Fitness Consequences of Genetically Engineered Herbicide and Antibiotic Resistance in *Arabidopsis thaliana*

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ABSTRACT

Researchers have often invoked the concept of metabolic drain to explain the lower growth rates of bacteria containing plasmids that confer antibiotic resistance. This idea posits that the energetic input needed to produce detoxifying enzymes diverts resources from clonal reproduction. In this paper we examine whether the concept of metabolic drain can be applied successfully to plants that differ from bacteria in several key aspects including their relative genome size and reproductive rate. We have conducted a field experiment using mutant and transgenic *Arabidopsis thaliana* that allows the comparison of genotypes differing by a single gene conferring resistance to either the herbicide chlorsulfuron or the antibiotic kanamycin. In addition to testing whether these traits reduce fitness, this experiment was conducted at two levels of resource availability to examine whether costs of resistance are sensitive to environmental quality. We found that herbicide-resistant individuals produced 26% fewer seeds than susceptible counterparts. However, contrasting published results in bacterial systems, the fecundity of individuals was completely unaffected by the expression of an introduced antibiotic resistance gene. The fitness cost associated with chlorsulfuron resistance was greater in nutrient-poor conditions relative to nutrient-rich conditions for comparisons involving mutant, but not transgenic, genotypes.

The evolutionary fate of a resistance gene depends largely on two properties: the benefit of the resistance conferred and the negative pleiotropic effect of the gene on fitness. If the fitness cost of a resistance gene is large, then the gene will be at a selective disadvantage relative to wild-type alleles in the absence of the selective agent. Although nobody questions that resistance traits act to reduce the detrimental effects of pathogens and herbivores, estimates of their pleiotropic costs on fitness in the absence of selective agents have been infrequently determined.

The most compelling demonstrations of fitness costs associated with resistance genes have come from bacterial systems, in which researchers have detected differences in growth rates between wild-type strains and those that contain plasmids conferring antibiotic resistance (e.g., Lee and Edlin 1985; Bouma and Lenski 1988; Nguyen et al. 1989; Bentley et al. 1990; Glick 1995). When researchers attempt to explain these fitness costs, one of three mechanisms is typically invoked. First, the growth rate of the resistant strain could be slowed due to the energy and resources needed to replicate the plasmid DNA. Second, expression of the antibiotic-resistance protein could entail a considerable metabolic load on the host cell, diverting energy and resources away from cellular functions involved in growth. Finally, introduced resistance genes could decrease growth rates by producing a toxic protein, by causing the buildup of toxic byproducts, or in some way interfering with proper metabolic functioning. There are well-documented examples that each of these mechanisms can be important in particular cases (Lee and Edlin 1985; Harrington et al. 1986; Cheah et al. 1987; Nguyen et al. 1989; Ryan et al. 1989; Skare et al. 1989; Bentley et al. 1990; Khosravi et al. 1990; Sano and Cantor 1990; Somerville et al. 1994; Valera et al. 1994).

In contrast to bacterial systems, our understanding of the costs of resistance genes in plants is far less sophisticated. Recent field experiments involving plants engineered to have resistance to herbicides, viruses, or insects have shown that resistance can be expressed with no decrease in fitness (Nelson et al. 1988; De Greef et al. 1989; Delannay et al. 1989; Kaniewski et al. 1990; Hilder and Gatehouse 1991). There are several potential reasons why costs of resistance might be less readily detected in plants. First, some of the mechanisms generating costs of bacterial resistance genes may not be present in eukaryotes such as plants. For example, the slowing of cell division in eukaryotes due to the replication of an extra gene is unlikely to be perceptible because of their relatively large genomes. Similarly, the metabolic cost of expressing an extra gene might be less noticeable in eukaryotes that contain and express far more genes than do bacteria. The action of metabolic interference, on the other hand, is presumably independent of genome size and is therefore likely to be of equal importance in bacteria and eukaryotes.
Second, bacterial systems provide more sensitive tests for costs of resistance because even minute fitness differences between resistant and susceptible strains can become amplified over many generations of growth. Whereas bacterial studies quantify population growth rates, experiments on plants instead are restricted to detecting potentially small differences among individuals. Previous studies in plants may have failed to detect differences in fitness merely because the sample sizes were inadequate for detecting fitness reductions due to a resistance allele that is only moderately costly.

