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Comparison of Triazine-Resistant and -Susceptible Biotypes of *Senecio vulgaris* and Their F_1 Hybrids[1]

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ABSTRACT

The relationship of triazine resistance to decreased plant productivity was investigated in *Senecio vulgaris* L. F_1 reciprocal hybrids were developed from pure-breeding susceptible (S) and resistant (R) lines. The four biotypes (S, S × R, R, R × S) were compared in terms of atrazine response, electron transport, carbon fixation, and biomass production. Atrazine response, carbon fixation rate, and PSII and whole-chain electron transport rates of hybrids were nearly identical to those of their respective maternal parents. Significant differences occurred between the two susceptible (S, S × R) and two resistant (R, R × S) biotypes in atrazine response (I_{50}), carbon fixation rate, and PSII and whole-chain electron transport rates; PSI rates were identical in all four biotypes. Coupled and uncoupled, whole-chain electron transport rates of thylakoids of the two susceptible biotypes were approximately 50% greater than those of the two resistant biotypes at photon flux densities greater than 215 micromoles per square meter per second. Carbon exchange rates of the two susceptible biotypes were 23% greater than those of the two resistant biotypes. Hybrid biotypes (S × R, R × S) were not identical to their maternal parents in biomass production. The S, S × R, and R × S plants all achieved greater biomass than R plants. These results suggest that while the resistance mutation influences thylakoid performance, reduced productivity of triazine-resistant plants cannot be ascribed solely to decreases in electron transport or carbon assimilation rates brought about by the altered binding protein. Since the F_1 hybrids differed from their maternal parents only in nuclear genes, it appears that the detrimental effects of the triazine resistance mutation on plant growth may be attenuated by interactions of the plastid and nuclear genomes.

Triazine resistance in weed populations was first reported by Ryan in 1970 (23). Numerous studies have been prompted by the discovery of this novel biotype of *Senecio vulgaris*, resulting in a general understanding of the mechanism of resistance to the triazine herbicides (18, 21). This mechanism has been described in detail recently. A mutation in the chloroplast psbA gene, which encodes the 32 kD quinone/herbicide binding (Qb^3) protein of PSII, results in an amino acid substitution in the 'binding pocket' of the mature polypeptide (8). The alteration markedly decreases the affinity of the Qb protein for triazine molecules and also modulates inherent redox properties of Qb essential for maintaining the flow of electrons from Qa to Qb during photosynthesis (4).

Many studies comparing the ecology and physiology of triazine-resistant weeds have demonstrated that in the absence of herbicides, resistant biotypes are less productive than susceptible biotypes. In both *Amaranthus* spp. and *S. vulgaris*, susceptible plants demonstrated more vigorous growth and greater seed production than resistant plants under competitive and noncompetitive conditions (1, 6, 11). Similarly, susceptible biotypes of *Chenopodium album* were shown to produce greater above-ground biomass than resistant biotypes (29). Lower photosynthetic rates have been measured in resistant biotypes of *Amaranthus retroflexus* (27), *Brassica* spp. (9), and *S. vulgaris* (12) compared to susceptible biotypes of the same species. Slower rates of whole-chain and isolated PSII electron transport in resistant thylakoids relative to susceptible ones have been documented in *Brassica campestris*, its backcross progeny *Brassica napus* ssp. Rapifera (Metzg.) Minsk. (2), and *Amaranthus hybridus* (17). Lower quantum yield of CO_2 fixation has been measured in triazine-resistant *A. hybridus* (17), *S. vulgaris* (12), and *B. campestris* (5) when compared to susceptible plants of the same species.

The inferior productivity and competitive ability of resistant plants relative to susceptible plants may be a result of growth limitations imposed by reduced photosynthetic capacity. The presence of the resistance mutation and the accompanying decrease in the rate of electron transfer from Qa to Qb (2, 18) have been suggested to be the cause of the observed fitness differences between susceptible and resistant biotypes (2, 5, 12). However, a causal relationship between the mutation conferring triazine resistance and decreased plant productivity has not been conclusively demonstrated (10). An understanding of the extent to which triazine resistance is correlated with plant productivity is essential to the future development of triazine-resistant crops. If the mutation directly limits plant growth, few benefits will be realized from its introduction into agronomically important species.

The objectives of this research were to investigate the nature of the relationship between triazine resistance and plant productivity and to determine if the alteration in the Qb protein that confers resistance also causes a reduction in productivity. Hybrid plants (F_1) were developed from pure-breeding S and R lines of *S. vulgaris*. These plants were used to describe the effect of the chloroplast mutation on plant performance in the absence of nuclear differences that may exist between parental biotypes. In this way, the role of the chloroplast genome in regulating growth and productivity of resistant plants could be clarified.

