

MULTIGENERATIONAL VERSUS SINGLE GENERATION STUDIES TO ESTIMATE HERBICIDE RESISTANCE FITNESS COST IN *ARABIDOPSIS THALIANA*

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Abstract.—The evolution of resistance in response to pesticide selection is expected to be delayed if fitness costs are associated with resistance genes. The estimate of fitness costs usually involves comparing major growth traits of resistant versus susceptible individuals in the absence of pesticide. Ideally, a measure of changes in resistance allele frequency over several generations would allow the best estimate of the overall fitness cost of a resistance gene. In greenhouse conditions, we monitored the dynamics of the evolution of the frequencies of six herbicide-resistant mutations (acetolactate synthase, cellulose synthase, and auxin-induced target genes) in the model species *Arabidopsis thaliana* in a multigenerational study covering five to seven nonoverlapping generations. The microevolutionary dynamics in experimental populations indicated a mean fitness cost of 38%, 73%, and 94% for the *ixr1-2*, *axr1-3*, and *axr2-1* resistances, respectively; no fitness cost for the *csr1-1*, and *ixr2-1* resistances; and a transient advantage for the *aux1-7* resistance. The result for the *csr1-1* resistance contrasts with a cost of 37% based on total seed number in a previous study, demonstrating that single generation studies could have limitation for detecting cost. A positive frequency dependence for the fitness cost was also detected for the *ixr1-2* resistance. The results are discussed in relation to the maintenance of polymorphism at resistance loci.

Key words.—Experimental evolution, fitness impairment, frequency dependence, herbicide resistance, pesticide management.

Received April 28, 2005. Accepted August 1, 2005.

In naturally occurring populations of weed species, the adaptation by genetic resistance to repeated treatment with a particular class of herbicides can be analyzed as an evolutionary process (e.g., Jasienuk et al. 1996). Numerous factors influence the dynamics of evolutionary processes in natural populations of plants and animals. For the evolution of pesticide resistance, the main evolutionary forces are those affecting demography and possibilities for local ecological adaptation such as gene flow among fields, relative carrying capacities, and selection pressures in both treated and untreated areas (Lenormand and Raymond 1998). The fate of resistance alleles depends on their selective advantage or disadvantage (fitness cost) in the presence or the absence of pesticides in the environment, as well as the dominance of these effects (reviewed by Maxwell and Mortimer 1994). Associating fitness costs to adaptive genes is a logical and in many cases valid assumption that is based on both theory and observations (Purrington 2000). First, genetic changes conferring adaptation to the new “environment” may involve large modifications of the previous phenotype and may therefore induce a fitness penalty in the previous environment. Second, polymorphisms at adaptive genes seem to persist in populations due to the counterselection of the adaptive genes in the absence of the corresponding selective pressure.

Two general groups of methods are used to determine the fitness cost of pesticide resistance alleles (e.g., Bourguet et

al. 2004). The first group is based on a direct comparison of major traits affecting fitness, among homozygous-resistant (RR) and homozygous-susceptible (SS) individuals. This approach can provide information about the specific trait modification endowing resistance but also responsible for a fitness cost. The second simply refers to the definition of fitness, that is, the average contribution of an allele to succeeding generations (Futuyma 1997). This involves quantifying the changes over several generations in the resistance allele frequencies in populations that are no longer treated with pesticides (Cochran 1993; Boivin et al. 2003; Gustafsson et al. 2003). The first approach is generally used to estimate fitness cost of herbicide resistance (reviewed in Bergelson and Purrington 1996) and more recently herbicide tolerance (Baucom and Mauricio 2004), whereas the second approach remains seldom used in plants to assess the fitness penalty of a gene conferring biotic or abiotic stress resistance. Because several small modifications between resistant and susceptible individuals can result in a significant cost when integrated over the whole life cycle, studying change in resistance allele frequencies over several generations should be more powerful to detect fitness costs and more reliable for giving the “real” cost value.