An alternative explanation for the scarcity of evidence for costs of resistance in plant systems is that experiments with plants are in some way methodologically flawed. One factor that may strongly affect the magnitude of fitness differences between resistant and susceptible plants is environmental variability (PARKER et al. Previous studies in plants may have failed to detect differences between resistant and susceptible strains can generally be flawed. One factor that may strongly affect the magnitude of fitness differences between resistant and susceptible plants is environmental variability (PARKER 1992; HAN and LINCOLN 1994). It has been suggested that when resources are abundant enough to satisfy the energetic requirements of reproductive function, excess resources can be channeled into resistance expression without significant tradeoffs between the two functions (BAZZAZ et al. 1987). Nevertheless, tests for costs of resistance in plants are frequently performed under nutrient rich, agricultural conditions (BERGELSON and PURRINGTON 1996). Experiments testing for fitness costs of resistance genes would be improved if tests were made with and without resource limitation, or, equivalently, with and without environmental stress. A second, potential methodological problem involves lack of genetic control. In studies measuring fitness differences between resistant and susceptible individuals from a segregating population, differences due to the expression of a resistance gene may be small relative to variation caused by segregation of other loci affecting fitness. The creation of near-isogenic resistant and susceptible lines by backcrossing to a recurrent parent can remove a substantial amount of this variation, but it is nearly impossible to eliminate linkage effects through conventional methods of plant breeding (BERGELSON and PURRINGTON 1996). Indeed, even after 11 generations of backcrossing, YOUNG and TANKSLEY (1989) found linkage blocks up to 51 cM around a resistance gene in tomato, highlighting the difficulty of attributing fitness differences to a resistance gene per se.

Transgenic technology, which allows one to introduce a gene without a linkage block, has been heralded by researchers in the field as the ultimate tool for addressing the pleiotropic fitness effects of resistance genes (e.g., ZANGERL and BAZZAZ 1992). Despite the technique’s potential, its adoption by evolutionary ecologists has been surprisingly slow. We have utilized the technique of genetic modification to create transgenic Arabidopsis thaliana (Brassicaceae) with and without a gene conferring resistance to the herbicide chlorsulfuron, and have grown these plants under field conditions in each of two levels of environmental stress. Our study incorporates a number of important controls. First, to control for the effect of the vector plasmid that is used to carry the resistance gene, we estimated fitness for transgenic lines transformed with the vector plasmid only. Second, we created multiple lines with each construct to control for the variability due to insertion position on the host chromosome. Third, we performed a backcrossing program followed by creation of near-isogenic lines to control for the effects of mutations induced during tissue culture. Unlike previous trials involving transgenic plants, the current experiment measures the effect of the resistance gene on fitness while simultaneously controlling for possible linkage, the effects of the vector plasmid, insertion position, and induced mutation. In addition, potential variation in costs due to environmental quality can be explored. This design represents a significant advance in the field of costs of resistance in plants, in which empirical research has suffered from an inability to separate fitness differences between resistant and susceptible plants into linkage and pleiotropic components (but see BERGELSON et al. 1996).

MATERIALS AND METHODS

Plant material: We obtained an herbicide-resistant mutant of A. thaliana isolated from a population of seeds of the ecotype, Columbia, that had been subjected to the mutagen EMS (HAUGHN and SOMERVILLE 1986). This mutant, named GH50, was provided by G. W. HAUGHN (University of Saskatoon) as material that had been backcrossed to Columbia for six generations. Resistance to the herbicide chlorsulfuron is due to the presence in GH50 of a mutant form of acetolactate synthase (ALS), the first common enzyme in the biosynthesis of the branched-chain amino acids, leucine, isoleucine, and valine (RAY 1984). ALS molecules in GH50 differ from wild-type molecules by a single amino acid, and this difference is due to a single point mutation at position 870 in the coding region of ALS (HAUGHN et al. 1988).

