

## FROM FLUORESCENCE TO FITNESS: VARIATION IN PHOTOSYNTHETIC RATE AFFECTS FECUNDITY AND SURVIVORSHIP

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**Abstract.** Genetic variation in photosynthetic traits within populations provides the potential for evolution, but few studies relate phenotypic variation in these traits to variation in fitness. We tested the prediction that a lower photosynthetic rate reduces fecundity and survivorship by comparing wild-type (WT) *Amaranthus hybridus* family lines to those having a single-gene mutation that confers resistance to atrazine (R) and lowers the rate of photosynthetic carbon assimilation. Wild-type and R family lines with nearly uniform nuclear genomes were used to minimize the confounding effects of other loci. We established experimental populations in agricultural and one-year-old field plots and measured chlorophyll fluorescence, gas exchange, and the fecundity and survivorship of WT and R genotypes for two generations.

The R genotype had a lower efficiency of electron transport through photosystem II, which translated into a 20–30% decrease in photosynthetic rate at light levels above 400  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . Compared to the WT, the R genotype also had lower water-use efficiency, higher specific leaf area, and greater leaf nitrogen concentration on a mass, but not area, basis. In five of six replicate populations, the R genotype had lower fecundity than the WT in the first generation. Survivorship of seed over winter was similar for the two genotypes, but survivorship of R seedlings during early establishment was lower than the WT in the agricultural field. The consistent pattern of selection against the R genotype during vegetative growth stages suggests that a lower photosynthetic rate reduces fitness. This selection, paired with heritable variation for photosynthetic traits within populations, provides a more complete scenario for the evolution of photosynthetic traits.

**Key words:** *Amaranthus hybridus*; atrazine resistance; genotype frequency; selection for photosynthesis; water-use efficiency.

### INTRODUCTION

The evolutionary potential of photosynthetic traits is evident at several levels of organization. Three divergent photosynthetic pathways ( $C_3$ ,  $C_4$ , and crassulacean acid metabolism) are evidence for the evolution of photosynthetic traits at the largest scale (Ehleringer and Monson 1993). Genetic differentiation of photosynthetic traits among populations of a single species (Garbutt 1986, Kalisz and Teeri 1986, Zhang et al. 1993, Dudley 1996, Jonas and Geber 1999) suggests that these traits evolve, but does not necessarily show that they are adaptive.

For a trait to evolve by natural selection two conditions must be met. First, heritable variation for the trait must exist within populations. In addition, phenotypic variation in the trait must result in differences in reproductive success (Falconer and Mackay 1996). Population differentiation in photosynthetic traits implies that heritable variation exists within a species, and that it may remain within populations. While less

evident than across populations, there is genetic variation within populations for photosynthetic (Teese 1995, Geber and Dawson 1997) and stomatal traits (Case et al. 1998), as well as for water-use efficiency (carbon gained by photosynthesis per water used; Zangerl and Bazzaz 1983, Geber and Dawson 1990, Schuster et al. 1992, Donovan and Ehleringer 1994a). This variation provides the potential for evolution, but few studies relate phenotypic variation in these traits to variation in fitness.

It might seem intuitive that a higher photosynthetic rate should increase carbon gain and in turn the accumulation of biomass, leading to an increase in fitness such as higher survivorship or fecundity. However, photosynthetic rate is one of many factors that can affect growth, just as numerous biochemical reactions influence photosynthetic rate. For example, relative growth rate can be modeled as a product of net assimilation rate, of which photosynthesis is a major component, and leaf area ratio (allocation to leaf tissue; see Lambers and Poorter 1992). Because variation in either component will contribute to relative growth rate and these effects can vary in magnitude (Dijkstra and Lambers 1989), it is not necessarily the case that a higher photosynthetic rate will confer a higher growth rate. Although these models do not extend to estimates

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of fitness such as survivorship or fecundity, it seems a logical extension that a higher photosynthetic rate should not necessarily confer increased fitness.

In natural populations, plant growth and architectural traits commonly have positive correlations with components of fitness (Maddox and Antonovics 1983, Lechowicz 1984, Samson and Werk 1986, Farris and Lechowicz 1990, Pigliucci et al. 1995). Relationships between physiological traits and fitness are studied much less commonly. Higher photosynthetic rates have been correlated with greater mean fruit mass (Lechowicz and Blais 1988) and total fruit mass (Dudley 1996). However, photosynthetic rate had a small, negative correlation with fruit production (Farris and Lechowicz 1990), and was not correlated with several fitness components (Lechowicz 1984) or with mean fruit mass (Farris and Lechowicz 1990). Water-use efficiency has been studied with greater intensity, and is positively correlated with vegetative size (Farris and Lechowicz 1990, Donovan and Ehleringer 1994a, b) and reproductive mass (Dudley 1996) in some cases, and negatively in others (Kalisz and Teeri 1986, Schuster et al. 1992, Donovan and Ehleringer 1994a). In either case, such correlations do not demonstrate causation, and can result from unmeasured effects of the environment on both the trait and fitness (Rausher 1992), or from correlations with other unmeasured traits that themselves affect fitness (Mitchell-Olds and Shaw 1987, Lande and Arnold 1983). Therefore, these correlative approaches preclude a strong test of whether or not higher rates of photosynthesis cause higher fitness.

