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Evolution of a herbicide-resistant population of *Alopecurus myosuroides* Huds. in a long-term cropping system experiment

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A B S T R A C T

Due to heavy reliance on herbicides and a lack of cultural control measures, herbicide-resistant populations of *Alopecurus myosuroides* Huds. (blackgrass) appeared in the early 1990s in winter cereal rotations in France and in Europe. The aim of the present study was to analyse the effects of different cropping systems on an aryloxy-phenoxypropionate herbicide-resistant population in a field trial. Two crop rotations, one consisting exclusively of winter crops and another including spring crops, were assessed over a six-year period. The rotations were combined with different cultural practices including mouldboard ploughing, delayed sowing, and efficient herbicides for controlling resistant plants. *A. myosuroides* densities decreased in all the cropping systems, but the weed management was most effective when herbicides were combined with non-chemical practices. Rotation with an alternation of spring and winter crops was the most efficient solution against *A. myosuroides*. Moreover, during the six years, the percentage of resistant plants in different crop rotations was estimated independently of the cropping systems. This proportion did not vary during the six years of the experiment, suggesting that the resistance gene persisted, despite the removal of selection pressure by the aryloxy-phenoxypropionate herbicides.

1. Introduction

For about 50 years, chemical weed control in crops has been widely used and agriculture has become highly dependent on herbicides. Nevertheless, the ecological pressures against the systematic use of herbicides may induce farmers to choose another approach to weed control (Liebman and Davis, 2000; Mortensen et al., 2000). Furthermore, systematic use of herbicides (Hole and Powles, 1997), sometimes at low rates (Gasquez, 2005; Neve and Powles, 2005), led to the selection of weed populations resistant to herbicides, and currently about 190 different weed species worldwide have been reported with populations that are resistant to one or more herbicide modes of action (Heap, 2008). These resistances compromise weed management based on herbicides, and the adoption of alternative cropping systems is of increasing importance for managing or avoiding these problems.

Different approaches have been suggested to reduce herbicide use. However, the most promising alternative practice to herbicides is integrated weed management (Buhler, 2002). A better understanding of weed population dynamics is necessary to facilitate a reasonably efficient transition from conventional to alternative management methods (Hurle, 1993; Mortensen et al., 2000; White et al., 2004). There are a large number of studies focusing on the effects of cultural practices on the dynamics and management of single weed species (Wilson, 1981; Widderick et al., 2002) or plurispecific weed communities (Derksen et al., 1993; Mayor and Dessaint, 1998; Singer et al., 2000). Integrated management strategies aiming at weed control require knowledge on the long-term effects of a large range of cultural practices on target species. Only a few studies on the long-term effects of cropping systems on weed population dynamics have been undertaken as these effects are more difficult to evaluate (Doucet et al., 1999; White et al., 2004).

In the case of *Alopecurus myosuroides* Huds. (blackgrass), a major annual grass weed of autumn-sown crops in Europe, the choices of control strategies have been based on information about weed biology and the possible effects and interactions of cultural practices to limit the development of this winter annual weed (Moss and Clarke, 1994; Chauvel et al., 2001; Moss et al., 2007).

The present experiment was initiated in 1996 to study the effects of different cropping systems on an aryloxy-phenoxyproponiate (APP) herbicide-resistant population of *A. myosuroides*. During the first three-year rotation of the study, the objective was to develop weed management strategies that were not exclusively based on herbicides, but also on integrated

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non-chemical practices based on knowledge of *A. myosuroides* population demography and its interaction with some cultural practices (Chauvel et al., 2001). In order to evaluate long-term effects, the experiment was continued for a second three-year rotation, particularly to determine the effects of the longevity of *A. myosuroides* seeds in soil on weed dynamics. Furthermore, when the results of the first study were presented to farmers, they often asked whether it would be possible to return to the use of APP herbicides in situations where the *A. myosuroides* density had been sufficiently reduced by other means. This herbicide family is often applied after sowing, and has – so far – the advantage of not being included in the European Environmental Directive on pesticides (Council Directive 91/414/CEE), banning or reducing the doses of other herbicides against grass weeds.

The objective of the present study, carried out during the second three-year rotation of the cropping system experiment, therefore was (i) to evaluate long-term (six years) effects of cropping systems on the management of a herbicide-resistant *A. myosuroides* population and (ii) to check the feasibility of using a herbicide that had previously been responsible for the *A. myosuroides* resistance in populations that, over time, had been reduced to very low densities.

