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*Short Communication*

# Chloroplast Development in 4-Chloro-5-(dimethylamino)-2-($\alpha,\alpha,\alpha$-trifluoro-*m*-tolyl)-3(2*H*)-pyridazinone (Sandoz 6706)-treated Wheat Seedlings

## A PIGMENT, ULTRASTRUCTURAL, AND ULTRACENTRIFUGAL STUDY[1]

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The first visible effect following the application of sublethal doses of 4-chloro-5-(dimethylamino)-2-($\alpha,\alpha,\alpha$-trifluoro-*m*-tolyl)-3(2*H*)-pyridazinone (hereafter referred to as 6706, manufacturer's code number) to germinating wheat grains was the formation of albinistic seedlings. These seedlings grew and developed as well as untreated seedlings for 9 days. Hilton *et al.* (12) studied the effect of 6706 on established green leaves and meristematic tissues of several different species. They found that Hill reaction and $CO_2$ fixation were inhibited in green leaves and that chloroplast development was blocked in embryonic cells.

The action of 6706 on wheat seedlings grown under high light intensities appears to be similar to that of 3-amino-*s*-triazole (4) and of 3,4-dichlorobenzylmethylcarbamate (5). These herbicides cause the loss of grana-fret membranes and chloroplast ribosomes. Burns *et al.* (9) suggested that the mode of action of these herbicides was the inhibition of carotenoid synthesis. We investigated the effect of 6706 on the ultrastructure, ribosome, and pigment composition of the aberrant plastids of wheat seedlings which were grown in dark only, light only, or dark followed by light.

## MATERIALS AND METHODS

Wheat seedlings (*Triticum vulgare* L. var. Maricopa) were germinated and grown in Petri dishes (15 grains per dish) containing either 10 ml of 0.1 mM 6706 or distilled water, under one of the following conditions: (*a*) 6 days in light at 1 ft-c, or 1500 ft-c, 16-hr photoperiod, 21 C; (*b*) 6 days in darkness except for exposure to dim green light during watering, 23 C; or (*c*) 6 days in darkness followed by 1, 4, or 12 hr of light. The light intensity used during greening was either 1 ft-c, 75 ft-c, or 1500 ft-c, 23 C. Following germination, plants were watered with distilled water. On the 6th day shoots were harvested, weighed, and prepared for either ultrastructural, sedimentation, or pigment studies.

Sedimentation and ultrastructural studies were performed as discribed in an earlier publication (5) except that (*a*) grinding media used to isolate ribosomes contained 4% Triton X-100 (Rohm and Haas, Philadelphia) and (*b*) fixing solutions used in electron microscope preparations contained 0.01 M cacodylate-HCl buffer (pH 7.2) instead of phosphate buffer.

The ratio of chloroplast ribosomes to cytoplasmic ribosomes was calculated by measuring the area under the curve. This ratio represents the proportion of each type of ribosome in the total population of the extracted ribosomes. The chloroplast pigments of wheat seedlings were extracted with 85% acetone, and the chlorophyll and carotenoid pigments were estimated by method of Röbbelen (16).

## RESULTS

The chlorophyll content of 6706-treated seedlings depended upon the light intensities under which the plants were grown. At 1500 ft-c, the chlorophyll content (Table I) was reduced by 97%, and these seedlings appeared red, indicating the presence of only anthocyanin pigments. In contrast, treated seedlings grown at 1 ft-c for 6 days appeared a blue-green color and contained about 60% as much chlorophyll as did the controls (Table I). When these plants were exposed to 1500 ft-c of light for 12 hr, they lost 80% of their original chlorophyll, while control plants accumulated three times more chlorophyll than they originally had. The chlorophyll content of 6706-treated, dark-grown plants was determined by the light intensity under which the plants were illuminated. If they were exposed to 1 ft-c of light for 12 hr, the seedlings became green and accumulated about 70% as much chlorophyll as controls (Table I), while those illuminated with 1500 ft-c of light failed to gain chlorophyll pigment.

