Status of resistance to acetyl-coenzyme A carboxylase inhibitors in French populations of black-grass (*Alopecurus myosuroides* Huds.)

Yosra Menchari1, Bruno Chauvel1, Jean Philippe Guillemin2 and Christophe Délye1

1 Institut National de la Recherche Agronomique, UMR Biologie et Gestion des Adventices, BP 86510, F-21065 Dijon CEDEX, France
2 Etablissement National d’Enseignement Supérieur Agronomique de Dijon, UMR Biologie et Gestion des Adventices, BP 87999, 21079 Dijon CEDEX, France

**Summary** We assessed the respective contributions of target-site and metabolism-based resistance to herbicides inhibiting acetyl-coenzyme A carboxylase (ACC) in 243 black-grass populations (*Alopecurus myosuroides* Huds.) collected across France using a herbicide sensitivity bioassay and ACC genotyping. Seedlings resistant to fenoxaprop were identified in 241 out of the 243 populations investigated. Among the five resistant ACC alleles known in black-grass, alleles containing an isoleucine-to-leucine substitution at codon 1781 were predominant, occurring in 59.5% of the plants containing resistant ACC alleles. Comparison of the respective results from herbicide sensitivity bioassays and from genotyping indicated that more than 75% of black-grass plants resistant to ACC-inhibiting herbicides in France would be resistant due to increased herbicide metabolism. Analysis of herbicide application records suggested that, in 15.9% of the populations studied, metabolism-based resistance to ACC-inhibiting herbicides was mostly selected for by herbicides with other modes of action. Our results revealed the importance of metabolism-based resistance in black-grass, an issue of practical importance when considering managing populations resistant to ACC-inhibiting herbicides by using herbicides with alternative modes of action.

**Keywords** Acetyl-CoA carboxylase, *A. myosuroides*, cultural practice survey, genotyping, herbicide resistance mechanism.

**INTRODUCTION**

Black-grass (*Alopecurus myosuroides* Huds.) is a major, annual grass weed of winter crops in Northern Europe. In France, herbicides targeting chloroplastic acetyl-coenzyme A carboxylase (ACC) have been extensively used to control this weed since the end of the 1980s. As a consequence, numerous black-grass populations have evolved resistance to these herbicides (Heap 2006). Resistance to herbicides inhibiting ACC can be due to one of at least five distinct amino-acid substitutions within ACC. The associated cross-resistance patterns of these amino acid substitutions have been characterised (reviewed in Délye 2005). Resistance to herbicides inhibiting ACC may also be due to various metabolic enzymes that detoxify herbicides (reviewed in Délye 2005). ACC-based resistance is now relatively well understood, but the genetic basis for metabolism-based resistance remains very poorly characterised (Délye 2005).

In this work, we assessed the respective contributions of the five resistant ACC alleles and of metabolism-based resistance to the global phenomenon of black-grass resistance to ACC-inhibiting herbicides in France by comparing results from herbicide sensitivity bioassay and from genotyping in a total of 243 black-grass populations.

**MATERIALS AND METHODS**

**Plant material** A total of 243 black-grass populations, collected in winter wheat fields, were used in this study. A first set of populations was sampled in 2000 from a total of 116 fields mostly issued from north-eastern France (Figure 1). A second set of populations was sampled in 2003 across the French administrative district of Côte d’Or (Figure 1) and was performed to check whether the results obtained at a local geographical scale were consistent with those obtained at the level of France.

**Herbicide sensitivity bioassay** Sensitivity of black-grass seedlings (100 per herbicide per population) was assessed to fenoxaprop (Fx, 30 µM) and/or clodinafop (Cd, 15 µM) using a seed-based bioassay (Letouzé and...
Formulations used were Puma LS (69 g L⁻¹ Fx, Aventis France, Gif-sur-Yvette, France) and Célio (100 g L⁻¹ Cd, Evolya, Rueil-Malmaison, France).

**DNA extraction and ACC genotyping** DNA extraction from 48 seedlings from each of the 116 populations from France and from 60 seedlings from each of the 127 populations from Côte d’Or was as described in Délye et al. (2002).

