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# Fitness costs associated with three mutant acetyl-coenzyme A carboxylase alleles endowing herbicide resistance in black-grass *Alopecurus myosuroides*

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## Summary

1. Pleiotropic effects associated with genes endowing resistance to herbicides are generally predicted to reduce plant fitness. Quantifying these effects is necessary to develop management strategies against herbicide-resistant weeds. We assessed the pleiotropic effects associated with three mutant alleles of the herbicide target enzyme acetyl-coenzyme A carboxylase (ACCase) on plant growth and seed production in black-grass *Alopecurus myosuroides*.

2. In each of two field experiments, black-grass populations segregating for Leu-1781 (five populations), Asn-2041 (three populations) or Gly-2078 (two populations) ACCase were produced to obtain several distinct, homogenized genetic backgrounds and to permit reliable comparisons among wild-type, heterozygous and homozygous mutant ACCase plants grown in competition with a wheat crop.

3. No significant differences from wild-type plants in vegetative biomass, height and seed production were observed in Leu-1781 or Asn-2041 ACCase plants.

4. Over both experiments, homozygous Gly-2078 ACCase plants displayed a significant reduction in biomass (42%), height (6%) and seed production (36%). Reduction varied with the segregating population and between field experiments.

5. *Synthesis and applications.* Our work illustrates the variation in fitness cost depending on the resistance gene, the plant genetic background and the environment. This underlines the necessity to identify the resistance gene(s) present in a weed population before designing resistance-management strategies. Competitive crops should be effective against Gly-2078 ACCase plants. The effect of resistant ACCase alleles on seed survival and germination needs to be studied in order to develop cultural practices creating or maximizing fitness costs in resistant plants. However, the variation of fitness cost with the environment and the weed population, as well as the likely absence of fitness cost associated with resistance genes such as Leu-1781 ACCase, renders the success of specific cultural control practices uncertain. A solution could be a weed control programme maximizing the diversity of cultural practices and including anti-resistance cultural practices to avoid or reduce selection for resistant ACCase alleles.

**Key-words:** ACCase, biomass production, evolution of resistance, grass weed, seed production, target-site mutations

## Introduction

Herbicide resistance is an intriguing and widespread trait in arable weeds. It has been selected to date in at least 183 species (Heap 2007). Herbicide resistance is directly selected for by human action on weed populations – by spraying herbicides in fields to kill weeds growing there. Herbicides exert an

extremely drastic selective pressure by killing up to 95–99% of wild-type, herbicide-sensitive individual plants (Foster, Ward & Hewson 1993). As a consequence, any gene enabling a weed individual to survive herbicide application is expected to be strongly selected for. The subsequent evolution of its frequency in weed populations will depend on its mode of inheritance, the reproductive biology of the weed species and its possible pleiotropic effects on the fitness of individual plants in the absence of the selecting herbicide(s) (Jasieniuk,

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Brûlé-Babel & Morrison 1996). Fitness, or success in the field, can be defined as the ability to establish, survive and reproduce successfully in a given environment. Fitness alterations can be exploited to predict population dynamics and to establish resistance-management strategies (Jordan *et al.* 1999).

Resistance to pesticides, including herbicides, is generally expected to be associated with reduced fitness (Coustau, Chevillon & French-Constant 2000). This has, for instance, been demonstrated in artificial mutants of *Arabidopsis thaliana* (Roux, Gasquez & Reboud 2004). However, observations of fitness costs associated with resistance to herbicides in natural weed populations are scarce, and mostly concern herbicides targeting photosynthesis (reviewed by Holt & Thill 1994). Yet understanding the pleiotropic effects of the genes endowing resistance to herbicides is essential to predict the evolution of resistance in weed populations.

A literature review (Bergelson & Purrington 1996) revealed that a greater control of genetic background increased the probability of detecting resistance costs, and that substantial variation in the cost associated with a given resistance trait was observed in different genetic backgrounds. This suggests that major traits in the life cycle of weeds, such as seed germination, vegetative growth or seed production, must be compared between resistant and sensitive plants with genetic backgrounds as similar as possible, and that this comparison must be performed with various genetic backgrounds. Furthermore, it is essential to identify the gene(s) endowing resistance, because different genes can be associated with different fitness costs (Roux, Gasquez & Reboud 2004). In studies where the genetic background was not controlled and the gene(s) endowing resistance were not identified, no fitness costs were identified (Marshall, Kirkwood & Leach 1994; Wiederholt & Stoltenberg 1996; Rashid *et al.* 2003).