This paper extends previous work by examining the consequences of a substantial, genetically based difference in photosynthetic rate over multiple episodes of selection. We investigated the effect of variation in leaf-level photosynthetic rate on a variety of fitness components for field-grown plants by comparing two genotypes that differ in their rates, and we tested the prediction that a lower rate of photosynthesis reduces fecundity and survivorship.

We used family lines of wild-type *Amaranthus hybridus* and others having a single-gene mutation in the *psbA* locus that codes for the plastoquinone binding site ( $Q_b$ ) in photosynthetic electron transport. This mutation is in the chloroplast genome and confers resistance to the herbicide atrazine, which binds to  $Q_b$  and halts photosynthesis in wild-type (WT) plants. In the absence of atrazine, plastoquinone binds to the  $Q_b$  site in resistant (R) plants but does so with reduced affinity, thereby slowing photosynthetic electron transport compared to the WT (Ort et al. 1983). This reduction in electron transport can translate into a 10–35% lower rate of photosynthesis in R plants depending on study species, temperature, and incident irradiance (Holt et al. 1981, Jursinic and Pearcy 1988, Stowe and Holt 1988, Dekker and Sharkey 1992). The magnitude of this difference in photosynthetic rate between geno-

types is similar to variation seen among populations in other systems (Teramura and Strain 1979, Dudley 1996, Geber and Dawson 1997). By using variation in photosynthesis that results from a single-gene mutation of large effect, we provide a strong test of whether higher rates of photosynthesis cause higher fitness.

We established experimental populations comprised of WT and R family lines in equal frequency and measured chlorophyll fluorescence, gas exchange, survivorship, and fecundity for two generations in two environments. Wild-type and R family lines with nearly uniform nuclear genomes were used to assess the effects of variation in photosynthetic rate on fitness while minimizing confounding effects of other loci. By following changes in the frequency of these genotypes over time we quantified selection and identified the life history stages when it was strongest.

## MATERIALS AND METHODS

### *Study species*

*Amaranthus hybridus* L. (smooth pigweed) is a highly self-pollinating annual with  $C_4$  photosynthetic metabolism and is common to disturbed sites (Weaver and McWilliams 1980). Seeds from wild-type (WT) and atrazine-resistant (R) *A. hybridus* were collected from agricultural populations and reciprocally crossed to produce family lines with nearly uniform nuclear backgrounds and distinct WT or R cytoplasmic genomes (Jordan 1996). There is no gene flow via pollen for this trait because the mutation conferring atrazine resistance is in the chloroplast genome. Therefore, maternal reproductive mass is likely to be an accurate measure of fitness in this system.

### *Experimental design*

Wild-type and R seeds (approximately equal numbers from seven family lines per genotype) were germinated in the greenhouse as in Arntz et al. (1998), and transplanted in May 1996 into an agricultural and an abandoned field located at the Phillips Tract Research Area, 8 km northeast of Urbana, Illinois, USA (40.06° N, 88.14° W). The agricultural field had been previously planted with corn and had a history of atrazine application. While atrazine can reside in the soil, residual herbicide would not affect transplant growth or seed germination because it must be applied directly to leaves. Prior to transplanting the WT and R seedlings, the agricultural field was tilled and the old field was tilled and left fallow for one year. Because of the high density of vegetation, plants in the old field received less light and water than in the agricultural field (see Arntz et al. 1998 for description of light environments). Predawn water potentials, measured on a typical midsummer morning with a pressure chamber (Soilmoisture Equipment, Santa Barbara, California, USA), were  $-0.04$  MPa in the agricultural environment and  $-0.17$  MPa in the old-field environment (ANOVA,  $P < 0.01$ ,  $N = 9$ ).

Three replicate populations were established in each environment. Each  $6 \times 6$  m population was enclosed by a 1-m-tall fence of 2.5-cm chicken wire and plots were separated by 5-m buffer zones. An adjacent fourth population was established only in the agricultural environment for physiological measurements. At the four-to-six-leaf stage, 50 WT and 50 R seedlings per population were transplanted 0.5 m apart in a grid and distributed randomly with respect to genotype. All *Amaranthus* sp. seedlings that germinated from the seed bank in the agricultural environment were removed. There were no seedlings in the old-field environment. Soil samples were collected midsummer in 1996 to measure the preexisting seed bank (collection as described for spring 1997, see *Methods: Generation 1: fecundity and survivorship*). Samples were germinated, the number of seedlings counted per sample, and the number of seeds per  $m^2$  estimated from the sample mean. In the agricultural environment there were  $\sim 45$  seeds per  $m^2$ . This density was considered negligible compared to seed input during the first generation (see *Results: Fecundity and survivorship*). There were no *Amaranthus* sp. seeds in the old-field seed bank.