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[3] Abbreviations: Qb, secondary electron acceptor of PSII; Qa, primary electron acceptor of PSII; S, susceptible parent in this study; R, resistant parent in this study; S × R, F_1 hybrid of S maternal and R paternal cross; R × S, F_1 hybrid of R maternal and S paternal cross; MV, methyl viologen (1,1′-dimethyl-4,4′-bipyridylium dichloride); FeCy, potassium ferricyanide; DAD, diaminodurene (2,3,5,6-tetramethyl-1,4-phenylenediamine); DBMIB, dibromothymoquinone (2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone); I_{50}, herbicide concentration producing 50% inhibition.

MATERIALS AND METHODS

Plant Material and Growth Conditions. R and S biotypes of *Senecio vulgaris* L. were originally collected in Washington and Oregon, respectively (21). These seed sources have been used extensively for research purposes (6, 10–12). Seeds collected from glasshouse-grown plants have been used to maintain the S and R parental lines. Since the flowers are cleistogamous, *S. vulgaris* genotypes remain pure through self-pollination unless manipulated artificially. The F_1 hybrids were obtained through reciprocal crosses of the parental biotypes via traditional methods (25). Because *S. vulgaris* is a protandrous species, crosses were readily achieved by emasculating flowers before stigma emergence and later brushing the receptive stigma with the desired pollen. Pairs of S and R biotypes grown from each of 12 selfed parental seed lines were grown in the glasshouse, as described below. Emasculated flowers left unpollinated were used as controls to verify that crosses were achieved; none of those flowers set seeds. Pollination of an emasculated R flower with S pollen produced R × S hybrid seed, and R pollen placed on an emasculated S flower produced S × R hybrid seed. The R × S and S × R hybrid plants possessed a common, mixed nuclear genome in a cytoplasm identical to that of the maternal parent.

Seeds of the four *S. vulgaris* biotypes were germinated in 10-cm pots of vermiculite in a bottom-heated (25°C) mist chamber under natural lighting. At the cotyledon stage, seedlings were transplanted into 10-cm pots containing a 1:3 mixture of vermiculite and UC potting mix No. 2 (50% soil, 25% peat, 25% wood shavings by volume). Plants were grown under natural lighting in a temperature-controlled glasshouse, where average daily temperatures ranged from 20 to 29°C with a maximum midday temperature of 32°C. Ambient photon flux density ranged from 0 to 1200 μmol m^{-2} s^{-1}, 400 to 700 nm, throughout the day. Pots were watered daily with half-strength Hoagland solution. Additional crosses were made from the parental types as necessary to maintain F_1 seed stocks.

Whole Plant Atrazine Response. Plant response to atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) was evaluated by the application of 10 kg ai (active ingredient) ha^{-1} Aatrex 80 W (Ciba Geigy) to 8-week-old *S. vulgaris* plants. Plants were sprayed over the top with Aatrex delivered in 935 L ha^{-1} H_2O using an 8004 nozzle at 40 psi with a backpack sprayer. Six treated replicates were compared to 2 untreated controls of each biotype (S, R, S × R, R × S) using a randomized complete block design. Visual ratings of percent phytotoxicity, where 0 = no injury and 100 = plant death, were assigned 7 d after treatment.

Electron Transport. Rates of oxygen evolution or consumption in isolated thylakoid membranes were quantified using an oxygen electrode (Rank Bros., Cambridge, England) with a continuously stirred reaction vessel held at 21°C. Samples were illuminated with a 300 W-slide projector lamp equipped with a Corning 3-67 cut-off filter (Corning Glass Works, Corning, NY). Photon flux densities ranging from 69 to 1650 μmol m^{-2} s^{-1} were generated with neutral density filters (Balzer Optical, Marlboro, MA) and monitored with a LiCor photometer model LI-185 with a 190s quantum sensor (LiCor, Inc., Lincoln, NE).

Stroma-free lamellar membranes were isolated from leaves of 8-week-old plants that had been placed in the dark for 12 h before harvesting to deplete cellular starch reserves. Fully expanded leaves were removed, weighed, washed twice in cold deionized H_2O, snipped into small pieces, and ground on ice in a 1:5 (w/v) tissue to buffer ratio using a mortar and pestle. The grinding buffer contained 300 mM NaCl, 50 mM Tricine-NaOH (pH 7.8), 3 mM $MgCl_2$, 10 mM ascorbic acid, and 2 mg ml^{-1} BSA. The mash was filtered through 16 layers of cheesecloth and centrifuged at 2200*g* for 3 min. Two ml of buffer containing 100 mM sorbitol, 10 mM Tricine-NaOH (pH 7.8), 3 mM $MgCl_2$, 10 mM KCl, and 1 mg ml^{-1} BSA were used to resuspend and osmotically disrupt the chloroplast pellets. The extracts were then filtered through a Kimwipe, brought to a final volume of 30 ml with the same solution, and centrifuged for 5 min at 3000*g*. The pelleted thylakoids were resuspended in 4 ml of buffer containing 400 mM sorbitol, 10 mM Tricine-NaOH (pH 7.8), 3 mM $MgCl_2$, 10 mM KCl, and 1 mg ml^{-1} BSA, and divided into four 1-ml volumes for storage at −80°C. Fresh or frozen and thawed preparations gave comparable results and were used interchangeably.