The carotenoid pigments of the 6706-treated seedlings were virtually absent, irrespective of whether the plants were grown at high or low light intensities or in darkness (Table I). The carotenoid pigments of 6706-treated seedlings were barely detectable in the acetone extract, indicating that 6706 inhibited the synthesis and accumulation of carotenoid pigments. The influence of 6706 on the ribosomal composition of wheat seedlings grown under 1500 ft-c was identical to that of aminotriazole- and sirmate-treated plants (4, 5). The ribosomal preparations (Table II) of 6706-treated seedlings had only a single 80 S peak while control plants contained two peaks with approximate sedimentation coefficients of 70 S, which represent chloroplast and cytoplasmic ribosomes, respectively (6). In contrast, the ribosomal composition (Table II) of 6706-treated seedlings grown for 6 days under 1 ft-c of light showed the presence of both 70 S and 80 S ribosomes; however, the ratio of cytoplasmic 80 S ribosomes to chloroplast 70 S ribosomes for 6706-treated plants was 4:1 as compared to a 80 S to 70 S ratio of 3:1 for controls, indicating a slight reduction of 70 S ribosomes. Some of these seedlings which were grown for 6 days at 1 ft-c were then exposed to 1500

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Table I. *Effect of Light Intensity of Chlorophyll Content of 6706-treated Plants*

Seedlings were grown in a 16-hr photoperiod at a temperature of 21 C. High light intensities were provided by fluorescent lamps supplemented with incandescent lamps and low light intensities by incandescent lamps. The concentration of 6706 used in these experiments was 0.1 mM.

| | Chlorophyll | | Carotenoids | |
|---|---|---|---|---|
| | Control | 6706 | Control | 6706 |
| | *μg/g fresh wt* | | | |
| Light 6 days at 1500 ft-c | 790 | 17 | 155 | 1 |
| Light 6 days at 1 ft-c | 175 | 105 | 45 | 2 |
| Light 6 days at 1 ft-c, then 12 hr at 1500 ft-c | 560 | 19 | 40 | 2 |
| Dark 6 days | ... | ... | 75 | 3 |
| Dark 6 days, then 12 hr at 1 ft-c | 55 | 41 | 48 | 2 |
| Dark 6 days, then 12 hr at 1500 ft-c | 62 | 11 | 40 | 1 |

Table II. *Effect of Light Intensity of Ribosomal Content of 6706-treated Plants*

Plants were grown as described in Table I.

| | 80 S to 70 S Ribosome Ratios | |
|---|---|---|
| | Control | 6706 |
| Light 6 days at 1500 ft-c | 2:1 | 80 only |
| Light 6 days at 1 ft-c | 3:1 | 4:1 |
| Light 6 days at 1 ft-c then 12 hr at 1500 ft-c | 3:1 | 80 only |
| Dark 6 days | 3:1 | 3:1 |
| Dark 6 days then 1500 ft-c for 1 hr | 3:1 | 4:1 |
| Dark 6 days then 1500 ft-c for 4 hr | 2:1 | 6:1 |
| Dark 6 days then 1500 ft-c for 12 hr | 2:1 | 80 only |
| Dark 6 days then 12 hr at 1 ft-c | 2:1 | 2:1 |
| Isolated ribosomes incubated with 6706 for 6 hr, 1500 ft-c | 2:1 | 2:1 |

ft-c of light for 12 hr, and a sedimentation analysis showed that the chloroplast ribosomes of these seedlings disappeared while 80 S ribosomes remained (Table II).

In contrast to these results obtained with illuminated plants, both 6706 and control, dark-grown plants were found to have identical ribosomal composition (Table II), and the ratio of cytoplasmic to chloroplast ribosomes was the same (3:1) in both, indicating that 6706 did not affect synthesis or accumulation of 70 S ribosomes in the dark. Illumination of these dark-grown plants wath 1500 ft-c of light for either 1, 4, or 12 hr caused the rapid reduction and finally the loss of the 70 S chloroplast ribosomes which were present prior to illumination. After 1 hr of illuminating the 6706-treated plants (Table II), their 80 S to 70 S ribosomal ratio changed to 4:1 as compared to a 3:1 ratio for dark-grown 6706-treated plants. Four hours of illumination of 6706-treated plants changed 80 S to 70 S ratio to 6:1 and after 12 hr of light, only a hint of 70 S ribosomes were observed (Table II) in the 6706 plants. The 80 S to 70 S ratios, 2:1, for control seedlings were about the same for each light treatment.

In contrast, when dark-grown plants were illuminated with low intensities of light (1 ft-c) for up to 12 hr, the 70 S ribosomes were not reduced and the 80 S to 70 S ratio (2:1) was the same as that of the controls (Table II). However, when the light intensity was again increased (75 ft-c), the 80 S to 70 S ratio dropped to 6:1.