#### *Physiological measurements*

To confirm that the altered *psbA* gene in R plants conferred a lower photosynthetic rate under field conditions, we measured photosynthesis on individual leaves from all plants in the agricultural population designated for physiological measurements (50 WT and 50 R). Measurements were made on a clear day at five intervals between the hours of 0600 and 1800 with a portable gas-exchange system (LICOR-6200 Portable Photosynthesis System, LICOR Incorporated, Lincoln, Nebraska, USA). At each interval 10 WT and 10 R plants were measured. To calculate stomatal conductance and intercellular  $CO_2$  concentrations, we assumed that leaves were amphistomatous and that boundary layer conductance inside the cuvette was  $1.3 \text{ mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  (von Caemmerer and Farquar 1981). Irradiance was measured inside the chamber with a quantum sensor (GaAsP photocell, Hamamatsu Photonics K. K., Bridgewater, New Jersey, USA; as in Pearcy 1989).

Immediately following each photosynthetic measurement, chlorophyll fluorescence from photosystem II (PSII) was measured using a portable fluorometer (PAM-2000, Heinz Walz GmbH, Effeltrich, Germany) to determine the effect of the mutation on energy partitioning in photosynthetic electron transport. The quantum yield of PSII ( $\phi_{\text{PSII}}$ ) was measured in ambient light and calculated as  $\Delta F/F_m'$  (Genty et al. 1989). Photochemical fluorescence quenching (qP) and the efficiency of open PSII reaction centers ( $F_v'/F_m'$ ) were measured by determining the fluorescence yield in actinic light under transient darkening of the leaf ( $F_0'$ ), and applying a 3-s pulse of far-red illumination.

Leaves were sampled after midday fluorescence mea-

surements, dried at  $60^\circ \text{C}$ , and analyzed for nitrogen and carbon content using a Carlo Erba CNS Elemental Analyzer NA 1500 (Fisons Instruments, Milan, Italy). Nitrogen content was expressed on an area basis using calculations of specific leaf area.

#### *Generation 1: fecundity and survivorship*

During the first generation we measured survivorship of WT and R transplants, their fecundity (seed production in fall 1996), and survival of seeds over winter. Populations were censused weekly to determine transplant survivorship. Fecundity was measured with seed traps in fall 1996. Prior to seed dispersal, 50 seed traps were installed at random locations in each population in the agricultural environment. Traps were constructed from plastic funnels ( $71\text{-cm}^2$  opening) with fine-mesh fabric covering the exit to capture the seed; this design collected seed  $\sim 16$  cm above the soil.

Plants were much smaller and produced fewer seeds in the old-field environment, so traps were designed to collect seed falling from individual plants. Traps were constructed from plastic funnels ( $314\text{-cm}^2$  opening) with a 15-cm-long tube of fine mesh fabric attached to the exit. They were fastened around individual plant stems with a twist-tie, and secured to the ground with a nail. Ten traps for each genotype were installed in each population in the old-field environment.

The contents of each seed trap were sieved through number 18 (1 mm) and number 35 (0.5 mm) USA Standard Testing Sieves (W. S. Tyler, Mentor, Ohio, USA) to remove seeds from other species, debris, and soil. Seeds in each sample were germinated on a plastic plate covered with 1 cm of Strong-lite® Germination mix (Horticultural Products, Seneca, Illinois, USA). To distinguish seedlings of the R genotype from the WT, seedlings were counted at the 3–6 leaf stage and then sprayed with a 0.10 mM atrazine solution (analytical standard Atrazine, PESTNAL, [Crescent Chemical, Hauppauge, New York, USA] dissolved in 5% ethanol and 1% Tween 20 [Aldrich Chemical, Milwaukee, Wisconsin, USA]). After one week, the proportion of surviving R seedlings in the total sample was taken as the relative frequency of the R genotype. The replicate samples were averaged to obtain population-wide estimates of the relative frequency of the R genotype in the population. Seeds of known WT or R genotype were germinated and assayed as controls; the assay was 100% accurate.

The overwinter survivorship of the seeds produced in Generation 1 was measured by collecting 50 soil cores per population in spring 1997; each core was  $12.5 \text{ cm}^2$  and 2.5 cm deep. Samples were air dried, homogenized, and assayed for the frequency of R seed as described for seed trap samples. The 50 soil samples were averaged to obtain a population-wide estimate of the relative frequency of the R genotype.

### Generation 2: survivorship

We used differences in chlorophyll fluorescence from WT and R leaves to identify the genotype of seedlings that germinated in spring 1997 in the field and to quantify their survivorship. When leaves are treated with atrazine,  $\phi_{\text{PSII}}$  of R plants is an order of magnitude greater than WT plants (A. M. Arntz, *unpublished data*). We used this difference to assay leaf samples from the field by soaking them in a 0.10 mM atrazine solution for one hour under natural lighting, sandwiching them between two glass plates, and measuring fluorescence with  $10 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  actinic irradiance. Leaves were then scored as WT or R to calculate the relative frequency of the R genotype in seedlings sampled from the field.