Resistance to the graminicide herbicides inhibiting chloroplastic acetyl-coenzyme A carboxylase (ACCase, EC 6.1.4.2), a key enzyme in the synthesis of fatty acids (Harwood 1988), has evolved in 35 grass weed species so far (Heap 2007). Its history has been reviewed elsewhere (Devine & Shimabukuro 1994). Black-grass, *Alopecurus myosuroides* Huds., is a very widespread annual and allogamous grass weed of cereal crops (Chauvel & Gasquez 1994), and one of the most troublesome grass weeds having evolved resistance to ACCase inhibitors (Heap 2007). The genetic basis of target site-based resistance of black-grass to ACCase inhibitors is well understood (reviewed by Délye 2005). Seven mutations in the gene encoding ACCase in black-grass were shown to confer resistance to herbicides inhibiting this enzyme. They cause amino-acid substitutions at positions 1781 (Ile-to-Leu, two mutations), 2027 (Trp-to-Cys, two mutations), 2041 (Ile-to-Asn), 2078 (Asp-to-Gly) or 2096 (Gly-to-Ala) (Délye 2005). A broad-scale survey conducted across 243 black-grass populations from France showed that mutant Leu-1781 ACCase alleles were predominant. The other four types of mutant ACCase alleles were detected in lower frequencies, with Gly-2078 alleles being observed least frequently (Menchari *et al.* 2006). This suggested that different fitness costs could be associated with the distinct mutant ACCase alleles.

To date, a single study (Vila-Aiub, Neve & Powles 2005) has assessed the fitness cost associated with Leu-1781 ACCase. This work considered one population of rigid rye-grass, *Lolium rigidum* Gaud., a grass weed, and found an absence of pleiotropic effects of Leu-1781 ACCase on plant growth and seed production in the absence of competition from a crop plant. Here we used segregating black-grass populations to control the genetic background of resistant and sensitive plants. A total of 20 such populations were used in two experiments (10 populations per experiment) to assess the possible pleiotropic effects of Leu-1781, Asn-2041 and Gly-2078 ACCase alleles in various genetic backgrounds on plant growth and seed production in field conditions, in competition with wheat. Our study reveals that the fitness cost associated with mutant ACCase depends on the mutant allele and on the environment.

## Materials and methods

### PLANT MATERIAL

Eight black-grass accessions (Table 1) collected in 2003 in the administrative district of Côte d'Or (eastern France) and previously analysed by ACCase genotyping (Menchari *et al.* 2006) were used to produce 10 populations, each one segregating for a given ACCase allele, so as to homogenize the genetic background of homozygous wild-type (W/W), heterozygous mutant (M/W) and homozygous mutant (M/M) plants. One segregating population was derived from each of the six accessions D24, D41, D57, D60, D98 and D143 (Table 1). Two populations, each segregating for one of the mutant ACCase alleles, were derived from each of accessions D83 and D121 that contained two different mutant ACCase alleles (Table 1). All black-grass accessions were collected within 120 km of the location of the field experiments.

Vernalization is necessary to ensure flowering of black-grass plants (Chauvel *et al.* 2002). Seeds from each accession were deposited onto two layers of 4-mm-thick artificial sponge sheets (Spontex, Paris, France) soaked with distilled water and placed in 19 × 13 × 4-cm

**Table 1.** Black-grass accessions used to produce the segregating populations analysed

Accession	Frequency (%) of ACCase alleles*			Derived segregating populations
	Leu-1781	Asn-2041	Gly-2078	
D24	68.3†	0.0	0.0	L1781-D24-04, -05
D41	0.0	0.0	25.0†	G2078-D41-04, -05
D57	33.3†	8.3	0.0	L1781-D57-04, -05
D60	1.6	8.3†	0.0	N2041-D60-04, 05
D83	0.0	84.9†	6.6†	N2041-D83-04, -05 G2078-D83-04, -05
D98	26.6†	0.0	0.0	L1781-D98-04, -05
D121	23.3†	40.0†	0.0	L1781-D121-04, -05 N2041-D121-04, -05
D143	25.0†	0.0	0.0	L1781-D143-04, -05

\*Frequencies obtained by Menchari *et al.* (2006).

†In each of two independent experiments, 30 plants heterozygous for this ACCase allele (e.g. Leu-1781/wild-type plants) were crossed to produce one segregating population.