All reaction mixtures contained thylakoid membranes equivalent to 20 μg Chl ml^{-1} in a 3-ml solution volume, as determined spectrophotometrically for each sample. Electron transport rates were measured for 90 s after turning on the light. Comparative light-saturated reactions were repeated six times; photosynthetic light curves were constructed in replicates of five.

The basic reaction mixture used for measuring rates of electron transport contained 50 mM sorbitol, 50 mM Tricine-NaOH (pH 7.8), 3 mM $MgCl_2$, and 10 mM KCl in a total 3-ml reaction volume. For whole-chain transport reactions, either 5 mM NaN_3 plus 0.1 mM MV or 1.5 mM FeCy were added to the basic mixture. Gramicidin D (2 μM) was used as an uncoupler in all assays except coupled light curves. Half-reactions employed oxidized DAD as the terminal electron acceptor for PSII ($H_2O \rightarrow$ DADox), or reduced DAD as the initial electron donor for PSI after inhibition by DCMU of PSII donation (DADred → MV). Freshly oxidized DAD was generated by including 1.2 mM FeCy and 0.4 mM DAD in the reaction mixture for isolated PSII transport. DBMIB (1 μM) was also added to inhibit electron transport beyond PSII. The 3-ml solution for PSI evaluation contained 1 mM ascorbate, 0.5 mM DAD, and 5 μM DCMU in addition to the basic reaction mixture for MV measurements described above.

Thylakoid Atrazine Response. The atrazine sensitivity of isolated thylakoids was determined for each biotype by measuring the rate of uncoupled electron transport from water to MV in the presence of a range of atrazine concentrations from 2 nM to 500 μM. The herbicide solution contained 99% technical atrazine in dimethylsulfoxide (DMSO). The maximum DMSO concentration did not exceed 1% of the 3-ml reaction volume. Controls (100% reaction rate) for each biotype were measured using DMSO without dissolved atrazine. The experiment was repeated five times.

Carbon Fixation. Instantaneous measurements of photosynthetic carbon assimilation were obtained from attached leaves of 8-week-old *S. vulgaris* plants using an open system, portable infrared gas analyzer and data logger, the ADC LCA-2 from P. K. Morgan Instruments, Inc., Andover, MA. Six replicate plants per biotype were used over 3 consecutive d. The CO_2-assimilation rate of one fully expanded leaf, third or fourth from the top of a separate axillary stem of each replicate plant, was measured each day. Single leaves were equilibrated in the 6.25 cm^2 assimilation chamber for approximately 90 s before measurements were taken. Leaves were harvested immediately after assaying, and leaf areas determined with a LiCor area meter, model LI-3000 (LiCor, Inc.). Experiments were conducted under natural lighting in a temperature-controlled glasshouse where temperatures ranged from 28 to 32°C with 40 to 50% RH. Assimilation-chamber temperatures ranged from 31 to 38°C with 20 to 40% RH. The flow rate in the assimilation chamber was provided by a mass flow unit (P. K. Morgan Instruments, Inc.) and fixed at 250 ml min^{-1}; intake CO_2 concentration was approximately 360 μl L^{-1}. Incident photon flux density on the leaf chamber ranged from 280 to 550 μmol m^{-2} s^{-1} during the course of the experiments.

Growth and Productivity. Growth and productivity of *S. vulgaris* over a period of 10 weeks were analyzed by means of destructive harvesting. Twenty-four replicates of each biotype (S, R, S × R, R × S) were started from seed in the spring of 1986 and arranged in the glasshouse in a randomized complete block design. Plants were grown as described above; eight replicates

of each biotype were harvested after 6, 8, and 10 weeks of growth. Individual plants for the 10-week harvest were transplanted into 2-L containers at 6 weeks to avoid restriction of root growth. Flowers were collected from each plant daily and pooled at each harvest date. At harvest, height was measured, plants were removed from the soil, and biomass was separated into above- and below-ground portions. Shoots were further divided into leaves and stems. Total leaf area per plant was obtained using an area meter (LiCor, Inc.). Roots, leaves, stems and flowers were placed in separate paper bags and dried to constant weight (72 h at 64°C) before weighing.

Statistical Analysis. Each experiment was arranged in a randomized complete block design with four qualitative treatments (biotypes). The number of replications for each experiment is indicated in the text and figure legends. Statistical analysis of each experiment included a one-way analysis of variance and mean separation by Duncan's multiple-range test. The analysis of variance for total vegetative biomass included nonbiased estimates calculated by the method of Yates (30) for missing data points in the 10-week harvest. Error bars in all figures represent 1 SE.

