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THE ACTION OF SIRMATE (3,4-DICHLOROBENZYL METHYLCARBAMATE) ON CHLOROPLAST RIBOSOMES OF *TRITICUM VULGARE* L. SEEDLINGS

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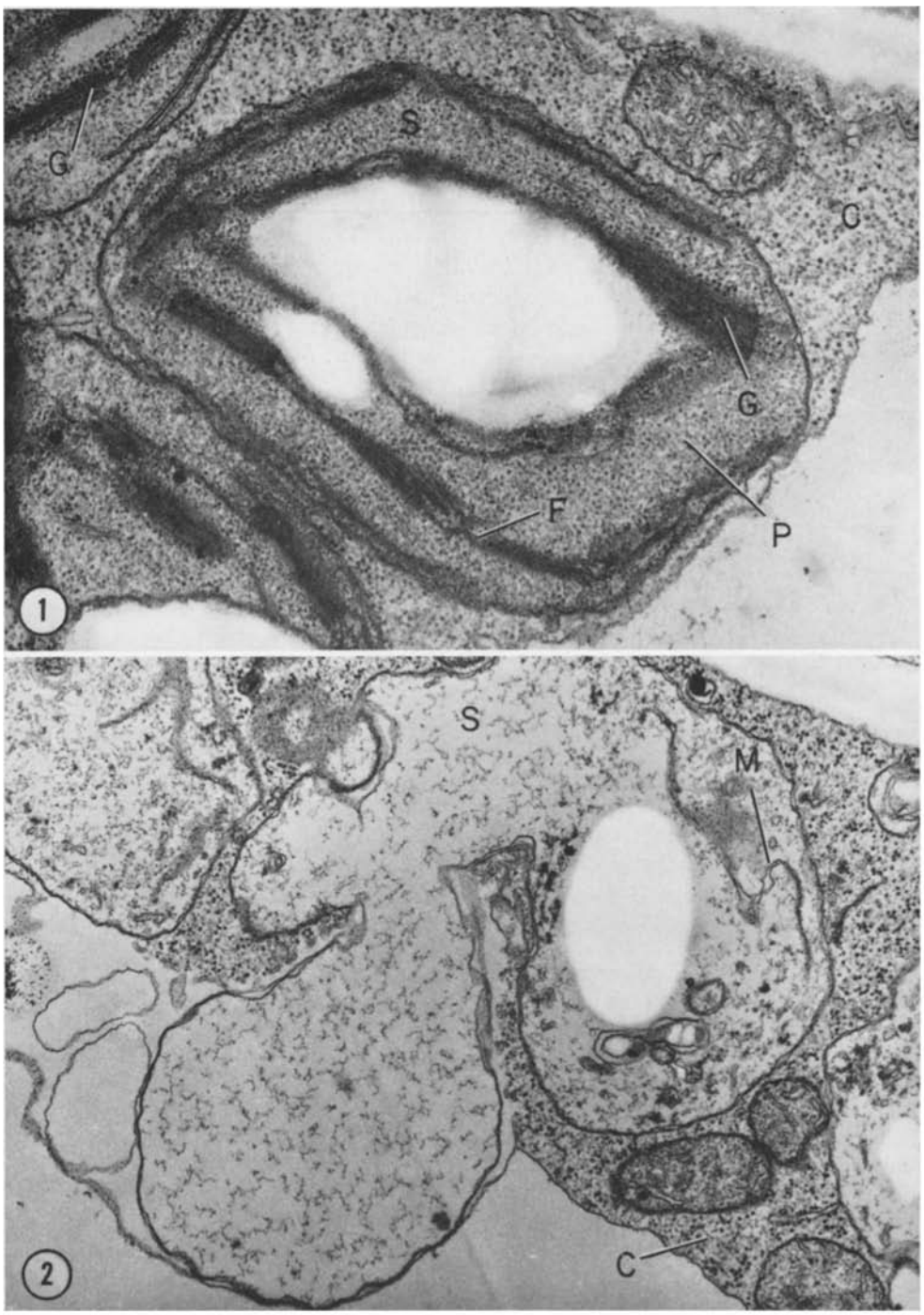
INTRODUCTION

