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Altered Leaf Structure and Function in Triazine-Resistant Common Groundsel (*Senecio vulgaris*)[1]

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ABSTRACT

Anatomical and physiological characteristics of leaves of triazine-susceptible and -resistant biotypes of common groundsel (*Senecio vulgaris* L.) were studied in order to explain the differences in light-saturated photosynthetic rates previously reported. Leaves were of uniform leaf plastochron index from greenhouse-grown plants. Susceptible plants had greater leaf fresh and dry weights and leaf areas, while resistant plants had greater specific leaf mass (mg fresh weight/cm^2). Susceptible plants had greater amounts of total chlorophyll per unit leaf weight and a higher chlorophyll *a*/*b* ratio. Soluble protein in leaves was higher in susceptible chloroplasts on a weight and area basis, but similar to resistant chloroplasts on a unit chlorophyll basis. Activity of ribulose 1,5-bisphosphate carboxylase was higher in resistant plants on a fresh weight, leaf area, and milligram chlorophyll basis. Stomatal frequency, length, and arrangement were similar between biotypes, as were transpiration and conductance. Resistant leaves had less air space (v/v), more cells in palisade and spongy mesophyll, and a greater volume of palisade tissue than spongy, when compared to susceptible leaves. Differences in leaf structure and function between biotypes are probably due to a complex of developmental adaptations which may be only indirectly related to modified photosystem II in resistant plants. These results indicate that the consistently lower rates of net photosynthesis and yield in resistant plants cannot be explained solely on the basis of these leaf characteristics. Several possible mechanisms to account for reduced productivity are suggested.

Triazine resistance in weed populations was first reported in 1970 (22). Since that time, the phenomenon has been reported for numerous weed species in many different genera, occurring in a number of locations around the world (4). The mechanism of resistance to the *s*-triazines appears to be similar for all of the species studied. It has been shown that in resistant biotypes, there is a conformational change in the 32-kD herbicide-receptor polypeptide of PSII, located in the region of the secondary electron acceptor, B, that prevents triazine binding (3, 21). Studies on the inheritance of triazine resistance in several species have confirmed that maternal inheritance occurs; therefore, the chloroplast genome probably controls this trait (24).

In the absence of triazine herbicides, susceptible and resistant biotypes collected from naturally occurring wild populations differ in a number of ecological, physiological, and morphological characteristics. Differences in plant size, leaf shape, and pigmentation were observed in *Amaranthus hybridus* (17). Susceptible *Senecio vulgaris* and *A. hybridus* had more vigorous growth and seed production than resistant biotypes in noncompetitive or competitive situations (1, 9, 11). Rates of net CO_2 fixation at all light levels and quantum yields were lower in resistant plants of these species (1, 17, 23). Rates of O_2 evolution and patterns in flashing light measured in isolated thylakoids differed between biotypes, as well (12).

It is clear that the biotypes differ in efficiency at the level of the light reactions, and that there is a parallel difference in light-saturated photosynthetic rates and yield. It is unclear whether the thylakoid membrane alteration which confers resistance is directly responsible for lowering the overall rate of photosynthesis, decreasing vigor, and reducing competitive fitness in resistant plants in the absence of all herbicides. The recent surge of interest in developing resistant crop plants makes this question particularly relevant today. Herbicide-resistant crops would be of value only if the alteration in the herbicide binding protein does not also cause the overall decrease in vigor typical of resistant plants.

Electron transfer through PSII is slower in resistant plants, most likely as a direct result of the altered 32-kD protein which confers resistance (7, 8, 12, 17). However, detailed studies of PSI and PSII activities in *A. hybridus* showed that biotypes were similar in *in vitro* whole-chain electron transport, and that these rates were sufficient to support the measured rate of light-saturated Co_2 fixation in susceptible plants (17). It has been suggested that impaired PSII in resistant plants is still likely to be more rapid than the normally rate-limiting oxidation of the plastoquinone pool, such that photochemical reactions are not the limiting step to CO_2 fixation in resistant biotypes (17). From these studies, it appears that the altered protein associated with the secondary electron acceptor of PSII in resistant plants which is regulated by the chloroplast genome may not be directly responsible for lower rates of light-saturated CO_2 fixation.

A number of leaf structural and functional characteristics have been shown to regulate CO_2 assimilation in the dark reactions of photosynthesis (6, 14). The capacity of light-saturated photosynthesis in most plants will be influenced by the resistances of stomatal and mesophyll components of leaves to CO_2 diffusion (r_s and r_m, respectively), carboxylation and other dark reactions (r_x), and overall photochemical efficiency (r_e) (6, 28). These resistances are functionally associated with stomatal structure and function, internal leaf anatomy, amount and activity of RuBP[2] carboxylase, and rate of electron transport and photophosphorylation. With the exception of photochemical efficiency, little information has been published regarding these leaf characteristics in the triazine-resistant and susceptible weed biotypes for which pronounced differences in photosynthetic capacity and yield have been reported.