Transformations: Chlorsulfuron-resistant transgenic plants were produced by cocultivation of root explants (VALVEKENS et al. 1988) with A. tumefaciens strain LBA4404 harboring the binary plasmid, pGH8 (Figure 1A), a fusion between the vector pBIN19 and a 5.8-kb Xba fragment (provided by G. W. HAUGHN, University of Saskatoon) containing the mutant form of the ALS gene. This mutant ALS gene, Car-1, was cloned from the car1 locus on chromosome 3 of GH50 (HAUGHN and SOMERVILLE 1986). The fused fragment contains the ALS coding region, with a 2.7-kb upstream and a 1.3-kb downstream flanking sequence. Of those plantlets surviving on kanamycin-containing media, we identified four lines with a single insertion position on the basis of Southern analysis (below). Control lines containing only the marker gene (NPT-II, conferring resistance to the antibiotic kanamycin) were generated by cocultivating root explants with A. tumefaciens harboring the binary vector pBIN19 (BRYAN 1984; FRISCH et al. 1995), and selecting for T-DNA integration by growth on media containing kanamycin sulfate. After Southern analysis (below), three independent lines showing a single insertion site of the T-DNA were selected from the available regenerants surviving on the selective medium. The selected regenerants were free from obvious morphological abnormalities.

To advance the four pGH8 lines and three pBIN19 lines,
selfed seeds from the original regenerants were surface sterilized and placed on germination medium containing 50 mg/L kanamycin + 100 nm chlorsulfuron (for plants transformed with pGH8) or containing 50 mg/L kanamycin sulfate (for plants transformed with pBIN19). Pollen from individuals surviving on these plates was applied to emasculated flowers of the wild-type parent, Columbia. After pollination, flowers were wrapped in plastic to prevent contamination by pollen from nearby plants. An additional cycle of seedling selection was completed, the progeny were selfed, and individuals homozygous for the transgene (null segregants) were selected by progeny analysis (Figure 2). In our experiments, we used the seed collected from a single homozygous-resistant and homozygous-susceptible individual derived from each transformant.

**Southern hybridizations:** To confirm that transformation had resulted in the integration of the transgene at a single locus, Southern hybridizations were performed using tissue from progeny of the original regenerants. Four mature leaves from individual plants were frozen in liquid nitrogen and ground to a fine powder by vortexing with ball bearings (COLLONE and SCHAAL 1993). A phenol:chloroform DNA extraction procedure adapted from COGGESHALL and COI (1993) was used. 0.01 ml of the purified genomic DNA was digested with Clnt-1 and Sall, and the probe labeling, hybridization, and detection were done according to the manufacturer’s protocols. Hybridization was done at 68°C and the final washes were with 0.2x SSC and 0.2% SDS at 60°C.

**Germination experiment:** For each of the 16 genotypes (Columbia, GH50, three transgenic pBIN19, three pBIN19 null segregants, four transgenic pGH8, four pGH8 null segregants), 10 replicate petri dishes containing 10 seeds each were placed in a growth chamber maintained at 22°C and 18-hr days. Seeds were placed on a single sheet of Whatman’s qualitative grade filter paper and moistened with deionized water, and then submersed in a solution of 50 mL of 13% PEG8000 and 1.6 mg/L chlorsulfuron (for plants transformed with pGH8 or pBIN19), plus 30 mL of 3 M NaOAc (adjusted to pH 5) and 600 mL of isopropyl alcohol. After spinning at 22°C for 3 min, the pellet was washed with 70% ethanol, allowed to dry, and was resuspended in 50 mL TE. DNA was precipitated by the addition of 50 ml 13% PEG8000 and 1.6 M NaCl, incubated on ice for 30 min, pelleted by centrifugation at 22°C, washed in 70% ethanol, and resuspended in 40 mL TE.