The most striking response of light-grown wheat seedlings to sublethal doses (0.1 mM) of the herbicide Sirmate (3,4-dichlorobenzyl methylcarbamate) was the formation of achlorophyllous leaves. This herbicide did not inhibit seed germination and the leaves emerged normally, except that they had a bleached appearance. After 9 days, these plants died, presumably of starvation due to depletion of endosperm reserves. Postemergent application of Sirmate directly on plants caused contact injury or necrosis but did not induce chlorosis of mature green leaves. The mechanism of Sirmate's herbicidal action is still unknown, although Herrett and Berthold (5) suggested that Sirmate inhibited chlorophyll synthesis in light-grown seedlings, whereas in dark-grown plants the carotenoid pigments were not affected, quantitatively or qualitatively, by the Sirmate treatment. In the present investigation, we examined the effect of Sirmate on chloroplast ultrastructure and on the ribosomal population of dark- and light-grown wheat seedlings.

MATERIALS AND METHODS

About 15 wheat grains (*Triticum vulgare* L. var. Federation) were germinated in a Petri dish containing 10 ml of 0.1 mM Sirmate or distilled water. 30 of these Petri dishes with the treated and control grains were placed in a Percival compact plant growth chamber, (Percival Refrigeration and Manufacturing Company, Des Moines, Iowa) and the seedlings were germinated and grown under the following conditions: (*a*) 6 days of fluorescent light (1000 ft-c, 16 hr photoperiod, 21°C); (*b*) darkness for 5 days (21°C); (*c*) 5 days of darkness followed by 20 hr of light (1000 ft-c, 21°C). After germination, all plants were watered with distilled water. The shoots were harvested on the sixth day, and then prepared for either ultrastructural or sedimentation studies. Any plants which appeared green following Sirmate treatment were not used.