The five amino-acid substitutions within ACC protein sequence that confer resistance to ACC-inhibiting herbicides are caused by seven point mutations within the gene encoding ACC (Délye 2005). The five resistant ACC alleles are listed in Table 1. Genotyping was performed by MALDI-TOF mass spectrometry using the ‘GOOD Assay’ SNP analysis procedure (Sauer et al. 2000).

**Survey of herbicide application** The record of herbicide applications was obtained for each of the 243 fields sampled.

**RESULTS**

**Frequencies of resistant seedlings** Out of a total of 24,300 seedlings assayed for sensitivity to Fx, 18,350 (75.5%) were identified as resistant in our herbicide sensitivity bioassay. They consisted of 8180 seedlings from the 116 populations from France (70.5% of the seedlings assayed) and 10,170 seedlings from the 127 populations from Côte d’Or (80.1% of the seedlings assayed). Bioassays performed with Cd identified a total of 5935 resistant seedlings out of the 24,300 assayed (24.4%). They consisted of 3570 seedlings from the 116 populations from France (30.8% of the seedlings assayed) and 2365 seedlings from the 127 populations from Côte d’Or (18.6% of the seedlings assayed).

Among the 243 populations studied, only two (0.8%) did not contain any seedlings resistant to Fx. Both were collected in France in 2000. The vast majority (221) of the 241 remaining populations contained more than 50% seedlings that were resistant to Fx in our bioassay. Thirty-five populations (14.4%) did not contain any seedlings resistant to Cd in our bioassay. These consisted of eight populations from Côte d’Or and 27 populations from France. The majority (136) of the remaining 208 populations contained less than 31% seedlings that were resistant to Cd in our bioassay.

**Occurrence of resistant ACC alleles** Out of a total of 13,188 seedlings genotyped, 10,594 (80.4%) did not contain any of the five known resistant ACC alleles (Table 1). One hundred and eighteen seedlings (0.9%) contained two distinct types of resistant ACC alleles (e.g., L1781/N2041). The remaining seedlings, either homozygous (e.g., L1781/L1781) or heterozygous (e.g., L1781/wild-type) contained a single resistant ACC allele. None of the five resistant ACC alleles were detected in 105 populations. In each of the 137 out of the 138 remaining populations, at least one seedling did not contain any of the five resistant ACC alleles. Among the seedlings genotyped, seedlings containing L1781 ACC alleles were largely predominant both in France and in Côte d’Or (Table 1).

**Contribution of resistant ACC alleles to herbicide resistance** It has been found previously that plants containing one or two L1781 ACC allele(s) were resistant to Fx but sensitive to Cd (Délye et al. 2002), while plants containing at least one of any other ACC alleles were resistant to both herbicides in seed-based bioassays (Délye 2005). The contribution of the five resistant ACC alleles known to date to the global resistance of black-grass to ACC-inhibiting herbicides was assessed by comparing the results of the seed-based herbicide sensitivity bioassays (observed frequencies of resistant seedlings within each population) to those of ACC genotyping (expected frequencies of resistant seedlings if the five ACC alleles were the only genes endowing resistance in the populations studied) (Figure 2). The seedlings used for ACC genotyping were not the same seedlings upon which sensitivity was assessed using seed-based bioassays. Comparison of the observed and expected proportions of seedlings resistant to Fx revealed an excess of observed resistant seedlings in 78 populations from France and in 124 populations from Côte d’Or (Figure 2), where 59.2%