During the second generation we measured the genotype frequency of WT and R seedlings that had germinated in spring 1997, and of seeds remaining in the seed bank in fall 1997. For seedlings, genotype frequencies were determined using the chlorophyll fluorescence assay on 100–200 individual leaf samples per population. A drought killed these second generation plants before they reproduced. Therefore, they did not contribute to the seed bank and adult plants were not assayed. An additional series of soil cores (50 per population) was collected to determine the genotype frequency of seeds remaining in fall 1997; these would be one-year-old seeds produced in fall 1996.

### Seed germination and early seedling establishment

To determine if there was differential mortality in the very early stages of seedling establishment, we planted seeds of known WT or R genotypes in the agricultural environment and monitored germination and survivorship. In May 1998, a section of buffer zone between two of the  $6 \times 6$  m plots was cleared and 200 seeds of each genotype were planted 10 cm apart in rows separated by 25 cm. They were planted randomly with respect to genotype and censused every 1–2 wk for 10 wk.

### Data analyses

Physiological data were plotted as a function of irradiance, and differences between the genotypes were tested by the interaction between genotype and a linear or quadratic (when appropriate) light-response term using analysis of covariance (PROC GLM; SAS 1996). The 95% CI of WT and R response curves were compared to estimate the irradiance at divergence.

Genotype frequencies were analyzed with a  $G$  test for goodness of fit for single classification frequency distributions (Sokal and Rohlf 1995). With the exception of the final seed bank in Generation 2, the expected frequencies were based on those observed at the previous time point. Because there was no seed production by seedlings in 1997 in the agricultural environment, the fall 1997 and spring 1997 seeds were compared. In

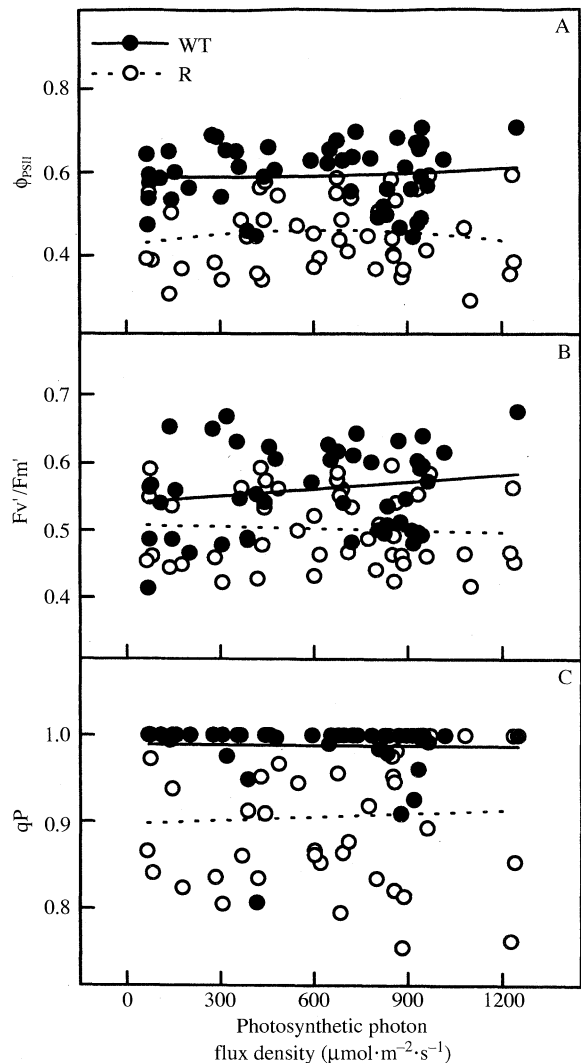


FIG. 1. Chlorophyll fluorescence parameters for WT and R genotypes of *Amaranthus hybridus* in a tilled agricultural field. (A) The quantum yield of photosystem II ( $\phi_{\text{PSII}}$ ), (B) the efficiency of open reaction centers ( $F_v'/F_m'$ ), and (C) the proportion of open reaction centers involved in photochemical quenching of chlorophyll fluorescence ( $q_P$ ) as a function of irradiance from measurements made on a typical sunny day between the hours of 0600 and 1800.

the old-field environment the fall 1997 seeds were also compared to all other stages. Germination and seedling survivorship distributions from 1998 were compared using PROC LIFETEST (SAS 1996).