RESULTS

Whole Plant Atrazine Response. The differential effect of atrazine on four biotypes of *S. vulgaris* was evaluated at the whole plant level. As expected, observed phytotoxic responses in *S. vulgaris* biotypes coincided with those of the seed parent. Individuals with a susceptible cytoplasmic component (S, S × R) did not survive the application of 10 kg ha^{-1} atrazine, while plants containing resistant cytoplasm (R, R × S) were unharmed. Seven d after atrazine application, all treated S and S × R plants received visual ratings of greater than 95% on a scale of 0 to 100% phytotoxicity (data not presented).

Electron Transport. Figure 1 depicts the rate of whole-chain photosynthetic electron transport in thylakoids of each biotype over a range of photon flux densities in the presence of gramicidin. The saturating photon flux density used in further experiments was determined from these results to be 1650 μmol m^{-2} s^{-1}. Thylakoids from the susceptible biotypes (S, S × R) supported nearly identical rates of oxygen consumption that were markedly higher than the rates produced by resistant thylakoids (R, R × S) at all photon flux densities (Fig. 1). Differences in the rates of uncoupled electron transport between the two resistant biotypes (R, R × S) were not significant ($P \geq 0.10$ at each photon flux density measured). Rates produced by S and S × R thylakoids were statistically distinct from those of R thylakoids at photon flux densities greater than 92 μmol m^{-2} s^{-1} and from those of R × S thylakoids when photon flux densities exceeded 215 μmol m^{-2} s^{-1} ($P = 0.01$). The hyperbolic shape of the uncoupled light curves (Fig. 1) describes the saturation of electron transport by light when electron flow through the photosystems was released from the restrictions of photophosphorylation by an uncoupler.

Figure 2 portrays the coupled rates of electron transport in the four biotypes. Coupled electron transport rates were restricted by buildup of the protonmotive force during photosynthesis and by the absence of ADP and Pi substrates in the reaction mixture. These restricted rates exhibited the same relative ranking among the four biotypes as the uncoupled rates and were used only as indicators of membrane integrity.

Whole-chain photosynthetic electron transport measurements using thylakoids isolated from the parental biotypes (S, R) produced equivalent rates of oxygen flux for each biotype using either MV or FeCy. Light-saturated rates of uncoupled, whole-chain electron transport were approximately 150 μmol O_2 mg Chl^{-1} h^{-1} in susceptible thylakoids and 100 μmol O_2 mg Chl^{-1} h^{-1} in resistant ones (Table I). MV was chosen for use in further experiments to compare whole-chain rates ($H_2O \rightarrow MV$) directly with partial-chain, single-photosystem measurements ($H_2O \rightarrow DAD$, $DAD \rightarrow MV$).

Electron flow rates through PSII ($H_2O \rightarrow DAD$) were significantly higher in the two susceptible biotypes (S, S × R) than in the two resistant biotypes (R, R × S) and were equivalent between plants with a common cytoplasm (S and S × R; R and R × S) (Table I). S and S × R thylakoids produced about 100 μmol O_2 mg Chl^{-1} h^{-1} in the presence of oxidized DAD, while R and R × S thylakoids attained rates that were approximately 25% of those of susceptible preparations. Rates of electron transport through PSI ($DAD \rightarrow MV$) were similar among the four biotypes and were approximately 300 μmol O_2 mg Chl^{-1} h^{-1} (Table I).

Thylakoid Atrazine Response. The inhibition of photosynthetic electron transport by atrazine in isolated *S. vulgaris* thylakoids

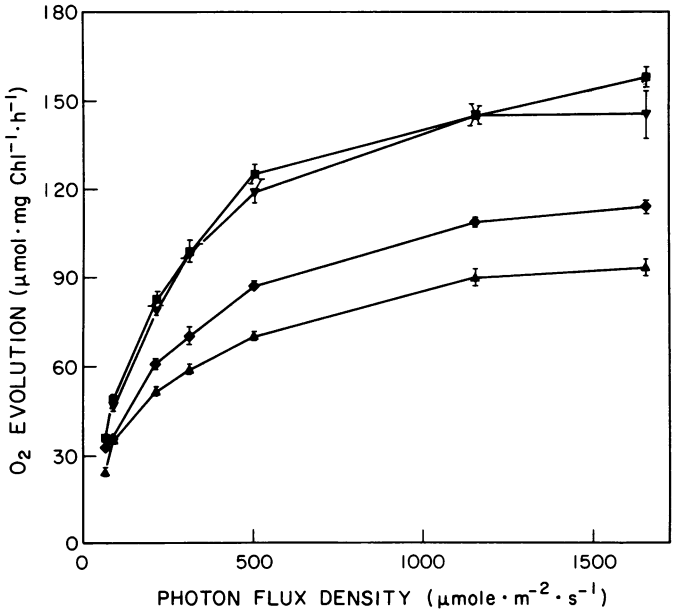

FIG. 1. Uncoupled whole-chain electron transport rates in triazine-S and -R biotypes of *S. vulgaris* and their F_1 hybrids (S × R, R × S) measured at photon flux densities ranging from 69 to 1650 μmol m^{-2} s^{-1}. Measurements were replicated five times. Error bars show 1 SE about the mean. (■), S; (▼), S × R; (♦), R × S; (▲), R.

