Inhibition by Sethoxydim of Chloroplast Biogenesis, Development and Replication in Barley Seedlings

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Sethoxydim not only blocks leaf growth and development of barley seedlings but also inhibits chloroplast biogenesis at all stages of development from proplastids to prochloroplasts, young and mature chloroplasts. Not only thylakoid synthesis, thylakoid multiplication and grana formation are affected, but also chloroplast replication. The chloroplasts of secondary leaves which before the sethoxydim application are in the stage of young, developing chloroplasts, remain in this differentiation stage when treated with sethoxydim. With their ultrastructural characteristics (*e.g.* lower stacking degree, higher proportion of exposed membranes, a lower thylakoid frequency etc.) they resemble sun-type chloroplasts. In the shoot meristem sethoxydim-treated plants contain only proplastids, whereas the plastids in the shoot meristem of control plants are already in the developmental stage between prochloroplasts to young chloroplasts. Mesophyll cells of sethoxydim-treated plants contain only one third of the chloroplasts found in the controls.

Introduction

Sethoxydim, a cyclohexendione derivative (NP 55; BAS-9052 OH), (Fig. 1) is a very efficient herbicide for postemergence control of annual and perenniel grass weeds (*Avena fatua, Agropyron repens, Alopecurus myosuroides, Echinochloa crus-galli, Cynpodon dactylon, Sorghum halepense* etc.) in a wide range of dicotyledonous crop plants (soybeans, flax, sugar beet, pea nuts, tomatos, cotton) [1]. The structurally related substance alloxydim (NP 48) exhibits very similar effects and is also applied in broad leaf crops [2]. Absorbed by the leaves the systemic herbicide sethoxydim is rapidly translocated (phloem) to the meristematic zones of leaves, roots and the shoot apex where it blocks growth and yields necrotic tissue.

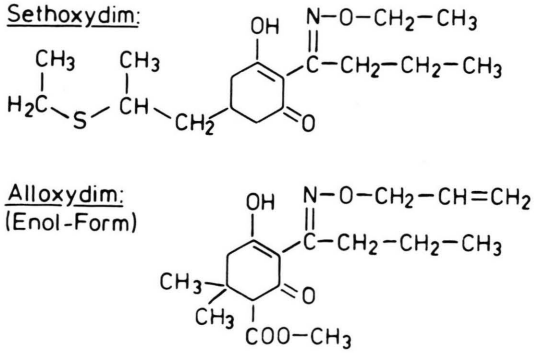

Fig. 1. Chemical structures of the two related herbicides sethoxydim and alloxydim.

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The reason for its selectivity towards grass weeds and also towards gramineous crop plants, seems to be a differential uptake and a rapid breakdown of sethoxydim in broadleaf crops. The exact mode of action of sethoxydim or alloxydim are at present, however, not known [2–4].

Plastid (chloroplast) differentiation and multiplication are basic parameters of leaf growth and development. In order to obtain more information on its possible mode of action we studied the effect of sethoxydim on chloroplast biogenesis and development in barley seedlings.

Materials and Methods

Barley seedlings (*Hordeum vulgare* L. cv. Breuns Villa) were germinated for 3 days in the dark then placed in a day-night rhythm (14 h + 10 h) and grown (22 °C, 65% relative humidity) at a medium light intensity of 20 $W \cdot m^{-2}$ (~ 6 klux). On the 8th day the plants were sprayed with sethoxydim in a dose of 300 $g \cdot ha^{-1}$, which corresponds to spraying

an area of 500 cm^2 with 10 ml of a ca. 4.4×10^{-4} M sethoxydim solution.

On day 11 leaf segments were infiltrated and fixed in a buffered 5% glutardialdehyde (pH 7.4) at 4 °C, postfixed in 2% OsO_4 and further prepared for the electron-microscope investigation as described [5]. Poststaining with 10% uranyl acetate and lead citrate was applied [6]. The biometrical analyses (Table 1) were performed on 30 median longitudinal chloroplast sections. The stacking degree of thylakoids was determined by measuring the total length of thylakoids and the length of stacked thylakoid regions. From the length and the width of median chloroplast sections we calculated the chloroplast volume by assuming an ellipsoidal rotation body, revolving on the axis *a*. $V = 4/3\,\pi \cdot a \cdot b^2$ (a = half width and b = half length).

