Structural and Physiological Comparisons of Triazine-Susceptible, -Resistant, and "Mixed" Biotypes of *Poa*

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Triazine-resistant (R) biotypes of several dicot weed biotypes have been shown to differ from triazine-susceptible (S) biotypes both structurally and biochemically. To this point, no R monocot has been examined, however. In this report, we examine the ultrastructure and physiology of three biotypes of *Poa annua* L.: a S biotype, a R biotype, and a "mixed" (M) biotype that displays a level of resistance just slightly less than the R biotype, as well as a unique fluorescence in the so-called M region of the fluorescence induction curve. Like the dicots investigated previously, the R and M biotypes have larger grana stacks, and more light-harvesting chlorophyll *a*/*b* protein than the S biotype. These are similar to the differences described between "shade-type" and "sun-type" chloroplasts although anatomical parameters, that normally vary between sun and shade type plants, do not appear to be different in the three biotypes. These data indicate that the structural and biochemical modifications of the chloroplast reported previously in R biotypes of dicots are also found in R biotypes of monocots and further indicate that these are natural consequences of the slower electron transport found in R biotypes.

Introduction

Previous studies in our laboratory [1, 2] and by others [3] have shown that chloroplasts from triazine-resistant (R) weed biotypes are both structurally and biochemically distinct from triazine-susceptible (S) weed biotypes of a given species. Chloroplasts of the R biotypes have more thylakoids/granum that those of the S biotype and, in accordance with the increase in grana stacking, the percentage of chlorophyll present as the chlorophyll *a*/*b* light harvesting complex (LHC) is greater in the R biotype than the S [1, 2]. Because of the lower photosynthetic efficiency of the R biotypes studied so far [4], starch is also present in much lower concentrations in the R biotype than the S [1–3]. The consistency of these results in a number of different species [1–3] and the observations that similar structural and biochemical alterations can be induced by treatment with sublethal levels of herbicides that effect electron flow through photosystem (PS) II [1] indicate the validity of relating these chloroplast alterations as secondary consequences of mutation in the Dl (herbicide-binding) protein. Recent data from our laboratory on iso-nuclear lines of canola differing in triazine resistance indicate that these structural and biochemical differences are not due to biotypic differences, unrelated to the mutation in triazine resistance but solely to the mutation in Dl [2].

All of the ultrastructural studies of R biotypes undertaken previously use dicot weeds. In this report, we extend these investigations to a monocot: *Poa annua*. Besides an R biotype that is similar in levels of resistance to the R biotype in the dicots investigated previously, a new biotype, "mixed" (M) has also been described [5]. Both R and M biotypes have the same mutation in the Dl protein as that found in dicots (Dyer and Dron, personal communication). This M biotype is distinct from the R biotype because of a unique pattern of fluorescence: the initial phase of fluorescence is slightly higher than the R biotype and the region of the fluorescence curve associated with carbon fixation (so-called M region) exhibits an abnormal "bump", not found in either the R or S biotypes [5]. The M biotype is also slightly less resistant to triazine and other PS II herbicides than the R biotype; mixtures of 3 parts R thylakoids: 1 part S thylakoids mimic the level of resistance of 100% M thylakoids to triazine herbicides in PS II assays. The M biotype is not a mixture of R and S