In a previous study on eight herbicide resistance alleles in the model species *Arabidopsis thaliana*, we presented results from a single generation analysis of morphological and productivity-related traits in a segregating F₂ R/S population in the absence of herbicide treatment (Roux et al. 2004). This method was first designed to measure the dominance of herbicide resistance cost. It belongs to the first group of methods described above and measures costs by comparing fitness

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traits in R/S lines in the absence of herbicide. Here, we designed a multigenerational experiment belonging to the second group of methods to examine the fitness cost of six herbicide resistance alleles in *A. thaliana*. In greenhouse conditions, the fitness cost for each resistance was inferred from the evolution of the resistance allele frequency in experimental populations in the absence of herbicide treatment over five to seven nonoverlapping generations. For each resistance and for each of the two methods, the seeds used originated from the same seed bulk and the experiments were conducted under identical growing conditions, thereby allowing an unambiguous comparison of the fitness cost estimates found with each method.

MATERIALS AND METHODS

Plant Materials and Experimental Populations

The six herbicide resistance lines used in our study are the *csr1-1* chlorsulfuron resistance; the *ixr1-2* and *ixr2-1* isoxaben resistances; and the *aux1-7*, *axr1-3*, and *axr2-1* 2,4-dichlorophenoxy acetic acid (2,4-D) resistances. A more detailed description of these resistances as well as the reasons for conducting the analysis using a Ler-Col crossing scheme or the genotyping methods used is available in Roux et al. (2004). Briefly summarized, a process of “genetic background randomization” was performed for each resistance by generating a segregating F₂ population from a cross between each mutant resistance line (Col genetic background) and the male sterile sensitive line NW77 (Ler genetic background). Any character differing between Col and Ler ecotypes would segregate freely from the resistance locus, thus providing a mean cost value of resistance over a large number of genetic backgrounds. Each cross was replicated (named family effect) by using two different plants from the same mutant line. For each family, six experimental populations were initiated with F₂ seeds issued from the same seed source that was used by Roux et al. (2004). Thus, 12 replicate populations, each with 120 F₂ seeds, were generated for each resistance to initiate the first generation, G1. In the absence of segregation distortion, the starting resistance allele frequency should therefore be 0.5. For each replicate experimental population, plants were grown in 17 × 27 × 7 cm plastic trays in an insect-free greenhouse and regularly watered with a standard nutrient solution. The plastic trays have elevated edges ensuring isolation among trays although plants may freely self-fertilize within a tray (Lavigne et al. 2001). These growing conditions are equivalent to 2600 plant/m², which represents a plant density in which there was substantial interplant competition. Throughout seven generations (G1 to G7) for the *csr1-1*, *ixr1-2*, *ixr2-1*, and *aux1-7* resistances, or five generations (G1 to G5) for the *axr1-3* and *axr2-1* resistances, seeds were carefully harvested and bulked separately from each population, and 120 seeds were used to initiate the next generation in a new tray of equal size. Throughout the experiment, generations were discrete, non-overlapping, and kept as isolated gene pools. Before conducting the final phase of simultaneous genotyping analysis over all the generations, the seeds were stored in the dark at 4°C, 10% humidity (Lavigne et al. 2001). Each generation was grown in herbicide-free conditions. To permit compar-

isons of fitness cost estimates between the two methods, the growing conditions were kept identical between this experiment and the one designed for the first method (Roux et al. 2004). Plants were grown under natural light supplemented by artificial light to provide a 16-h photoperiod. The temperature was maintained between 20°C and 25°C in the greenhouse. To avoid microenvironmental heterogeneities, the trays were regularly rotated throughout the growing period.

