



HAL
open science

High gene flow promotes the genetic homogeneity of arable weed populations at the landscape level

Christophe Délye, Julie a J Clément, Fanny Pernin, Bruno Chauvel, Valérie Le Corre

► To cite this version:

Christophe Délye, Julie a J Clément, Fanny Pernin, Bruno Chauvel, Valérie Le Corre. High gene flow promotes the genetic homogeneity of arable weed populations at the landscape level. *Basic and Applied Ecology*, 2010, 11 (6), pp.504-512. 10.1016/j.baae.2010.06.008 . hal-04474478

HAL Id: hal-04474478

<https://hal.science/hal-04474478>

Submitted on 23 Feb 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



ELSEVIER

GfÖ

GfÖ Ecological Society of Germany,
Austria and Switzerland

Basic and Applied Ecology 11 (2010) 504–512

Basic and
Applied Ecology

www.elsevier.de/baae

High gene flow promotes the genetic homogeneity of arable weed populations at the landscape level

Christophe Délye*, Julie A.J. Clément¹, Fanny Pernin, Bruno Chauvel, Valérie Le Corre

INRA, UMR 1210 Biologie et Gestion des Adventices, 17 rue Sully, F-21000 Dijon, France

Received 6 November 2009; accepted 27 June 2010

Abstract

To determine whether weed populations growing in neighbouring fields were genetically isolated, we investigated the genetic contamination of *Alopecurus myosuroides* populations in organic fields by populations in conventional fields. Herbicide resistance was used as a marker for gene flow to organic populations, which are not under herbicide selective pressure. Organic fields contained on average 74.5% herbicide-resistant plants (80.1% in conventional fields). Identical resistance alleles were found in neighbouring organic and conventional fields. AFLP analysis revealed that populations from organic and conventional fields had similar neutral genetic diversity. Massive pollen flow from conventional fields is likely chiefly responsible for the genetic makeup of *A. myosuroides* populations from organic fields. Using a demo-genetic model, we propose that demographic collapses of populations due to effective weed control enhance gene flow towards these populations. Fields with a low weed density could act as 'genetic sinks' that would facilitate the diffusion of genes from neighbouring, dense weed populations. Populations of allogamous wind-pollinated weed species like *A. myosuroides* occurring in neighbouring fields are therefore clearly not independent units. Adaptive evolution and management of such weeds should thus be considered on a scale broader than the field.

Zusammenfassung

Um zu bestimmen, ob Unkrautpopulationen, die in benachbarten Feldern wachsen, genetisch voneinander isoliert waren, untersuchten wir die genetische Kontamination der *Alopecurus-myosuroides*-Populationen in ökologisch bewirtschafteten Feldern durch die Populationen von konventionellen Felder. Die Resistenz gegenüber Herbiziden wurde als ein Maß für den genetischen Fluss in die ökologischen Populationen benutzt, die nicht unter dem selektiven Druck durch Herbizide standen. Die ökologischen Felder enthielten im Durchschnitt 74,5% herbizidresistente Pflanzen (80,1% in den konventionellen Feldern). In benachbarten ökologisch und konventionell bewirtschafteten Feldern wurden identische Resistenzallele gefunden. AFLP-Analysen ließen erkennen, dass die Populationen der ökologisch und konventionell bewirtschafteten Felder eine ähnliche neutrale genetische Diversität besaßen. Der massive Pollenflug von den konventionellen Feldern ist wahrscheinlich kurzfristig für die genetische Zusammensetzung der *A. myosuroides* Populationen in den biologischen Feldern verantwortlich. Unter Verwendung eines genetischen Demo-Modells sagen wir vorher, dass demografische Zusammenbrüche von Populationen aufgrund einer effektiven Unkrautkontrolle den Genfluss in diese Populationen verstärkt. Felder mit geringen Unkrautdichten könnten als genetische

*Corresponding author. Tel.: +33 380 693 185; fax: +33 380 693 262.

E-mail address: delye@dijon.inra.fr (C. Délye).

¹ Present address: INRA, UMR 1099 Biologie des Organismes et des Populations Appliquée à la Protection des Plantes, F-35650 Le Rheu, France.

Senken funktionieren und dies würde die Diffusion von Genen aus den benachbarten Populationen mit hohen Unkrautdichten fördern. Die Populationen von allogamen, windbestäubten Arten wie *A. myosuroides*, die in benachbarten Feldern auftreten, sind damit deutlich keine unabhängigen Einheiten. Die adaptive Evolution und Management dieser Unkräuter sollten deshalb auf einer Skala betrachtet werden, die größer als die eines Feldes ist.

© 2010 Gesellschaft für Ökologie. Published by Elsevier GmbH. All rights reserved.

Keywords: Acetyl-coenzyme A carboxylase (ACCase); Amplified fragment length polymorphism (AFLP); Black-grass (*Alopecurus myosuroides*); Concerted weed management; Herbicide resistance; Organic farming; Wind-pollinated species; Demography

Introduction

Weeds are plants adapted to the highly disturbed agricultural ecosystems (Baker 1974). For agricultural production to take place, weeds demography needs to be restrained in arable fields. This has traditionally been achieved by growers designing and using a range of cultural practices at the individual field level. However, weeds are able to propagate pollen or seeds beyond the borders of a field (Thill & Mallory-Smith 1997; Busi, Yu, Barrett-Lennard, & Powles 2008). Estimating the magnitude of gene flow among weed populations growing in different fields is important to determine whether adaptations occurring under one particular agricultural system may spread to other systems. This may concern traits conferring 'weediness' (Baker 1974) as well as adaptation to specific cultural practices. It is thus an issue with ecological and agricultural consequences. Yet, no data is available regarding gene flow connecting weed populations growing in different fields.

