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Gene flow increases the initial frequency of herbicide resistance alleles in unselected *Lolium rigidum* populations

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ABSTRACT

In two different locations of the Western Australian "wheatbelt", *Lolium rigidum* (rigid ryegrass) seeds were collected from organic fields (no herbicide use) and neighbouring conventional fields (persistent herbicide use), the latter infested with herbicide-resistant plants, to investigate the occurrence of gene flow among field populations as revealed by herbicide resistance gene transfer. Herbicides targeting acetyl-CoA carboxylase (ACCase) or acetolactate-synthase (ALS) were used to detect herbicide-resistant plants. Overall, the frequency of plants resistant to ACCase- or ALS-inhibiting herbicides was, respectively, 21% and 74% in the conventional fields and 2% and 37% in neighbouring organic fields. Mutant, herbicide-resistant *ACCase* and *ALS* alleles were detected in 16% and 38% of plants from conventional fields and in 0.53% and 3.7% of plants from organic fields. Identical mutant, herbicide-resistant *ALS* haplotypes were detected both in conventional and organic fields, supporting the occurrence of gene flow between *L. rigidum* populations in different fields. Gene flow can thus substantially increase the frequency of herbicide-resistant plants in unselected *L. rigidum* populations. Although gene flow cannot be prevented, it can be limited or managed. Hygiene tactics such as clean crop seed, weed seed removal at harvest and seed destruction post-harvest should be considered in order to minimize gene transfer among farms.

1. Introduction

Weeds can adapt to different environments and evolve strategies to escape control in cultivated crops (Baker, 1974). The evolution of herbicide resistance in modern agro-ecosystems reliant on herbicide use is not only a prominent example of weed adaptation, but is also an increasing problem (Powles and Yu, 2010).

Genetic and bio-ecological factors interplay to determine the dynamics of resistance evolution in weed populations (Warwick, 1991). Herbicide resistance evolution is mainly driven by population genetic principles, and can be considered as a trade-off between the intensity of the herbicide selection, the mutation rate towards resistance alleles and the possible pleiotropic effects decreasing plant fitness (fitness cost) that may be associated with resistance-endowing alleles (Diggle and Neve, 2001; Vila-Aiub et al., 2009). Furthermore, migration of alleles (gene flow) between populations can affect the dynamics of herbicide resistance evolution by connecting plant populations and increasing the initial frequency of resistance alleles in unselected populations (Jasieniuk et al., 1996). In cross-pollinated species with high pollen longevity, pollen is expected to have a greater contribution to resistance dispersal than seeds (Darmency, 1996). However, the impact of gene flow on the initial frequency of resistance in unselected populations is still not well assessed or understood (Jasieniuk et al., 1996). It was suggested that in predominantly self-pollinated plant species, gene flow frequencies should be extremely low (i.e., ranging between 10^{-9} and 10^{-6}), whereas in cross-pollinated species, gene flow frequencies could be as high as 10^{-3} (Levin and Kerster, 1974). Studies have shown pollen-mediated gene flow at low frequency with limited gene migration in self-pollinated crops such as rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.) or weeds such as wild oats (*Avena fatua* L.) (Murray et al., 2002; Gaines et al., 2007b; Rong et al., 2007). Conversely, in cross-pollinated weed species, pollen-mediated flow of herbicide resistance genes can occur over considerable distances at relatively high frequency (Watrud et al., 2004; Zapiola et al., 2007; Beckie and Hall, 2008; Busi et al., 2008).

The intensity of gene exchange between plant populations mainly depends on the plant breeding system (Darmency, 1996), the respective demographic and pollen migration dynamics of donor and recipient populations (Damgaard and Kjellsson, 2005; Délye et al., 2010a), and the distances among populations (Busi et al., 2008). Multiple, independent selection of resistance alleles under persistent herbicide application in geographically heterogeneous environments has been reported in the cross-pollinated weed species *Alopecurus myosuroides* Huds. (Délye et al., 2010b). Thus, at a broad geographical scale, the selection of resistance alleles from within the local existing genetic variation seems to play the most important part in the evolution of herbicide resistance.

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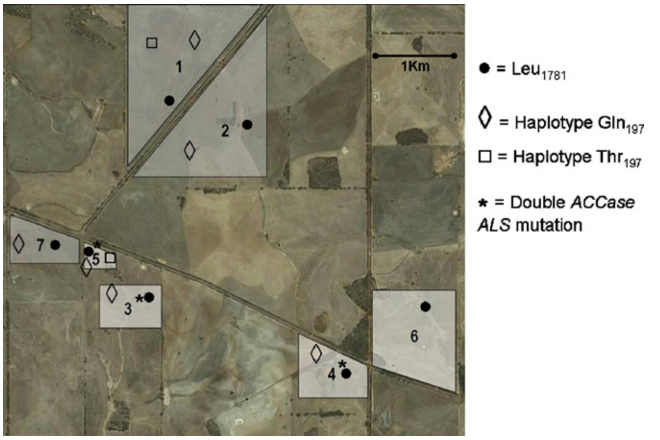

Fig. 1. Aerial Google Earth® photo of the Dumbleyung site showing the sampled conventional (3, 4, 5 and 6 in light grey) and organic fields (1, 2 and 7 in dark grey) and the spatial distribution of resistant *ACCase* and *ALS* alleles. Only resistant alleles and haplotypes found in both conventional and organic fields are shown. Mean field size was 60 ha.