## RESULTS

### Physiological measurements

The quantum yield of chlorophyll fluorescence from photosystem II ( $\phi_{\text{PSII}}$ ) was greater for the WT than the R genotype across all light levels (Fig. 1A,  $P < 0.01$ ;  $\phi_{\text{PSII}}$  is the product of  $F_v'/F_m'$  and  $q_P$ , which represent the efficiency of light capture by oxidized PSII reaction

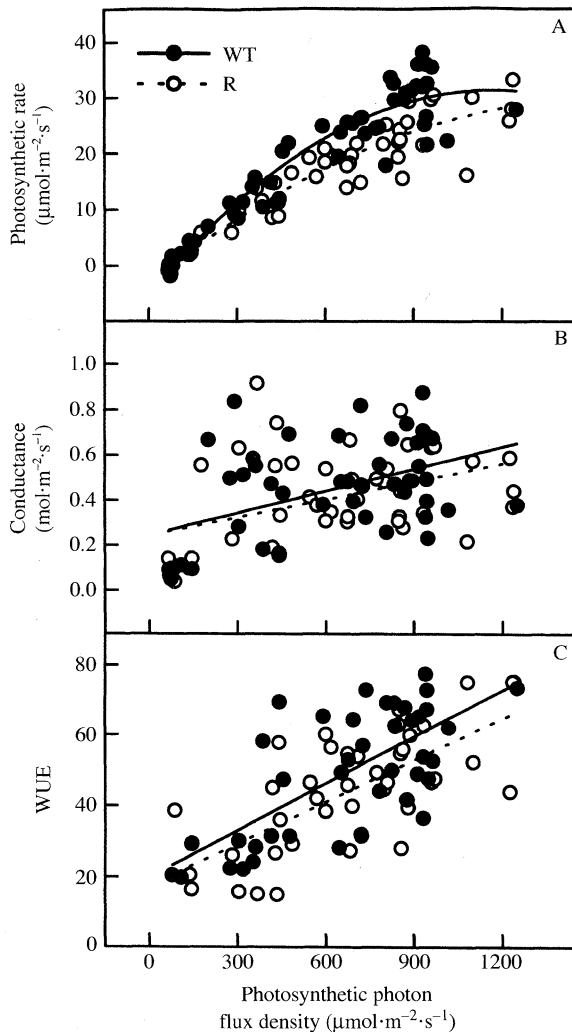


FIG. 2. Gas-exchange measurements for WT and R genotypes of *Amaranthus hybridus* in a tilled agricultural field: (A) photosynthetic rate, (B) stomatal conductance, and (C) water-use efficiency (WUE) as a function of irradiance from measurements made on a typical sunny day between the hours of 0600 and 1800.

centers and the fraction of reaction centers that are fully oxidized, respectively). The WT had more efficient reaction centers (Fig. 1B,  $P < 0.01$ ) as well as more reaction centers open throughout the day (Fig. 1C,  $P < 0.01$ ).

Higher  $\phi_{PSII}$  supported a higher rate of photosynthetic carbon uptake for the WT compared to the R genotype (Fig. 2A). As is typical of many  $C_4$  species, net photosynthesis of both genotypes increased almost linearly with irradiance. The R genotype had a 20% lower photosynthetic rate at irradiances approximately  $>400 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  (interaction between the quadratic light response term and genotype:  $P < 0.05$ ). The genotypes did not differ in conductance (Fig. 2B); and, thus, wa-

ter-use efficiency (WUE) was lower for the R genotype compared to the WT (Fig. 2C,  $P = 0.051$ ).

At midday the R genotype had a 28% lower photosynthetic rate (Table 1). Leaves of the R genotype also had a higher SLA (specific leaf area) and more nitrogen on a percent (mass) but not an area basis. Genotypes did not differ significantly in percent carbon (data not shown), and R leaf tissue had a lower C:N ratio. Despite this greater concentration of nitrogen, the genotypes did not differ in photosynthetic nitrogen-use efficiency (PNUE:  $\text{CO}_2$  fixed per mole of N).

#### Fecundity and survivorship

There was no mortality of WT or R transplants during the first generation in the agricultural environment (data not shown). In all three agricultural populations, adult plants produced abundant seed in fall 1996 (Fig. 3B). However, the relative frequency of the R genotype in these seeds was 0.38, 0.35, and 0.34 in the three replicate populations (Fig. 3A), a significant decrease from the parental frequency of 0.50. The total number of seeds in the soil the following spring (1997) was higher than the estimated seed production (Fig. 3B). This discrepancy may have resulted from loss of seed from the traps. The relative frequency of R seed in the soil in spring 1997 did not change from that of seed produced the previous fall (Fig. 3A). For seedlings that germinated in the spring, the relative frequency the R genotype was 0.24, 0.19, and 0.18, significantly lower than the frequency of the R genotype in the seeds in the soil from which they germinated (Fig. 3A). Neither WT nor R seedlings survived to reproduce in 1997; the summer was unusually dry and both genotypes died prior to reproduction. In the absence of new seed production very few seeds remained in the soil in fall 1997, and the relative frequency of R seed did not differ from that in spring 1997 (Fig. 3A).

During the first generation in the old-field environment there was no mortality of WT or R transplants (data not shown) and plants produced substantially fewer seeds than the agricultural population (Fig. 4B).