Here, we took advantage of the coexistence of two different farming systems in an agricultural landscape to investigate gene flow among weed populations growing in different fields. In organic agriculture, herbicide-based weed control is not permitted (European Community regulations 834/2007, 889/2008 and 1254/2008). In conventional agriculture, weed control is chiefly based on herbicide use. Above 195 weed species to date (Heap 2010) have adapted by evolving resistance mechanisms enabling them to withstand herbicide application. Herbicide resistance is an adaptive trait specific to conventional farming systems.

Alopecurus myosuroides Huds. (black-grass) is an annual, diploid, highly allogamous, wind-pollinated (Naylor 1972) grass weed very frequently growing in winter cereals in north-western Europe. In conventional fields, herbicides inhibiting acetyl-CoA carboxylase (ACCase) have been intensively used to destroy it (reviewed in Délye 2005; Moss, Perryman, & Tatnell 2007). As a consequence, resistance to these herbicides is now extremely frequent in conventional *A. myosuroides* populations (Délye et al. 2007; Délye et al. 2010; Moss et al. 2007). Resistance to ACCase inhibitors can be due to seven different mutant resistant ACCase alleles, and/or to other, unknown genes (Délye 2005).

To determine whether gene flow occurred among *A. myosuroides* populations in neighbouring fields, we examined whether the resistance to ACCase-inhibiting herbicides occurring in populations in conventional fields was trans-

ferred to populations in organic fields, and compared the neutral genetic diversity of populations in organic and conventional fields.

Materials and methods

A. myosuroides populations

Three samplings of populations collected across the French administrative district of Côte d'Or were used in this study (Fig. 1). In Côte d'Or, 1.4% of the cultivated agriculture area is under organic farming. The first sampling consisted of 17 populations collected in 2006 in organic wheat fields across Côte d'Or. The second sampling consisted of 127 populations collected in 2003 across a network of conventional wheat fields chosen at random and distributed so that coverage of *A. myosuroides* range in Côte d'Or was as exhaustive and homogeneous as possible (Menchari et al. 2006). These populations had previously been used to monitor the resistance to ACCase inhibitors and mutant ACCase alleles in conventional *A. myosuroides* populations from Côte d'Or (Menchari et al. 2006; Délye et al. 2007). Populations in this sampling were located close to most of the organic fields sampled (Fig. 1). In 2006, we re-sampled 22 of the 127 conventional fields sampled in 2003 to check whether the image of the resistance in conventional populations provided by the 2003 sampling was still accurate in 2006, and therefore whether the 2003 sampling could serve as a reference for comparison with populations in the organic sampling collected in 2006. The 22 fields re-sampled in 2006 were selected so that they were cultivated with wheat in 2006, distributed across Côte d'Or and, when possible, located close to an organic field in the second sampling.

Seeds were collected by zigzagging across the whole field and harvesting all ripe seeds from one or two heads on each of 50–150 plants. Seeds samples were stored at room temperature for 4 months to allow for a sufficient after-ripening period and thoroughly mixed to minimise sampling variance before a subsample was used for subsequent analyses.

Herbicide resistance assessment

Fenoxaprop is the ACCase inhibitor that has been most frequently used in France. The sensitivity to fenoxaprop of

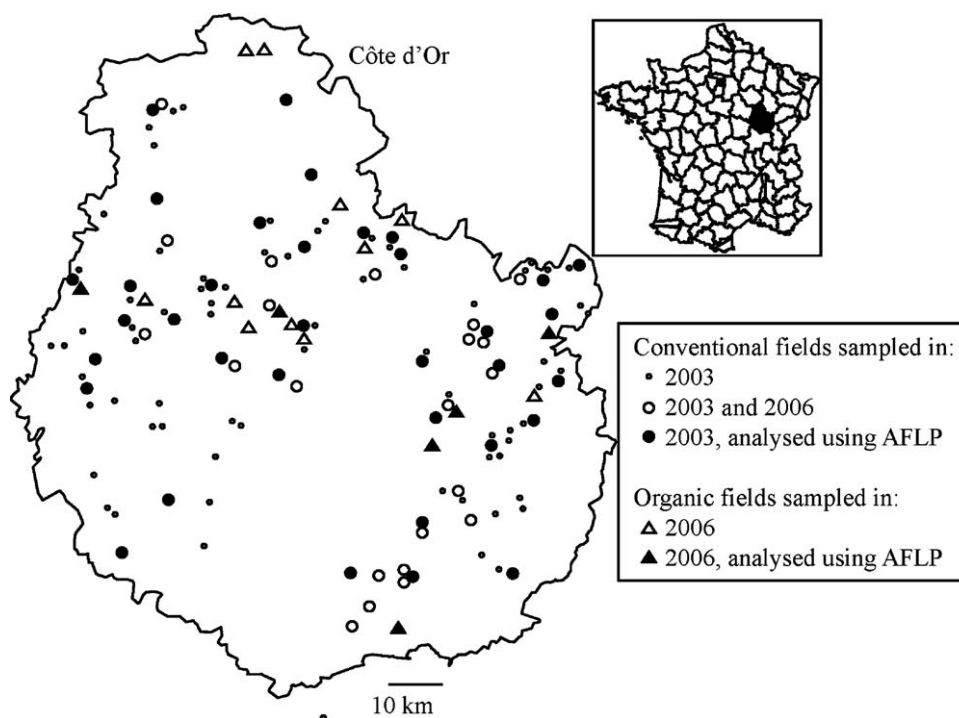


Fig. 1. *A. myosuroides* populations used in this study. The conventional populations collected in 2003 had previously been analysed using herbicide sensitivity bioassays (Délye et al. 2007) and ACCase genotyping (Menchari et al. 2006). Among these, 35 populations had also previously been analysed using AFLP (Menchari et al. 2007). All populations collected in 2006 were analysed in this study using herbicide sensitivity bioassays and ACCase genotyping. Among these, six organic populations were analysed in this study using AFLP.