Results and Discussion

The development of the leaf blade of the secondary leaf of 8 day old barley seedlings is blocked by spraying of the plants with sethoxydim. This refers to growth in length and in width of the leaf blade (Fig. 2). There is little effect on the primary leaf, which at the time of herbicide treatment had almost fully been developed. The tertiary leaf being formed in the control plants from day 8 to day 11 after germination does not appear in the herbicide-treated plants. This indicates that sethoxydim primarily affects the growing parts of leaves.

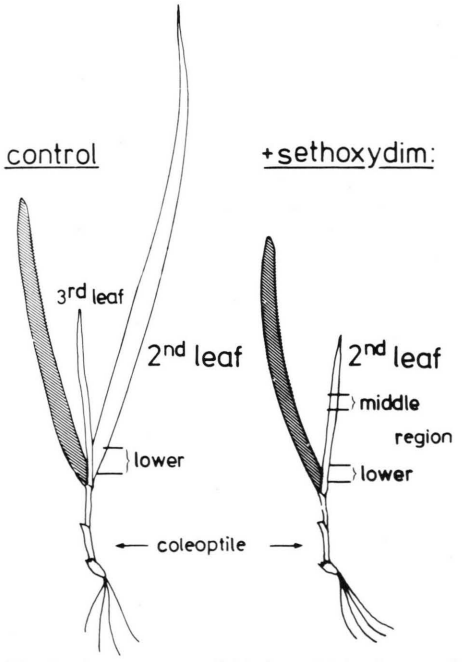


Fig. 2. Appearance of 11 day old barley seedlings without (control) and with sethoxydim-treatment (300 g · ha^{-1}) on the 8th day after germination.

Leaf development of barley proceeds like in other gramineous plants, via an intercalaric meristem on the leaf basis. At the time of herbicide treatment the top of the secondary leaf blade contains the older mature leaf cells with fully developed chloroplasts. In the middle leaf region of the control plants there occurs from day 8 to 11 the final cell elongation and the plastids go through the final steps of chloroplast development (further thylakoid multiplication and grana formation). In the lower and younger parts of the blade of the secondary leaf the cells and chloroplasts are in a much earlier stage of development.

Since adult green leaf cells contain a much higher number of chloroplasts per cell than the younger, more meristematic cells [7], one can expect in this youngest part of the leaf blade (2nd leaf) not only developmental chloroplast stages between proplastids to young chloroplasts, but also a significantly lower number of plastids (chloroplasts) per cell. In order to study the effect of sethoxydim on chloroplast development we analysed the leaf mesophyll cells in the top, middle and lower region of the second leaf blade.

In the control plants the chloroplasts from the upper, middle and lower part of the second leaf blade on 11 day old barley seedlings showed a similar ultrastructure with well developed stroma and grana thylakoids of mature chloroplasts (Fig. 3). The leaf tip (2nd leaf) of the sethoxydim-treated plants did not show any difference to those of control plants. The middle and lower parts of the leaf blade (2nd leaf), however, contained chloroplasts which were not elongated as in controls but rounded off (Fig. 3 b). They also showed a lower thylakoid frequency (µm thylakoids per 10 μm^2 chloroplast section), a lower stacking degree of thylakoids, as well as a lower width and height of grana stacks. This was as expected more pronounced in the younger, lower part of the leaf blade than in the middle region (Table 1).