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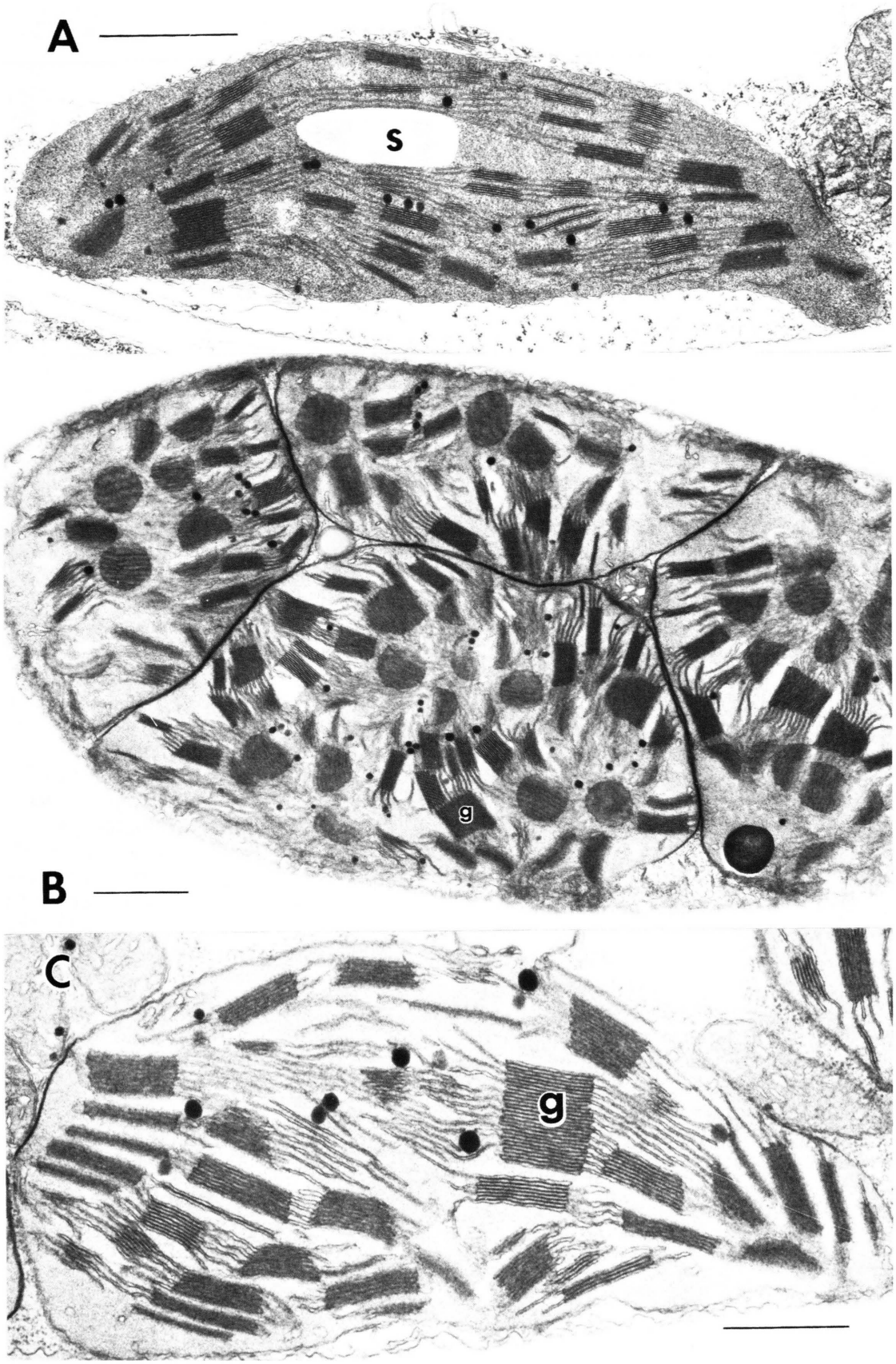
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plastids, however, as the progeny of the M biotype are all M progeny rather than a mixture of R and S biotypes [5]. Thus, the M biotype appears to be a unique triazine resistance type with some of the characteristics of R biotypes as well as a few properties that are unique to this M biotype. In this report, we describe structural, ultrastructural, and biochemical alterations in the R, S, and M biotypes of *Poa*. Our data indicate that, although the M biotype has several differences from the R biotype of this species, the structural modifications noted previously in other triazine-resistant biotypes are noted in both the R and M biotypes of *Poa*.

Materials and Methods

Seeds of the R, M, and S biotypes of *Poa annua* L. (see description in 5) were germinated in a soilless potting mixture (3 parts peat: 1 part perlite: 1 part vermiculite) and resultant plants were grown in growth chambers at a constant illumination of 250 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ photosynthetically active radiation at 20C. Plants were grown for 1 month, the top 2–3″ of growth cropped, and the plants allowed to regrow for 1 week before harvesting. Procedures for microscopy, morphometry, electrophoresis, immunological procedures, and fluorescence measurement are as described previously [1, 2].