Measuring Resistance Allele Frequencies

To assess the evolution of resistance allele frequencies over the generations, a sample of 100 seeds was randomly selected from each population seed bulk. DNA was extracted from 48 10-day-old germinated seedlings per population and generation. Because the germination rate approached 100% under our petri dish conditions, there was no possible germination bias among SS, RS, and RR seeds. Thus the seeds produced at generations 2, 4, and 6 could be used to estimate resistance allele frequencies for generations 3, 5, and 7, respectively. For each resistance type, R/S segregation distortion in initial frequencies (G1) was also checked by randomly analyzing 100 F₂ seedlings per family.

For the *ixr2-1*, *aux1-7*, and *axr2-1* resistances, an allele-specific polymerase chain reaction method was used to discriminate among the three genotypes SS, RR, and RS (details in Roux et al. 2004). For the three other resistance loci, genotyping was obtained more quickly with newly available single nucleotide polymorphism (SNP) technologies: either Amplifluor (Serologicals Corporation, Norcross, GA; Myakishev et al. 2001) for the *csr1-1* and *axr1-3* resistances, or TaqMan (Applied Biosystems, Foster City, CA; Livak 1999) for the *ixr1-2* resistance.

Frequency Deviations Arising from Genetic Drift

Allele frequency deviations may result from both the directional selection on fitness differences among individuals and the random genetic drift in a population of finite size. Because our experimental populations were of relatively small nominal size, genetic drift could induce substantial randomly distributed deviations among the 12 replicates. To determine the magnitude of allele frequency deviations in the experimental populations that could be attributable to genetic drift alone, 95% confidence limits were therefore derived from theoretical distributions of drift variance in a population. Experimental results were then compared to these confidence intervals to identify the deviations due to either advantage or disadvantage of the resistance allele over the susceptible one. Following Falconer and Mackay (1996), after *t* generations, the expected variance in allele frequencies among replicate populations due to genetic drift alone is:

$$V_{qt} = p_0q_0 \left[1 - \left(1 - \frac{1}{2N_e} \right)^t \right], \quad (1)$$

where *p*₀ and *q*₀ are initial susceptible and resistant allele frequencies, respectively; and *N*_{*e*} is the multigeneration effective population size. No family effect on resistance allele frequencies was observed by a nested analysis of variance using the model *y* = generation + family(generator) + error, where the relevant term was the nested term (*csr1-1*: *F* =

TABLE 1. Standard diploid model for selection in a selfing population. Genotype frequencies add to unity: $P + Q + R = 1$. The mean zygote fitness is $w = 1 - Qsh - Rs$. a , b , and c are the frequencies of SS, RS, and RR seeds produced by a RS plant, respectively.

| Genotype | SS | RS | RR |
|--------------------------|----------------------|----------------|-----------------------------|
| Fitness | 1 | $1 - sh$ | $1 - s$ |
| Frequency in adult | P | $\frac{Q}{b}$ | $\frac{R}{c}$ |
| Segregation of RS plants | a | b | c |
| Frequency in progeny | $[P + aQ(1 - sh)]/w$ | $bQ(1 - sh)/w$ | $[R(1 - s) + cQ(1 - sh)]/w$ |

0.20, $P = 0.894$; *ixr1-2*: $F = 0.09$, $P = 0.963$; *ixr2-1*: $F = 0.96$, $P = 0.424$; *aux1-7*: $F = 0.20$, $P = 0.897$; *axr1-3*: $F = 0.23$, $P = 0.796$; *axr2-1*: $F = 0.22$, $P = 0.803$). As a consequence, the expected variance for the mean of 12 populations in generation t , Vx_{qt} , was calculated as $V_{qt}/12$. A 95% probabilistic confidence interval for deviations attributable to genetic drift is thus expressed as initial susceptible allele frequency $\pm (1.96 \times \sqrt{V_{qt}/12})$ against initial resistance allele frequency $\pm (1.96 \times \sqrt{V_{qt}/12})$. Over multiple generations, the effective population size can be approximated by its harmonic mean as

$$\frac{1}{N_e} = \frac{1}{t} \left(\frac{1}{N_{e1}} + \frac{1}{N_{e2}} + \frac{1}{N_{e3}} + \dots + \frac{1}{N_{et}} \right) \quad (2)$$