These results suggest that 6706 in conjunction with light causes rapid destruction of 70 S chloroplast ribosomes. To determine if the destruction of 70 S ribosomes was caused by the photoactivation of the herbicide, isolated 70 S and 80 S ribosomes were incubated with 6706 *in vitro* under 1500 ft-c of light for 6 hr. The 70 S ribosomes were not destroyed and 80 S to 70 S ratios were identical in both the control and treated extracts (Table II). The ultrastructural effect of 6706 on light-grown (1500 ft-c) and dark-grown seedlings appeared to be identical to that of aminotriazole and sirmate (4, 5). The plastids of light grown plants lacked grana and ribosomes but contained thylakoids which were unbranched and unusually long and were arranged parallel to the edge of the plastid envelope. The etioplasts of 6706 dark-grown plants were morphologically identical to the etioplast of control plants, each having prolamellar bodies and ribosomes.

When 6-day-old, dark-grown plants were exposed to high light intensitiy (1500 ft-c) for 12 hr, the control seedlings became green whereas the 6706-treated plants remained white. Prolamellar body (P) of the etioplast from this white tissue changed during the greening process into an aggregated unit of highly disorganized, interconnecting membranes rather than the normal structure of grana-thylakoids and the stroma became devoid of ribosomes. The plastid appeared to be morphologically similar to sirmate-treated plants grown under the same conditions (5).

When seedlings were grown under 1 ft-c of light for 6 days, the plastids (Fig. 1) of control plants contained prolamellar bodies (P), ribosomes (R), and grana (G) structures while plastids of 6706-treated plants also had prolamellar bodies (P) and ribosomes (R) but lacked grana structures (Fig. 2).

## DISCUSSION

Many recent studies have demonstrated that several different factors such as mineral or vitamin deficiencies (7, 18), treatment with unnatural pyrimidine (11, 22), treatment with herbicides (2, 10), senescence (17), and genetic alternation (3, 15, 21) can cause similar aberrant ultrastructural changes in the chloroplast structure. It is evident from our results that 6706 also disrupts the chloroplast morphology and development.

We feel, at this time, that the best explanation for the mode of action of 6706 on chloroplast development is the inhibition of carotenoid synthesis or accumulation. This explanation seems to accomodate all of our data. Burns *et al.* (9) have proposed this as the mode of action for three other herbicides which also bleach plants. Table I shows that carotenoid pigments are essentially absent in all the 6706-treated seedlings regardless of whether they were grown in the dark, dim light, or high light intensities, indicating that 6706 blocked carotenoid synthesis. This was not the case for chlorophyll pigments since 6706 plants grown under 1 ft-c light contained 70% as much chlorophyll as controls.

Anderson and Robertson (1) suggested that the carotenoid pigments act as "chemical buffers" to protect the chloroplast and chlorophyll pigment from photodestruction. They reported that a carotenoidless albino mutant of corn (*Zea mays*) (white-3) when grown in dim light (0.5 ft-c) produced chlorophyll; however, exposure of this mutant to bright light for only 1 hr in the presence of air resulted in the destruction of the chlorophyll pigments. Wallace and Schwarting (19) discovered a carotenoidless albino mutant of the sunflower (*Helianthus annuus*) which also accumulated maximum chlorophyll at about 0.5 ft-c, then faded above this light intensity. Our results show that the 6706 plants responded to low and high light intensities much like these albino mutants; *i.e.*, at 1500 ft-c of light the treated plants failed to accumulate chlorophyll (Table I) or 70 S ribosomes.