TABLE 1. Photosynthetic rate and leaf properties of WT and R genotypes of *Amaranthus hybridus*.

Variable	WT	R	<i>P</i>
Photosynthetic rate ( $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ )	$28.4 \pm 6.4$	$22.1 \pm 6.3$	$<0.05$
Specific leaf area ( $\text{cm}^2/\text{g}$ )	$49 \pm 11$	$66 \pm 18$	$<0.05$
Nitrogen (%)	$3.0 \pm 0.30$	$3.5 \pm 0.20$	$<0.001$
Nitrogen content ( $\text{mmol N}/\text{m}^2$ )	$460 \pm 112$	$396 \pm 93$	NS
C:N ratio	$13.4 \pm 1.1$	$11.6 \pm 0.60$	$<0.001$
PNUE ( $\mu\text{mol}/\text{mol}$ )	$66.4 \pm 25.9$	$59.1 \pm 23.1$	NS

Notes: Samples are from mid-day measurements of photosynthetic rate ( $N = 10$ ). Values shown are means  $\pm 1$  SD, with *P* values from a one-way ANOVA.

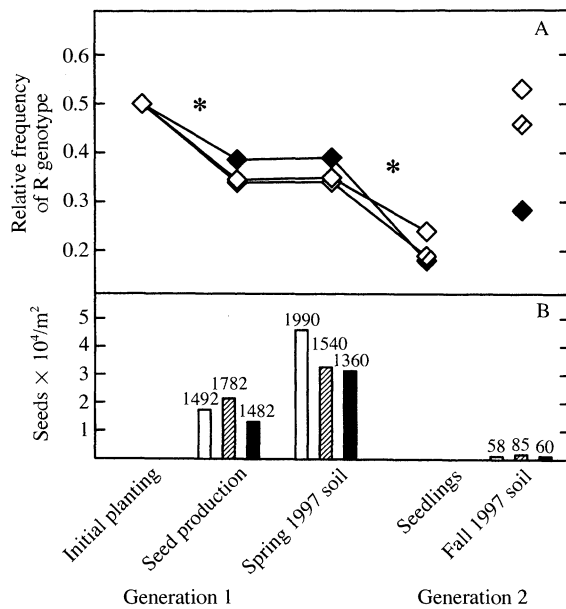


FIG. 3. (A) Temporal changes in the relative frequency of the R genotype and (B) the total density of WT and R seed in experimental populations of *Amaranthus hybridus* in a tilled agricultural field. The three replicate populations are shown as open (Population 1), hatched (Population 2), or closed (Population 3) symbols and bars. Asterisks indicate a significant change ( $P < 0.05$ ) from the previous time point. Seedlings did not contribute seed to the fall 1997 soil. Therefore, a line connecting these time points is omitted. Data were analyzed with chi square tests; numbers of seeds in samples used to estimate densities and expected frequencies are given above each bar.

The relative frequency of the R genotype decreased from the initial planting frequency of 0.50 to 0.40 and 0.41 in two populations, but increased to 0.58 in one (Fig. 4A). In spring 1997, the number of seeds in the soil remained at levels similar to the previous fall and the relative frequency of the R genotype in the seed bank did not change. No seedlings of either genotype were found in the second generation. Few seeds remained in the soil in fall 1997 and their genotype frequency was not significantly different from spring 1997 or from frequencies at any other time point (Fig. 4A).

#### Early establishment

The percentage of field-planted seed germinating in 1998 was 32% and 33% for the WT and R genotypes, respectively. Seedlings survived  $\sim 2$  mo and survivorship was virtually identical (data not shown; LIFE-TEST,  $P = 0.85$ ).

#### DISCUSSION

##### Genotypic differences in photosynthetic physiology

Differences in photosystem II (PSII) photochemistry between WT and R leaves were comparable to those observed for triazine-resistant mutants of *Brassica* species (Dekker and Burmester 1992, Sundby et al. 1993,

Plowman and Richards 1997). The lower quantum yield of chlorophyll fluorescence ( $\phi_{PSII}$ ) in the R genotype is attributable to a lower efficiency of light capture by PSII reaction centers ( $Fv'/Fm'$ ) and a smaller fraction of open centers (qP). A lower  $Fv'/Fm'$  indicates that the R plants were less effective at capturing light energy and using it for carbon fixation, which could possibly be related to transient photoinhibition. Decreases in the fraction of open centers in the R genotype resulted directly from the mutation in the D1 protein, which alters the kinetics of electron transfer within PSII (Bowes et al. 1980). Reductions in the quantum yield of PSII are known to lower rates of photosynthesis (Baker and Ort 1992). When resulting from photoinhibition, decreases in the quantum yield of photosystem II and of  $CO_2$  uptake correlate with decreased growth in crop species (Farage and Long 1991).