(Falconer and Mackay 1996), where t is the number of generations, and N_{e1} , N_{e2} , N_{e3} , ..., N_{et} are effective population sizes in successive generations. To account for the selfing breeding system of *A. thaliana*, following Pannell and Barrett (2001) we used

$$N_{et} = \frac{N_t}{1 + F_t}, \quad (3)$$

where N_t is the population size and F_t is the inbreeding coefficient. Under partially self-fertilization, F_t changes each generation according to the recursion equation $\frac{1}{2}z(1 + F_{t-1})$, with z being the probability that an individual is produced by selfing (Pollak 1987). Assuming an outcrossing rate of 2% in *A. thaliana* (Snape and Lawrence 1971), z equals 0.98 in our study. In this study, sizes of each experimental pop-

ulation were assumed to be equal to the number of seeds sown; that is, 120 plants. Under our calculations, effective population size N_e was calculated to drop from 120 to about 61 "Fisher-Wright" individuals in seven generations.

Estimating Fitness Cost

The value of the selection coefficient, here assumed to estimate the fitness cost necessary to produce allele frequency changes of a given magnitude, was determined using the recursion equation;

$$q_{t+1} = \frac{R_t(1 - s) + cQ_t(1 - sh) + (1/2)[bQ_t(1 - sh)]}{1 - Q_tsh - R_ts}, \quad (4)$$

where q_{t+1} is the frequency of the resistance allele in generation $t + 1$, R_t and Q_t are RR and RS genotype frequencies in generation t (see Table 1), s and h are the selection coefficient and dominance deviation, and b and c are the frequencies of RS and RR seeds produced by an RS plant, respectively (see Table 1). Iterations of recursion equation (4) were performed over seven generations over the maximum range 0–1 for s (in 0.001 increments) and for values of h as estimated in Roux et al. (2004); that is, 0.05, 0.13, 0.5, 0.3, and 0.91 for the *csr1-1*, *ixr1-2*, *ixr2-1*, *axr1-3*, and *axr2-1* resistances, respectively. In the case of the *aux1-7* resistance, an overdominance effect is observed: $(1 - sh)$ in equation (4) had to be changed into $(1 + h)$ accordingly, and h fixed as 0.48 in numerical applications.

RESULTS

Initial Frequencies

In total, 9792 seedlings were genotyped covering the three generations G3, G5, and G7 for the *csr1-1*, *ixr1-2*, *ixr2-1*, and *aux1-7* resistances (1824 seedlings each) and the two generations G3 and G5 for the *axr1-3* and *axr2-1* resistances (1248 seedlings each). The genotypic frequencies observed in the initial generation (G1) are given in Table 2. As previously found, a significant distortion from Mendelian segregation was again detected for the *axr1-3* resistance with fewer RR plants than expected. The initial resistance allele frequency q_0 was thus established to be 0.40 for the *axr1-3* resistance and 0.50 for all the other resistances. As a consequence, the values of the parameters a , b , and c used in equation (4) were 0.25, 0.5, and 0.25, respectively, for all the resistances except the *axr1-3* one; and 0.301, 0.591, and 0.108 for the *axr1-3* resistance (values based on the mean of two families) to account for the distortion of segregation observed in the initial generation.

TABLE 2. Genotypic frequencies at the initial generation (G1) for each of the six resistances. χ^2 was calculated based on the number of individuals in each of the three classes under a Mendelian hypothesis of $\frac{1}{4}$ SS, $\frac{1}{2}$ RS, $\frac{1}{4}$ RR with 2 df.