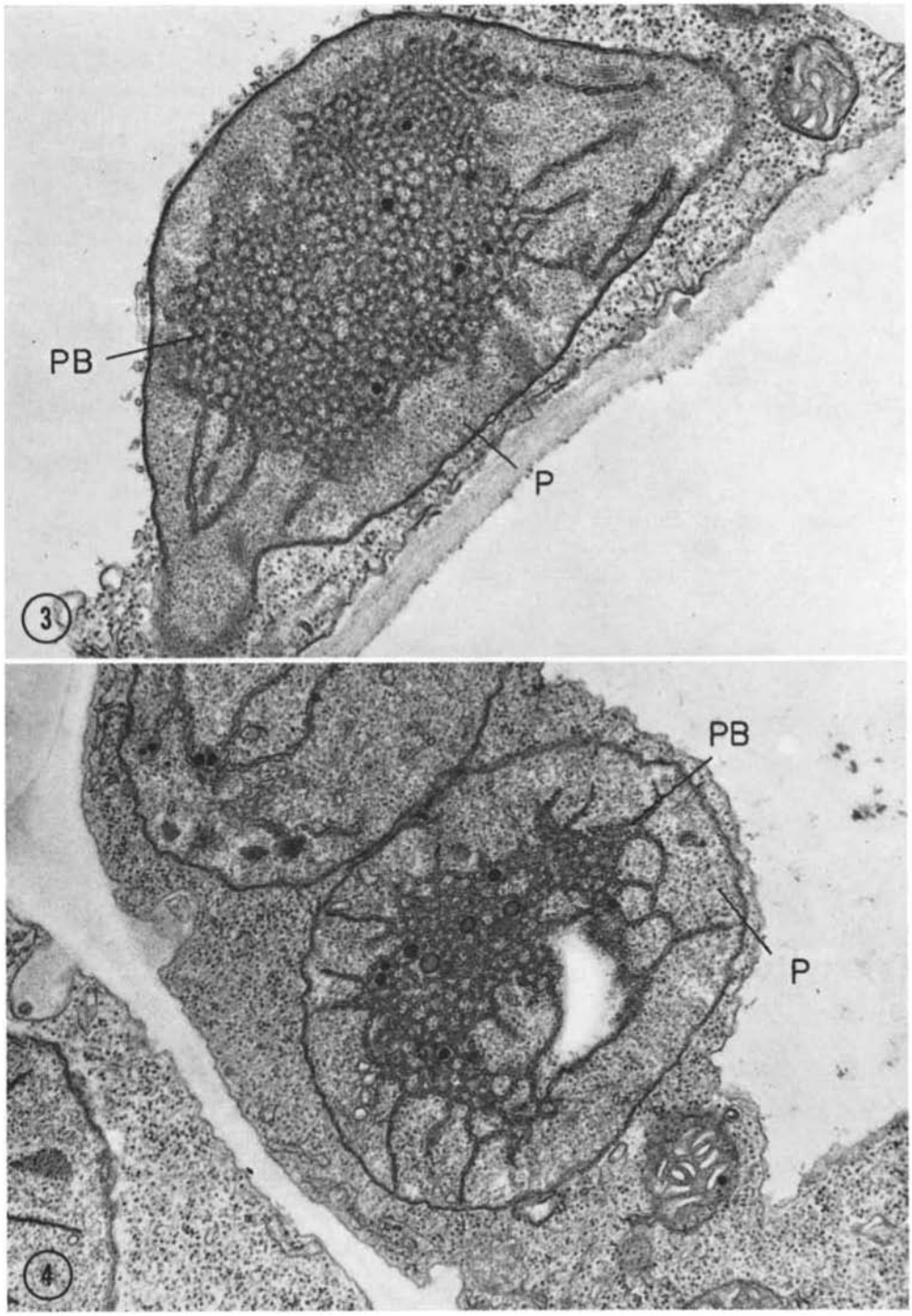
For sedimentation studies, approximately 10 g of fresh leaf tissue were chilled and ground in a mortar and pestle (2°C) with an equal volume of sucrose-tris buffer at pH 8 (6). The homogenate was strained through two layers of cheesecloth and centrifuged for 30 min at 23,000 *g* (1°C) in a Servall SS-34 rotor; the resulting supernatant was centrifuged for 1½ hr at 226,000 *g* in a Spinco 50 Ti rotor. The pellet was resuspended in a buffer (pH 7.5) composed of 5 mM Tris (Sigma Chemical Co., St. Louis, Mo.), 7 mM magnesium acetate, and 5 mM mercaptoethanol, and then was clarified by centrifugation for 10 min at 8,000 *g* (SS-34 rotor). The ribosomal suspension (supernatant) was subjected to ultracentrifugal analysis on a Spinco model E analytical ultracentrifuge, with a standard 12 mm, 4° sector cell in an



Figs. 1–2 are sections of chloroplasts from 6-day-old wheat seedlings grown at 21°C and under 1000 ft-c of light. Fixed with glutaraldehyde and Dalton's fixative.

FIGURE 1 Chloroplast from control seedling contains grana (*G*), frets (*F*), plastid ribosomes (*P*), cytoplasmic ribosomes (*C*), and stroma (*S*). × 35,500.

FIGURE 2 Chloroplast from Sirmate-treated seedling with only a few scattered membranes (*M*). Cytoplasmic ribosomes (*C*) are abundant but chloroplast ribosomes are absent. *S*, stroma. × 27,900.



Figs. 3–4 show proplastids from 5-day-old wheat seedlings grown in complete darkness (21°C). Fixed with glutaraldehyde and Dalton's fixative.