<table>
<thead>
<tr>
<th>Resistant ACC alleles detected</th>
<th>Plants (%)</th>
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<tbody>
<tr>
<td></td>
<td>116 populations from France</td>
</tr>
<tr>
<td>None</td>
<td>65.7</td>
</tr>
<tr>
<td>L1781</td>
<td>19.9</td>
</tr>
<tr>
<td>C2027</td>
<td>5.3</td>
</tr>
<tr>
<td>N2041</td>
<td>2.6</td>
</tr>
<tr>
<td>G2078</td>
<td>1.3</td>
</tr>
<tr>
<td>A2096</td>
<td>3.6</td>
</tr>
<tr>
<td>Two</td>
<td>1.6</td>
</tr>
<tr>
<td>Total</td>
<td>100.0</td>
</tr>
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*Plants containing two distinct resistant ACC alleles.*
and 89.7% of the Fx-resistant seedlings were respectively expected not to contain any of the five ACC alleles conferring resistance to Fx. Similar results were obtained for Cd in 47 populations from France and in 61 populations from Côte d’Or (Figure 2), where we found that 67.8% and 88.5% of the Cd-resistant seedlings, respectively, were expected not to contain any of the four different ACC alleles conferring resistance to Cd. Overall, 75.1% of the Fx-resistant seedlings and 78.6% of the Cd-resistant seedlings were expected not to contain any ACC alleles conferring resistance to these herbicides.

**Impact of herbicide application upon resistance**

Herbicide applications obtained for the 243 fields ranged across six years in average. Ten surveys were too incomplete to be analysed. A total of 19 and 14 herbicide modes of action comprising 10 and six ACC-inhibiting molecules were sprayed across 196 of the fields surveyed in France and in Côte d’Or respectively. No ACC-inhibiting herbicide application was recorded in the 37 remaining fields (five from France, 32 from Côte d’Or).

**DISCUSSION**

We describe here the largest study ever conducted to investigate the extent and the genetic basis of resistance to herbicides in a weed species. Assessment of the sensitivity of a total of 48,600 seedlings using seed-based bioassay revealed that resistance to the two major ACC-inhibiting herbicides used in France, Fx and Cd, was very widespread. However, resistance to Cd was less widespread with a higher frequency of Fx-resistant seedlings than Cd-resistant seedlings. This difference suggests that mechanisms conferring resistance to Fx do not necessarily confer resistance to Cd. This is consistent with our previous finding that plants containing L1781 ACC alleles are resistant to

![Figure 2](image-url)
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Fxs but not to Cds. Also, metabolism-based resistance to ACC-inhibiting herbicides belonging to the same chemical family can be due to distinct metabolic enzymes (e.g., McFadden et al. 1989).

Metabolism-based resistance to ACC-inhibiting herbicides has already been observed in black-grass populations from France, UK and Spain (reviewed in Délye 2005). In the 243 black-grass populations we analysed, 75.1% of the Fx-resistant seedlings and 78.6% of the Cd-resistant seedlings were expected not to contain any of the five known resistant ACC alleles. This highlights the considerable significance of other resistance mechanisms in black-grass. These can be unknown, or rare, resistant ACC alleles, or enhanced herbicide metabolism, or both. Several distinct genes endowing resistance to ACC-inhibiting herbicides can coexist in a single black-grass population. Several distinct resistance genes can also coexist in a single black-grass plant (Table 1). Such multiple resistance has been reported elsewhere in black-grass (Letouzé and Gasquez 2001) and other grass weeds (Maneechote et al. 1997; Vila-Aiub et al. 2005).

A high number of seedlings resistant to Fx and some seedlings resistant to Cd were observed in black-grass populations sourced from a total of 37 fields where no application of ACC-inhibiting herbicides was recorded. Most seedlings did not contain any resistant ACC alleles. Selection for genes endowing cross-resistance to ACC-inhibiting herbicides and to herbicides with other modes of action has already been observed in black-grass and was shown to be due to enhanced herbicide metabolism (Letouzé and Gasquez 2001). Thus, our results strongly suggest that, in the 37 populations in question, resistance to Fx or Cd was mostly a side effect of the selection for metabolism-based resistance to herbicides with mode(s) of action other than ACC inhibition. Another possible explanation would be that ACCase resistance has arrived to these fields via gene flow, either pollen or seeds.

In contrast with ACC-based resistance, metabolism-based resistance is poorly understood, especially at the level of the genes endowing it. Given its widespread occurrence, its major role in resistance and the possibility of cross-resistance to herbicides with distinct modes of action, metabolism-based resistance to herbicides is clearly a facet of resistance that needs to be thoroughly investigated in the near future in order to develop resistance diagnosis tools and to propose efficient resistance management strategies.

REFERENCES