| Resistance | Family | No. of plants | | | χ^2 | |
|---------------|--------|---------------|-------|-------|----------|-------|
| | | Total of 48 | SS 12 | RS 24 | | RR 12 |
| <i>csr1-1</i> | 1 | 48 | 13 | 24 | 11 | 0.17 |
| | 2 | 47 | 9 | 28 | 10 | 1.77 |
| <i>ixr1-2</i> | 1 | 44 | 15 | 18 | 11 | 2.18 |
| | 2 | 45 | 14 | 22 | 9 | 1.13 |
| <i>ixr2-1</i> | 1 | 45 | 12 | 25 | 8 | 1.27 |
| | 2 | 46 | 16 | 21 | 9 | 2.48 |
| <i>aux1-7</i> | 1 | 43 | 13 | 17 | 13 | 1.88 |
| | 2 | 46 | 16 | 21 | 9 | 2.48 |
| <i>axr1-3</i> | 1 | 46 | 15 | 27 | 4 | 6.65* |
| | 2 | 46 | 14 | 28 | 4 | 6.52* |
| <i>axr2-1</i> | 1 | 45 | 13 | 21 | 11 | 0.38 |
| | 2 | 46 | 12 | 27 | 7 | 2.48 |

* 0.05 > P > 0.01.

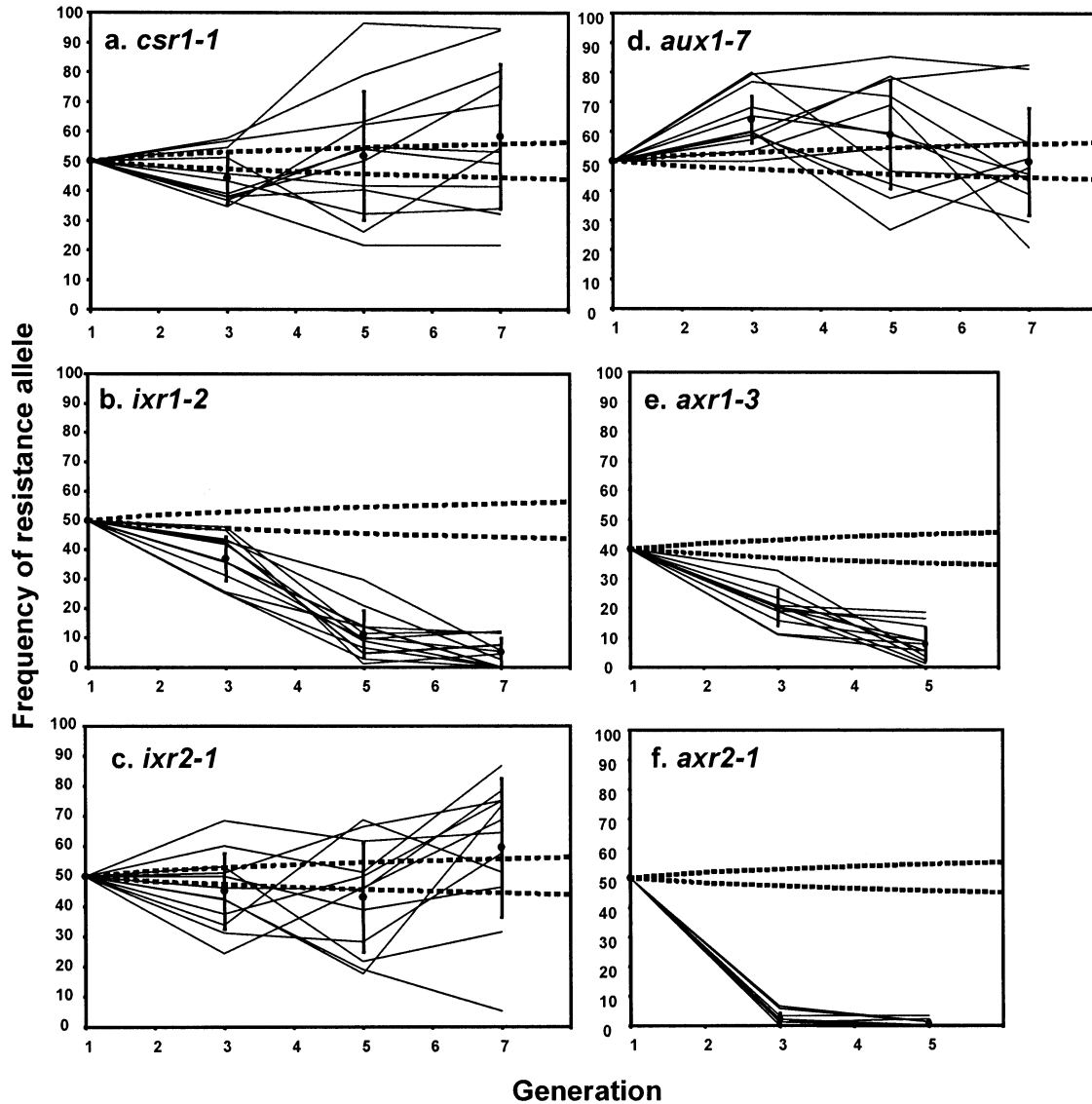


FIG. 1. Evolution of the six resistance allele frequencies in the absence of herbicide in experimental *Arabidopsis thaliana* populations. (a–d) represent the frequency of the *csr1-1*, *ixr1-2*, *ixr2-1*, and *aux1-7* resistance alleles, respectively, after three, five, and seven generations. (e) and (f) represent the frequency of the *axr1-3* and *axr2-1* resistance alleles, respectively, after three and five generations. Dots represent mean allele frequencies (with associated standard errors) of 12 populations (48 seedlings genotyped for each population). Where error bars are not indicated, they are less than the height of the dot. Solid lines represent the resistance frequency trajectories in each of the 12 populations. Dashed lines represent the 95% confidence intervals around the expected mean frequency in the absence of selection (0.5 for the *csr1-1*, *ixr1-2*, *ixr2-1*, *aux1-7*, and *axr2-1* resistance alleles and 0.4 for the *axr1-3* resistance allele) on the basis of theoretical predictions of drift variance.