FIGURE 3 Proplastid from control seedling contains a prolamellar body (*PB*) and plastid ribosomes (*P*). × 29,000.

FIGURE 4 Proplastid from Sirmate-treated seedling has a prolamellar body (*PB*) and plastid ribosomes (*P*). × 29,000.

AnD rotor. The sedimentation coefficients were calculated by the procedure of Markham (7).

For electron microscopic studies, the lower half of the leaf was sliced into 1 mm sections in the presence of 6% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2, 4°C) and fixed in this solution for 9 hr. The sections were washed three times over a 3 hr period with phosphate buffer (pH 7.2) and then postfixed with Dalton's fixative for 9 hr (4). A graded acetone series was used to dehydrate the leaf sections. These sections were embedded in Maraglas (10), cut with a DuPont diamond knife on a Porter-Blum MT-1 ultramicrotome, and doubly poststained with aqueous uranyl acetate and lead citrate. A Phillips 200 electron microscope was used to examine the sections.

RESULTS

Fig. 1 shows a section of a chloroplast from a control, light-grown seedling. This chloroplast has a membrane system of compartmented grana (*G*) connected by anastomosing frets (*F*). The stroma (*S*) contains many 170–200-A particles (*P*) which conform to the electron microscopical criteria for ribosomes, since they were preserved with glutaraldehyde and OsO_4, stained with uranyl acetate. Bartels and Weier (1) showed that these particles of wheat plastids were removed by ribonuclease. In contrast, a section of a chloroplast from a Sirmate-treated leaf (Fig. 2) shows that the normal plastid membrane system of grana and frets is absent, and only a few scattered membranes (*M*) are visualized in the plastids. Also, the plastid stroma (*S*) lacked ribosomal particles whereas the cytoplasm contained an abundance of ribosomes (*C*).

Fig. 6 shows an ultracentrifugal pattern obtained with the ribosomal fractions from leaf tissue identical with that used in each ultrastructural study. The direction of sedimentation was from left to right. Fractions from the untreated, light-grown leaves (lower curve) showed three peaks with approximate sedimentation coefficients of 18S, 70S, and 80S; they represent fraction 1

