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Current status in herbicide resistance in *Lolium rigidum* in winter cereal fields in Spain: Evolution of resistance 12 years after

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ABSTRACT

Lolium rigidum Gaud. is the most prevalent and damaging grass weed of winter cereals in Spain. *L. rigidum* infestations are frequently treated with herbicides and, consequently, populations have evolved resistance. In 2012–2013 a random survey was conducted across cereal cropping areas of the Castilla-León and Cataluña regions to establish the distribution and frequency of herbicide resistance in *L. rigidum* populations to chlortoluron (Photosystem II inhibitor), chlorsulfuron (Acetolactate synthase inhibitor) and diclofop-methyl (Acetyl CoA Carboxylase inhibitor), commonly used herbicides for *L. rigidum* control in Spain. The results of this survey were compared with the results of a previous survey conducted in 2000–02. Resistance to PSII and ALS-inhibiting herbicides was common: 51% and 92% of the accessions collected from Castilla-León and Cataluña respectively were resistant to chlortoluron, while 75% of accessions from both regions were resistant to chlorsulfuron. Resistance to ACCase was more widespread in Cataluña, where 83% of accessions were classified as resistant, than in Castilla-León where 74% of the populations were still classified as susceptible to diclofop-methyl. These results show that resistance levels to all three herbicides had increased in Castilla-León since 2000 and to chlortoluron and chlorsulfuron in Cataluña. The accessions were also treated with the double dose (2X) of each herbicide. The percentage of *L. rigidum* that now exhibits multiple herbicide resistance has increased considerably, especially in Cataluña where 75% of the accessions were resistant to multiple herbicides, therefore herbicide sustainability and resistance management present a great challenge.
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1. Introduction

The appearance of herbicide resistance is a major concern in agriculture because resistance alters weed control based on herbicides and makes weed control more difficult and more expensive. Weeds adapt to the repeated use of herbicides through the selection of resistance mechanisms that allow the survival of resistant plants. The evolution of herbicide resistance depends on several factors such as the intensity of selection pressure, the biology of weed species and various genetic factors including the frequency of resistance alleles in weed populations, mode of inheritance of resistance and fitness costs associated with resistance. *Lolium rigidum* Gaud. is a native Mediterranean species and is the most important *Lolium* species in Spain where it is commonly found as a major weed in winter cereal crops (Recasens et al., 1996; González-Andujar and Saavedra, 2003). *L. rigidum* exhibits several ecological factors such as high genetic variability, plasticity, fecundity and seed survival which have contributed to its success as a major grass weed (Gill, 1996). For years, it has been controlled by various pre- or post-emergence herbicides with different mechanisms of action such as of acetyl CoA carboxylase (ACCase) inhibitors (group A), acetolactate synthase (ALS) inhibitors (group B), and urea/amide photosynthetic inhibitors (group C). HRAC (Herbicide Resistance Action Committee) mode of action (MOA) group classifications are used. The excellent efficacy of these herbicides encouraged their widespread repeated use in several countries. This selection pressure led *L. rigidum* to become a major problem to evolve resistance to several herbicides (Powles and Yu, 2010). To date, *L. rigidum* has evolved resistance to 15 herbicide modes of action in 12 countries

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(Heap, 2017). Surveys conducted in Australia (Owen et al., 2007, 2014; Malone et al., 2014), USA (Rauch et al., 2010) and Spain (Loureiro et al., 2010) have revealed widespread occurrence of herbicide-resistant *Lolium* species, with biotypes resistant to almost all herbicides available for its control.

In Spain, the first cases of failures in *L. rigidum* control with diclofop-methyl (fop, ACCase inhibitor) and chlortoluron (substituted urea, PS II inhibitor) were cited in fields from Cataluña (Recasens et al., 1996), while failures in the control with chlorsulfuron were found in the river Duero region in Castilla-León (de la Carrera et al., 1999). A farmer survey conducted in Castilla-León 20 years ago revealed *L. rigidum* control failures in 4.2% of the fields from the region treated with chlortoluron and in 3.3% of those treated with chlorsulfuron (Fernández-García, 1998). By that time, chlortoluron, diclofop-methyl and chlorsulfuron herbicides were the most commonly used active ingredients for *L. rigidum* control in cereals (Taberner, 2001). Previously, a survey of herbicide resistance in *L. rigidum* conducted in 2000–2002 in these two cereal growing regions (Loureiro et al., 2010) demonstrated that herbicide resistance was not widespread, but there were numerous accessions with certain degree of resistance to chlortoluron (4.7% in Castilla-León and 10% in Cataluña), chlorsulfuron (10.5% in Castilla-León and 60% in Cataluña) and also to glyphosate (6.9% in Castilla-León).