plastic boxes. Boxes were placed at 4 °C constant temperature in darkness for 3 weeks (Chauvel *et al.* 2002). Vernalized seeds were subsequently sown into individual mini-pots in a glasshouse. At the three-leaf stage, the presence of all known mutations at ACCase was investigated in seedlings by genotyping, as described elsewhere (Délye, Matějček & Gasquez 2002; Délye *et al.* 2003, 2005). This ensured that all mother plants used subsequently for producing a segregating population were all M/W for the same mutant ACCase allele (e.g. Leu-1781/wild-type) and did not contain any other known mutant ACCase allele. To obtain a segregating population, 30 mother plants from the same accession, all M/W for the same mutant ACCase allele, were planted in mid-April in a field in Dijon. Each group of 30 plants was isolated within a pollen-proof enclosure. All plants flowered simultaneously. Open pollination was allowed within each enclosure. The period critical for growth and seed production of the mother plants ran from May to August. During this period, the cumulative rainfall in Dijon was 289 mm in 2004 and 185 mm in 2005 (<http://www.meteociel.fr>). Ripe seeds were harvested in August as a bulk sample from all 30 plants and stored for 2 months at room temperature to enable after-ripening. Segregating populations were labelled after the type of ACCase allele they contained, the accession they were derived from (Table 1), and the year they were obtained (e.g. L1781-D121-04). Five segregating populations containing Leu-1781 ACCase, three containing Asn-2041 ACCase, and two containing Gly-2078 ACCase (Table 1), a total of 10 segregating populations, were produced in 2004 for the 2004–05 field experiment. The whole procedure was repeated in 2005 so that 10 new segregating populations were used in the 2005–06 field experiment.

## FIELD EXPERIMENTS

Seeds from the segregating populations were germinated as described (Menchari *et al.* 2006). After 7 days, 700 one-leaf seedlings per population were transplanted in multipot plates in a glasshouse. The transplanted seedlings came from the seeds that germinated first. At the three-leaf stage, a 1-cm section was cut from the first leaf. DNA was extracted and the presence of all known mutations at ACCase was investigated in each of at least 200 seedlings per population by genotyping, as before. This ensured that all seedlings in a given segregating population exclusively contained one given type of mutant ACCase allele. Each genotyped seedling was classified as W/W, M/W or M/M for the mutant ACCase allele it contained. Thirty-three plants were then randomly chosen per genotype and per segregating population, in order to obtain a total of 990 plants in the field experiment (33 plants × 3 genotypes × 10 populations). Plants were transplanted just after tilling in a field sown with winter wheat at the INRA experimental farm in Bretenières, 13 km south-east from Dijon, following a fully randomized design. Plants were placed between wheat rows (17 cm apart) and were spaced by 30 cm in the row to avoid black-grass/black-grass competition. A coloured plastic label was placed close to each plant to ensure proper identification at harvest. The standard cultivation practices used in Côte d'Or were performed subsequently, except that no herbicide active on grass weeds was sprayed after black-grass plants were planted. In the following year, just before wheat harvest, the whole above-ground part of each black-grass plant was collected.

During the 2004–05 growing season, black-grass plants were transplanted on 15 January 2005 into a field that had been sown with wheat cultivar Apache (333 seeds m<sup>-2</sup>) on 8 November 2004. The field experiment was repeated during the 2005–06 growing season in a neighbouring field sown with wheat cultivar Cap Horn (324

seeds m<sup>-2</sup>) on 1 October 2005. Black-grass plants were transplanted to the field on 15 December 2005. At the time of transplantation, transplanted black-grass plants from all segregating populations and black-grass plants naturally occurring in neighbouring fields had one or two developed tillers. Black-grass was collected on 6 June 2005 and 12 June 2006. Wheat was harvested on 30 June 2005 and 12 July 2006. Wheat yield at harvest was about 8000 kg ha<sup>-1</sup> in both experiments. The period encompassing March to June is crucial for black-grass growth and seed production in the field. During this period, the cumulative rainfall in Bretenières was 277 mm in 2005 and 359 mm in 2006, as recorded by a station located about 1 km from the field experiments.

## MORPHOLOGICAL AND REPRODUCTIVE DESCRIPTORS

Three morphological descriptors with ecological significance were measured on each plant at the end of the experiment. They were plant height, measured as length of the longest flowering tiller, reflecting the capacity for inflorescences to appear above the crop canopy; total vegetative dry biomass after discarding all inflorescences and seeds, reflecting plant competitive ability; and total seed production, a major component of plant fitness. It was demonstrated previously that the number of seeds is highly correlated to length of the inflorescence (Chauvel 1996). For each plant, the length of one inflorescence was measured and the seed number determined by counting the number of spikelets on the inflorescence. The total seed production was estimated subsequently for each plant from the sum of the lengths of all inflorescences of the plant (Chauvel 1996).

## STATISTICAL ANALYSES

As a first approach to detecting a possible effect of plant genotype at ACCase on the variation of plant descriptors, an ANOVA using a general hierarchical model was performed using the GLM procedure of SYSTAT 10 (Systat Software Inc., Richmond, CA, USA). The model analysed the effects of growing season (year), population nested within year, and genotype nested within population within year. Segregating populations were produced independently each year from different mother plants with different genetic backgrounds, and were therefore compared within year. Because each type of mutant ACCase allele appeared several times independently (Délye *et al.* 2004), and because each segregating population may also contain distinct wild-type ACCase haplotypes, genotypes were compared within populations within years. All factors were considered random in this exploratory analysis because year is clearly a random factor; accessions from which mother plants were selected were randomly selected among black-grass field populations containing mutant ACCase alleles; and mother plants with their specific ACCase haplotypes were randomly selected within accessions. In addition, this conferred a general value to the results for all possible years, populations and genotypes. As the Levene test for heteroscedasticity was significant for all descriptors, all data were log-transformed before analysis.