Results and Discussion

Chloroplasts of the R and S biotypes of *Poa* display the same structural differences as did the R and S dicotyledonous weeds studied previously in our laboratory [1, 2] and by others [3]. The R biotype has larger and more prominent grana stacks than the S biotype (Fig. 1 and 2). Because of the large variation in starch from sample to sample and chloroplast to chloroplast, no significant variation in starch were obvious between the samples when these were analyzed by morphometric techniques (not shown), even though the absolute percentage of starch is lower in the R biotype than the S. The M biotype appears similar structurally to the R biotype (Fig. 1 and 2) and has a similar distribution of grana size also shows a striking similarity between the R and M biotypes, with the S biotype having much smaller grana stacks (Fig. 2).

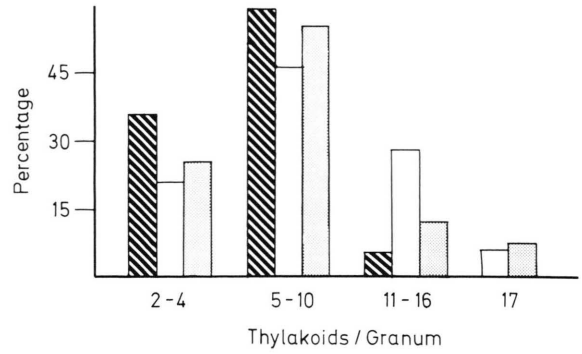

Fig. 2. Distribution of grana stack sizes in the S (diagonal bars), R (clear bars), and M (hatched bars) biotypes. Grana stacks from over 100 chloroplasts were counted for these determinations.

Light microscopic comparison of the three biotypes reveal no striking differences in the tissue organization or the contribution of a given cell type to the whole leaf anatomy (Table I). Previous observations on the anatomical differences between the R and S biotypes of *Senecio* [6], however, indicate that the leaf anatomy of the two biotypes is quite different. These anatomical differences observed in *Senecio* may represent biotypic differences that are unrelated to triazine resistance. Recent investigations in our laboratory on isonuclear lines of canola also reveal no anatomical differences between the R and S biotypes even though the chloroplast ultrastructural differences are striking [2].

"Green" gels [7] of the three *Poa* biotypes reveal a similar distribution of chlorophyll-protein complexes as noted previously in other S and R biotype comparisons [1, 2]; much more of the total chlorophyll is

Table I. Distribution of cell type contribution of leaf anatomy in the three *Poa* biotypes. None of the values for a given cell type or air space are significantly different between the biotypes.

Biotype	Epidermis	% Volume Parenchyma	Free space
S	33.23	50.06	16.69
R	33.32	50.64	16.04
M	35.97	47.74	16.20

Fig. 1. Ultrastructure of typical chloroplasts of *Poa annua* L. biotypes A. S biotype with prominent starch grains (s) and less prominent grana stacking than is noted in the R or M biotypes. B. R biotype. C. M biotype. g = granum. Bar = 1.0 μm.

present as a light-harvesting complex in the R biotype that the S. A distribution similar to the R biotype is noted in the M biotype (Fig. 3 and Table II). Thus, with the increase in grana-stacking, there is also observed an increase in the principle chlorophyll-protein complex of grana lamellae, the LHC [8]. Although this chlorophyll-protein complex is associated with the PS II reaction center, there appears to be no increase in the CPa (another component of the PS II reaction-center complex) in either the R or M biotypes. Coomassie-blue stained gels reveal no differences in the presence of any thylakoid protein (not shown), although obvious quantitative differences between several of the protein bands, such as noted for the LHC, were noted.