These results clearly show that the final stages of chloroplast differentiation are blocked by sethoxydim. Similar differences in chloroplast ultrastructure between control and sethoxydim-treated plants

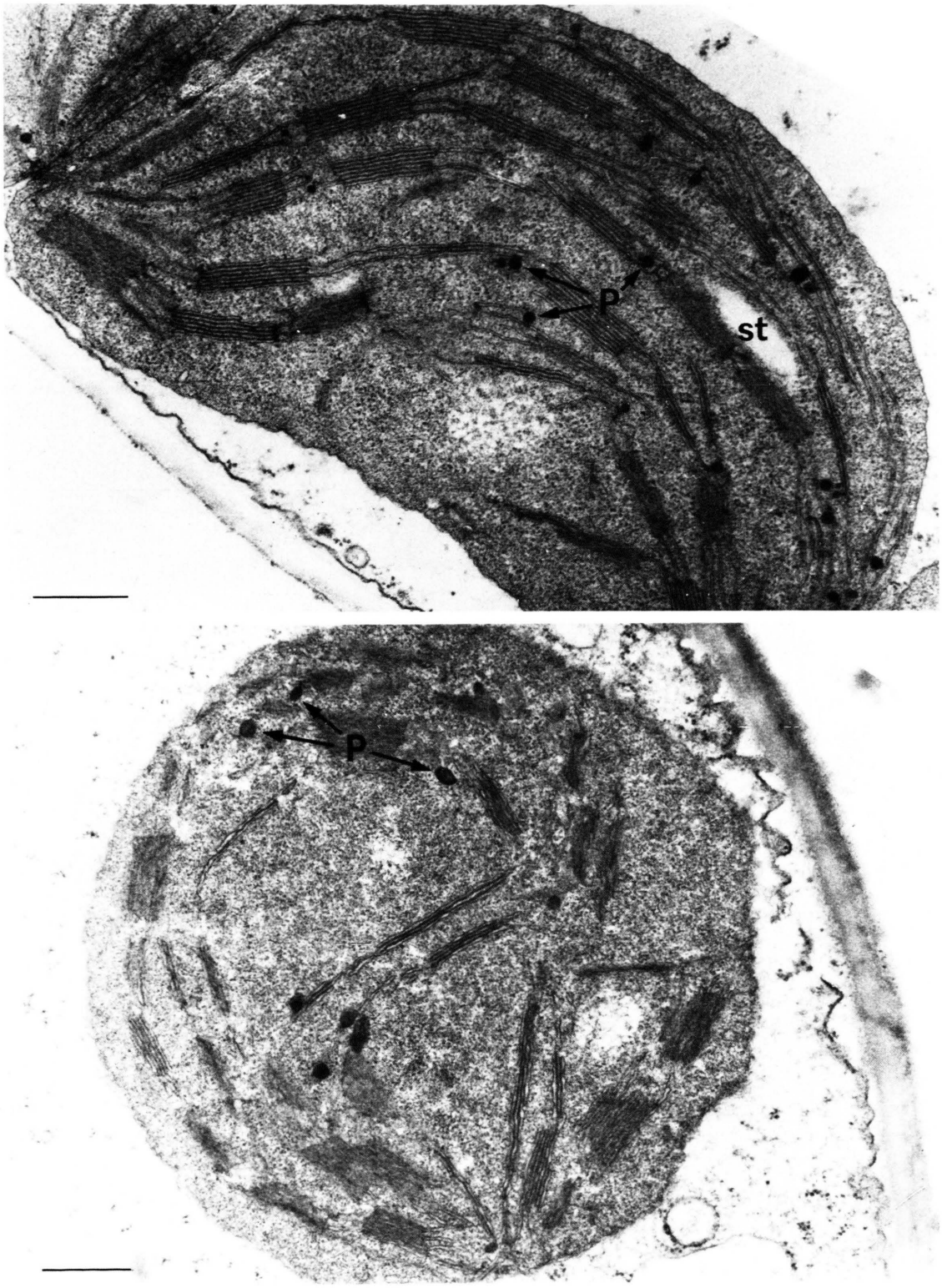


Fig. 3. Chloroplasts of the secondary leaf (lower region) from 11 day old barley seedlings a) controls and b) three days after treatment with sethoxydim. P = plastoglobuli; st = starch; bar = 0.5 μm.