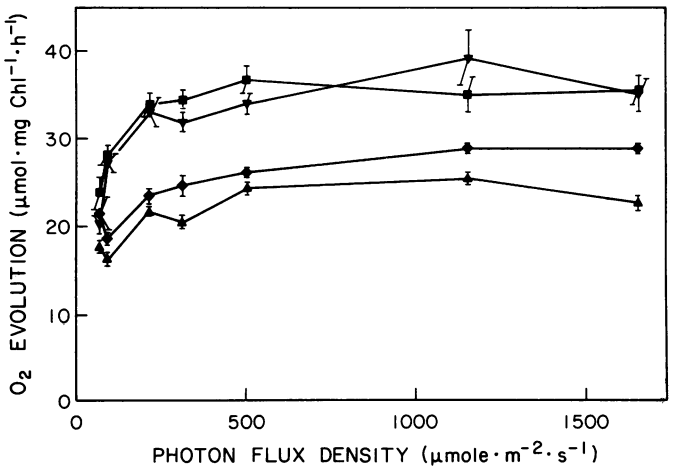

FIG. 2. Coupled whole-chain electron transport rates in triazine-S and -R biotypes of *S. vulgaris* and their F_1 hybrids (S × R, R × S) measured at photon flux densities ranging from 69 to 1650 μmol m^{-2} s^{-1}. Measurements were replicated five times. Error bars show 1 SE about the mean. (■), S; (▼), S × R; (♦), R × S; (▲), R.

Table I. *Light-Saturated Electron Transport Rates in Thylakoid Membranes of Triazine-Susceptible (S) and -Resistant (R) Biotypes of Senecio vulgaris and their F_1 Hybrids (S×R, R×S)*

Electron transport rates were measured as O_2 evolution or uptake with an oxygen electrode. Reaction components are given in the text. Numbers within a column followed by the same letter are not significantly different at the P = 0.01 level according to Duncan's multiple-range test for all reactions except $H_2O \rightarrow MV$, where P = 0.05. Number of replicates = 6 for all reactions except $H_2O \rightarrow MV$, where $n = 5$.

Biotype	Assay			
	PSII + PSI		PSII	PSI
	$H_2O \rightarrow MV$	$H_2O \rightarrow FeCy$	$H_2O \rightarrow DAD$	$DAD \rightarrow MV$
	$\mu mol\ O_2\ mg\ Chl^{-1}\ h^{-1}$			
S	158 + 17a	161 + 18a	103 + 12a	319 + 12a
S×R	145 + 40a		96 + 15a	319 + 27a
R×S	114 + 10b		26 + 3b	306 + 14a
R	93 + 11b	89 + 16b	26 + 10b	281 + 29a

is displayed in Figure 3. I_{50} values of 0.17 and 0.40 μM were calculated for the S and S×R biotypes, respectively, using regression analysis of the linear portion of the appropriate curve. The rates of oxygen consumption in R and R×S samples were completely unaffected at these atrazine concentrations (Fig. 3). Decreased transport rates were apparent in the resistant biotypes at concentrations above 100 μM. I_{50} values of 310 and 380 μM were calculated for R and R×S thylakoids, respectively. I_{50} values between plants with a common cytoplasm (S and S×R; R and R×S) were not statistically different.

Carbon Fixation. Susceptible plants (S, S×R) displayed higher rates of carbon fixation than resistant plants (R, R×S) when individual leaves were measured (Table II). Carbon assimilation rates for S and S×R plants were about 8.1 μmol CO_2 m^{-2} s^{-1}, while those for R and R×S plants were approximately 6.6 μmol CO_2 m^{-2} s^{-1}. Differences in CO_2 assimilation between susceptible and resistant biotypes were significant at the $P = 0.01$ level. Differences between plants of similar cytoplasm (S and S×R; R and R×S) were not significant (Table II).

Growth and Productivity. Plants of the S biotype outperformed *S. vulgaris* plants of the R biotype in all growth parameters measured including plant height, leaf number, leaf area, and biomass. Growth data were significantly different at the $P = 0.01$ level between plants of the parental biotypes (S, R) at all harvests (Table III). In contrast, growth measurements of the two hybrid biotypes (S×R, R×S) converged over the timecourse of the experiment. At the 6-week harvest, all growth parameters except leaf number were greater for S×R plants than for R×S plants. Differences in total leaf area and root and flower weights were no longer apparent at the 8-week harvest. By the 10th week, most parameters measured were not significantly different between S×R and R×S plants (Table III).

