using Fisher's least significant difference (LSD test).

3. Results and Discussion

3.1. Evaluation of the efficacy of herbicides on ryegrass

Statistical analysis revealed significant differences in the efficacy of the thirteen tested herbicides (p= 0.0067). The results showed that only one of tested herbicide treatments had satisfactory controlled rigid ryegrass in field trials. This herbicide was Tolurex (Clortoluron), which has a mode of action (PSII inhibitor) different from the twelve other herbicides (ALS inhibitors and ACCase inhibitors). Tolurex showed satisfactory efficacy (83.6%) on ryegrass. However, all other herbicides (Amilcar OD, Amilcar WG, Apyros, Evrest, Pallas OD, Axial, Dopler plus, Grasp, Illoxan CE, Puma Evolution OD, Topik, Traxos) showed insufficient efficacy on ryegrass. Herbicide ALS inhibitors and Herbicide ACCase inhibitors efficacy varied from 28 to 56% with an average of 41.5%. With these herbicides (ALS inhibitors and ACCase inhibitors), about 60% of the ryegrass populations have escaped herbicide treatments (Fig. 2). Ryegrass (*Lolium rigidum*) is a very common weed in Tunisia that cause significant damage to cereals in terms of yield. Ryegrass has caused problems for farmers, especially in the northern areas of Tunisia (Bizerte). The ryegrass infesting over 480,000 hectares (32% of the cereal cropped areas) with densities ranging from 0 to more than 600 plants/m² (Khammassi et al. 2016). The region of Bizerte was characterized by the abundance of rainfall are among the main factors that can contribute to the development of ryegrass and therefore serious infestations with this weed. Furthermore, the absence of crop rotation, the succession of winter cereals, simplified tillage techniques, late weeding and abundant rainfall rains favored development and infestation with ryegrass (Khammassi et al. 2013). The control of ryegrass in cereal crops is the key to cereal success which improves grain yields (Lumb and McPherson, 1964; Reeves and Tuohey, 1972; Reeves and Smith, 1975). This success linked to the chemical control of ryegrass encouraged farmers to use effective herbicides, sometimes at low doses (Gasquez, 2005; Neve and Powles, 2005). In the field experiment, our study showed that several populations of ryegrass (60%) ryegrass were escaped herbicide inhibiting ACCase and herbicide inhibiting ALS. So, these herbicides, commonly used by farmers for grass weed control in wheat, did not provide a satisfactory control of rigid ryegrass. On the other hand, herbicide (Chlortoluron) with another site of action (PSII inhibitor) controlled the ray grass well and showed satisfactory efficacy on ryegrass. Factors influencing herbicide efficacy have been documented. These include weed and crop densities (Radford et al. 1980; Scursoni *et al*. 2012), which can further be influenced by other factors such as soil type, period of weed emergence and weed growth stage (Jensen, 1985; O'Donovan et al. 1985). Bizerte region is known by high density with ryegrass which can reach 600 plants/m² (Khammassi et al. 2016). Although, the experimental field at Fritissa, belonging to Bizerte region, was highly infested with rigid ryegrass, but low herbicide efficacies could not be attributed to the high weed densities. Even with a high density of ryegrass, chlortoluron has shown good efficacy on ryegrass.

3.2. Detection of resistance in ryegrass populations

3.2.1. Evaluation of the surviving plants of ryegrass populations

The results of the count of the surviving plants of ryegrass from pot experiment method after 30 DAT have shown that 70.1% of ryegrass populations are resistant to ACCase inhibitor herbicides against 76.4% to ALS inhibitor herbicides. However, over 70% of the ryegrass population survived for both herbicide treatments [Topik® (ACCase inhibitors) and Amilcar WG® (ALS inhibitors)]. The ryegrass populations that have developed resistance (DR), they have only 15.1% and 12.8%, respectively, for ACCase and ALS inhibitors. Percentages of susceptible populations are almost identical for ACCase inhibitor herbicides (14.8%) and ALS (10.8%) (Table 3). In the region of Bizerte, herbicides have been the primary weed control mean in wheat-based cropping systems for several years. Likewise, the increase in cultural techniques favorable to increased ryegrass populations among Tunisian farmers was accompanied by the application programs of weeding based on the almost systematic and repeated use of grass herbicides. The repeated use of these herbicides has probably resulted in the evolution of herbicide resistance. These results were confirmed by the results of the surviving plants of ryegrass populations from a pot experiment method which showed that over 70 % of the populations of ryegrass tested are resistant to both herbicides inhibitors [Topik® (ACCase inhibitors) and Amilcar WG® (ALS inhibitors)]. These results are also confirmed by the work of Gasquez (2000) and Souissi et al. (2004) that reported in 1996 the presence of the first cases of resistance to ACCase inhibitors in ryegrass populations collected from the north of Tunisia.