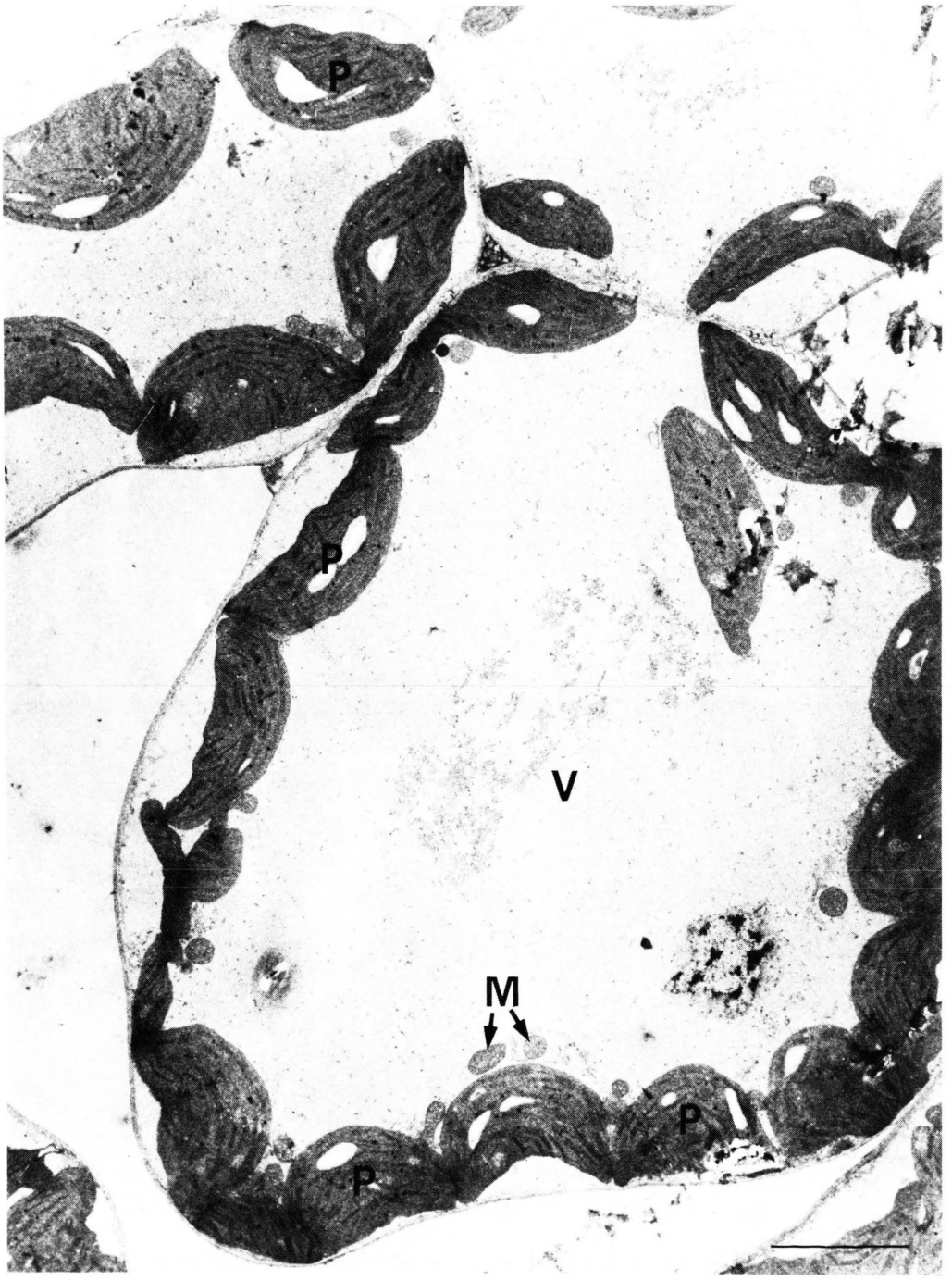


Fig. 4. Section through mesophyll cells of the secondary leaf (lower region) of 11 day old barley seedlings (control plants) with many chloroplasts. M = mitochondria; P = chloroplasts; V = vacuole; bar = 5 μm.