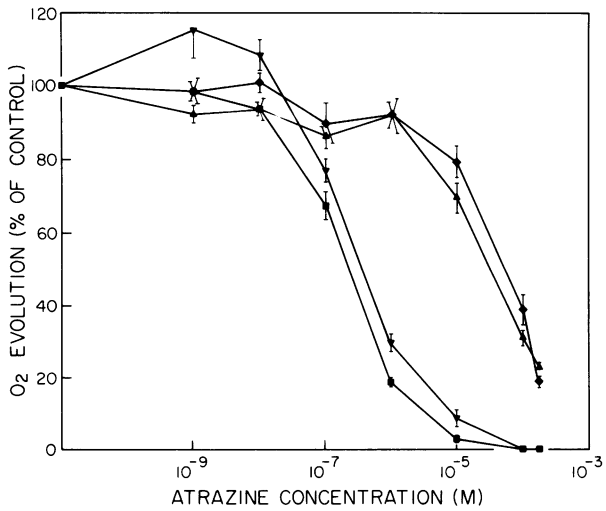

FIG. 3. Inhibition of photosynthetic electron transport by atrazine in chloroplast thylakoids isolated from triazine-S and -R biotypes of *S. vulgaris* and their F_1 hybrids (S×R, R×S). Measurements were replicated five times. Error bars show 1 SE about the mean. (■), S; (▼), S×R; (◆), R×S; (▲), R.

Table II. *Steady State Rates of Carbon Assimilation in Triazine-Susceptible (S) and -Resistant (R) Biotypes of Senecio vulgaris and their F_1 Hybrids (S×R, R×S)*

Numbers followed by the same letter are not significantly different according to Duncan's multiple range test using P = 0.01. Values presented are averages from measurements of three leaves from each of six plants per biotype; SE ≤ 6% for each mean.

Biotype	Carbon fixation
	$\mu mol\ CO_2\ m^{-1}\ s^{-1}$
S	8.21a
S×R	8.09a
R×S	6.53b
R	6.68b

In general, growth measurements were more similar at each harvest between plants of the susceptible biotypes (S, S×R) than between those of the resistant biotypes (R, R×S). At 6 weeks, most parameters measured were statistically similar between each hybrid biotype and its maternal parent (Table III). After 8 weeks of growth, S plants had greater leaf area, root and leaf weights, and total vegetative biomass than S×R plants. By the 10th week, however, the two susceptible biotypes did not differ statistically in any growth parameter measured (Table III). In contrast, R×S plants achieved greater height, leaf number, leaf area, shoot weight, and vegetative biomass than R plants after 8 weeks of growth. Differences between the two resistant biotypes remained distinct with time (Table III). At 10 weeks, R×S plants had greater leaf area and biomass of all parts than R plants and approached the level of productivity attained by plants of the two susceptible biotypes (Table III).

DISCUSSION

The relationship between the chloroplast mutation conferring triazine resistance and productivity of resistant plants is not fully understood, in part because most research has been conducted with weed populations of uncertain genetic backgrounds. Ecotypic differences between biotypes of the same species that are

Table III. *Growth and Productivity in Triazine-Susceptible (S) and -Resistant (R) Biotypes of Senecio vulgaris and their F_1 Hybrids (S×R, R×S)*

Numbers within a column at each harvest date followed by the same letter are not significantly different at the P = 0.01 level according to Duncan's multiple-range test. Number of replications for the 6th- and 8th-week harvests = 8; replications for the 10th-week harvest were height = 7, leaf number = 2, leaf area = 2, root weight = 8, shoot weight = 6, and leaf weight = 6.

Week	Biotype	Height	Leaves	Leaf Area	Dry Weight			
					Root	Shoot	Leaf	Flower
		cm	*No.*	*cm²*		*g*		
6	S	32.88a	79.50a	692.88a	6.98a	1.90a	2.14a	1.08a
	S×R	31.06a	90.88ab	526.69b	7.78a	1.92a	1.70b	1.37a
	R×S	23.25b	65.50bc	321.04c	2.80b	0.72b	1.04c	0.50b
	R	13.63c	50.63c	129.85d	1.75b	0.41b	0.51d	0.32b
8	S	46.63a	228.25a	954.69a	4.88a	7.09a	3.65a	4.83a
	S×R	47.13a	229.75a	667.31b	2.95b	6.60a	2.71b	5.37ab
	R×S	42.25b	162.25b	605.16b	2.57b	3.94b	2.07c	3.96bc
	R	32.75c	135.13c	264.24c	1.61b	2.03c	1.09c	2.80c
10	S	47.71a	569.50a	1133.41a	10.95a	14.44a	5.38a	
	S×R	47.14a	573.00a	904.73a	9.67a	12.15ab	3.88a	
	R×S	45.57b	626.00a	1193.15a	10.65a	10.53b	4.23a	
	R	41.00b	322.50a	431.46b	4.70b	4.42c	1.65b	