This occurrence of resistance has been increasing as a result of the reliance on the same modes of actions. In 2013, a survey conducted on the use of phytosanitary products in Spain revealed that chlortoluron (260,000 ha), chlorsulfuron (220,000 ha) and diclofop-methyl (550,000 ha) were among the most used active ingredients for grass weed control in cereals (wheat and barley), together with others with the same mode of actions as fenoxaprop-ethyl (group A), metsulfuron-methyl, thifensulfuron-methyl, iodosulfuron-methyl and mesosulfuron-methyl (group B) or isoproturon (group C) (MAPAMA, 2013). Currently there are *L. rigidum* populations that are resistant to the herbicides containing active ingredients of the groups A, B and C2 (CPRH, 2015). In some cases biotypes with cross- and multiple-resistances have been detected. This resistance can be conferred by two mechanisms, mutation(s) in the gene encoding the herbicide target, which decreases the affinity of the target for herbicides (TSR, target-site resistance), or by other mutations that enhance herbicide metabolism and cause a reduction in the amount of herbicide reaching the target (NTSR, non-target site resistance) (Tranel and Wright, 2002; Délye, 2005, 2013). Both mechanisms occur alone or together creating complex genetic linkages for numerous herbicide mode of actions at plant and population level (Petit et al., 2010; Busi et al., 2013). Those situations complicate resistance management and threatens the productivity and sustainability of the cereal farming systems. The problem is even greater considering that no major new site-of-action herbicide has been introduced into the market for more than 20 years (Duke, 2012).

In the present study, a second large-scale random survey of *L. rigidum* across the cereal cropping regions of Castilla-León and Cataluña was conducted to update and quantify the geographical extent and spectrum of herbicide resistance.

2. Materials and methods

2.1. Plant material

Mature seed samples of a total of 89 *L. rigidum* accessions were collected in field surveys of grass weeds conducted annually between 2012 and 2014. The surveys were conducted randomly across the cereal fields (wheat and barley) of several provinces in Castilla-León (Ávila; Burgos; León; Palencia; Salamanca; Segovia; Soria; Valladolid and Zamora) and in the province of Lérida (L) in Cataluña, two of the main cereal areas in Spain (Fig. 1). *L. rigidum* accessions were collected by driving throughout the regions and stopping at least at 5 km intervals (geo-referenced) to sample the nearest cereal field (Loureiro et al., 2010). A representative random sample of spikes from different plants (25–50) of each accession were harvested at maturity from different field patches. Seeds from all of these plants were manually threshed, bulked and stored for several months before being planted.

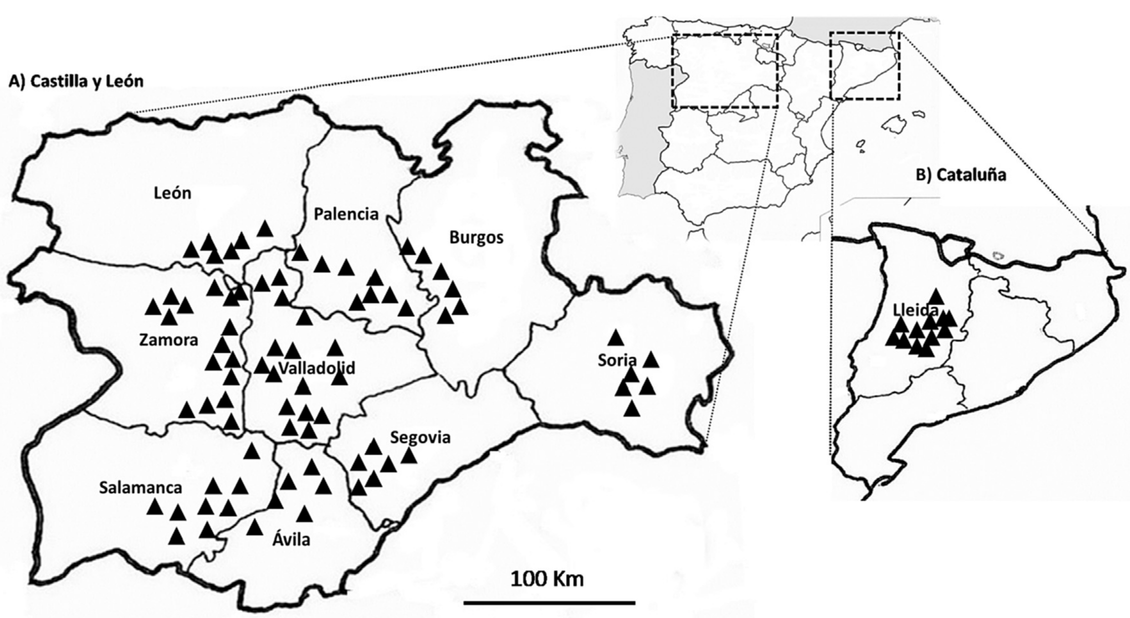

Fig. 1. Geographical origin of the *Lolium rigidum* populations. 77 populations were collected in Castilla-León and 12 in Cataluña (in the province of Lleida). The location of each surveyed population is indicated by a point.

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