In a second step, we focused on the possible pleiotropic effects of the presence of no (W/W plants), one (M/W plants) or two (M/M plants) copies of a given type of mutant ACCase allele on plant descriptors. Data were thus analysed separately for each of the three types of mutant ACCase allele (Leu-1781, Asn-2041 or Gly-2078) using an ANOVA mixed model with copy number of the mutant allele as a fixed factor, and year, population nested within year, and interaction between copy number of the mutant allele and population within year as random factors. Copy number of the mutant allele

**Table 2.** Deviation from the expected proportions of W/W, M/W and M/M plants in the four black-grass populations segregating for Gly-2078 ACCase alleles

Population	Genotype at ACCase (%)			Total genotyped	<i>P</i> value ( $\chi^2$ )*
	W/W	M/W	M/M		
G2078-D83-04	31.3	51.3	17.4	201	0.02
G2078-D83-05	30.6	58.3	11.1	297	<0.001
G2078-D41-04	24.0	55.5	20.5	200	0.21
G2078-D41-05	23.4	71.1	5.5	603	<0.001

\**P* values for observed numbers of plants with W/W, M/W and M/M genotype at ACCase differing significantly from expected numbers of such plants if proportions of these genotypes were 25, 50 and 25%, respectively.

was fixed, as fixed effects apply to an entire population or to well defined and repeatable subsets of a population, which is the case here: using genotyping, sampling W/W, W/M and M/M plants can be carried out repeatedly, and all possible levels of this factor (0, 1 or 2) were sampled. As in the first model, and for the same reasons, the other factors were considered random. Descriptor values for all levels of factors were compared using Student's *t*-test with Bonferroni adjustment of pairwise comparisons.

Fitness cost was computed as  $(M_{W/W} - M_{M/M})/M_{W/W}$  where  $M_{W/W}$ ,  $M_{M/W}$  and  $M_{M/M}$  are the descriptor raw mean values measured for W/W, M/W and M/M individuals, respectively. With genotypic fitness, a fitness dominance index *d* can be computed for each descriptor as  $d = |(M_{W/W} - M_{M/W})/(M_{W/W} - M_{M/M})|$ . Following convention, the mutant allele is dominant toward cost when  $d = 1$ , semidominant when  $d = 0.5$  and recessive when  $d$  approaches 0 (Roux, Gasquez & Reboud 2004).

## Results

### DETERMINATION OF PLANT GENOTYPES AT ACCASE PRIOR FIELD EXPERIMENT

Plants from the segregating populations produced in 2004 and in 2005 were genotyped before transplanting to the field experiment. No deviation from the expected proportions of W/W plants (25%), M/W plants (50%) and M/M plants (25%) was detected in nine out of the 10 populations for the 2004 seed production (not shown). A significant deficit in M/M Gly-2078 ACCase plants was observed in population G2078-

D83-04 (Table 2). For the seeds produced in 2005, no deviation from the expected proportions was observed in eight segregating populations: five containing Leu-1781 ACCase and three containing Asn-2041 ACCase (not shown). A significant deficit in M/M Gly-2078 ACCase plants was observed in the segregating population G2078-D83-05 (Table 2). This deficit was similar to the one observed in the previous year ( $\chi^2 = 4.6$ , 2 df,  $P = 0.11$ ). A significant deficit in M/M Gly-2078 ACCase plants was also observed in the segregating population G2078-D41-05 (Table 2), in contrast with the previous year ( $\chi^2 = 42.0$ , 2 df,  $P < 0.001$ ).

### FIELD EXPERIMENTS

Most transplanted plants developed successfully in the field and could be harvested and measured (plant numbers are given in Table S1 in Supplementary Material). The details of the raw mean descriptor values are also provided in Table S1. Results of the 2005–06 experiment were very homogeneous, while most differences appeared in the 2004–05 experiment. The general hierarchical, random model run on log-transformed data indicated no significant year effect on plant height (Table 3). A significant year effect was detected for biomass and seed production (Table 3), explaining 50 and 42% of the total variation, respectively, of these descriptors. Plants in the 2005–06 experiment weighed three times more and produced twice as many seeds as plants in the 2004–05 experiment (Table S1). Population nested within year always had a significant effect (Table 3), but accounted for less than 4% of the total variation of each plant descriptor. Genotype nested within population and year had a significant effect on seed production but not on the other descriptors (Table 3). The proportion of variance due to population and genotype effects computed from the raw or log-transformed plant descriptor values was always less than 4%. This indicated a very limited genetic component in the variation of plant height, vegetative biomass or seed production.