Southern hybridizations (SAMBROOK et al. 1989) were used to detect the DNA fragments in restriction digests containing the ALS gene. We used 0.01 ml of the purified genomic DNA in 0.1 mL restriction digestion reactions. The DNA fragments generated by the restriction digests were separated by electrophoresis in 0.7% agarose gels. The DNA fragments were transferred to nylon membranes by capillary transfer. Membranes were probed with a PCR-generated DNA fragment from the coding region of the A. thaliana ALS gene (for pGH8 transfectants) and the NPT II gene (for pBIN19 transfectants). The probe labeling, hybridization, and detection were done using an Illuminator nonradioactive kit (Stratagene, San Diego, CA) according to the manufacturer’s protocols. Hybridization was done at 68°C and the final washes were with 0.2x SSC and 0.2% SDS at 60°C.

**Figure 1.**—Disarmed binary vectors used in Agrobacterium tumefaciens-mediated transformations of A. thaliana root explants, showing the contents of the T-DNA regions (between the left and right borders) that become integrated in the host nuclear DNA. Successful transfer of the T-DNA region of the 11,777-bp pBIN19 (A) confers kanamycin resistance (KanR) on the recipient showing the content of the T-DNA regions (between the left and right borders) that become integrated in due to the constitutive expression of neomycin phosphotransferase II by the nopaline synthase promoter of A. tumefaciens. The 5.8kb Crl-I fragment, cloned from the herbicide-resistant A. thaliana mutant, GH50, was fused to pBIN19 at the EcoRI site within the polylinker region, creating the 17,577-bp pGH8 (B). Plants transformed with pGH8, therefore, contained both the gene conferring kanamycin resistance and the gene conferring chlorsulfuron resistance (ChlorR).

**Figure 2.**—Breeding design followed to produce the twice-backcrossed, homozygous seed used in the field experiment. Transgenic progeny (T,) derived from the original transformant were backcrossed twice to the wild type, Columbia. After two generations of selfing, nonsegregating lines of BC2S2 with (-R) and without (-S) the transgene were selected by germinating subsamples of seed on selective agar media.
H₂O. Percentage germination was calculated on the number of seeds germinated as of day 11.

Field experiment: Eighty seeds of each of the 16 genotypes were sown in 1.5 × 1.5 in disposable plug trays in the University of Chicago greenhouse. When seedlings possessed four true leaves (3 weeks after sowing), the flats were transported to a recently plowed field in Downer’s Grove, Illinois. On 30–31 May 1995, we transplanted 80 seedlings of each genotype into randomly selected positions in a grid consisting of plants spaced at 0.5 m within rows and 0.9 m between rows. We watered seedlings daily for one week, and approximately weekly thereafter. Half of the plants (640) received bi-monthly, 100-ml additions of a 0.95 g/liter fertilizer solution (Stern’s 15-30-15 Miracle Gro), and the remaining plants received an equivalent volume of H₂O. Plants were not protected from insect herbivores or diseases. At no point during this experiment was chlorosulfon applied to plants.

On 21–22 June 1995, we collected five fruits from each plant and computed an average number of seeds produced per fruit. This early collection enabled us to estimate the lifetime seed production of senesced plants, on which we could count the total number of seeds produced during the season. Before collection, we confirmed that plants were senesced and had no remaining shoot meristems hidden beneath the soil level. All plants were collected for determination of fruit production by 15 July 1995. Lifetime fitness for each plant was calculated by multiplying the total number of fruits (as indicated by septa remaining attached to pedicels) and the number of seeds per fruit. We also censused early survivorship to detect mortality differences among lines.

Statistical analysis: The effects of the herbicide- and antibiotic-resistance transgenes were estimated in separate ANOVAs in which nutrient treatment, resistance class, and line were treated as fixed effects. An additional ANOVA was performed on data from the nontransgenic genotypes, GH50 and Columbia. For all ANOVAs, data were square-root transformed before analysis to improve homogeneity of variances. Planned comparisons between means of resistant and susceptible genotypes within control and nutrient addition conditions were performed using the SuperANOVA statistical package (ABACUS CONCEPTS 1989).