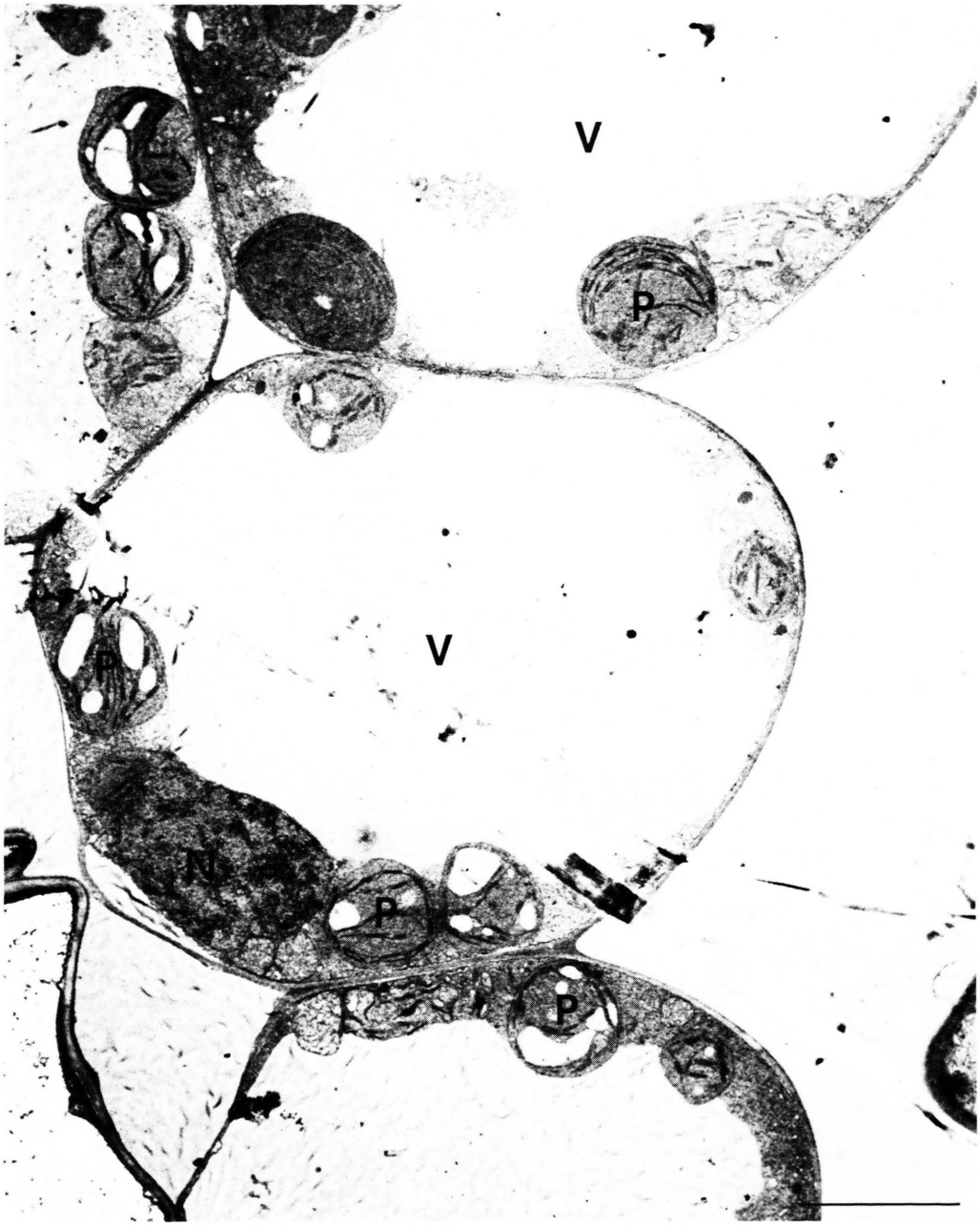


Fig. 5. Section through mesophyll cells of the secondary leaves of 11 day old barley seedlings, three days after treatment with sethoxydim (only few chloroplasts) N = nucleus; P = choroplasts; V = vacuole; bar = 5 μm.

are found between shade (low-light) chloroplasts and sun (high-light) chloroplasts of plants or leaves grown at different light quanta fluence rates [6, 8, 9]. The sethoxydim-induced block of the final thylakoid multiplication and grana formation thus results in the appearance of sun-type-like chloroplasts in younger parts of the secondary leaf which upon herbicide application are not further developed.