The mixed model used in a second step for analysing the possible pleiotropic effect of the copy number of a mutant allele in a plant showed significant year and population nested within year effects for the three descriptors, except for plant height in populations segregating for Asn-2041 or Gly-2078 ACCases (Table 4). Copy number of the mutant allele had no effect in populations segregating for Leu-1781 or Asn-2041 ACCase. In contrast, in populations segregating for

**Table 3.** *F* values from ANOVA on log-transformed data using the general, hierarchical, random model

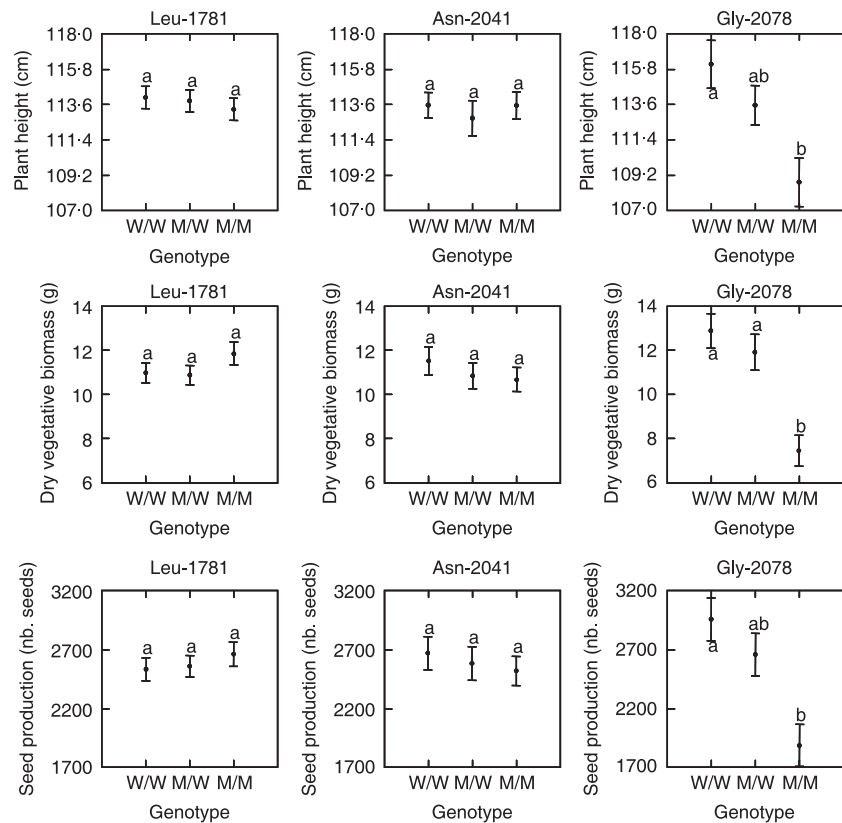
Factor	Test	Plant descriptor		
		Plant height	Dry vegetative biomass	Total seed production
Year	$F_{1,1853}$	2.17 <sup>NS</sup>	2000.44***	1479.69***
Population (Year)	$F_{18,1853}$	3.58***	5.54***	7.08***
Genotype (Population(Year))	$F_{40,1853}$	1.35 <sup>NS</sup>	1.26 <sup>NS</sup>	1.41*

<sup>NS</sup>, Not significant; \*, significant effect at 0.05 threshold; \*\*\*, significant effect at 0.001 threshold.

**Table 4.** *F* values from ANOVAs on log-transformed data using the mixed model for each type of mutant ACCase allele separately

Factor	Mutant ACCase allele	Test	Plant descriptor		
			Plant height	Dry vegetative biomass	Total seed production
Year	Leu-1781	$F_{1,958}$	17.08***	935.61***	702.80***
	Asn-2041	$F_{1,568}$	0.24 <sup>NS</sup>	684.23***	504.00***
	Gly-2078	$F_{1,348}$	2.20 <sup>NS</sup>	399.90***	274.91***
Mutant allele copy number	Leu-1781	$F_{2,16}$	0.40 <sup>NS</sup>	1.71 <sup>NS</sup>	0.34 <sup>NS</sup>
	Asn-2041	$F_{2,8}$	0.25 <sup>NS</sup>	0.59 <sup>NS</sup>	0.65 <sup>NS</sup>
	Gly-2078	$F_{2,4}$	9.66*	23.23**	6.95*
Population (year)	Leu-1781	$F_{8,958}$	3.23**	3.95***	8.06***
	Asn-2041	$F_{4,568}$	5.24***	9.13***	11.79***
	Gly-2078	$F_{2,348}$	2.01 <sup>NS</sup>	4.18*	4.58*
Mutant allele copy number × population (year)	Leu-1781	$F_{16,958}$	1.21 <sup>NS</sup>	0.67 <sup>NS</sup>	0.87 <sup>NS</sup>
	Asn-2041	$F_{8,568}$	2.18*	1.20 <sup>NS</sup>	0.89 <sup>NS</sup>
	Gly-2078	$F_{4,348}$	0.36 <sup>NS</sup>	0.34 <sup>NS</sup>	1.14 <sup>NS</sup>

<sup>NS</sup>, No significant effect; \*, significant effect at 0.05 threshold; \*\*, significant effect at 0.01 threshold; \*\*\*, significant effect at 0.001 threshold.