RESULTS

Effect of herbicide resistance: Pooled over treatment and lines, mean seed production of transgenic, herbicide-resistant plants was 28% less than the susceptible null segregants (Table 1). The effect of resistance was also evident in a significant interaction with line (Table 1), indicating that costs varied among the four lines, but these line effects did not vary across nutrient treatment. Planned comparisons between means of resistant and susceptible plants within each treatment revealed significant costs under both conditions (Table 1, Figure 3A).

Although mean seed production of GH50 was less than that of Columbia, the percentage difference was about half the magnitude as the difference observed between transgenic-resistant and susceptible genotypes. The effects of resistance class as a main effect (F₁,₁₃₈ = 1.02, P = 0.3131) and as part of an interaction with treatment (F₁,₁₃₈ = 2.57, P = 0.1109) were not significant in a two-way ANOVA. Planned comparisons between seed production of GH50 and Columbia in both treatments revealed marginally significant costs among control plants (F₁,₁₃₈ = 3.40, P = 0.0675) but not among plants receiving nutrient additions (F₁,₁₃₈ = 0.18, P = 0.6748) (Figure 3B).

Because percentage emergence was 100% for all genotypes, there was zero variation on which to perform statistical analyses. These data demonstrate that resistance class did not affect the probability of emergence.

Effect of antibiotic resistance: Plants containing the vector sequence performed as well as plants lacking it in both control and nutrient-addition conditions (Figure 4, Table 2). As with the ANOVA of pGH8 genotypes, the ANOVA involving pBIN19 lines contained a two-order interaction between resistance class and line (Table 2), indicating that the effect of the plasmid on lifetime seed production varied in the different lines. Of the three lines, two showed a benefit of possessing antibiotic resistance and one showed a cost, although none of these differences was significant (t = −1.65, P = 0.1014; t = 1.74, P = 0.0845; t = −1.19, P = 0.2364 for lines 1, 2, and 3).

The percentage germination data were too invariant (99.8% overall) to justify a statistical analysis.

DISCUSSION

We obtained opposite results for the two resistance traits that we engineered into A. thaliana; expression of antibiotic resistance had no detectable consequence while herbicide resistance caused large reductions in fitness. The cost of herbicide resistance is a convincing demonstration that the addition of a single resistance gene can have an effect in plants similar to that observed in bacterial systems. The negative result for antibiotic resistance (see also DALE and MCPARTLAN 1992; CRAWLEY et al. 1993) however requires explanation, especially because the antibiotic-resistance transgene is under the control of a powerful, constitutive promoter and because antibiotic resistance frequently reveals a cost in bacterial systems (reviewed in GLICK 1995). It appears that the combined energetic costs of replicating the extra DNA in dividing cells (i.e., carriage costs) and synthesizing an extra enzyme is negligible in plants, a situation that would not be true for bacteria. The cost associated with resistance to the herbicide chlorosulfuron in A. thaliana presumably stems from different mechanisms, as discussed below.

Our experimental design enabled us to eliminate two potential sources for the observed fitness cost of herbicide resistance. First, the yield reductions observed in plants transformed with pGH8 cannot easily be attributed to gene disruption by T-DNA because we did not see fitness reductions in the lines containing the vector plasmid alone. Even if the majority of plasmids containing the herbicide resistance gene had integrated into regions with active transcription, the criteria for advancing regenerants (e.g., normal growth, normal
phenotype) would have favored the selection of those regenerants with insertions that did not disrupt genes of major effect.