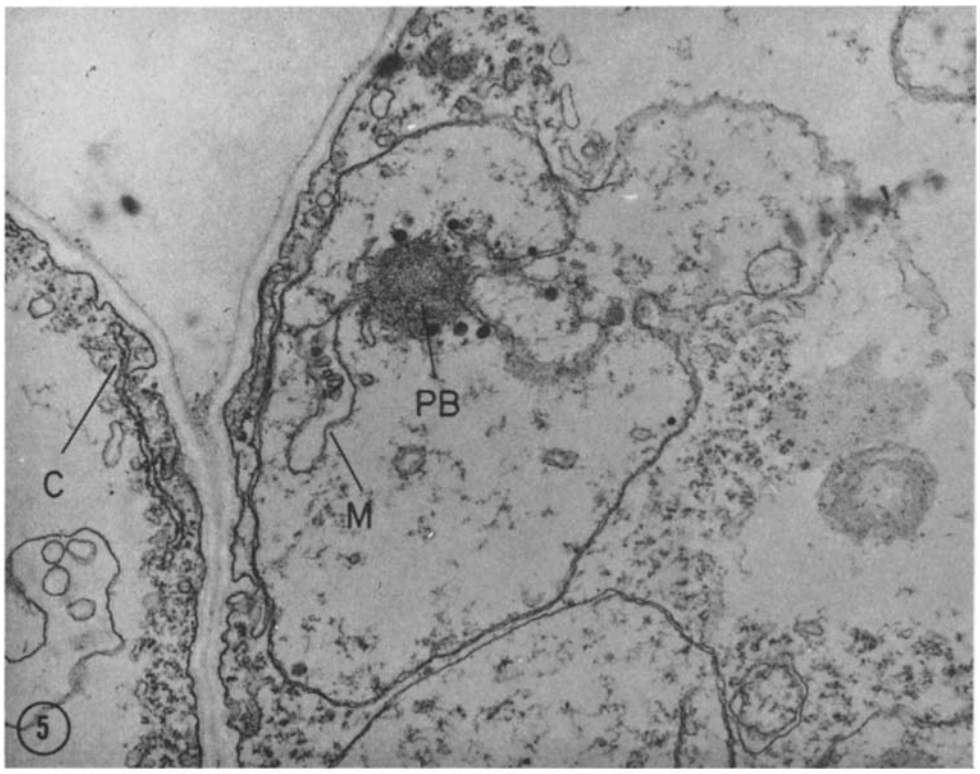
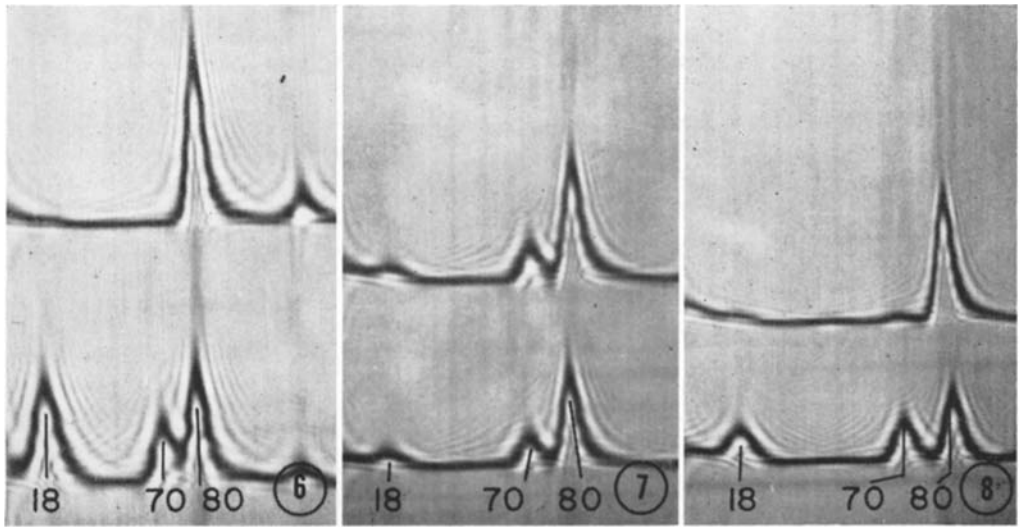


FIGURE 5 Chloroplast from a Sirmate-treated plant grown in the dark for 5 days and then exposed to 20 hr of light. Plastid may contain a disorganized prolamellar body (*PB*), and a few membranes (*M*). Note that chloroplast ribosomes are absent whereas cytoplasmic ribosomes (*C*) are present. × 24,500.



Figs. 6–8 represent schlieren patterns for ribosomal preparations from control and Sirmate-treated wheat seedlings. Sedimentations were carried out in AnD rotor at 34,000 rpm at approximately 22°C. Photographs were taken 21 min after start of centrifugation. Direction of sedimentation, left to right. Protein concentration, 3–5 mg/ml.

FIGURE 6 Ultracentrifugal pattern for light-grown, treated (upper curve) and control (lower curve) seedlings. Bar angle, 55°.

FIGURE 7 Ultracentrifugal pattern for dark-grown, treated (upper) and control (lower) seedlings. Bar angle, 70°.

FIGURE 8 Ultracentrifugal pattern for seedlings grown in dark for 5 days and then exposed to 20 hr of light. Treated, upper curve; control, lower curve. Bar angle, 65°.

protein, chloroplast ribosomes, and cytoplasmic ribosomes, respectively (2, 9). In contrast, the ribosomal preparations from Sirmate-treated, light-grown leaves (upper curve) yielded only an 80S peak; the 18S and 70S peaks were completely absent. The combined results of the ultrastructural and ultracentrifugal studies indicated that the herbicide caused the loss of chloroplast ribosomes and fraction 1 protein, but not cytoplasmic ribosomes.