The objective of this study was to investigate the possible

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[2] Abbreviations: RuBP, ribulose 1,5-bisphosphate; Chl *a*-*b* LHC, chlorophyll *a*-*b* light harvesting complex; LPI, leaf plastochron index; SLM, specific leaf mass.

origin of lower light-saturated CO_2 fixation rates in triazine-resistant plants from field-collected populations. The method employed was to evaluate a number of leaf anatomical and physiological characteristics, previously shown to determine photosynthetic capacity in other plants, for the two biotypes of *S. vulgaris*. These same characteristics, therefore, may be responsible for lower rates of light-saturated net photosynthesis, biomass production, and competitive performance observed in the resistant biotype of this and other species. Since these and other biotypes studied to date are not isonuclear, any differences between them cannot be attributed solely to the modified chloroplast genome conferring resistance, but may be caused by variations in other traits under nuclear control. The possible relationships between these leaf characteristics and the altered herbicide binding protein are discussed.

MATERIALS AND METHODS

Plant Material. Seeds of triazine-resistant and susceptible *Senecio vulgaris* from the same seed source as used previously (11) were germinated in soil in trays in a bottom-heated mist chamber (23°C). At the two- to four-leaf stage, seedlings were transplanted into 10-cm pots. Plants were grown in a temperature-controlled greenhouse with natural lighting under a photoperiod of 12 to 14 h. Light levels reached a maximum of 1050 μE cm^{-2} s^{-1} during the day. Mean daily maximum and minimum temperatures of 25° and 18°C, respectively, were recorded during the time in which the study was conducted. Plants were watered daily with half-strength Hoagland solution.

Whole Leaf Characteristics. Plants used for these measurements were 6 weeks old from the time of seed sowing. Three young, fully expanded leaves from each of 12 plants per biotype were selected. Fresh weight and leaf area[3] were measured on each leaf. Leaves were then dried at 65°C for 48 h, and dry weights were determined.

Leaf Surface Characteristics. Six-week-old plants were used for these measurements. One leaf with an LPI of 5 was selected from each of 10 plants per biotype for measurements. Duco cement was applied to the abaxial and adaxial surfaces of leaves (avoiding the midrib), and allowed to dry for 30 s. Epidermal imprints were then removed with forceps, mounted on glass slides in glycerine, covered with cover slips, and sealed with clear nail lacquer. Under light microscopy, 10 fields of view per imprint were analyzed for number of subsidiary cells per stoma, stomatal length, number of cells per unit area, and number of stomata per unit area.

Conductance and Transpiration. Intact leaves with an LPI of 5 from 8- to 9-week-old plants were used for porometry measurements. Readings were made at 0900, 1100, 1300, and 1600 Pacific Standard Time on a clear day on individual leaves from four plants per biotype. Midday readings (1300) were replicated on eight more leaves, each from a different plant, a few days later. Conductance to water vapor and transpiration were measured using a steady-state porometer.[4]

Chl Contents. Chl determinations were made on leaves of 8-week-old plants of resistant and susceptible biotypes. One young, fully expanded leaf per plant constituted a sample, and determinations were replicated 8 times per biotype. Samples were kept on ice in the dark while experiments were conducted. Leaf area and fresh weight were determined for each sample, then samples were cut into small pieces and ground in a mortar and pestle with 10 ml of grinding solution consisting of 90% (v/v) acetone and 10% dilute (1%) NH_4OH containing a few grains of $MgCO_3$. Samples were then spun at 3000*g* for 15 min, and the supernatants collected. The pellets were resuspended in 10 ml of 80% (v/v) acetone, mixed with a vortex mixer, and spun again at 3000*g* for 15 min. Supernatants were collected, combined with those of the first spin, and filtered through No. 1 Whatman filter paper. Chl concentrations were determined according to the method of Arnon (2). The experiment was replicated 3 times.

Protein Contents. Plants used for protein determinations were 8 weeks old from seed sowing. Approximately 1 g of leaf tissue (LPI 6 and 7) was removed from each of four plants per biotype, and leaf area and fresh weight were determined for each sample. Samples were kept on ice in the dark during experimentation. Leaf samples were homogenized in a mortar and pestle in 20 ml grinding medium (pH 7.8) consisting of 0.04 M Tris-HCl, 0.01 M $MgCl_2$, 0.25 mM EDTA, 5 mM DTT, and 5 mM L-isoascorbate. Homogenates were filtered through three layers of cheesecloth and spun at 13,000*g* for 15 min. The pellets were resuspended in 80% (v/v) acetone and refrigerated; the supernatant was spun at 80,000*g* for 30 min to remove any remaining membranes. Aliquots of this supernatant were assayed in triplicate for soluble protein content by the Peterson method (5, 20). The pellets were resuspended in 80% acetone, added to pellets from the first spin, and Chl determinations made according to the method of Arnon (2). The experiment was replicated 4 times.