unrelated to the resistance mutation may account for some of the differential vigor observed in S and R biotypes collected from the field (17). In all documented cases of triazine-resistant weeds, including *S. vulgaris*, the resistance trait is transmitted via the maternal chloroplast (25, 26, 28). Maternal inheritance of the trait ensured that F_1 hybrids from reciprocal crosses of pure-breeding S and R *S. vulgaris* biotypes possessed identical, mixed nuclear genomes but unique cytoplasms, depending upon the direction of the cross.

As expected, both whole plants and isolated thylakoid membranes of the S and S×R biotypes proved susceptible to atrazine toxicity. In contrast, resistant (R, R×S) plants were unharmed by treatment with atrazine. Thylakoids isolated from leaves of resistant plants exhibited only a partial inhibition of electron transport at atrazine concentrations in the 100 μM range, as reported previously (18). The inhibition of electron flow observed in resistant biotypes at extreme herbicide concentrations may reflect the very low affinity of atrazine for the altered herbicide-binding protein (19). Alternatively, atrazine may interact with a second low-affinity binding site in resistant chloroplasts (16).

The rate-limiting step of uncoupled photosynthetic electron transport to artificial acceptors occurred in PSII for all four biotypes of *S. vulgaris*. This limitation was much greater in the two resistant biotypes than in the two susceptible ones, as evidenced by their lower rates of PSII. A similar reduction in the rate of PSII electron transfer in R plants relative to S plants has been observed in *B. campestris* (5), *Brassica* spp. (2), *C. album* (13), and *A. hybridus* (17). Rates of electron transport through PSI were similar among the four *S. vulgaris* biotypes, indicating that thylakoid components and photosynthetic capacities for the latter portion of the transport chain were equivalent, as has been reported for other species.

These results support the hypothesis that the altered Qb protein in PSII of triazine-resistant plants reduces overall photosynthetic efficiency in the light reactions, since in both resistant biotypes (R, R×S), decreased electron transport rates through PSII were accompanied by slower whole-chain rates. Similar reductions in the rate of whole-chain electron transport in resistant biotypes relative to susceptible have not been consistently observed, however. Whole-chain rates were equivalent in resistant and susceptible biotypes of some species (13, 17), yet reduced in resistant biotypes of others (5, 12). Although our data suggest that the altered Qb protein imposes a restriction on both PSII and whole-chain electron transport in chloroplasts of resistant plants, the mechanism of this limitation is not fully understood and could not be resolved by the methods employed here.

In addition to electron transport, carbon fixation differed among the biotypes of *S. vulgaris*; resistant plants (R, R×S) had a 20% reduction compared to susceptible plants (S, S×R). These data are consistent with reductions in carbon fixation rates ranging from 14 to 23% in R compared to S biotypes of other species (1, 9, 17). Reductions in photosynthetic rate have also been correlated with diminished biomass production in triazine-resistant biotypes of *Amaranthus* spp. (1), *Brassica* spp. (9), and *Setaria italica* (22). In the absence of herbicides, growth and productivity of S biotypes were greater than those of R biotypes in *A. hybridus* and *S. vulgaris* (6), *B. campestris* (14), and *C. album* (15). Thus, the resistance mutation appears to have a detrimental effect on the light reactions of photosynthesis, carbon fixation, and on productivity, as well.

In assessing relative productivity among plants, results may be biased by experimental conditions. Growth restrictions imposed by pots, for example, may affect final yield of each biotype differentially. Roots of faster-growing susceptible plants (S, S×R) may be restricted earlier than those of resistant plants (R, R×S), resulting in reduced growth rate, alterations in biomass allocation, or earlier senescence. In addition, the timing of each harvest will influence apparent differences between biotypes. Susceptible biotypes developed faster than resistant biotypes in *B. campestris* (14), *C. album* (15), and *S. vulgaris* (11); however, differences between biotypes in plant size diminished with time. These results suggest that productivity of young plants in the exponential growth stage may be strongly limited by the resistance mutation, while growth of older plants may also be regulated by developmental phenomena, as well as by environmental limitations. These factors may have contributed to the similarities observed in growth parameters of the two hybrid biotypes of *S. vulgaris* at 10 weeks.