Evolution of Resistance Allele Frequency

The change in resistance allele frequency is illustrated in Figures 1a–f. The higher the cost, the stronger the deviation of average resistance allele frequency (and associated error bars) outside the 95% confidence intervals covering genetic drift only. Both the *csr1-1* and *ixr2-1* resistance alleles did not significantly deviate from the expected frequency of 0.5 at each generation (see Figs. 1a and 1c, respectively). The *csr1-1* and *ixr2-1* resistance alleles showed a significant (Levene's test, $F_{1,22} = 12.92$, $P = 0.02$) and a nonsignificant (Levene's test, $F_{1,22} = 2.98$, $P = 0.097$) increase of variance between

generations 3 and 7, respectively. Such an increase of variance over time is consistent with the cumulative effect of genetic drift alone. Thus, there was no fitness cost found to be associated with the *csr1-1* and *ixr2-1* resistances.

The *ixr1-2*, *axr1-3*, and *axr2-1* resistance alleles deviated negatively from the initial frequency. The *ixr1-2* resistance allele exhibited a significant slight decrease at generation 3 and then a strong divergence from its initial frequency of 0.5 at generations 5 and 7 (see Fig. 1b). The *axr1-3* and *axr2-1* resistance alleles were at lower-than-expected frequencies (negatively selected), with a high divergence from their initial

TABLE 3. Estimated fitness costs for the six resistances. For each resistance, *t*-tests were used to compare fitness cost estimates across generations. Within a row, different letters indicate different values at $P = 0.05$. ns, fitness costs not significantly different from zero.