RuBP Carboxylase. Leaves of LPI 6 and 7 were collected from each of four plants per biotype, and leaf area and fresh weight were determined for each of the eight samples. Each sample (0.8–1.0 g) was ground in a glass homogenizer with 5 ml extraction buffer consisting of 50 mM Hepes (pH 7.6), 50 mM Bicine, 20 mM $MgCl_2$, 10 mM $NaHCO_3$, 1 mM DTT, and 1% PVP. Two ml of the resulting homogenate were removed for Chl determination in 80% (v/v) acetone as described above. The remaining homogenate was spun at 12,000*g* for 15 min, and the supernatant used for assay of RuBP carboxylase activity. The freshly prepared assay mixture contained 50 mM Hepes (pH 8.2), 50 mM Bicine, 20 mM $MgCl_2$, 22.5 mM $NaH^{14}CO_3$ (0.5 Ci/mol; 1 Ci = 3.7×10^{10} Bq), and 0.75 μM RuBP. Assays were carried out at 25°C in glass scintillation vials. Total activity was measured by adding 100 μl of leaf homogenate to 400 μl of assay mixture. The reaction was stopped after 0, 30, or 60 s by the addition of 0.2 ml HCl. Reaction mixtures were dried at 45°C overnight, then 0.5 ml H_2O and 10 ml scintillation cocktail were added to each vial. Radioactivity was measured by liquid scintillation counting. Assays were replicated four times per biotype in each experiment, and the entire experiment was repeated 5 times.

Internal Leaf Anatomy. Leaves used for this portion of the study were of LPI 5 from 7-week-old plants. One leaf was removed from each of 10 plants per biotype. Several 5-mm discs were cut from each leaf, avoiding the midrib, and fixed in FAA (13). FAA-fixed leaf discs were dehydrated in a graded series of ethanol solutions and embedded in glycol methacrylate (Polysciences, Inc.). Median cross-sections 4 μm thick perpendicular to the midvein were made from 10 discs per biotype using a Bright ultramicrotome. Sections were mounted in H_2O on glass slides, heated until dry, stained by flooding with a solution of toluidine blue in borate (pH 4.4) for 1 min, and washed in distilled H_2O (16). Drawings prepared with the aid of a camera lucida were made of six sections per biotype for quantitative measurements using an overall magnification of × 200 (27). Based on preliminary counts and analysis, this number was determined to be a sufficient sample size at $P = 0.05$ (26). Using computer-generated test grids as overlays, relative volumes of mesophyll tissue (spongy and palisade layers and intercellular space) and mesophyll cell surface areas were calculated according to the stereological methods of Parkhurst (18, 27, 30).

Statistical Analysis. For all measurements except leaf surface characteristics, one-way analysis of variance was used to compare data for the two biotypes. Leaf surface characteristics for the two

[3] LI COR area meter model LI-3000, LI COR, Inc., Lincoln, NE.

[4] LI COR steady-state porometer model LI-1600, LI COR, Inc., Lincoln, NE.

biotypes were analyzed as a 2 × 2 factorial arrangement using analysis of variance, where biotype and position (abaxial or adaxial) were considered factors for each variable measured. Means were separated using the LSD test (26).

RESULTS

Whole Leaf Characteristics. Leaf fresh weight, dry weight, and leaf area were all significantly greater for susceptible than resistant leaves (Table I). Specific leaf mass, or weight per unit area, however, was significantly greater for resistant leaves, whether on a fresh or dry weight basis (Table I). Since fresh weight and leaf area were also measured for all Chl and protein determinations, numerous replications of SLM were made; all are consistent with results in Table I. This indicates that resistant leaves are either thicker or denser, or both, than susceptible leaves.

Leaf Surface Characteristics. Although leaf surface features were measured on abaxial and adaxial surfaces of both biotypes, factorial analysis of variance showed no significant interaction between biotype and leaf position. Therefore, data from both surfaces of each leaf were combined, and only main effect means were compared. The only significant difference measured between biotypes was in stomatal length (Table II). Mean stomatal lengths were 47.81 and 43.12 mm for resistant and susceptible leaves, respectively. However, in relating stomatal structure to photosynthetic capacity, the important parameter is stomatal length/unit area, F × L, which is a measure of the total pore area through which CO_2 may diffuse into the leaf. The lack of a significant difference in this value between biotypes reflects the fact that size and frequency of stomata were inversely proportional in *S. vulgaris* biotypes. In resistant leaves, stomata were larger and less numerous (63.50/mm^2), whereas in susceptible leaves, they were smaller and more numerous (75.30/mm^2). Hence, no significant difference in functional stomatal length was detected.