The lower photosynthetic rate of the R genotype compared to the WT at irradiances  $>400 \mu mol \cdot m^{-2} \cdot s^{-1}$  was not accompanied by decreases in stomatal conductance. Therefore, water-use efficiency of the R genotype was significantly lower. Dekker and Burmester (1992) found that stomatal conductance of R genotypes of *Brassica napus* either did not change or was greater when photosynthesis declined, also resulting in de-

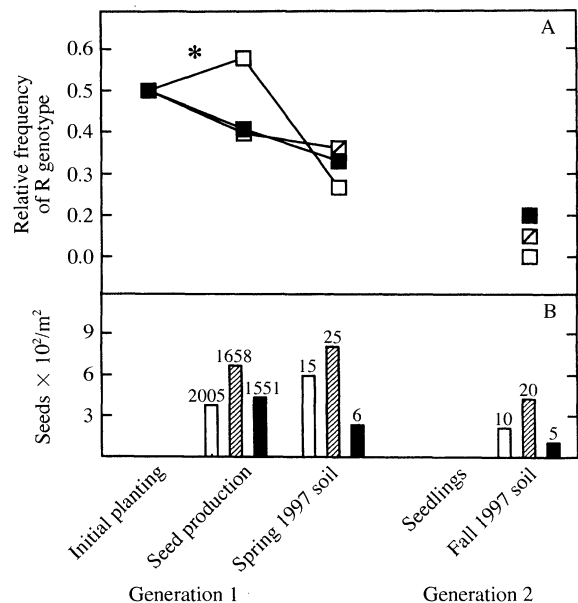


FIG. 4. (A) Temporal changes in the relative frequency of the R genotype and (B) the total density of WT and R seed in experimental populations of *Amaranthus hybridus* in a one-year-old field. The three replicate populations are shown as open (Population 1), hatched (Population 2), or closed (Population 3) symbols and bars. Asterisks indicate a significant change ( $P < 0.05$ ) from the previous time point. Seedlings did not emerge from the soil in spring 1997; therefore, lines connecting to this time point are omitted. Data were analyzed with chi square tests; numbers of seeds in samples used to estimate densities and expected frequencies are given above each bar.

creased water-use efficiency of the R genotype. Changes in water-use efficiency appear to be an important effect of the resistance mutation. If these changes result in differential whole-plant water use, they may explain growth differences between the genotypes in water-limited environments (Arntz et al. 1998). This effect on water use is secondary, arising indirectly from the mutation in PSII, and supports the notion that genetic variation in physiological traits is likely to have a range of indirect consequences, including those on fitness (Jordan 1996, Arntz et al. 1998).

#### *Genotypic differences in fitness*

The differences in photosynthesis between WT and R family lines were similar in magnitude to variation in these traits in natural populations (Teramura and Strain 1979, Dudley 1996, Geber and Dawson 1997). Therefore, these genotypes provide a reasonable proxy for measuring the fitness consequences of variation in photosynthetic rate. An episode of relatively strong selection against the R genotype occurred during the reproductive stage in all three agricultural populations and in two populations in the old-field environment. This result agrees with reports of reduced reproductive biomass for R genotypes of *Senecio vulgaris* in greenhouse experiments (McCloskey and Holt 1990) and field studies with the *A. hybridus* genotypes used here (Jordan 1996, Arntz et al. 1998). The consistent pattern among replicate populations suggests that changes in genotype frequency are attributable to selection.

An episode of selection against the R genotype also occurred during the seedling establishment phase in the agricultural environment. The reduced establishment of R plants could result from lower germination of R seeds or decreased seedling survival. Of these two possibilities the latter seems more likely. Very few seeds remained in the soil after germination and the frequency of R seeds in fall and spring 1997 soil not differ, suggesting that germination of WT and R genotypes was similar. This result is also supported by greenhouse observations (A. M. Arntz, *personal observation*) and by the data from spring 1998 which demonstrate that germination of WT and R seed did not differ. Germination of WT and R seed in non-isonuclear *Amaranthus retroflexus* does not differ (Weaver and Thomas 1986); however, it was higher in the R genotype of *Solanum nigrum* (Kremer and Lotz 1998) and lower in *A. retroflexus* and *A. powelli* (Weaver and Thomas 1986) depending on temperature and irradiance. Although the 1998 experiment did not reveal differential seedling survivorship, that summer was excessively moist while in 1997 it was relatively dry; these environmental differences likely influenced survivorship.

Because germination in our system does not appear to differ between genotypes, selection against the R genotype in 1997 was likely the result of seedling mortality during establishment. N. Jordan (*unpublished data*) has also found survivorship of R seedlings to be

lower than the WT. Increased R mortality may be partially explained by significantly smaller seed sizes. For WT and R seed from a previous study, the mass of R seeds was 0.365 mg/seed and 0.388 mg/seed for the WT (A. M. Arntz, *unpublished data*;  $N = 10$ ,  $P < 0.001$ ). We do not know if R individual seed mass was lower than the WT in this study, but given that R parents have less available photosynthate, it is feasible that they would allocate less to each seed. Whether this enabled the production of more seed, through a trade-off between seed number and size, is unknown. Any trade-off of this sort would not have compensated for the effect of the mutation on seed production because the R genotype produced fewer seeds than the WT. Even a small reduction in seed size could affect survivorship during the seedling, and perhaps later, stages.