100 seedlings in every population was assessed as described by Délye et al. (2007). Briefly, seeds from each population were placed into Petri dishes onto blotting paper soaked with a solution of KNO_3 (20 mM) and allowed to germinate for 1 week in controlled environment chambers (20 °C, 12 h light). Germinated seeds were then planted into plastic trays (1 L volume, 17 × 12.5 × 5.5 cm) filled with soil from an *A. myosuroides*-free field in Dijon. Thirty germinated seeds were planted in each tray, and thinned down to 25 before herbicide spraying. Fenoxaprop formulated as Puma LS (69 g L⁻¹ fenoxaprop) was applied at the recommended field rate (69 g ha⁻¹) onto seedlings at the 3–4-leaf stage using a custom-built sprayer. Herbicide sensitivity of the seedlings was assessed 3 weeks after treatment. Dead plants and surviving plants expanding a new green leaf were classified sensitive and resistant, respectively.

ACCase genotyping and sequencing

The seven mutant, resistant ACCase alleles will be referred to as C-L1781, T-L1781, C-C2027, T-C2027, N2041, G2078 and A2096. Their respective frequencies were assessed in all populations collected in 2006 by genotyping 60 plants/field as described by Menchari et al. (2006).

Mutation events being rare, it is very unlikely that two mutant ACCase alleles with identical sequences observed in neighbouring populations result from two independent

mutation events. To ascertain the fact that mutant ACCase alleles detected in an organic field were transferred from neighbouring field(s), we sequenced a highly polymorphic 1257-bp fragment from the gene encoding ACCase that enables an easy discrimination of distinct mutant haplotypes corresponding to distinct appearances of the allele (Délye, Straub, Michel, & Le Corre 2004). Sequencing was performed when the same allele was present in frequency higher than 3% in an organic field and in neighbouring conventional field(s). The ACCase fragment was sequenced on both strands from 5 plants/field. Homozygous mutant plants were directly sequenced. Amplicons from heterozygous plants were cloned in plasmid p-Drive (Qiagen). Three different inserts were sequenced per haplotype. PCR and sequencing were as described by Menchari et al. (2006).

AFLP genotyping

Six populations from the organic fields investigated (Fig. 1) that had been under organic farming for the longest time (8–20 years, Table 1) were used for AFLP following the procedure described in Menchari, Délye, and Le Corre (2007) and using 60 plants/population. Results were compared to those previously obtained (Menchari et al. 2007) from 35 of the populations collected in 2003 in conventional fields.

Table 1. *A. myosuroides* populations collected in organic fields in 2006.

Code	Years under organic farming	% fenoxaprop-resistant plants detected ^a	% mutant ACCase alleles detected ^b
O1 ^c	8	77	4.2
O2	8	88	0.0
O3	5	77	5.8
O4	5	82	0.0
O5	6	74	0.8
O6 ^c	10	73	0.0
O7 ^c	8	74	1.7
O8 ^c	8	80	18.3
O9	4	79	11.7
O10	5	61	0.8
O11	5	86	3.3
O12 ^c	16	79	3.3
O13	5	60	0.8
O14 ^c	20	59	0.0
O15	8	67	0.0
O16	8	74	0.0
O17	5	76	0.0

^a100 seedlings assayed.^b120 ACCase alleles genotyped (2 alleles/plant, 60 plants genotyped).^cPopulations used for AFLP analysis.

Statistical analyses and modelling

Analyses and modelling were performed using R (Anonymous 2009). A simple analytical model was used to investigate the effects of demography and gene flow on the evolution of resistance in a population not sprayed with herbicides that receives pollen from a population containing a high frequency of resistant plants. The source population size was set to a constant value ($N_s = 10^6$ individuals) corresponding to a density of 5 plants m^{-2} in a 20-ha field, which is representative of winter cereal fields in north-eastern France (Chauvel, unpublished data; Agreste 2009). The frequency of resistant plants within the source population was set to 80% according to previous observations (Délye et al. 2007). Resistance was supposed to be monogenic and dominant.

$N(t)$, the number of individuals in generation t in the recipient population, was modelled according to a logistic growth function. The average annual rate of increase in *A. myosuroides* varies between 2- and 15-fold depending on

the cultivation system (Moss 1990). Considering the average organic cultivation system for wheat in Côte d'Or, the logistic growth rate was set to 5 and the field carrying capacity to 10^7 individuals.

The model was iterated for eight generations, starting with a recipient, untreated population containing $N(0)$ herbicide-sensitive individuals. $N(0)$ was set to vary between 10^3 and 10^7 . The highest $N(0)$ value simulated a constant population size. The lowest values simulated a population that had collapsed, as can be observed after the intercalation of highly competitive crops in the rotation (Moss 1985).

Each individual plant contributed equally to the fertilizing pollen cloud. Mating was random, with the fertilizing pollen cloud containing a proportion m of migrant pollen issued from the source population. m was a function of P_m , the probability that a viable pollen grain from the source population travels to the untreated population: at a given generation t , $m(t) = P_m \times N_s / [N(t) + P_m \times N_s]$. We tested P_m values ranging from 10^{-6} to 10^{-1} . The proportion of resistant plants in the recipient population at each generation after pollen flow and reproduction was computed after Hardy–Weinberg law.