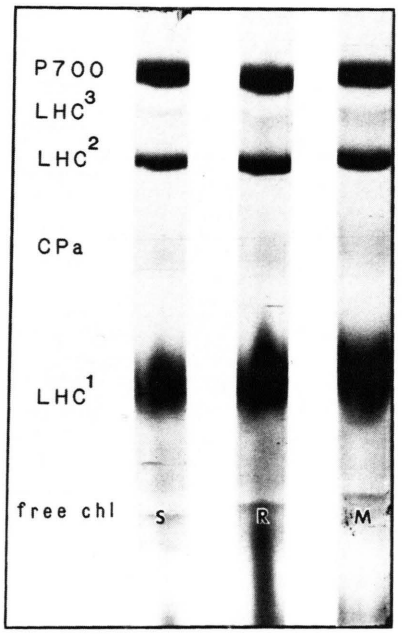

Fig. 3. Chlorophyll-proteins of "green" gels of the three *Poa* biotypes reveal an increase in the amount of chlorophyll present as light-harvesting complex. P700 = P700 chlorophyll *a* protein complex, LHC = chlorophyll *a*/*b* light-harvesting complex, CPa = chlorophyll *a* protein associated with photosystem II, Free chl = chlorophyll dissociated from chlorophyll complexes.

Table II. Comparison of chlorophyll-protein complex and ribulose bisphosphate carboxylase levels in the S, R and M biotypes of *Poa*. Chlorophyll proteins are the average of four separations and ribulose bisphosphate carboxylase levels are from 12 separate immunodiffusion readings. Abbreviations for chlorophyll complexes are as in Fig. 3. Only the relative concentrations of the P700 chlorophyll a protein and the light-harvesting chlorophyll *a*/*b* protein are significantly different between the R and S biotypes.

Chlorophyll Protein (% total)	Biotypes S	R	M
P700	29	20	19
CPa	8	8	8
LHC (all)	51	61	60
Free chl	12	11	13
Ribulose bisphosphate carboxylase (% S)	100	106	102

Although Holt and Goffner [6] reported that the R biotype of *Senecio* had more RuBisCo activity than the S biotype, no apparent differences were noted in RuBisCo concentration as determined by radial immunodiffusion (Table II). Similarly, the molecular weight of the chloroplast-encoded large subunit, as determined by Western blotting, appears to be identical in all three biotypes (not shown). Thus, the differences in RuBisCo activity in the *Senecio* biotypes may be unrelated to triazine resistance, is more of an ecotypic difference than one that is a primary or secondary consequence of the mutation in the Dl protein. Likewise, the difference in the fluorescence induction curve observed in the M biotype [5] is probably unrelated to any gross CO_2 fixation difference between the biotypes because of RuBisCo large subunit differences.

Chlorophyll fluorescence was used as a sensitive and rapid indicator of alterations in photosynthetic electron transport through PS II in the three *Poa* biotypes. Several known PS II inhibitors (diuron, pyrazon, atrazine, bentazon, and dinoseb) were used to determine if the cross-resistance pattern of the R biotype was similar to the S biotype. The R biotype is resistant to atrazine and pyrazon but is sensitive to diuron, bentazon, and dinoseb. This same pattern of resistance and sensitivity was noted for the M biotype. Although the S biotype was sensitive to all of the herbicides, 10-fold more bentazon was required to cause a detectable fluorescence rise in the S biotype than in either the R or M biotypes. These are the same sorts of cross-resistance and sensitivity patterns noted in the dicots studied by others [9], indicating that the mutation in this monocot is similar to that found in the dicots.

Like the dicot weeds investigated previously [1–3], the R and M biotypes of *Poa* have much larger grana stacks (Fig. 1 and 2) and more LHC (Fig. 3) than the S biotype. Along with the data from

isonuclear lines of canola that vary in triazine-resistance [2] and the data from the monocot *Poa* described in this report we can conclude that the structural and physiological changes described herein are natural consequences of triazine-resistance. Kyle *et al.* [10] have found that the degree of membrane phosphorylation (and hence grana stacking) is related to the state of Q. All of the triazine-resistant biotypes investigated to date have a less efficient electron transport through Q, due to the mutation in the D1 protein, and thus the decreased membrane phosphorylation would naturally lead to increased grana stacking. It would be interesting to investigate other triazine-resistant mutants such as those in *Chlamydomonas*, in which the mutation is less devastating to electron transport or the resistance less complete [11], with the procedures in this report.

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