| Resistance | Fitness cost (\pm SE) ¹ | | | Fitness cost at F2 generation ² |
|---------------|---------------------------------------|----------------------------------|--------------------------------|--|
| | Generation 3 | Generation 5 | Generation 7 | |
| <i>csr1-1</i> | -17.1 \pm 7.4 ^{ns} | 5.5 \pm 10.7 ^{ns} | 9.1 \pm 6.9 ^{ns} | -36.9 |
| <i>ixr1-2</i> | -37.9 \pm 6.9 ^a | -65.0 \pm 6.6 ^b | -63.4 \pm 8.1 ^b | -43.2 |
| <i>ixr2-1</i> | -12.8 \pm 9.3 ^{ns} | -8.8 \pm 6.4 ^{ns} | 6.9 \pm 5.9 ^{ns} | none |
| <i>aux1-7</i> | 53.0 \pm 9.9 ^a | 20.6 \pm 11.8 ^{b, ns} | 0.6 \pm 6.6 ^{b, ns} | none |
| <i>axr1-3</i> | -73.3 \pm 5.7 ^a | -67.4 \pm 6.6 ^a | — | -78.2 |
| <i>axr2-1</i> | -93.8 \pm 1.7 ^a | -89.7 \pm 4.4 ^a | — | -89.0 |

¹ Fitness costs and associated standard errors correspond to selection coefficients s in equation (4) (see Materials and Methods). They are expressed as percentages and are based on the mean frequency deviations of the resistance alleles.

² Fitness costs are expressed as percentages and are based on the comparison of the total seed number between homozygous susceptible SS and homozygous resistant RR plants (Roux et al. 2004).

frequencies after generation 3 (see Figs. 1e,f). Thus, a fitness cost was found to be associated with the *ixr1-2*, *axr1-3*, and *axr2-1* resistance alleles.

The *aux1-7* resistance allele showed a more complicated trajectory. It first deviated positively from the expected frequency at generation 3 (see Fig. 1d). However, this transient slightly positive selection of the *aux1-7* resistance allele was no longer detectable at generations 5 and 7 where the measured frequencies did not significantly deviate from neutrality.

Estimates of Fitness Cost

The effect of each type of herbicide resistance on fitness as measured in this study was compared with the one based on the direct comparison of the approximated total seed number produced by homozygous-susceptible SS and homozygous-resistant RR plants at the F₂ generation (see Table 3). Both methods showed no significant fitness cost for the *ixr2-1* resistance. The *ixr1-2*, *axr1-3*, and *axr2-1* resistances all conferred strong fitness costs, as previously found when comparing the RR and SS fitness at the F₂ generation (Roux et al. 2004). At generation 3, the values of fitness cost conferred by those resistances were close to those already found at the F₂ generation. Interestingly, the fitness cost conferred by the *ixr1-2* resistance significantly increases from 37.9% at generation 3 to about 64% at generation 5 (*t*-test, $F = 7.99$, $P = 0.01$) and generation 7 (*t*-test, $F = 5.73$, $P = 0.026$), indicating a possible positive frequency dependence of the fitness cost associated to the *ixr1-2* resistance. For the *axr1-3* resistance, when taking into account the segregation distortion, the measure of the fitness cost is 54.3% and 60.8% at generations 3 and 5, respectively. Whatever the method used to measure the fitness costs, the highest fitness penalty found among the six resistances tested here was the one conferred by the *axr2-1* resistance.

Comparing SS and RR plants at the F₂ generation, the *csr1-1* resistance led to a decrease of 36.9% in the total seed set in the homozygous state, although no fitness cost was detected when studying the evolution of the *csr1-1* resistance allele over a longer period of time. The *aux1-7* resistance in the homozygous state exhibited a nonsignificant advantage of 31% in the seed production when measured at the F₂ generation, whereas it led to a transient significant increase

of 53% when estimated by the second method but only at generation 3.