Results

Relevance of the 2003 conventional population sampling as a reference for resistance in conventional fields in 2006

Overall, the 22 conventional fields re-sampled in 2006 (Fig. 1) contained 85.4% fenoxaprop-resistant seedlings and 18.9% mutant ACCase alleles. The average differences in the proportions of resistant seedlings and mutant ACCase alleles between 2003 (Délye et al. 2007) and 2006 (this study) computed for these 22 populations were -0.9% and 0.7% , respectively. The average proportions of fenoxaprop-resistant seedlings and of mutant ACCase alleles in the 22 populations did not differ between 2003 and 2006 (Wilcoxon's rank sum test for paired samples with continuity correction, $w = 105.0$, $p = 1.00$ and $w = 123.0$, $p = 0.81$, respectively). This is consistent with a previous study conducted on conventional fields sampled from 2001 to 2005 across the range of *A. myosuroides* that showed no effect of the sampling year on the observed resistance to ACCase inhibitors (Délye et al. 2010). Thus, the 127 populations collected in 2003 reli-

Table 2. Frequencies of plants resistant to the herbicide fenoxaprop detected in *A. myosuroides* populations collected in organic fields in 2006 and conventional fields in 2003 (reference sampling for conventional populations).

Populations	# populations	Proportion of plants resistant to fenoxaprop			
		Minimum	Average	Median	Maximum
Organic	17	59.0	74.5 ^a	76.0	88.0
Conventional ^b	127	13.0	80.1 ^a	82.0	100.0

^aValues significantly different according to Wilcoxon's rank sum test with continuity correction ($w = 684.5$; $p = 0.0193$).^bPopulations analysed in a previous study (Délye et al. 2007).

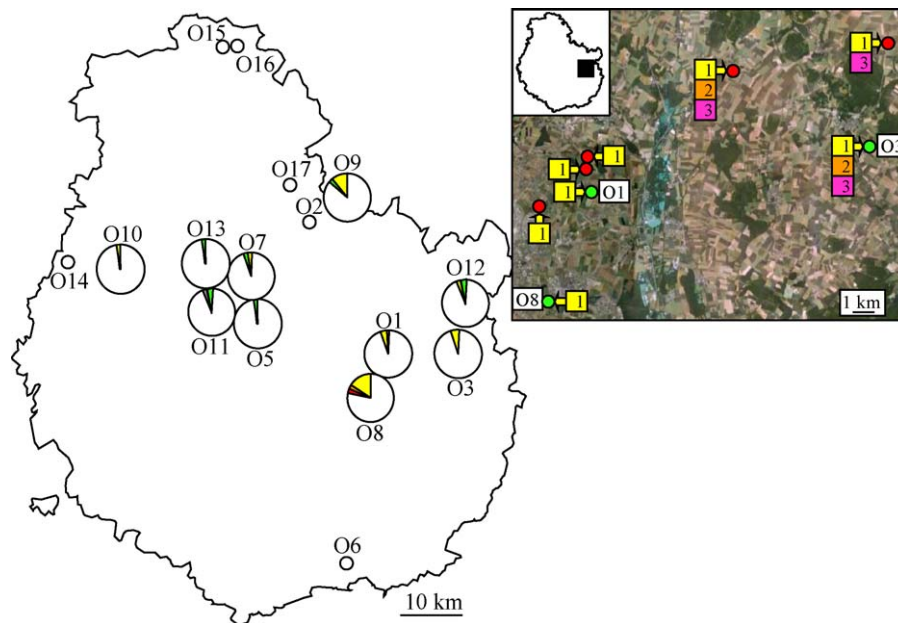


Fig. 2. Mutant ACCase alleles detected in 17 fields. Each field is represented by a pie chart centred on its geographical location that shows the respective frequencies of the ACCase alleles detected. White, wild-type allele; yellow, C-L1781; amber, T-L1781; red, C-C2027; green, N2041; grey, A2096. Fields where no mutant resistant ACCase allele was detected are shown as smaller, white circles. The insert on the right shows the distribution of C-L1781 ACCase haplotypes across three organic (green dots) and five conventional (red dots) fields. The number(s) indicated in boxes located closest to a dot refer to the code of the haplotype(s) found in the corresponding population (see text).

ably represented the resistance situation among conventional fields in Côte d'Or in 2006. The 2003 conventional sampling was used for comparisons with the organic fields sampled in 2006 because it contained more fields located close to the organic fields studied (Fig. 1).

Occurrence of herbicide resistance in organic fields

All 17 organic fields contained fenoxaprop-resistant seedlings (59–88% of the seedlings analysed; Tables 1 and 2). There was no significant correlation between the number of years a field had been under organic farming and the proportion of fenoxaprop-resistant seedlings detected (Spearman's rank correlation test, $\rho = -0.212$, $p = 0.41$). The average frequency of fenoxaprop-resistant plants was significantly lower in organic fields than in conventional fields (Table 2). This was due to an absence of organic fields containing above 88% resistant plants (Tables 1 and 2), while 93 of the 127 conventional fields contained above 88% resistant plants.

Occurrence of mutant, herbicide-resistant ACCase alleles in organic fields

Mutant ACCase alleles were detected in 10 of the 17 organic fields, in frequencies ranging from 0.8% to 18.3% of the alleles genotyped (Table 1 and Fig. 2). C-L1781 was the most frequent allele detected (2.3% of all alleles genotyped and 77.0% of the mutant alleles detected; Fig. 2; Appendix A:

Table 1). C-L1781 was mostly detected in population O9 and in populations O1, O3 and O8 in the East of Côte d'Or (Fig. 2), an area where it had been detected in substantial frequencies in conventional fields (Menchari et al. 2006) (Appendix A: Fig. 1).