**Fig. 1.** Mean values with SE of plant height (top), dry vegetative biomass (centre) and seed production (below) computed for each level of copy number of a mutant allele (genotype) and each type of mutant ACCase allele using all populations segregating for a given type of ACCase allele: Leu-1781 (10 populations); Asn-2041 (six populations); Gly-2078 (four populations). Identical letters within one graph indicate no significant differences at the 0.05 threshold after Student's *t*-test with Bonferroni adjustment of pairwise comparisons on log-transformed data.

Gly-2078 ACCase, M/M plants were significantly smaller and produced fewer seeds than W/W plants (Table 4; Fig. 1). M/M plants also produced less biomass than both M/W and W/W plants in these populations (Table 4; Fig. 1). The interaction between copy number of the mutant allele and population within year was significant in only one case, for Asn-2041 ACCase (Table 4). This was due to W/W plants being smaller than M/W and M/M plants in the segregating population N2041-D83-04 only (Table S1, Supplementary Material).

Over both field experiments, our results showed a significant reduction in vegetative biomass (42%) and seed production (36%) in M/M Gly-2078 ACCase plants in comparison with W/W plants. The reduction in plant height in M/M plants was about 6% in comparison with W/W plants from the same populations. The fitness cost dominance index *d* computed in populations segregating for Gly-2078 ACCase was 0.35 for plant height, 0.18 for vegetative biomass and 0.28 for seed production. The *d*-values indicated a possible weak semidominance. However, no significant effect on plant descriptors

was observed for M/W plants in comparison with W/W plants (Fig. 1), suggesting that the fitness cost associated with Gly-2078 ACCase is more likely to be recessive.

## Discussion

Assessing fitness costs associated with genes endowing herbicide resistance is of importance in predicting resistance dynamics and proposing resistance-management strategies (Maxwell, Roush & Radosevitch 1990; Jasieniuk, Brülé-Babel & Morrison 1996). This is the first study where weed individuals with a characterized genotype at a locus endowing herbicide resistance have been used to assess fitness costs associated with herbicide resistance, and where fitness cost dominance was assessed.

Mutant ACCase alleles have been shown to appear independently in distinct black-grass populations (Délye *et al.* 2004). Given that there may be occurrences when some of the differences observed could be due to linkage disequilibrium of a mutant ACCase allele with deleterious genes, and given that fitness cost associated with a resistance gene can also vary depending on the genetic background in which it occurs (Bergelson & Purrington 1996), a total of five, three and two segregating populations were used across each of two field experiments to assess possible fitness costs associated with Leu-1781, Asn-2041 and Gly-2078 ACCase alleles, respectively. Fitness cost was also reported to vary with environment (Bergelson & Purrington 1996; Jordan *et al.* 1999; Martin & Lenormand 2006), which is the reason why we chose to assess it in the situation where resistance genes are selected, in competition with a crop plant rather than in the absence of competition.

### FITNESS COST DEPENDS ON THE MUTANT ACCASE ALLELE

We did not observe any fitness cost on plant growth and seed production associated with Leu-1781 ACCase in 10 different black-grass populations segregating for this allele. Our results confirmed and extended those of previous studies where the fitness cost associated with Leu-1781 ACCase on plant growth and seed production was investigated in rye-grass in the absence of competition (Vila-Aiub, Neve & Powles 2005). These authors observed a fitness cost associated with enhanced herbicide metabolism, and an absence of additional fitness cost in plants with both enhanced herbicide metabolism and Leu-1781 ACCase, which enabled them to conclude indirectly that there was an absence of fitness cost associated with Leu-1781 ACCase in the rye-grass population they studied (Vila-Aiub, Neve & Powles 2005). They also found that Leu-1781 ACCase may confer differences in germination in plants from the particular population they studied (Vila-Aiub, Neve, Steadman & Powles 2005), but this finding needs to be confirmed across a broader range of genetic backgrounds before its general value can be established. Literature suggests that the absence of fitness cost associated with Leu-1781 ACCase is a general feature. First, Leu-1781