2. Material and methods

The experiment was carried out on a 6-hectare field infested with an APP herbicide-resistant population of *A. myosuroides* (Chauvel et al., 2001) in Lux, Burgundy (47°29′N, 12°41′E; altitude: 255 m). The field was located on a loamy clay soil (approx. 0.90 m deep) and the average rainfall was 600–650 mm per year. In 1996, 2 hectares with an almost uniform infestation of *A. myosuroides* (300 plants per m^2 after spraying in June 1996) were divided into seven permanent plots of 110 m × 20 m. The farmer owning the field carried out all the agricultural tasks. In order to study changes in the densities of resistant *A. myosuroides* in this commercial field managed with ordinary farm equipment, large plots were required and no replication was possible.

2.1. Cropping systems

2.1.1. Initial three-year experiment (1st stage – 1996–1999)

As the details of the initiation of the field trial are given by Chauvel et al. (2001), only the main characteristics are described here. During the first three years, two rotations were used on each of the seven plots. The local crop rotation (conventional rotation or CV) consisted exclusively of winter crops and the second rotation (new rotation or NW) alternated spring-sown and winter crops. For both rotations, three different weed management strategies were tested (Table 1):

(1) Strategy 1 is a classic strategy, similar to the local practices, comprising intensive herbicide use, chisel ploughing, usual sowing dates, high nitrogen rates aiming at maximum yield (CV1 and NW1).
(2) Strategy 2 is a strategy aimed at minimizing *A. myosuroides* that included cultivation practices affecting the aspects of the *A. myosuroides* life-cycle that are crucial for weed dynamics. This system consisted in deep mouldboard ploughing with a skim-coulter after winter crops and chisel ploughing after spring crops, delayed sowing dates, and an intensive herbicide programme specifically aimed at controlling resistant *A. myosuroides* (CV2 and NW2). In the new rotation, two levels of nitrogen inputs were applied (classic input NW2a and low-input NW2b).
(3) Strategy 3 is a low-input strategy consisting of chisel ploughing, delayed seeding, and low nitrogen fertiliser levels. Herbicide spraying was limited to a single application of a herbicide chosen according to the crop (CV3 and NW3) whenever possible.

2.1.2. The continuation of the experiment (2nd stage – 1999–2002)

The aim of each system was retained during the second stage of the experiment but the frequencies of mouldboard ploughing and spring crops were decreased to be consistent with the practices used by the farmers in this area (Table 1). As no effect of nitrogen was observed during the first stage, the system with the low nitrogen input (NW2b) in the new rotation was discontinued. The plot taken up by this system during the first experiment stage was used to test the re-use of APP herbicides (designated NW4). This practice was also tested in one winter rotation plot (CV4) which had not been used during the first three years of the experiment, as at the onset of the study in 1996, *A. myosuroides* density was slightly lower and slightly less homogeneous in that plot than in the remaining experimental plots.

Consequently, during the second three-year stage of the experiment (1999–2002), *A. myosuroides* density was studied on eight plots, four with the conventional rotation (CV1, CV2, CV3, CV4) and four with the new one (NW1, NW2, NW3, NW4). The main cultivation practices (sowing dates, tillage, herbicides) are given in Tables 2A and 2B.

2.2. Assessments of A. myosuroides plants

Before the sowing of winter and spring crops, *A. myosuroides* density was assessed by counting plants during the non-cultivation period (Ncp) before the subsequent crop was sown and twice during the cropping season (S1 and S2). The assessment of the non-cultivation period (Ncp) was carried out from 16 quadrats (0.25 m^2) placed in a "W" pattern.

In winter crops, the first assessment (S1) was carried out just after crop emergence by counting seedlings in 0.04 m^2 quadrats distributed on a systematic 5 m × 5 m grid consisting of four 23-quadrat rows (92 quadrats per plot). The second assessment (S2) was carried out after the second herbicide application and before *A. myosuroides* senescence, by counting plants in 16 quadrats (0.25 m^2) placed in a "W" pattern in each plot.

In the spring crops, two assessments were carried out, the first (S1) using 92 quadrats per plot after the first herbicide, and the second (S2) before harvest of the crop, using 16 quadrats.

The weed densities were used to compare the different cropping systems. For the statistical analysis, *A. myosuroides* densities in crops were log-transformed (Neperian logarithm) and analysed with the following linear model:

$$\ln(y+K) = \text{constant} + a \times \text{YEAR} + b_{CS} \times \text{YEAR} + [\text{cropping system effect}] + [\text{assessment date effect}] + \text{error}$$

- where y is the weed density (plant/m^2),
- $K = 0.0001$ is a constant added to the density to make possible the log-transformation (ln) of zero values.
- [cropping system effect] and [assessment date effect] are qualitative variables with eight cropping systems and two levels (i.e. post-sowing and spring assessments), respectively;
- YEAR is a quantitative variable and gives the number of years since the onset of the experiment (1996);
- a and b_{CS} are parameters with b_{CS} depending on the cropping system. b_{CS} × YEAR represents the interaction between the year and the cropping system.