Conductance and Transpiration. No significant differences between biotypes were detected in transpiration rate or stomatal conductance at any time during the day (Table III). In both biotypes, values for conductance and transpiration increased from 0900 to a maximum at 1300, as was expected for a C_3 species. Similarities in stomatal structure (Table II) and function (Table III) indicate that limitations to CO_2 assimilation in the resistant biotype are not at the stomatal level.

Chl Contents. On a fresh weight basis (mg/g), Chl *a*, *b*, and total Chl were significantly greater in susceptible than in resistant leaves (Table IV). Expressed as a function of leaf area (μg/cm^2), values for Chl *a*, *b*, and total Chl were all similar in leaves of the two biotypes. These data reflect the fact that resistant leaves have a higher SLM (mg/cm^2) than do susceptible leaves (Table I). The higher weight per unit area in resistant leaves compensates for lower amounts of Chl/g and results in amounts of Chl/cm^2 similar to susceptible leaves. The Chl component showing the least amount of difference between resistant and susceptible leaves, on a leaf area as well as a fresh weight basis, was Chl *b*. As a consequence, the Chl *a*/*b* ratio was significantly lower in resistant leaves (2.74) than in susceptible leaves (3.01).

Protein Contents. Susceptible leaves had significantly higher amounts of leaf-soluble protein than resistant leaves on both a weight and area basis (Table V). Susceptible leaves contained 11.60 mg soluble protein/g fresh weight as compared to 8.74 mg/g for resistant leaves. Likewise, susceptible leaves had 0.34 mg soluble protein/cm^2 *versus* 0.27 mg/cm^2 for resistant leaves. On a per unit Chl basis, however, amounts of soluble protein in

Table I. *Characteristics of Leaves of Senecio vulgaris Biotypes*

Each measurement was performed on three individual leaves from each of 12 plants per biotype. Mean values are presented on a per-leaf basis. Plants were 6 weeks old at the time measurements were made.

Parameter	Biotype		LSD (0.05)
	Resistant	Susceptible	
Fresh wt (mg)	252.2	481.5	47.6
Dry wt (mg)	17.1	30.5	3.0
Leaf area (cm^2)	6.7	15.0	1.4
SLM (mg fresh wt/cm^2)	38.0	32.3	1.4
SLM (mg dry wt/cm^2)	2.6	2.0	0.1

Table II. *Leaf Surface Anatomy of Senecio vulgaris Biotypes*

Each number is an average of 10 fields of view on each of 10 epidermal imprints from the adaxial and 10 from the abaxial leaf surface per biotype. Leaves measured were of LPI=5 from 6-week-old plants. Factorial analysis of variance showed no significant interaction between biotype and leaf position (adaxial or abaxial). Therefore, data from adaxial and abaxial surfaces were combined and only main effect means were compared.

Parameter	Biotype		LSD (0.05)
	Resistant	Susceptible	
Subsidiaries per stoma (No.)	4.06	4.02	NS
Stomatal length, L (mm)	47.81	43.12	2.09
Cells/unit area, C (No./mm^2)	152.13	163.93	NS
Stomata/unit area, F (No./mm^2)	63.50	75.30	NS
Stomatal length/unit area, F × L (mm/mm^2)	3.00	3.12	NS
Stomata to cell ratio, F ÷ C	0.42	0.44	NS
Cell size, 1 ÷ C (mm^2)	0.01	0.01	NS

Table III. *Transpiration and Conductance to Water Vapor by Leaves of Susceptible (S) and Resistant (R) Senecio vulgaris Biotypes*

Each value is an average of measurements from a single attached leaf per plant, replicated 4 times per biotype. Measurements at 1300 were repeated on eight leaves per biotype, but no significant differences were detected between biotypes. Leaves measured were of LPI=5 from 8- to 9-week-old plants.

Time of Day	Leaf Temperature	Conductance		Transpiration	
		R	S	R	S
h	°C	$cm^2 \cdot s^{-1}$		$\mu g \cdot cm^{-2} \cdot s^{-1}$	
0900	26	0.69	0.67	7.50	7.28
1100	30	1.02	0.97	15.35	14.43
1300	30	1.34	1.26	19.53	19.15
1600	30	0.87	0.91	14.50	14.13
LSD (0.05)		0.25		2.59	

Table IV. *Chl Contents of Leaves of Senecio vulgaris Biotypes*

Each measurement was performed on one leaf per plant and replicated 8 times per biotype. Plants used were 8 weeks old. The experiment was replicated 3 times; since results from all three were nearly identical, data shown are from one experiment.