To determine whether they had been transferred from conventional to neighbouring organic fields, C-L1781 alleles were sequenced for a total of 40 plants from organic fields O1, O3 and O8 and from five neighbouring conventional fields (Fig. 2). Twenty plants were heterozygous. A total of 60 sequences were thus obtained. On the basis of the 10 nucleotide substitutions observed, they clustered into three haplotypes. The predominant haplotype (1781-1, 46 sequences) was observed in all fields investigated, organic or conventional (Fig. 2). Haplotype 1781-2 (5 sequences) that differed from haplotype 1781-1 by six substitutions was present in organic field O3 and in one conventional field 9 km apart (Fig. 2). Haplotype 1781-3 (9 sequences) that differed from each of haplotypes 1781-1 and 1781-2 by seven nucleotide substitutions was present in organic field O3 and in two conventional fields 6 km and 9 km apart (Fig. 2).

Neutral genetic variation

AFLP genotyping generated 116 markers, of which 102 (87.9%) were polymorphic. Genetic variation in the six organic fields was compared to data previously obtained for 35 conventional fields sampled in 2003 (Menchari et al. 2007). The expected genetic diversity within population

(H_W) and the overall genetic differentiation among populations (F_{ST}) were not significantly different between organic and conventional fields (Table 3).

Although populations from organic and conventional fields were collected in 2006 and in 2003, respectively, the average pairwise F_{ST} values computed for all possible pairs including one organic and one conventional population (0.0206, S.E. = 0.0090), all pairs of organic populations and all pairs of conventional populations (Table 3) were not significantly different (Kruskal–Wallis rank sum test, $\chi^2 = 3.980$, $df = 2$, $p = 0.1367$). Thus, the six populations from organic fields were not different from populations from conventional fields in terms of neutral genetic diversity.

Modelling

When the untreated recipient *A. myosuroides* population had a large initial size that remained constant ($N(0) = 10^7$, Fig. 3), pollen flow from a population containing high frequencies of resistant plants resulted in a noticeable increase in the frequency of resistant plants only when the probability of pollen migration was unrealistically high ($P_m = 10^{-1}$). When the initial size of the recipient population was strongly reduced ($1000 \leq N(0) \leq 10^5$, Fig. 3), frequencies of resistant plants similar to those observed in organic fields (Tables 1 and 2) were obtained under more plausible probabilities of pollen migration ($10^{-3} \leq P_m \leq 10^{-2}$). When P_m was set to 5.10^{-3} , the proportions of resistant plants obtained in the untreated population after eight generations were 80%, 76% and 40% for $N(0)$ values of 10^3 , 10^4 and 10^5 , respectively.

When $N(0)$ was low, the demographic imbalance of the recipient with the source population was important. Thus, the pollen from the source population was initially dominant in the pollen cloud fertilizing the recipient population. In the subsequent generations, the proportion of pollen from the source population rapidly decreased as the recipient population recovered a size similar to that of the source population. In our model, the time lag to demographic recovery was at most six generations. However, the time lag before a stable resistance frequency was attained in the recipient population was much shorter. With $P_m = 5.10^{-3}$, 95% of the frequency of resistant individuals observed after eight generations was

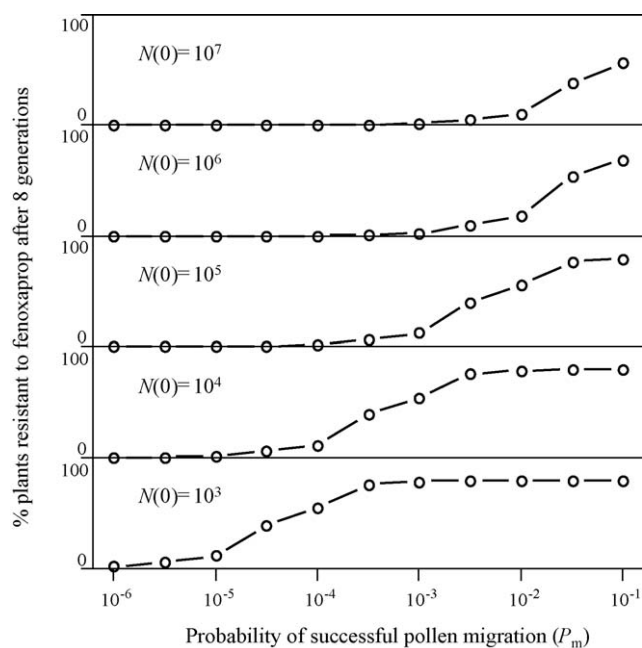


Fig. 3. Outputs of a simple demo-genetic model simulating the evolution of resistance in a recipient population not sprayed with herbicides receiving migrant pollen from a source population containing a high frequency of resistant plants (see text for details). The frequency of resistant individuals in the recipient population predicted after eight generations is plotted as a function of the probability for pollen migration (P_m). $N(0)$, initial size of the recipient population.

attained after only 1, 2 or 3 generations for $N(0)$ values of 10^3 , 10^4 and 10^5 , respectively.

Discussion

Herbicide resistance is an adaptive trait specific to weeds growing under conventional farming systems. Here, we used it as a marker to detect gene flow among *A. myosuroides* populations. We found that organic fields contained very high frequencies of fenoxaprop-resistant plants (Tables 1 and 2), whereas this trait does not confer any known advantage under organic farming systems. This implies that either resistance genes have been selected by herbicide applications performed

Table 3. Expected genetic diversity within population (H_W), genetic differentiation among populations (F_{ST}) and average pairwise genetic differentiation (F_{STpair}) computed from AFLP analysis of *A. myosuroides* populations collected in organic fields in 2006 and in conventional fields in 2003 (reference sampling for conventional populations).

Populations	# populations	H_W	S.E.	F_{ST}	S.E.	F_{STpair}	S.E.
Organic	6	0.2396 ^a	0.0031	0.0234 ^b	0.0989	0.0233	0.0074
Conventional ^c	35	0.2406 ^a	0.0014	0.0201 ^b	0.0779	0.0200	0.0091

^a Values not significantly different according to a Student's *t*-test ($t = 0.769$; $p = 0.44$).