3.2.2. Evaluation of the fresh weight of ryegrass populations

Results of a percent reduction in fresh weight of ryegrass populations compared to untreated control showed that 81.5% were resistant to ACCase inhibitor herbicides and 86.4% were resistant to ALS inhibitor herbicides. However, over 80% of the ryegrass population survived for both herbicide treatments [Topik® (ACCase inhibitors) and Amilcar WG® (ALS inhibitors)]. The ryegrass populations that have developed resistance (DR), they have only 11.4% and 8.5%, respectively, for ACCase and ALS inhibitors. The sensitive populations are of the order of 7.1% and 5% respectively for ACCase and ALS (Table 4). However, over 80% of the ryegrass populations were resistant to ACCase inhibitor herbicides and to ALS inhibitor herbicides. Studies by Gasquez (2000) and by

Souissi et al. (2004) showed resistance in ryegrass to ACCase inhibitor herbicides. Other studies have also shown resistance to ALS inhibitor herbicides (Beldi, 2005). Likewise, Manchari et al. (2009) were confirmed the presence of resistance to ACCase inhibitors in Lolium and Phalaris genres. Worldwide, there are currently 494 unique cases of herbicide resistant weeds globally, with 254 species (148 dicots and 106 monocots). Weeds have evolved resistance to 23 of the 26 known herbicide sites of action and to 163 different herbicides. Herbicide resistant weeds have been reported in 92 crops in 70 countries (Heap 2018). Rigid ryegrass is among the top 10 weeds which have evolved resistance to many herbicides with different modes of action. Resistance to the ACCase and ALS inhibiting herbicides and in some cases the cross or multiple resistance patterns are documented from the main cereal cropping regions worldwide (Heap and Knight, 1986; Powles and Matthews, 1992; Preston et al. 2009; Owen et al. 2014). In Australia, ryegrass populations collected from the Western wheat belt have evolved high levels of resistance to commonly used herbicides (Llewellyn and Powles, 2001; Owen et al. 2007). Our study showed resistance on ryegrass populations collected from Bizerte was detected based on the use of field experiments and the pot experiments by the count of the surviving plants of the ryegrass (Llewellyn and Powels, 2001) and by the determination of the percentage of reduction of the fresh weight of ryegrass (Moss et al. 1999). These results demonstrated that resistant ryegrass populations are more common than susceptible populations in Bizerte. Considering the impact of this resistance on cereal production, the pot experiment method could be a very useful management tool for Tunisian growers to rapidly detect resistant populations and to take rapid decisions for the implementation of appropriate weed management options in the region.

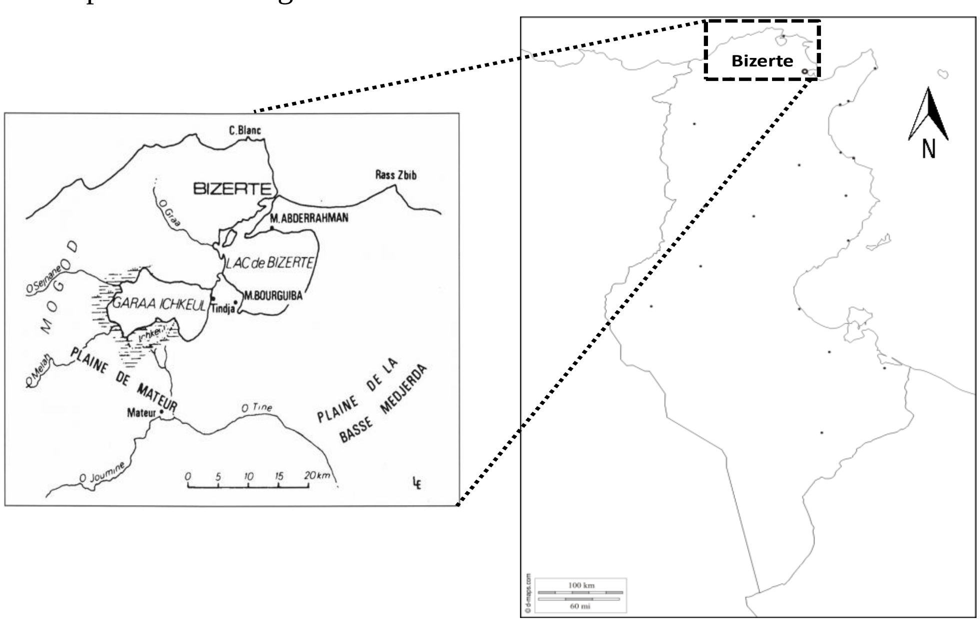

Figure 1- Prospected area for the collection of ryegrass seeds at Bizerte region.