The relative productivity of susceptible and resistant biotypes of a single species may also depend upon environmental conditions, in particular, temperature. Resistant plants of *Setaria* spp. produced more biomass under cooler temperatures than warmer and yield was inversely related to increasing temperature, while susceptible plants grew best at higher temperatures (22). Growth was also related to temperature in *B. napus* cultivars raised in Canada. In most locales, yields of the resistant cultivar were 80% those of susceptible cultivars, while at more southern latitudes, the resistant cultivar produced 94% of typical susceptible rapeseed yields (3). A resistant biotype of *Phalaris*

paradoxa displayed more vigorous growth than the susceptible biotype when grown under conditions of early winter (24). These data support the hypothesis that growth temperature influences the relative fitness of triazine-resistant weeds.

Temperature sensitivity may be directly related to the resistance mutation. High temperature has been shown to inhibit Qa to Qb electron transfer, perhaps through a conformational change in the Qb protein (7). The mutation in Qb in triazine-resistant plants may increase the thermal sensitivity of the Qb protein. Changes in membrane lipid composition observed in chloroplasts of R plants may also influence temperature response (20). These observations may partially explain differences in carbon fixation between the susceptible and the resistant biotypes of *S. vulgaris*, since chamber temperatures during measurements varied by as much as 7°C. Thus, differential vigor between triazine-resistant and -susceptible plants appears to be directly related to the resistance mutation and the effects of the altered Qb protein on productivity.

Reduced vigor of triazine-resistant biotypes cannot be attributed solely to the chloroplast mutation that confers resistance, however, since some differences in biomass production occurred between maternal and F_1 hybrid biotypes of *S. vulgaris* with identical chloroplast complements. Differential productivity between parental and hybrid biotypes with the same chloroplast type was also observed in *Setaria italica* (22). Therefore, although the resistance mutation exerts a predominate influence on potential productivity, other factors contribute to final biomass yield. Such factors as temperature sensitivity, light response, carbohydrate allocation, maintenance respiration, and the duration of active photosynthesis throughout the day may be regulated by the nuclear genome and influence growth and productivity. Thus, the traits of decreased plant vigor and triazine resistance may be separable, or at least interactive, since it appears that the detrimental effects of the triazine resistance mutation on plant growth may be attenuated by other factors.

If the nuclear component of the cell exclusively determined plant growth potential, productivity of both hybrids (S × R, R × S) would be equivalent and intermediate in relation to that of the parental (S, R) biotypes. However, the hybrids were not identical and both were more comparable to the S biotype than to the R biotype in measured growth parameters. That a symmetrical effect of the mixed nuclear genome was not expressed in the hybrids was most likely due to interactions between the nuclear and chloroplast genomes. In R × S plants, interaction of the resistant chloroplast with factors derived from the susceptible nuclear genome compensated in some way for the detrimental effects of the resistance mutation by partially restoring productivity. The basis for the partial reduction in productivity of the S × R hybrid relative to the S parent is not clear. Some level of nuclear/cytoplasmic incompatibility may have impaired the performance of the S × R plants. Alternatively, the nucleus of R plants may have developed compensatory mechanisms that allow R plants to grow, albeit in a limited fashion, in the presence of the altered Qb protein. Such mechanisms would be unnecessary in plants with normal chloroplasts, such as the S × R hybrids, and could divert energy from more productive uses.

Differences in growth between susceptible (S, S × R) and resistant (R, R × S) *S. vulgaris* plants used in this research may also be related to temperature responses that are not under chloroplast control. Since seeds of the S biotype were originally collected from a more southern latitude than seeds of the R biotype (21), S plants may be better-adapted than R plants to environmental conditions imposed in southern California where these experiments were conducted. The contribution of nuclear components from the S biotype may have contributed some form of temperature tolerance to the R × S hybrid that enhanced the growth potential of these resistant plants. Conversely, the contribution of nuclear factors from the R biotype may have impaired the performance of S × R hybrid plants by increasing plant sensitivity to high temperature.

The results reported here suggest that reduced vigor is not inseparably linked with triazine resistance. Nevertheless, isolation and introduction of the psbA gene into a crop plant remains an enigmatic problem for the biotechnology and crop breeding industries. Insertion of a gene carrying the mutation into any foreign genetic background will almost certainly cause an initial decrease in electron transport and probably result in yield loss. Co-adaptation of the nuclear and plastid genomes through the selection process is likely to be required before a vigorous resistant plant can be produced. Furthermore, various combinations of cytoplasmic and nuclear components from related species may be expected to yield differential results. The compatibility of particular plastome/genome combinations, and the extent to which their interactions compensate for the deleterious effects of the resistance mutation, must be further characterized if triazine-resistant crop cultivars with acceptable yields are to be achieved.

The results of this study suggest that the resistance mutation has a distinct, detrimental effect on thylakoid performance which is translated into decreased plant yields. This deleterious effect may be attenuated by the interaction of the plastid and nuclear genomes, but the limits of this compensation are not known. Future research using backcrossed lines should delineate more clearly the nature of the interaction and its potential use in separating triazine resistance from reduced plant yield.

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