^b Values not significantly different according to a Student's *t*-test ($t = 0.078$; $p = 0.94$).

^c Populations analysed in a previous study (Menchari et al. 2007).

before the fields were converted to organic farming and have persisted since then, or that resistance genes have been transferred to populations in organic fields by gene flow from populations in neighbouring conventional fields.

High frequencies of resistant plants have been shown to persist for at least 6 years in *A. myosuroides* populations in the absence of the selecting herbicides (Chauvel, Guillemin, & Colbach 2009). However, the first severe cases of resistance to ACCase-inhibiting herbicides in Côte d'Or occurred in just a few fields in 1996 (Gasquez 1996). The occurrence of high frequencies of resistant plants in conventional fields generalised between 2000 and 2003 (Chauvel, unpublished data; Délye et al. 2007). Thus, it is very unlikely that fields under organic farming for 8 years and more at our sampling date all contained high frequencies of herbicide-resistant plants prior to conversion. Persistence of resistance does not explain the occurrence of identical herbicide-resistant ACCase alleles in organic and conventional fields a few kilometres apart either (Fig. 2). It is also conflicting with the absence of genetic differentiation between populations in organic and conventional fields revealed by AFLPs (Table 3), which suggests current or recent extensive gene flow.

Dissemination of genes in plants is mediated by seed and pollen flow. Due to their low weight, *A. myosuroides* seeds have been efficiently removed from cereal seeds in France since at least 70 years. Besides, organic growers are not allowed to sow conventional seeds. Contaminated seed batches thus cannot explain the occurrence of resistant *A. myosuroides* plants in organic fields. In the absence of any physical obstacle, *A. myosuroides* seeds are dispersed up to 4 m by wind and gravity (Colbach & Sache 2001), which is unlikely to allow high levels of contamination even among adjacent fields. *A. myosuroides* seeds can be carried by agricultural machinery (Wallinga, Kropff, & Rew 2002). Organic growers are allowed to share machinery with conventional growers, but generally possess their own machinery. Thus, machinery-mediated dispersal of seeds is also unlikely to account alone for the proportion of resistant plants observed in all organic fields.

Pollen is considered the predominant vehicle of gene flow in many plant species (Levin & Kerster 1974), especially highly allogamous and wind-pollinated grasses like *A. myosuroides* (Naylor 1972). Pollen-mediated gene flow was detected in grasses at up to 21 km from the pollen source in studies considering the agricultural landscape level (Watrud et al. 2004; Busi et al. 2008). In Côte d'Or, field size is relatively small (10–20 ha on average). Organic fields are scattered in an agricultural landscape essentially made up of conventional fields. Thus, every organic field investigated herein was adjacent to or a few dozen metres away from conventional field(s). *A. myosuroides* flowering is synchronic in organic and conventional fields. Therefore, the occurrence of pollen flow from conventional to organic fields is certain.

Modelling showed that pollen dispersal rates around 10^{-1} were necessary to obtain a noticeable frequency of resis-

tant individuals in populations in organic fields with a large constant census size (Fig. 3). Such an intensity of pollen-mediated gene flow seems difficult to fit with *A. myosuroides* biology. Indeed, most of the pollen from grasses is deposited within a few meters from the source (van Treuren, Goossens, & Sevcikova 2006). In cereals, *A. myosuroides* population sizes can reach several hundred plants m^{-2} (Moss 1983; Chauvel et al. 2009). At high densities, plants are expected to essentially cross with their closest neighbours (Rognli, Nilsson, & Nurminiemi 2000). This is expected to greatly hamper pollen-mediated gene flow, especially in the centre of organic fields, where most of the seeds analysed were collected. However, *A. myosuroides* population sizes fluctuate. During the 8 years preceding our sampling, all organic fields investigated were grown at least once for 2 or 3 consecutive years with highly competitive fodder crops that almost suppress *A. myosuroides* development (De Gournay 1963; Moss 1985). *A. myosuroides* seeds do not persist for long in the soil, with 97% seed loss in 3 years (Moss 1985). Thus, all *A. myosuroides* populations from organic fields investigated underwent at least one strong demographic collapse in the 8 years before seed collection, while substantial populations were always present in neighbouring conventional field(s). Our model indicated that under these conditions, a pollen dispersal rate of about 10^{-3} from a population in a conventional field to a population in an organic field could account for frequencies of resistant plants in organic fields consistent with those observed in the field within a few generations. Demographic collapse followed by pollen flow from populations in conventional fields is thus a likely cause for the presence of resistant plants in organic fields in frequencies similar to those in neighbouring conventional fields, as well as for the absence of genetic differentiation observed among populations in organic and conventional fields. Given that populations in conventional fields also underwent demographic collapses because of practices such as herbicide applications, the same process is also a likely explanation to the absence of genetic differentiation observed among populations in conventional fields.

Conclusions

Each arable field has a specific history of agricultural practices. Agricultural landscapes are thus spatial and temporal mosaics of diverse selective pressures, with the individual field being the largest spatial scale where selective pressures are relatively homogeneous. Thus, the individual field can be considered the primary spatial scale at which weed adaptive evolution takes place.

Our data demonstrate that massive pollen flow connects *A. myosuroides* populations occurring in neighbouring fields. This conclusion most likely also applies to other allogamous and wind-pollinated weed species, that is, a large number of weeds (Baker 1974). Our results further suggest that demographic collapses of weed populations resulting

from agricultural practices likely enhance gene flow towards these populations. Isolated weed plants or fields with a low weed density can thus be considered as ‘genetic sinks’ that facilitate the diffusion of genes from neighbouring, dense weed populations. It ensues that populations of allogamous, wind-pollinated weed species in individual fields are not independent units in an agricultural landscape.