In contrast to the results obtained with light-grown plants, Sirmate treatment of dark-grown seedlings appeared not to alter the proplastid ultrastructure, plastid ribosomal content, or fraction 1 protein content. An ultrastructural examination of either the untreated (Fig. 3) or treated (Fig. 4) seedlings indicated that the proplastids of each plant were morphologically identical with respect to the ribosomes (*P*) and prolamellar bodies (*PB*). To confirm these electron microscopial observations, we performed an ultracentrifugal study of ribosomal fractions from tissue identical with that used in the ultrastructural study. The schlieren pattern in Fig. 7 (Sirmate-treated, upper curve; control, lower curve) showed that both treated and untreated dark-grown leaves had fraction 1 protein (18S), plastid ribosomes (70S), and cytoplasmic ribosomes (80S).

When 5-day-old, dark-grown plants were exposed to light (1000 ft-c) for 20 hr, the control seedlings became green whereas the Sirmate-treated plants remained yellow. Fig. 5, an example of a chloroplast from this yellow leaf tissue, shows that the prolamellar body (*PB*), present in the dark, was changed during the greening process into an aggregated unit of highly disorganized, interconnecting membranes rather than the normal grana-fret system shown in Fig. 1. Note also the absence of ribosomes in the chloroplast. An ultracentrifugal investigation of this tissue revealed that untreated plants had fraction 1 protein and also 70S and 80S ribosomes (Fig. 8, lower curve)

whereas the Sirmate-treated seedlings lacked fraction 1 protein and 70S ribosomes (Fig. 8, upper curve). These treated plants, which were grown for 5 days in dark then exposed to 20 hr of light, had a ribosomal composition similar to that of the treated plants grown for 6 days in continuous light. The results suggest that chloroplast ribosomes and fraction 1 protein are destroyed in the Sirmate-treated seedlings during the greening process.

Fig. 5 also shows that the cytoplasm of these cells appeared disorganized and that cytolysis had started. Blisters or blebs (Figs. 1, 5) of the chloroplast envelope project into the vacuolar area of the cell and displace the vacuolar membrane.

DISCUSSION

This investigation revealed that the grana-fret membranes, fraction 1 protein and ribosomes were absent in chloroplasts of Sirmate-treated, light-grown plants. The loss of these chloroplast structures may be caused by the absence of chloroplast ribosomes. In support of this suggestion, Smillie et al. (8) reported that certain Calvin cycle enzymes, including fraction 1 protein (ribulose-1,5-diphosphate carboxylase), were synthesized on chloroplast ribosomes and not on cytoplasmic ribosomes. On the other hand, proplastids from Sirmate-treated, dark-grown plants contained 70S ribosomes, normal appearing prolamellar bodies, and fraction 1 protein. The reason for the different response of dark- and light-grown plants to Sirmate treatment is still unknown, but we would like to suggest that Sirmate blocks a process in which the chloroplast precursors of the meristematic cells become competent to green upon exposure to light but that Sirmate does not inhibit dark development. This competence is probably physiological rather than morphological and involves some of the chloroplast nucleic acids. Bogorad (3) reported that, despite the fact that there are large numbers of ribosomes in the proplastids of dark-grown plants, massive production of plastid ribosomal RNA and, presumably, plastid ribosomes preceded the greening of the dark-grown plants upon exposure to light. Sirmate may in some manner block light-stimulated RNA synthesis either for plants initially grown in the dark and then exposed to light or for plants germinated and grown under continuous light. During the greening process, the proplastid ribosomes, 70S, of Sirmate-treated, dark-grown seedlings disappeared. The cause of the ribosomal disappearance is the subject of current investigation.

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