Table 1- List of herbicides used in field trials.

Site of action	Trade name	Active substance	Concent. (a.i.)	Recommanded rate (ha^{-1})
ALS inhibitors	Amilcar OD	Mesosulfuron-methyl+iodosulfuron-methyl-sodium	7.5 g/l+7.5 g/l	1 l
	Amilcar WG	Mesosulfuron-methyl+iodosulfuron-	30 g/kg+30 g/kg	330 g
	Apyros	Sulfosulfuron	75 %	26.6 g
	Evrest	Flucarbazone sodium	70 %	43 g
	Pallas OD	Pyroxsualm	46.6 g/l	0.5 l
ACCase inhibitors	Axial	Pinoxaden	45 g/l	1 l
	Dopler plus	diclofop-méthyl+fénoxapropo-p-ethyl	250 g/l + 20 g/l	2.5 l
	Grasp	Tralkoxydim		0.8 l
	Illoxan CE	Diclofop-methyl	360 g/l	2 l
	Puma Evolution OD	Fenoxaprop-P-ethyl+ iodosulfuron-methyl-sodium	64 g/l + 8 g/l	1 l
	Topik	Clodinafop-propargyl	100 g/l	0.5 l
	Traxos	Pinoxaden+ Clodinafop-propargyl	22.5 g/l+22.5 g/l	1.2 l
PSII inhibitor (Ureas and amides)	Tolurex	Clortoluron	500 g/l	4.8 l

Table 2- Origin of the collected ryegrass populations.

Region	Origin of ryegrass seed	Number of Population
Bizerte	Bizerte sud	10
	Mateur	09
	Ghazala	04
	Tinja	01
	Menzel bourguiba	01
	Utique	06
	Total	31

Table 3- Distribution of ryegrass populations under each class of resistance according to the surviving plants of ryegrass populations from pot experiment method.

Class of resistance	Percentage (%) of ryegrass population	
	ACCase	ALS
S	14.8	10.8
DR	15,1	12.8
R	70,1	76,4
Total	100,0	100,0

Classification according to Llewellyn et Powels (2001) [Sensitive (S) : 0%, Development of Resistance (DR) : 1-20% et Resistance (R) : > 20%].

Table 4- Distribution of ryegrass populations under each class of resistance according to the fresh weight of ryegrass populations from the pot experiment method.

Class of resistance	Percentage of population	
	ACCase	ALS
S	07.1	05.0
DR	11.4	08.5
R (RRR+RR)	81.5	86.4
Total	100.0	100.0

Classification according to Moss et al, (1999) [fresh weight <40%, Resistance confirmed (RRR), fresh weight between 40% and 80%, Resistance confirmed (RR), fresh weight between 80% and 90%, Development of Resistance (DR), fresh weight > 90% sensitive (S)].