ACCase enzyme activity did not differ from that of wild-type ACCase in the grass species where it has been studied (rye-grass, Yu *et al.* 2007; green foxtail *Setaria viridis*, Shukla, Leach & Devine 1997; confirmation that the mutant ACCase studied was Leu-1781 was provided by Délye, Wang & Dar-mency 2002). Second, Leu-1781 ACCase is clearly the most widespread mutant ACCase allele in black-grass populations in France (Menchari *et al.* 2006). Third, Leu-1781 ACCase is the mutant ACCase allele most frequently identified across grass species where populations evolved mutant ACCase (five species, Délye 2005; Zhang & Powles 2006a; Liu *et al.* 2007; Yu *et al.* 2007). Finally, this allele became fixed in at least three grass species, annual blue-grass *Poa annua*, prostrated blue-grass *Poa supina* and red fescue *Festuca rubra* (Délye & Michel 2005). Among these species, prostrated blue-grass and red fescue are not arable weeds, and have consequently not been subjected to the selective pressure of ACCase-inhibiting herbicides. All these data are clearly in favour of the absence of fitness cost associated with Leu-1781 ACCase.

To date, there has been no published study addressing the possible fitness costs associated with Asn-2041 or Gly-2078 ACCase. Asn-2041 ACCase from black-grass displayed a moderately reduced enzyme activity when compared with wild-type ACCase (Délye *et al.* 2003). It was significantly less frequent than Leu-1781 ACCase in France (Menchari *et al.* 2006). Asn-2041 ACCase has been reported in only two other weed species so far: rye-grass (Délye *et al.* 2003; Zhang & Powles 2006b; Yu *et al.* 2007) and wild oat *Avena sterilis* (Liu *et al.* 2007). However, we did not detect any fitness cost on plant growth or seed production associated with Asn-2041 ACCase across six black-grass populations segregating for this allele grown in competition with wheat. Because our study did not address all key parameters of the black-grass life cycle, a possible deleterious pleiotropic effect of Asn-2041 ACCase on seed germination or survival in the soil cannot be excluded, and needs to be investigated. Additionally, the broad phenotypic plasticity of black-grass and the phenotypic variability observed across the 2 years in which the field experiments were conducted (Table S1) may render undetectable any subtle fitness cost, nevertheless having a cumulative, deleterious effect in the field over many growing seasons.

Sequencing showed that two distinct Gly-2078 ACCase haplotypes occurred in both populations G2078-D41-04 and -05. Both haplotypes were distinct from the one occurring in populations G2078-D83-04 and -05 (not shown). This enabled us to rule out the occurrence of an unknown gene linked to Gly-2078 ACCase that would be responsible for the significant reduction in plant height, vegetative biomass and seed production observed in M/M Gly-2078 ACCase plants across both field experiments (Table 4; Fig. 1). Gly-2078 ACCase was the mutant, resistant ACCase allele detected least frequently across black-grass populations in France (Menchari *et al.* 2006). It has been reported only in black-grass and rye-grass so far (Délye *et al.* 2005; Yu *et al.* 2007). Its enzyme activity was about half that of wild-type ACCase (Délye *et al.* 2005; Yu *et al.* 2007). The data suggested that there may be a fitness cost associated with Gly-2078 ACCase, which our study

confirms: we found that the fitness cost associated with Gly-2078 ACCase would be recessive or weakly semidominant. Characterizing the dominance of fitness cost is very important because the spread and persistence of resistance genes initially depends on the fitness of the first mutant resistant individuals to appear, which are M/W plants in diploid species such as black-grass. The diffusion of a resistant ACCase allele will be much faster if the associated fitness cost is recessive (Jasieniuk, Brûlé-Babel & Morrison 1996). As a consequence, the fitness cost associated with Gly-2078 ACCase should only moderately limit the frequency and alter the initial evolutionary dynamics of this type of alleles in black-grass populations.

The low (<4%) genetic contribution to variation in vegetative biomass, plant height and seed production observed here across genotypes and populations suggests that it is unlikely that black-grass will be selected for higher or lower vegetative and reproductive productivity. Thus any fitness cost reducing vegetative and reproductive productivity is expected to have a dramatic effect on the survival and reproduction of the plants. Large individual plants gain more resources (water, light, nutrients) and produce more seeds than smaller ones. Smaller individuals thus tend to be eradicated in plant populations (Weiner 1986). In addition, the reduction in M/M, Gly-2078 plant height observed (7 cm, about the average length of an inflorescence) may prevent inflorescences from emerging from the wheat canopy, reducing the possibility of pollen exchange that is crucial for seed production in the allogamous black-grass. M/M, Gly-2078 plants are therefore expected to be at a disadvantage in competitive crops or in competition with plants containing Leu-1781 ACCase, Asn-2041 ACCase or, in the absence of herbicide treatment, with wild-type plants. This disadvantage might be exacerbated by a segregation distortion against M/M, Gly-2078 ACCase plants (Table 2), although the deficit observed in this genotype may also reflect differences in the dynamics of germination among genotypes. ACCase being a key enzyme in lipid biosynthesis, and lipid being important storage compounds in black-grass seeds (Darmency, Landry & Mossé 1981), pleiotropic effects of the reduced enzyme activity of Gly-2078 ACCase on seed germination and/or survival in the soil may exist, and need to be investigated.