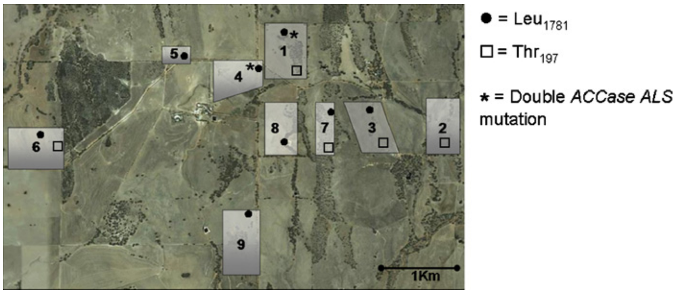

Fig. 2. Aerial Google Earth® photo of the Moora site showing the sampled conventional (2, 4, 5, 6, 7, 8 and 9 in light grey color) and organic fields (1 and 3 in dark grey color) and the spatial distribution of resistant *ACCase* and *ALS* alleles. Only resistant alleles found in both conventional and organic fields are shown. Mean field size was 25 ha.

Yet, at a local farm scale, the movement of resistance alleles by pollen flow has been reported to substantially affect the evolution of resistance (Délye et al., 2010a).

Highly effective herbicides inhibiting key enzymes of fatty acid (acetyl-coenzyme A carboxylase – ACCase) or branched-chain amino acid (acetolactate synthase – ALS) biosynthesis were introduced to world agriculture in 1980 and have been adopted for weed control in major crops worldwide, which has led to the selection of resistance mechanisms in at least 149 species (Heap, 2011). Resistance to ACCase- or ALS-inhibiting herbicides is often conferred by mutant alleles of the *ACCase* or *ALS* gene, respectively. Resistance to each class of herbicides has been characterized in several plant species at the phenotypic, enzymatic and molecular level (Tranel and Wright, 2002; Délye, 2005; Powles and Yu, 2010). In particular, molecular markers to identify resistance-endowing mutant *ACCase* or *ALS* alleles have been developed for several weed species and can provide an excellent tool to investigate the migration of resistance alleles in weed populations (Délye et al., 2010a).

In this study, the frequency of plants resistant to ACCase and ALS inhibitors was assessed in populations of the cross-pollinated grass weed *L. rigidum* Gaud. collected from conventional fields where these herbicides have regularly been used, and from nearby organic fields where herbicides have not been used in the last 20 years, or at all. The aim was to investigate the occurrence of gene flow from *L. rigidum* populations in which resistance genes are present in substantial frequencies, to those growing in neighbouring fields without herbicide selection, and to understand whether gene flow could affect the frequency of herbicide resistance genes in these latter populations.

2. Materials and methods

2.1. Sample collection from conventional and organic fields

Mature seed samples were collected in early summer 2007 (December) as described by Owen et al. (2007) from two locations approximately 430 km apart in the Western Australian wheat belt: Dumbleyung (33°18′38.11″S, 117°49′11.79″E) and Moora (30°33′55.47″S, 116°3′52.41″E). The mean field size was 60 ha in Dumbleyung (Fig. 1) and 25 ha in Moora (Fig. 2). *L. rigidum* seed samples were collected from 11 fields under conventional farming with intensive use of herbicides (source populations for resistance alleles) and from five fields where organic crops have been grown without herbicide use for at least 20 years (recipient populations for resistance alleles) (see Table 1). In Dumbleyung seeds from organic recipient fields were collected both from field margins and the centre. In each location seed samples were collected from organic receptor fields of one organic farm (i.e. managed by one grower), whereas conventional source fields were part of different conventional farms (Figs. 1 and 2). A total of 20 seed samples (12 from conventional and 8 from organic fields, Table 1) were collected. Each seed sample consisted of 100 *L. rigidum* spikes (one spike/plant). Each seed sample was assigned with a specific code referring to the site, the field number on our sampling list, the position of collection point within the field, and the management strategy used (Table 1). Geographical coordinates for each seed sample were recorded by a GPS.

2.2. Identification of herbicide-resistant plants

In 2008 during the winter growing period (May–August), seedlings from all the samples collected from the Moora and Dumbleyung fields were simultaneously tested for resistance to ACCase- or ALS-inhibiting herbicides. As resistance to herbicides can be due to mutation(s) at the herbicide target site and/or to other genes causing a reduction in the amount of herbicide reaching its target, in particular by causing enhanced herbicide metabolism by the plant (Délye, 2005; Powles and Yu, 2010), screening for resistant plants was conducted with two types of herbicides. Diclofop-methyl (an ACCase inhibitor) and chlorsulfuron (an ALS inhibitor) can be metabolized by *L. rigidum* plants (Christopher et al., 1992) and were used to assess the total frequency of resistant plants. Sethoxydim and sulfometuron are respectively ACCase and ALS inhibitors for which metabolism-based resistance has never been reported, and survival of treatment with these herbicides is thus likely due to the occurrence of mutant alleles.

Plants were grown in pots containing a standard potting mixture (50% sand, 30% peat moss and 20% pine bark). Plants were kept in an outdoor environment simulating field conditions well watered and fertilized. A total of 7609 plants were treated with herbicides to identify resistant individuals. Plants from each collection point were treated at the two-leaf stage with the label rate of one of the following herbicides: diclofop-methyl at 375 g ha^{-1} (Hoegrass 50% EC, Bayer CropScience), sethoxydim at 93 g ha^{-1} (Sertin 18.6% EC, Bayer CropScience), chlorsulfuron at 20 g ha^{-1} (75% WDG, 4Farmers Ltd.) or sulfometuron at 20 g ha^{-1} (Oust 75% WDG, Dupont). Plants from the well-characterized *L. rigidum* population VLR1 are susceptible to all herbicides. This population was included in all