Parameter	Biotype		LSD (0.05)
	Resistant	Susceptible	
Chl *a* (mg/g)	1.12	1.54	0.08
Chl *b* (mg/g)	0.41	0.51	0.03
Total Chl (mg/g)	1.52	2.06	0.11
Chl *a* (μg/cm^2)	35.37	38.21	NS
Chl *b* (μg/cm^2)	12.91	12.69	NS
Total Chl (μg/cm^2)	48.27	50.88	NS
Chl *a*/Chl *b*	2.74	3.01	0.06

Table V. *Soluble Protein in Leaves of Senecio vulgaris Biotypes*

Each determination was performed on approximately 1 g of leaf tissue. Protein assays were performed in triplicate on four samples per biotype and the experiment was replicated 4 times. Results from all experiments were nearly identical; data shown are from one experiment.

Parameter	Biotype		LSD (0.05)
	Resistant	Susceptible	
Soluble protein (mg/g fresh wt)	8.74	11.60	1.85
Soluble protein (mg/cm^2)	0.27	0.34	0.05
Soluble protein/Chl (mg/mg)	13.54	14.06	NS

Table VI. *RuBP Carboxylase Activity in Leaves of Senecio vulgaris Biotypes*

Each determination was performed on four samples per biotype and the experiment was replicated 5 times. Results from all experiments were identical in magnitude of differences between biotypes; data shown are from one experiment. Values are means ± 1 SE.

Parameter	Biotype	
	Resistant	Susceptible
μmol CO_2 fixed/min·mg Chl	3.5 ± 0.3	1.5 ± 0.2
μmol CO_2 fixed/min·g fresh wt	2.1 ± 0.2	0.7 ± 0.1
μmol CO_2 fixed/min·dm^2 leaf area	5.9 ± 0.5	2.2 ± 1.3

the two biotypes were similar, reflecting the higher amounts of Chl found in susceptible leaves. More soluble protein in susceptible leaves is possibly indicative of a greater quantity of RuBP carboxylase, which constitutes over 50% of the soluble protein fraction of most mature leaves (5, 6).

RuBP Carboxylase. The *in vitro* activity of RuBP carboxylase per mg Chl of resistant leaves was over 2-fold higher than that of susceptible leaves (3.5 μmol CO_2/min·mg Chl *versus* 1.5 for susceptible) (Table VI). Expressed on the basis of both fresh weight and leaf area, the resistant biotype also showed higher activity of nearly 3-fold magnitude. Higher RuBP carboxylase activity in resistant leaves indicates a greater potential for light-saturated CO_2 fixation than in susceptible leaves.

Internal Leaf Anatomy. At the light microscope level, qualitative differences in leaf characteristics between biotypes were readily observed (Fig. 1). Resistant leaves were somewhat thicker than susceptible ones (0.262 mm *versus* 0.235 mm, respectively), although this difference was not statistically significant. Volume fractions of tissue components varied greatly, as seen in Table VII. The resistant leaves had a greater percentage of palisade tissue (44.97% *versus* 37.98% for susceptible), whereas susceptible leaves contained more spongy tissue (44.20% *versus* 39.20% for resistant). In both the palisade and spongy tissues, susceptible leaves contained significantly more air space compared to resistant leaves. The overall volume of air space in the mesophyll tissue was 43.97% for susceptible and 30.05% for resistant leaves.

In view of the lower percentage of air space in resistant leaves, it follows that the amount of Chl-containing cells in both the palisade and spongy layers should be greater than in susceptible leaves, resulting in a more densely packed mesophyll. This is seen to be the case in Table VII, where the percentage of palisade cells per leaf was significantly higher in resistant leaves (34.45% *versus* 24.53% in susceptible leaves). Since the two biotypes did not differ in internal cell size (data not shown), the higher percentage of palisade cells in resistant leaves must be due to an increased number of cells, relative to the susceptible. The percentage of spongy cells per leaf was somewhat higher, but not significantly so, in resistant leaves as well (24.35% *versus* 21.63% in susceptible leaves). These results explain the higher specific leaf mass found in resistant than susceptible leaves. Furthermore, a denser mesophyll accounts for the observation that resistant leaves have less Chl per unit weight but similar Chl per unit area compared to susceptible leaves.

Another means of quantifying internal leaf anatomy as it relates to photosynthetic capacity is cell wall surface area of Chl-containing cells exposed to intercellular air spaces per unit volume of mesophyll (S/V) (Table VIII). The two biotypes were similar in palisade tissue S/V ratios. This result implies that even though resistant leaves have a greater percentage of cells and a smaller volume of air space in the palisade mesophyll than do susceptible ones, this denser packing of cells results in proportionately more cell-cell contacts rather than cell-air space contacts. As a result, the palisade tissues of the two biotypes have similar internal areas across which CO_2 can diffuse into cells. In the spongy mesophyll, however, the S/V ratio is significantly higher in susceptible leaves, presumably due to a greater volume of air space, a similar volume of cells, and therefore, less cell compacting relative to resistant leaves.