While a deleterious effect of Gly-2078 ACCase was clearly detected in M/M plants in the 2004–05 field experiment, it was not evident in the 2005–06 experiment (Table S1). The 2005–06 field experiment was performed in a context of lower competition for water resources than the previous experiment, as can be seen clearly in the higher total vegetative dry biomass and seed production values (Table S1). Also, the mother plants used to produce the segregating populations were not the same as in the previous year. Fitness cost on plant growth and seed production associated with Gly-2078 ACCase is thus clearly dependent on the population and environmental conditions experienced during the growing season. This illustrates the roles of the genetic background and of the environment in the expression of fitness cost.

From the literature and our results, Leu-1781 ACCase appears to be the 'best' herbicide-resistant ACCase allele to

be selected for in black-grass, and probably in other grass weed species. Given that mutant, resistant ACCase alleles arise by multiple, independent appearances in black-grass populations (Délye *et al.* 2004; Menchari *et al.* 2006), a replacement of the less fit mutant ACCase alleles by Leu-1781 ACCase in black-grass populations is expected, especially if herbicide selective pressure is maintained and all ACCase alleles confer a similar degree of protection against the selecting herbicide.

#### MANAGEMENT OF RESISTANCE ENDOWED BY MUTANT ACCASE

The different mutant ACCase alleles do not confer identical cross-resistance patterns to ACCase-inhibiting herbicides. These herbicides essentially belong to two chemical families, 'fops' and 'dims' (reviewed by Délye 2005). Leu-1781, Asn-2041 and Gly-2078 ACCase alleles all confer resistance to the two fop herbicides (fenoxaprop and clodinafop) used most commonly in France over the past 15 years (Délye 2005; Menchari *et al.* 2006). However, Leu-1781 ACCase confers cross-resistance to other, but not all, fops and to some, but not all, dims. Asn-2041 ACCase confers cross-resistance to all fops assayed but not to dims. Gly-2078 confers resistance to all fops and to all dims assayed (reviewed by Délye 2005), which may be a reason why this allele is still present in a number of black-grass populations (Menchari *et al.* 2006) despite its association with a significant fitness cost. The broad cross-resistance spectrum of Gly-2078 ACCase makes it the most potentially troublesome resistant ACCase allele in terms of resistance management. This allele is fortunately associated with a recessive or weakly semidominant fitness cost such that the use of competitive crops (rye, wheat sown at higher density, wheat cultivars with a strong biomass development) should strongly hamper the development, seed production and pollen exchange of M/M plants, and could perhaps reduce those of M/W plants containing Gly-2078 ACCase. Alternatively, including a grass or alfalfa meadow in the crop rotation should have a similar, or more drastic, effect on the development and seed production of plants containing Gly-2078 ACCase.

To obtain a complete picture of fitness costs associated with mutant ACCase alleles, we need to study the whole life cycle of black-grass, and to investigate the possible pleiotropic effects of Cys-2027 and Ala-2096 ACCase alleles. This may reveal differences in the biology and ecology of plants containing these alleles that can be exploited by cultural practices to create or to maximize fitness costs, as demonstrated elsewhere (Jordan *et al.* 1999). However, given that fitness costs associated with resistant ACCase alleles may vary with black-grass genetic background and with the environment, as observed here for Gly-2078 ACCase, the continued success of such cultural practices is uncertain. Also, it is possible that, as is very likely the case for Leu-1781 ACCase, a significant part of the genes endowing resistance to ACCase-inhibiting herbicides are not associated with pleiotropic effects on the black-grass life cycle. A solution could then be to use diversified



management practices, as has been suggested to manage triazine-resistant *Amaranthus hybridus* plants (Jordan *et al.* 1999). This would involve combining cultural practices such as ploughing, which disrupts black-grass proliferation (Chauvel *et al.* 2001), with cultural practices found to create or maximize the fitness cost for some resistant ACCase alleles, and herbicides with diverse modes of action. Such a diversified black-grass management programme should prevent selection for resistant ACCase alleles, or reduce the speed with which it spreads. However, as Jordan *et al.* (1999) noted, 'many social, economic and agronomic barriers stand in the way of diversified integrated weed management'.

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### Supplementary material

The following supplementary material is available for this article.

**Table S1.** Mean values of the three plant descriptors of blackgrass plants computed for each genotype in each blackgrass segregating population studied.

This material is available as part of the online article from:  
<http://www.blackwell-synergy.com/doi/full/10.1111/j.1365-2664.2008.01462.x>  
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