Second, metabolic drain due to the physiological cost of producing excess acetolactate synthase does not sufficiently explain the cost observed in either the conventionally bred genotype (GH50) or in plants transformed with pGH8. In GH50, there is little potential for metabolic drain through overproduction of ALS because both resistant and susceptible plants possess a single ALS locus, the resistant allele codes for an enzyme with the same number of amino acids as the enzyme produced by the susceptible allele, and activity levels of the two enzymes are equivalent (HAUGHN and SOMERVILLE 1986). In contrast, pGH8 transformants have two ALS loci, which suggests a potential for metabolic drain through overexpression. However, several studies have found that ALS activity in tobacco plants transformed with the chlorsulfuron-resistance transgene (under the transcriptional control of the native A. thaliana promoter) is less than or equal to ALS activity in wild-type plants (HAUGHN et al. 1988; TOURNEUR et al. 1993), suggesting that the addition of an extra ALS gene does not result in measurable overexpression. Even if the pGH8 transformants lack mechanisms such as cosuppression that could prevent ALS overexpression, the energetic cost of doubling ALS transcript levels, which in tobacco is normally at 0.1% of mRNA (KEELER et al. 1993), would be insufficient to explain the 26% reduction in seed production that we observed. Finally, it is important to note that because the introduced ALS gene is under control of the endogenous promoter, it is relatively unlikely to cause large accumulation of transcript. In contrast, the promoter for NPT-11, the antibiotic resistance gene on the vector plasmid, is linked to a strong, constitutive promoter. That pBIN19 showed no fitness cost is further evidence that the metabolic costs of enzyme production in eukaryotic systems may be undetectable.

Even though evidence suggests that the fitness cost of resistance was not due to overproduction of ALS molecules, resistant plants may have suffered from metabolic drain indirectly. Results from in vitro and in vivo studies of chlorsulfuron-resistant ALS have indicated that in several species the mutant form is relatively insensitive to feedback inhibition by one or more of the branched-chain amino acids (RATHINASABAPATHI et al. 1990; SINGH et al. 1992). As a result, one would predict chlorsulfuron-resistant plants to possess levels of

### TABLE 1

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>1</td>
<td>181637.57</td>
<td>181637.57</td>
<td>69.54**</td>
</tr>
<tr>
<td>Resistance class</td>
<td>1</td>
<td>82945.01</td>
<td>82945.01</td>
<td>31.76**</td>
</tr>
<tr>
<td>Line</td>
<td>3</td>
<td>28189.95</td>
<td>9396.65</td>
<td>3.60*</td>
</tr>
<tr>
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<td>26.32</td>
<td>26.32</td>
<td>0.01</td>
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<tr>
<td>Treatment $\times$ line</td>
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<td>1576.76</td>
<td>525.59</td>
<td>0.20</td>
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<tr>
<td>Resistance class $\times$ line</td>
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<td>42829.20</td>
<td>14209.73</td>
<td>5.44*</td>
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<tr>
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<td>12644.90</td>
<td>4214.97</td>
<td>1.61</td>
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<td>543</td>
<td>1418263.32</td>
<td>261</td>
<td>1.90</td>
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</tbody>
</table>

Planned comparisons:
- Control: transgenic vs. null segregant
- Nutrients: transgenic vs. null segregant

Analysis performed on square-root transformed data. *P < 0.05; **P < 0.001.

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**FIGURE 3.**—Lifetime seed production of chlorsulfuron-resistant (■) and susceptible lines (□) for transgenic (A) and nontransgenic (B) lines. Plants were grown outdoors in two environments without application of herbicide. Bars, 1 SE of the mean.
TABLE 2

Effect of antibiotic resistance on lifetime seed production

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<tr>
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<td>108944.53</td>
<td>108944.53</td>
<td>36.57**</td>
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<tr>
<td>Resistance class</td>
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<td>0.35</td>
</tr>
<tr>
<td>Line</td>
<td>2</td>
<td>6707.40</td>
<td>3353.70</td>
<td>1.13</td>
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<td>Treatment × resistance class</td>
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<td>641.93</td>
<td>641.93</td>
<td>0.22</td>
</tr>
<tr>
<td>Treatment × line</td>
<td>2</td>
<td>13913.47</td>
<td>6956.73</td>
<td>2.34</td>
</tr>
<tr>
<td>Resistance class × line</td>
<td>2</td>
<td>19179.42</td>
<td>9589.71</td>
<td>3.22*</td>
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<td>Treatment × resistance class × line</td>
<td>2</td>
<td>4208.50</td>
<td>2104.25</td>
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<tr>
<td>Error</td>
<td>387</td>
<td>1152970.72</td>
<td>2979.25</td>
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</tr>
</tbody>
</table>

Planned comparisons:

<table>
<thead>
<tr>
<th></th>
<th>d.f.</th>
<th>SS</th>
<th>MS</th>
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<tbody>
<tr>
<td>Control: transgenic vs. null segregant</td>
<td>1</td>
<td>1627.35</td>
<td>1627.35</td>
<td>0.55</td>
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<td>23.18</td>
<td>23.18</td>
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</tbody>
</table>

Analysis performed on square-root transformed data. *P < 0.05; **P < 0.001.

branched-chain amino acids in quantities higher than observed in wild-type individuals. Indeed, valine, leucine, and isoleucine concentrations in leaves and seeds have been found to be substantially higher in resistant plants (DYER et al. 1993; GUTTIERI et al. 1993; C. V. EBERLEIN, personal communication). Therefore, decreased seed production in resistant plants may be at least partially explained by an increased amount of resources diverted to the production of branched-chain amino acids. Interestingly, high levels of leucine, isoleucine, and valine in seeds of resistant plants is correlated with faster emergence times, suggesting that resistant biotypes may have an advantage in environments where recruitment is limited by early competitive interactions (DYER et al. 1993; THOMPSON et al. 1994a).

This indirect metabolic burden may be coupled with additional deleterious effects caused by abnormal amino concentration and low activity of ALS, particularly in rapidly growing tissues where ALS expression is high (ROST 1984; ROST et al. 1990; GUTTIERI et al. 1993; KEELER et al. 1993; RICHTER and POWLES 1993). The low activity of ALS may additionally cause the build up of α-amino butyric acid, a toxic compound that is produced when ALS activity is reduced by chlorsulfuron application (CHRISTIANSON 1991). Together, these mechanisms may explain the fitness reductions observed in our experiment, in the original studies on the A. thaliana mutant (HAUGHN and SOMERVILLE 1986), in other chlorsulfuron-resistant weeds (CHRISTOFFOLETI and WESTRA 1991; ALCOGER-RUTHLING et al. 1992; MALLORY-SMITH et al. 1992), and in recent trials of crop plants that have been engineered to express the A. thaliana Csrl-1 allele (BRANDLE et al. 1992; LI et al. 1992; BRANDLE and MIKI 1993).

There have been general predictions that the fitness effects of mutations will be most easily detected under novel, potentially stressful conditions (DYKHUIZEN and WTL 1980; DYKHUIZEN et al. 1987; SILVA and DYKHUIZEN 1993). A similar prediction made by physiological ecologists is that the production of chemical defenses will be relatively more costly when resources are scarce (COLEY et al. 1985). This prediction is consistent with our results from the nontransgenic genotypes, in which the cost of resistance is absent when plants are fertilized, but not for the transgenic genotypes, in which costs are present under both conditions. It is possible that the complete disappearance of costs in the mutant line in the fertilizer treatment may indicate the presence of mutations, induced during the initial mutagenesis, that still remained within the linkage block. This possibility is entirely speculative without knowledge of the size of the linkage block and the function of the genes it might contain, thus illustrating the uncertainty involved in assessing fitness differences in nontransgenic systems. Alternatively, the difference in responses of the transgenic and nontransgenic genotypes may be due to the absence of unidentified regulatory sequences that were not included within the 5.8-kb sequence cloned from the herbicide-resistant mutant.

One avenue of future research on this topic is to better understand why chlorsulfuron-resistant biotypes derived from certain populations of weeds do not dis-
play fitness reductions relative to susceptible individuals (THOMPSON et al. 1994b). These reports may reflect the importance of the genetic background in which the ALS mutation exists, or, alternatively, variation in fitness effects among ALS alleles that confer chlorosulfuron resistance. Preliminary genetic analysis has shown that ALS mutations are diverse both within and among species (GUTTIERI et al. 1992, 1995), indicating that a complete understanding of variation in fitness tradeoffs may require knowledge of both how different mutations affect seed production in single species, and how differences in genetic background modify this effect. With ALS mutations identified in Kochia scoparia, Lactuca serriola, Salsola iberica, Stellaria media, Loliun perenne, and Lolium rigidum, chlorosulfuron resistance is an attractive system in which to investigate the evolution of resistance genes in natural populations.

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LITERATURE CITED


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