At the end of the second generation in the old-field environment, the relative frequency of the R genotype was very low (0.10–0.20) in the three populations, and about half of the seeds remained from the spring seed bank. This decline suggests increased mortality of either R seed or of seedlings that were not detected. However, the decreases in R frequencies were not significant and small sample sizes may have made changes hard to detect.

All significant episodes of selection occurred during vegetative or reproductive stages, which was expected for a mutation that affects photosynthesis. There are no apparent effects of the mutation on dormant (seed) stages. These results underscore the value of using experimental material that allows the effects of genetic variation in photosynthesis to be distinguished from other genetic differences between genotypes. Other studies using non-isonuclear material have found reduced seed survival in association with triazine resistance (Kremer 1998). However, this effect probably resulted from differences between WT and R plants at other loci.

#### *From fluorescence to fitness*

We have shown a genetically based dichotomy in the ability of WT and R genotypes to capture light energy and assimilate carbon on an area basis. In addition to the effects of the mutation on electron transport, the higher SLA (specific leaf area) of the R genotype, if accompanied by fewer cells and chloroplasts, may also contribute to a decreased rate of photosynthesis. However, photosynthetic rate alone does not determine the potential for carbon gain. Rather, carbon gain is more closely estimated as a function of photosynthetic rate (on a leaf area basis) times the total leaf area, minus respiration (Lambers and Poorter 1992). In a previous field study of the same WT and R family lines, the genotypes had similar leaf areas and rates of respiration, suggesting that whole plant carbon gain is lower in the R genotype (Arntz et al. 1998). While other growth differences were small, reproductive biomass was significantly reduced. This result is consistent with

the lower fecundity of the R genotype observed in this study.

Given similar leaf areas, differences in photosynthetic rate can contribute to reproduction directly by immediate partitioning of photoassimilate to seeds during the reproductive stage and indirectly through small and cumulative effects on growth throughout development. Costs of resistance in terms of fecundity are typically greater than costs in terms of biomass (Bergelson and Purrington 1996), supporting the idea that small growth differences may accumulate to produce larger fitness differences (Jordan 1996, Arntz et al. 1998). The lower fecundity of the R genotype could have resulted from both direct and indirect contributions of photosynthesis.

While a 10%–30% lower photosynthetic rate may reduce seedling carbon gain, this might not be expected to substantially affect seedling survivorship. However, reduced carbon gain at very early stages can be compounded over time, and seedlings may be particularly sensitive to an alteration in carbon balance. The effects of the lower photosynthetic rate of the R genotype on survivorship may have also been exaggerated by a decrease in water-use efficiency, given the dry conditions of 1997. In addition, a potentially lower size of R seed may have contributed to a reduction in R carbon gain and subsequent survivorship.

Fitness differences between the WT and R genotypes we used vary among years (Jordan 1996, Arntz et al. 1998, Jordan et al. 1999). While this study was the first to characterize fluorescence and gas-exchange differences for these family lines, all four studies used the same genetic material. Collectively they show that within a single environment the fitness costs of the R genome can fluctuate within a season. However, across studies in Missouri and Illinois the atrazine-resistance mutation causes major impairments to fitness in terms of seed production that is fairly consistent, with an average relative fitness of the R genotype of 0.60. Some of the variation in relative fitness may arise from variation in levels of competition and other changes in resource availability. In addition, the location of the populations from which seeds were collected to create isogenic lines appears to influence differences in fitness between genotypes (Jordan et al. 1999). This suggests that plants may be able to compensate for mutations that reduce photosynthetic rate, given sufficient time and genetic variation.

Relationships between photosynthesis and growth have been assessed in crop species using isogenic and transgenic mutants (Stitt and Schulze 1994), yet plant and animal ecophysiologicalists have called for the development of more explicit linkages between physiological performance and fitness. Calow and Forbes (1998) note a striking lack of studies that simultaneously measure both types of traits, and it is the contribution of individual traits to fitness as well as the importance of environmental variation and correlations

with other traits that need to be developed. These missing links prevent a complete understanding of the importance of natural selection in the evolution of ecophysiological traits.

This study demonstrates selection for higher photosynthetic rates. The contribution of photosynthetic rate to fitness in more correlative studies may have been previously obscured because it appears to act indirectly (Arntz et al. 1998). Our demonstration of selection in combination with correlative selection studies (Lechowicz and Blais 1988, Dudley 1996) and evidence for genetic variation within populations (Donovan and Ehleringer 1994a, Teese 1995, Geber and Dawson 1997, Case et al. 1998) provides a more complete scenario for the evolution of photosynthetic traits by natural selection.

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