DISCUSSION

There is good evidence that *s*-triazine-resistant weeds from field populations have lowered photosynthetic capacity and yield relative to susceptible weeds of the same species (1, 8, 9, 11, 17, 23). It is still uncertain whether these detrimental characteristics are a direct consequence of the modified herbicide-binding protein and, therefore, are inseparable from the trait of resistance. This study is a first attempt to explain the underlying cause of lower CO_2 fixation rates in resistant plants on the basis of leaf characteristics not directly related to PSII. Results from this study indicate that *Senecio vulgaris* biotypes differ significantly in several anatomical and physiological features, some of which may affect photosynthetic performance. However, these differences do not clearly favor higher CO_2 fixation by susceptible plants and, therefore, cannot fully explain lowered photosynthetic capacity and yield in resistant plants.

In relation to stomatal resistance, r_s, functional stomatal length per unit area and other leaf surface characteristics were comparable in the two biotypes. Stomatal functioning on a diurnal basis was also similar between biotypes. These factors determine the area through which CO_2 can diffuse into a leaf, and are primary determinants of photosynthetic capacity in many plants (6, 14). However, stomatal structure and function cannot account for differences in rates of net CO_2 fixation between biotypes of *S. vulgaris*. This finding supports the conclusions from gas exchange studies performed under saturating CO_2 concentrations with *Amaranthus hybridus* (1).

Substantial differences in leaf morphology and anatomy were observed between triazine resistant and susceptible *S. vulgaris* biotypes. Fresh weight and dry weight were greater in susceptible than in resistant leaves. Reduced biomass production may be attributed directly to lower rates of CO_2 fixation in resistant plants relative to susceptible plants (1, 23). However, resistant leaves also had higher SLM, or weight per unit area, than susceptible leaves, a greater cell density in palisade and spongy mesophyll, and smaller leaf area. These results indicate that leaf expansion rate may be reduced in resistant leaves, similar to that in shaded leaves of the sun plant, *Helianthus annuus* (10). The fact that SLM did not differ between biotypes of the C_4 species *A. hybridus* (1) most likely reflects the individual genetic makeup of these two species and different degrees of phenotypic plasticity in response to growth conditions.