DISCUSSION

The estimate of a fitness cost conferred by a resistance gene is of primary importance when dealing with pesticide resistance management strategies (Maxwell and Mortimer 1994). Without multigenerational data on allele frequencies, one difficulty for assessing the fitness cost of a resistance gene is to assume that total fitness can be correctly approximated by a set of measurable traits. For outcrossing species, measuring total fitness would require the measure of offspring produced via pollination. Seed quality is often approximated by a germination rate in optimal conditions and not in terms of resource quality; also, it is possible that seed reserves are not in sufficient quantity to guarantee high competitive ability in a normal competitive plant environment. The total seed number is, however, a rather common measure of fitness in plants, especially for strictly annual and selfing species and seems adequate to identify a fitness cost for the *ixr1-2*, *ixr2-1*, *axr1-3*, and *axr2-1* resistance alleles. For the *axr1-3*, and *axr2-1* resistances, a complementary analysis suggested that the resistance cost was primarily expressed as a change in resource acquisition rather than a modification of the allocation pattern between traits (Roux and Reboud 2005). However, contrasting results between direct and indirect measures of a cost were found with the *csr1-1* resistance (Roux et al. 2004). The fitness penalty of *csr1-1* homozygous RR plants based on total seed number was estimated as 37%, while the evolution of the *csr1-1* allele frequency over several generations suggests no fitness cost. One explanation for this apparent discrepancy between results could be that only the second method would integrate a compensating higher and faster germination rate found in some weed populations resistant to the chlorsulfuron herbicide compared to susceptible ones (Tranel and Wright 2002). In addition, the amelioration of pleiotropic fitness costs over time by epistatic modifier loci could be an explanation for failure to detect fitness cost associated with the *csr1-1* resistance (Cohan et al. 1994).

When conducting a field experiment with plots containing a 50:50 mixture of *csr1-1* homozygous RR and homozygous SS plants, Bergelson and Purrington (2002) found that the

resistant genotypes quickly dropped in frequency (from 50 to approximately 25%) after one generation in the absence of herbicide. The difference between this experiment and our study could come from different growing conditions mainly due to assemblage of naturally occurring weeds in field plots. Moreover, the removal of competitors was shown to be sufficient to remove detectable fitness costs of the *csr1-1* resistance (Bergelson and Purrington 2002).

Although our experiments were not performed under natural conditions, the results obtained for the *csr1-1* resistance seem to indicate that multigenerational data on allele frequencies are sometimes necessary to assess a fitness cost of a resistance gene, as previously discussed by Asmussen et al. (1998). In contrast to the first approach, studying microevolutionary dynamics in experimental populations takes into account the cumulative effects of a mutation on both viability and fertility as well as the effects on meiotic drift (a parameter rarely integrated in the fitness cost), as illustrated here by the *axr1-3* resistance case.

A multigenerational study has some limitations though. Detecting low fitness costs still necessitates huge population sizes or numerous replicates to overcome the general stochastic effect of genetic drift. The magnitude of a fitness cost conferred by any resistance allele needs to exceed the 95% confidence intervals attributable to genetic drift. In this study, using 12 population replicates with a size of 120 selfing individuals and assuming additive gene action ($h = 0.5$), a resistance allele with initial frequency of 0.5 would have required a fitness cost of at least 10.9%, 6.4%, and 4.8% at generations 3, 5, and 7, respectively, to be detectable. Many generations, numerous replicates, and bigger populations all contribute to evaluate low fitness cost conferred by a resistance allele. When studying the probability of fixation of slightly advantageous or deleterious mutations when selection and genetic drift interact, Whitlock (2000) came to a similar conclusion.

ACKNOWLEDGMENTS

We are grateful to S. Vernhettes for the *ixr2-1* seeds. Special thanks are given to J. Bergelson, J. Willis, R. Mauricio, and two anonymous reviewers for helpful comments on an earlier version of this manuscript. We also thank H. McKhann, S. Powles, and Shane Friesen for correcting the English and A. Matějček, L. Jacquens, and M. Schoutith for their technical assistance. This study was supported by a grant to FR from Bayer Crop Sciences. The SNP genotyping was largely financed by a special grant from the Institut National de la Recherche Agronomique, Santé des Plantes et Environnement.

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Corresponding Editor: R. Mauricio