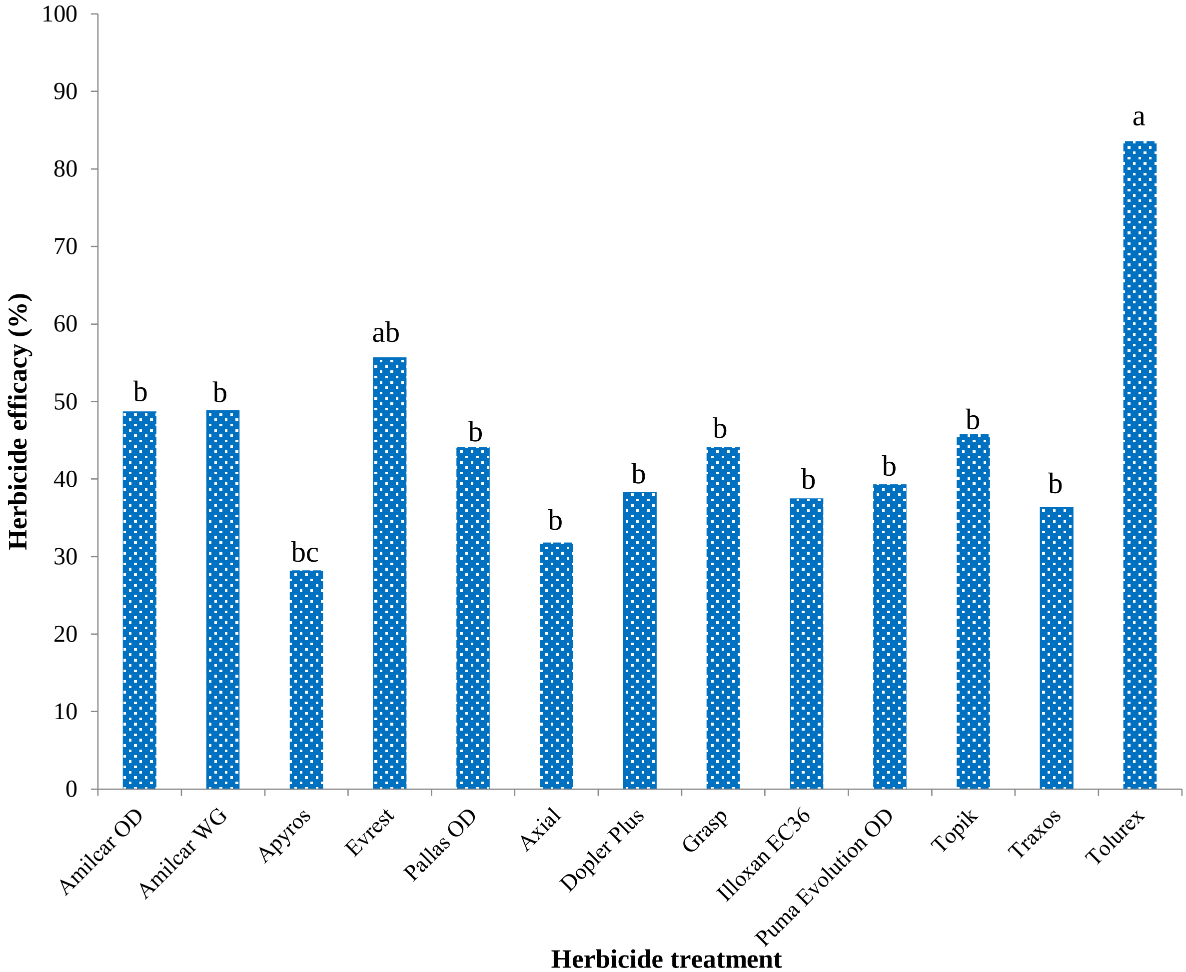

Figure 2- Efficacy of herbicides on ryegrass in the field farm of Fritissa at Bizerte region.

Conclusion

In the field at Fritissa, belonging to Bizerte region (north of Tunisia), the ACCase and ALS inhibiting herbicides showed low and unsatisfactory efficiencies for the control of ryegrass (*Lolium rigidum*). But PSII inhibiting herbicides have the best efficacy in this weed. This is explained by the development of resistance on ryegrass.

Ryegrass seeds collected from Bizerte region seeded in a pot experiment showed that over 70% of the populations of ryegrass are surviving to the ACCase and ALS inhibiting herbicides. The reduction in fresh weight of ryegrass populations compared to untreated control showed over 80% of the rye-grass populations were resistant to both herbicides. The resistance in populations of ryegrass (*Lolium rigidum*) was confirmed by the field experiment, the pot experiment and by the reduction in fresh weight. This can lead to control problems and also to an increase in production costs. The control of the ryegrass in cereals should therefore be conducted by integrated management methods (crop rotation,

rotation of herbicide with different modes of action, tillage, sowing date and sowing density).

Acknowledgements

We thank the National Institute of Field Crops, Syngenta Agroscience and Bioprotection for participation and financing of this work. We also thank all the farmers of the areas surveyed and Mrs Ahlem DRIDI (Weed Science Laboratory INAT).

Conflict of interest

Authors declare no conflicts of interest for this study.

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Cite this article as:

Khammassi M, Chaabane H, Souissi T. 2018. The occurrence of resistance to ALS and ACCase-inhibiting herbicides in ryegrass (*Lolium rigidum* Gaudin) in Bizerte region. Journal of Research in Weed Science. 1(2): 110-122.