In general, SLM, leaf thickness, tissue compaction, palisade

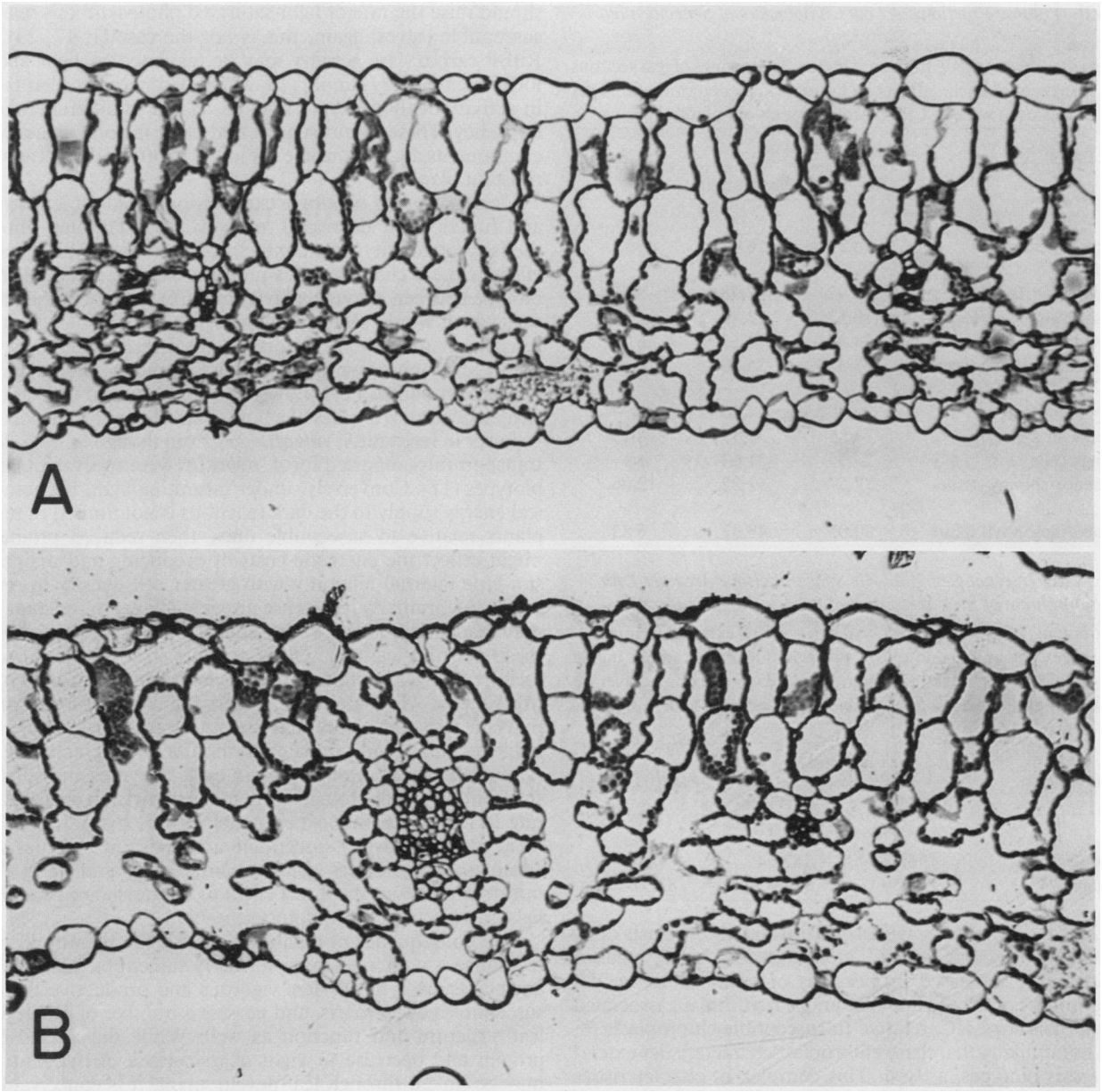

FIG. 1. Cross-sections of leaves from triazine-resistant (A) and triazine-susceptible (B) biotypes of *S. vulgaris*. × 100.

tissue development, and mesophyll cell-air space interface area/volume ratio are greater in plants from high-light environments relative to plants from low-light environments and are accompanied by higher photosynthetic rates, as well (10, 14, 18). Differences in leaf anatomy between *S. vulgaris* biotypes were similar to those reported for sun and shade plants, although the direction of differences was not consistent. A greater volume of intercellular spaces and a higher S/V ratio in the spongy mesophyll of susceptible leaves, similar to sun leaves, provide more internal area across which CO_2 can diffuse through the mesophyll and into cells, as compared to resistant leaves. Consequently, mesophyll resistance, r_m, is lower and photosynthetic rate should be higher in susceptible plants relative to resistant (14). This explanation is supported by gas exchange data from studies of *S. vulgaris* (23) and *A. hybridus* (1, 17). Triazine-resistant *S. vulgaris* leaves more closely resembled sun leaves in internal anatomy, however. This phenomenon may be a result of slow PSII electron transfer which exposes resistant plants to excess excitation energy and, therefore, provides an 'apparent' high light environment at the cellular level. The resulting well-developed palisade tissue and increased volume of photosynthesizing cells per unit leaf area in resistant leaves theoretically should optimize the utilization of light and CO_2 and raise photosynthetic rates relative to those of susceptible leaves (6, 14, 18). This is not the case, however (1, 17, 23). These results suggest that differences in leaf anatomy and expansion rate between biotypes are developmental phenomena indirectly related to the trait of resistance, possibly through the effect of PSII on apparent light environment. Other physiological or biochemical factors besides leaf structure must be primarily responsible for lowering photosynthetic rates in resistant leaves *S. vulgaris*, relative to susceptible ones.

In addition to leaf morphology and internal anatomy, cellular components differed between biotypes. Lower Chl *a*/*b* ratios in resistant relative to susceptible leaves have been reported for *A. hybridus* (1, 29), *Brassica campestris* (8, 29), *Chenopodium*

Table VII. *Volume Fractions of Leaf Components of Senecio vulgaris Biotypes*

Measurements were made from camera lucida drawings of leaf sections fixed for microscopy. Each value is an average of six determinations per biotype. Leaves used were of LPI=5 from 7-week-old plants.

Parameter	Biotype		LSD (0.05)
	Resistant	Susceptible	
	%		
Epidermal tissue/leaf	14.23	16.47	NS
Palisade tissue/leaf	44.97	37.98	5.87
Palisade air space/leaf	10.52	13.45	2.13
Palisade cells/leaf	34.45	24.53	6.77
Air space/palisade tissue	23.33	35.80	6.23
Spongy tissue/leaf	39.20	44.20	4.77
Spongy air space/leaf	14.85	22.57	4.15
Spongy cells/leaf	24.35	21.63	NS
Air space/spongy tissue	37.57	51.22	12.06
Air space/mesophyll tissue	30.05	43.97	6.83

Table VIII. *Internal Cell Wall-Air Space Interface Area per Unit Tissue Volume of Leaf Mesophyll of Senecio vulgaris Biotypes*

Measurements of mesophyll cell wall surface area exposed to intercellular air spaces per unit volume (S/V) were made from camera lucida drawings of leaf sections fixed for microscopy. Each value is an average of six determinations per biotype. Leaves used were of LPI=5 from 7-week-old plants.