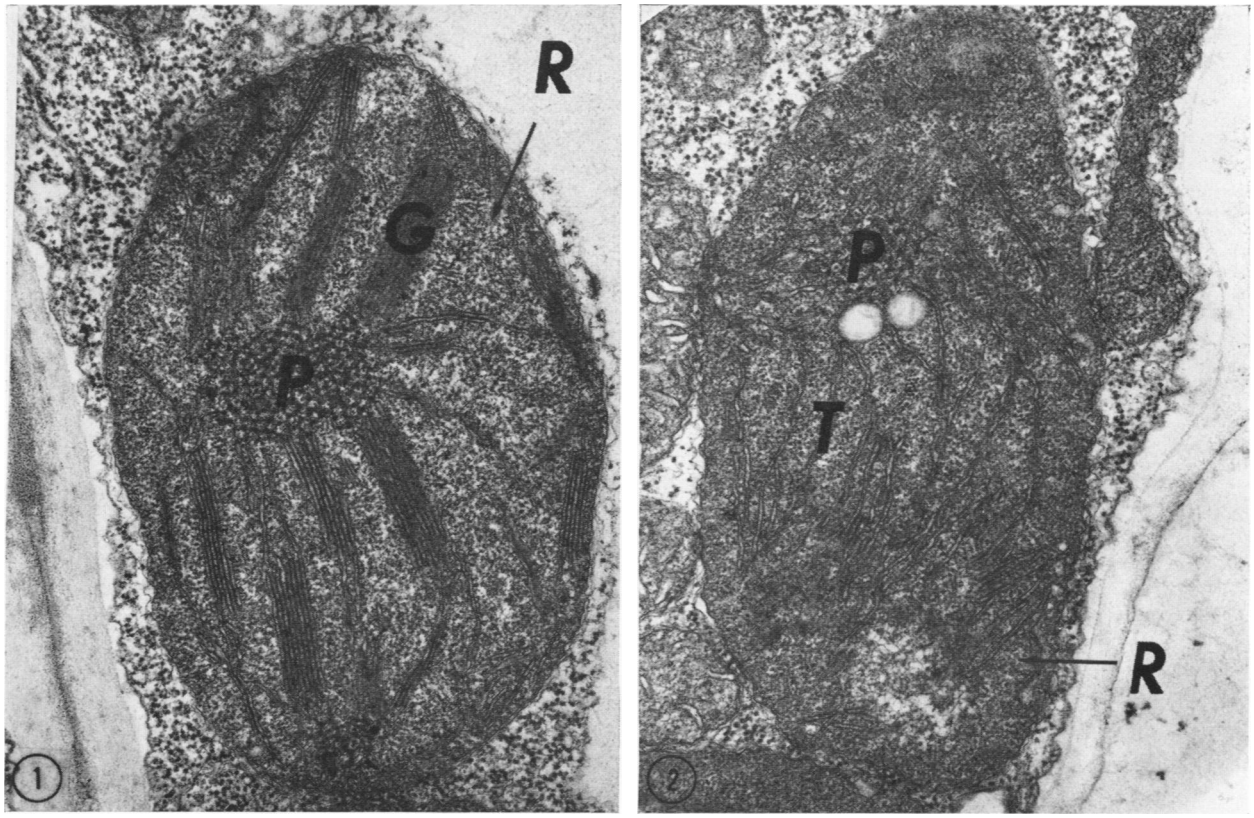


FIG. 1. Chloroplast from control plant grown under 1 ft-c of light for 6 days. Note the grana (G) structures surrounding the prolamellar body (P). × 31,400.

FIG. 2. Chloroplast from 6706-treated plant grown under 1 ft-c for 6 days. Note the absence of grana. Note single thylakoids (T) radiate from the prolamellar body (P). × 22,000.

while plants grown at 1 ft-c of light contained chlorophyll and 70 S ribosomes. The chlorophyll pigments and 70 S ribosomes that occurred in 6706 plants grown at 1 ft-c of light were still susceptible to photodestruction by high light intensities, as was the case for albino corn. Walles (20) found that carotenoidless, albino sunflower seedlings grown in weak light formed grana but that these are rapidly destroyed by bright light. In contrast, 6706 seedlings failed to develop grana in dim light (Fig. 2) and had only stroma thylakoids extending from the prolamellar body. The etioplast of 6706 and albino mutant plants resemble normal eitoplasts of control plants. Walles (20) reported that the absence of carotenoid pigments had no influence on the synthesis and organization of the prolamellar body.

Millerd *et al.* (14) reported that the maize mutant (M-11) sensitive to low temperatures and deficient in carotenoids lacked 70 S ribosomes. A chlorophyll-less plant of *Vicia faba* was reported to lack chloroplast ribosomes also (8). These studies show that mutants deficient in or lacking chloroplast pigments also lack 70 S ribosomes, as was the case for 6706 plants.

We inferred from our results that chlorophyll pigments in the absence of carotenoids become susceptible to photooxidation. These pigments may be converted to highly reactive molecular species, which then interact with and destroy other chloroplast components. Our data suggest that unstabilized photosensitized chlorophyll or precursors of chlorophyll react directly or indirectly with 70 S ribosomes and thylakoid to oxidize and destroy them. Some evidence for this inference is supported by the work of Leff and Krinsky (13). They reported that photooxidized chlorophyll could function as a photosensitizer which reacts with chloroplast DNA, resulting in genetic alternation.

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