From an agronomic point of view, a grower efficiently managing populations of such weeds and keeping their density at low levels in his fields can nevertheless ‘catch’ herbicide resistance from neighbouring field(s) where these weeds proliferated after evolving resistance. Thus, resistance to herbicides and by extension any adaptive trait that may be selected for in a weed species cannot be considered a private property problem. Our results support previous proposals that weed management should be considered at the agricultural landscape level, that is, by promoting grower co-operation over broad geographical areas (Llewellyn & Allen 2006; Dauer, Luschei, & Mortensen 2009). From a more fundamental point of view, our results underline the need to consider weed adaptation and ecology at two nested, interacting geographical scales: the individual field, where adaptation to agricultural practices occurs under different regimes of selective pressures, and the landscape, across which adaptive genes migrate to and from each field depending on weed demography.

Acknowledgements

We thank Aurélie Bérard and Dominique Brunel (INRA-CNG Évry) for skilled assistance in genotyping, INRA (Département Santé des Plantes et Environnement) for financial support (grant #2007-1210-01), and the Chambre d’Agriculture de Côte d’Or and all growers for help in collecting *A. myosuroides* populations.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.baae.2010.06.008.

References

- Agreste. *French official agricultural statistics*. (2009). <www.agreste.agriculture.gouv.fr> accessed 21.10.2009.
- Anonymous. *The R project for statistical computing*. (2009). <http://www.r-project.org/> accessed 21.10.2009.
- Baker, H. G. (1974). The evolution of weeds. *Annual Review of Ecology and Systematics*, 5, 1–24.
- Busi, R., Yu, Q., Barrett-Lennard, R., & Powles, S. (2008). Long distance pollen-mediated flow of herbicide resistance genes in *Lolium rigidum*. *Theoretical and Applied Genetics*, 117, 1281–1290.
- Chauvel, B., Guillemain, J.-P., & Colbach, N. (2009). Evolution of a herbicide-resistant population of *Alopecurus myosuroides* Huds. in a long-term cropping system experiment. *Crop Protection*, 2, 343–349.
- Colbach, N., & Sache, I. (2001). Blackgrass (*Alopecurus myosuroides* Huds.) seed dispersal from a single plant and its consequences on weed infestation. *Ecological Modelling*, 139, 201–219.
- Dauer, J. T., Luschei, E. C., & Mortensen, D. A. (2009). Effects of landscape composition on spread of an herbicide-resistant weed. *Landscape Ecology*, 24, 735–747.
- De Gournay, X. (1963). La lutte contre le Vulpin des champs (*Alopecurus myosuroides* Huds.) dans les cultures de blé d’hiver. *Annales de Physiologie Végétale*, 5, 229–247.
- Délye, C. (2005). Weed resistance to acetyl-coenzyme A carboxylase inhibitors: an update. *Weed Science*, 53, 728–746.
- Délye, C., Menchari, Y., Guillemain, J.-P., Matejicek, A., Michel, S., Camilleri, C., et al. (2007). Status of black grass (*Alopecurus myosuroides*) resistance to acetyl-coenzyme A carboxylase inhibitors in France. *Weed Research*, 47, 95–105.
- Délye, C., Michel, S., Bérard, A., Chauvel, B., Brunel, D., Guillemain, J.-P., et al. (2010). Geographical variation in resistance to acetyl-coenzyme A carboxylase-inhibiting herbicides across the range of the arable weed *Alopecurus myosuroides* (black-grass). *New Phytologist*, 186, 1005–1017.
- Délye, C., Straub, C., Michel, S., & Le Corre, V. (2004). Nucleotide variability at the acetyl-coenzyme A carboxylase gene and the signature of herbicide selection in the grass weed *Alopecurus myosuroides* (Huds.). *Molecular Biology and Evolution*, 21, 884–892.
- Gasquez, J. (1996). État de la résistance en France, INRA Dijon (Ed.). In *Comptes-rendus du Xème colloque international sur la biologie des mauvaises herbes* (pp. 173–180). Paris: AFPP.
- Heap, I.M. *International survey of herbicide resistant weeds*. (2010). <http://www.weedresearch.com> accessed 21.02.2010.
- Levin, D. A., & Kerster, H. W. (1974). Gene flow in seed plants. *Evolutionary Biology*, 7, 139–220.
- Llewellyn, R. S., & Allen, D. M. (2006). Expected mobility of herbicide resistance via weed seeds and pollen in a Western Australian cropping region. *Crop Protection*, 25, 520–526.
- Menchari, Y., Camilleri, C., Michel, S., Brunel, D., Dessaint, F., Le Corre, V., et al. (2006). Weed response to herbicides: regional-scale distribution of herbicide resistance alleles in the grass weed *Alopecurus myosuroides*. *New Phytologist*, 171, 861–874.
- Menchari, Y., Délye, C., & Le Corre, V. (2007). Genetic variation and population structure in black-grass (*Alopecurus myosuroides* Huds.), a successful, herbicide-resistant, annual grass weed of winter cereal fields. *Molecular Ecology*, 16, 3161–3172.
- Moss, S. R. (1983). The production and shedding of *Alopecurus myosuroides* Huds. seeds in winter cereals crops. *Weed Research*, 23, 45–51.
- Moss, S. R. (1985). The survival of *Alopecurus myosuroides* Huds. seeds in soil. *Weed Research*, 25, 201–211.
- Moss, S. R. (1990). The seed cycle of *Alopecurus myosuroides* in winter cereals: a quantitative analysis. In: European Weed Research Society (Ed.). In *Proceedings of the EWRS Symposium 1990* EWRS: London., (pp. 27–36).
- Moss, S. R., Perryman, S. A. M., & Tatnell, L. V. (2007). Managing herbicide-resistant blackgrass *Alopecurus myosuroides*: theory and practice. *Weed Technology*, 21, 300–309.