Parameter	Biotype		LSD (0.05)
	Resistant	Susceptible	
	cm^2/cm^3		
Palisade S/V	196.68	182.64	NS
Spongy S/V	195.50	287.63	83.37

album (29), and now for *S. vulgaris*. Resistant chloroplasts of *A. hybridus*, *B. campestris*, and *C. album* possess highly stacked thylakoid grana, increased proportions of grana lamellae, decreased amounts of stroma lamellae and starch, and an associated increase in Chl *a-b* LHC, relative to susceptible chloroplasts (8, 29). It is not unlikely that these chloroplast characteristics extend to *S. vulgaris* biotypes, as well. This complex of characteristics found in *s*-triazine-resistant biotypes is typical of shade leaves where it is generally correlated with enhanced light-harvesting capacity and lower light-saturated rates of photosynthesis (6, 25). Preferential dephosphorylation of the Chl *a-b* LHC, regulated by the relative rates of PSII and PSI turnover, is thought to increase grana stacking in low light (25). Slower PSII electron flow in resistant chloroplasts, seen as fewer reaction center turnovers per flash (12, 17), may provide an apparent low light environment at the chloroplast level, which increases grana stacking. Alternately, increased grana stacking may be the result of slower turnover of the 32-kD thylakoid protein (15), and/or a greater proportion of unsaturated fatty acids in resistant thylakoids (8, 29).

In general, enhanced light-harvesting capacity in shade leaves is maintained at the expense of RuBP carboxylase and other dark reaction enzymes (5, 6). This may also be the case in *s*-triazine-resistant *S. vulgaris*, as suggested by lower amounts of leaf soluble protein on a fresh weight and leaf area basis, relative to susceptible leaves. However, RuBP carboxylase activity is an equally important determinant of the capacity for CO_2 fixation in the dark reactions of photosynthesis (5, 6, 19, 28). The higher activity of this enzyme measured in resistant leaves theoretically should raise the rate of light-saturated photosynthesis relative to susceptible leaves; again, this is not the case (1, 17, 23). Since RuBP carboxylase activity may be regulated by light and photochemical energy supply (19, 28), differences between biotypes in activity of this enzyme may be related to differences in PSII efficiency. These results suggest that other factors besides cellular components are responsible for lower photosynthetic capacity in resistant plants.

Clearly, there is no single explanation based on leaf structure and function for decreased levels of light-saturated photosynthesis, productivity, and competition in resistant biotypes of *S. vulgaris*. Leaf characteristics reflect a balance achieved in each biotype between enzyme activity and CO_2 supply to the reaction sites which would seem to favor higher rates of CO_2 fixation in resistant plants. Several factors not yet investigated may contribute to lower photosynthesis in resistant plants, including subtle effects of modified PSII. Lowered quantum yield of CO_2 uptake suggests that *in vivo* rates of whole chain electron transport might be lower in resistant *S. vulgaris* (23), even though *in vitro* electron transport rates reported for *A. hybridus* were equivalent between biotypes (17). Conversely, under saturating light, if photochemical energy supply to the dark reactions is not limited in resistant plants relative to susceptible ones, then reduced productivity might reflect the energetic costs of producing and maintaining sun-type internal anatomy with greater cell density in resistant leaves (6). Similarly, the dense upper leaf layer in resistant plants may shade lower layers, with resulting decreases in net photosynthesis per unit leaf area. Other possible effects of modified PSII include changes in photophosphorylation and in stromal pH and Mg^{2+} levels. Alternately, the underlying cause of lower productivity in resistant plants may be other factors which are under nuclear control and unrelated to modified PSII, including respiration rate, levels of other Calvin cycle enzymes and substrates, susceptibility to photooxidation, and cell division and expansion rate in leaves. Further experiments with F_1 hybrids from reciprocal crosses between susceptible and resistant biotypes should eliminate any nuclear genome differences, and yield further information about the direct effect of the resistance mutation on light-saturated rates of photosynthesis.

As a consequence of a chloroplast protein alteration, triazine resistance is conferred upon formerly susceptible plants. Resistant plants are typically less vigorous and productive than their susceptible counterparts, and possess a number of differences in leaf structure and function as well. While the altered 32-kD protein and decrease in vigor characteristic of resistant plants may be linked through leaf development, it is conceivable that lowered CO_2 fixation and productivity are largely under nuclear control in these field strains, and could be overcome by expanding the genetic blueprint of the leaf through an altered nuclear genome, using either conventional breeding or genetic engineering techniques. Further research into the nature of triazine resistance should yield important information about the feasibility of developing resistant yet vigorous crop plants.

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