- Naylor, R. E. L. (1972). Biological flora of the British isles. *Alopecurus myosuroides* Huds. (A. Agrestis). *Journal of Ecology*, *60*, 611–622.
- Rognli, O. A., Nilsson, N. O., & Nurminiemi, M. (2000). Effects of distance and pollen competition on gene flow in the wind pollinated grass *Festuca pratensis* Huds. *Heredity*, *85*, 550–560.
- Thill, D. C., & Mallory-Smith, C. A. (1997). The nature and consequence of weed spread in cropping systems. *Weed Science*, *45*, 337–342.
- van Treuren, R., Goossens, P. J., & Sevcikova, M. (2006). Variation in effective pollination rates in relation to the spatial and temporal distribution of pollen release in rejuvenated perennial ryegrass. *Euphytica*, *147*, 367–382.
- Wallinga, J., Kropff, M. J., & Rew, L. J. (2002). Patterns of spread of annual weeds. *Basic and Applied Ecology*, *3*, 31–38.
- Watrud, L. S., Lee, E. H., Fairbrother, A., Burdick, C., Reichman, J. R., Bollman, M., et al. (2004). Evidence for landscape-level, pollen-mediated gene flow from genetically modified creeping bentgrass with CP4 EPSPS as a marker. *Proceedings of the National Academy of Sciences of the USA*, *101*, 14533–14538.

Available online at www.sciencedirect.com



Table 1

Frequencies of mutant ACCase alleles in the 17 *A. myosuroides* populations collected in 2006 in organic fields.

Code	Years under organic farming	% mutant ACCase alleles detected ^a							Total
		C-L1781	T-L1781	C-C2027	T-C2027	N2041	G2078	A2096	
O1	8	3.3	0.8	0.0	0.0	0.0	0.0	0.0	4.2
O2	8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
O3	5	5.8	0.0	0.0	0.0	0.0	0.0	0.0	5.8
O4	5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
O5	6	0.0	0.0	0.0	0.0	0.8	0.0	0.0	0.8
O6	10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
O7	8	0.8	0.0	0.0	0.0	0.8	0.0	0.0	1.7
O8	8	16.7	0.8	0.8	0.0	0.0	0.0	0.0	18.3
O9	4	10.8	0.0	0.0	0.0	0.8	0.0	0.0	11.7
O10	5	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.8
O11	5	0.0	0.0	0.0	0.8	1.7	0.0	0.8	3.3
O12	16	0.8	0.0	0.0	0.0	2.5	0.0	0.0	3.3
O13	5	0.0	0.0	0.0	0.0	0.8	0.0	0.0	0.8
O14	20	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
O15	8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
O16	8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
O17	5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

^a 120 ACCase alleles genotyped (two alleles per plant, 60 plants genotyped)

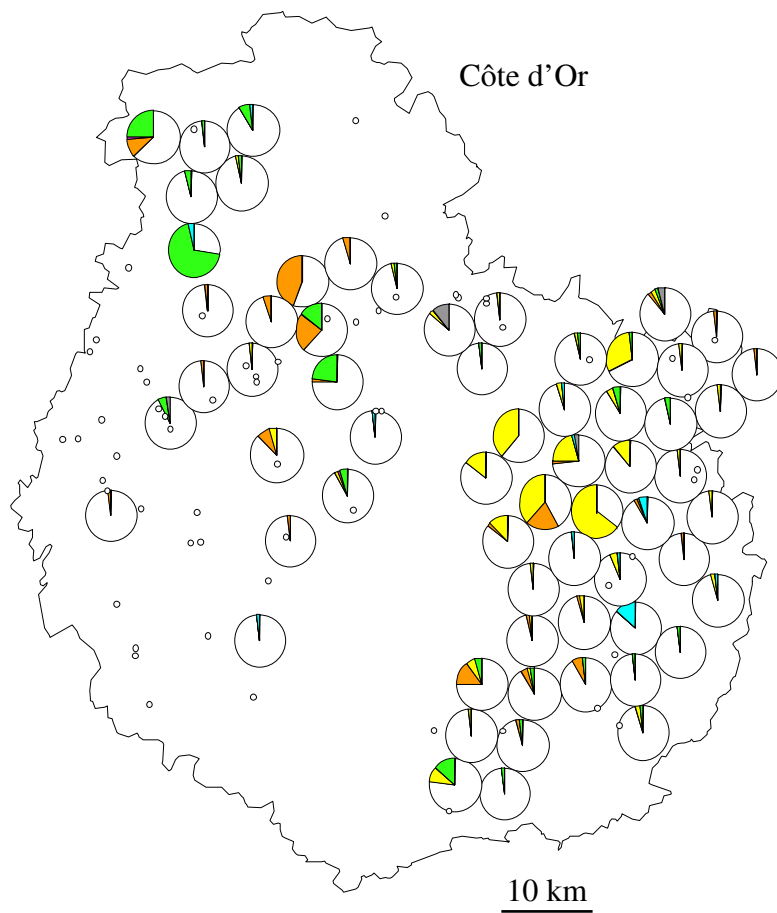


Figure 1. Mutant ACCase alleles detected in the 127 conventional fields sampled in 2003 and used as a reference sampling for this study. This data was obtained in a previous study (Menchari et al. 2006). Each field is represented by a pie chart centred on its geographical location that shows the respective frequencies of the ACCase alleles detected. White, wild-type allele; yellow, C-L1781; amber, T-L1781; red, C-C2027; magenta, T-C2027; green, N2041; blue, G2078; grey, A2096. Fields where no mutant resistant ACCase allele was detected are shown as smaller, white circles.