Sethoxydim also affects chloroplast replication. The mesophyll cells of the leaf blade (2nd leaf) of control plants (Fig. 4) also contain more chloroplasts than those of the herbicide-treated plants

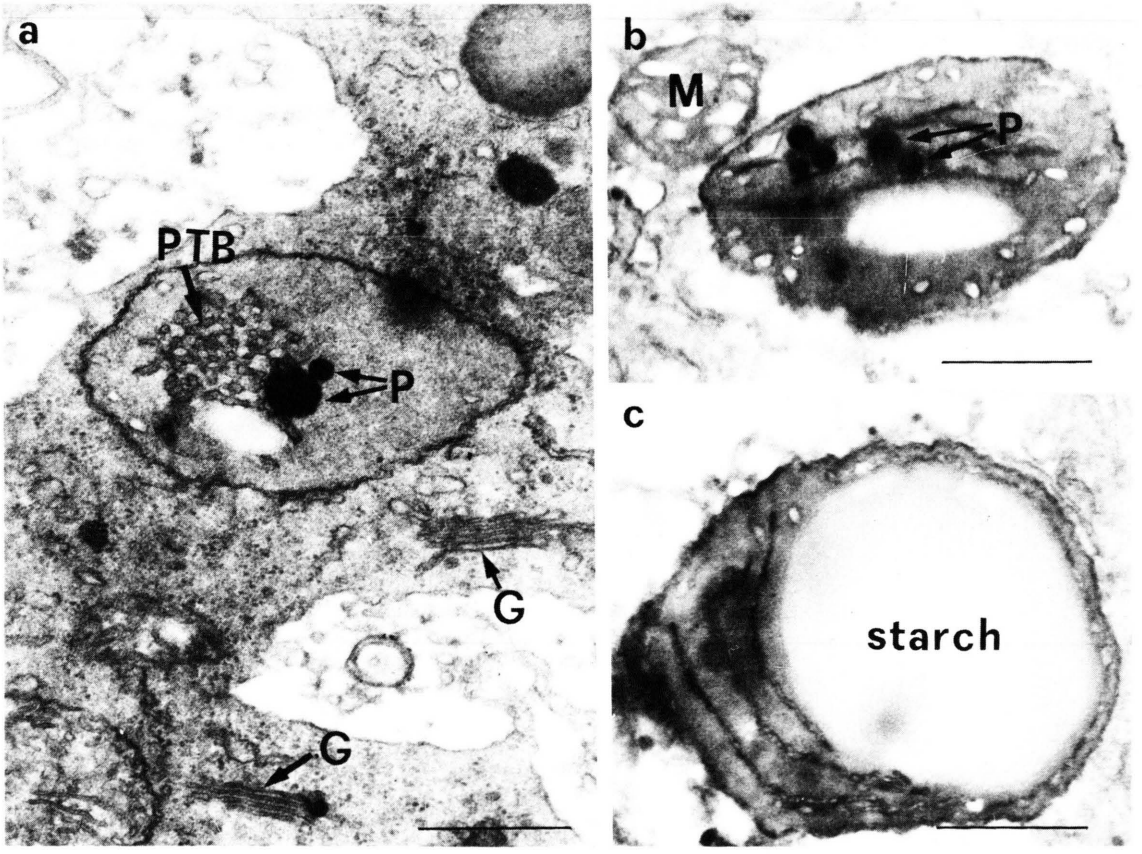
(Fig. 5). As compared to control plants in the average only one third of the chloroplast numbers per sectioned leaf cell are detected. In control plants similary small numbers of chloroplasts are found only in the still growing zones of the developing tertiary leaf. These results thus demonstrate that sethoxydim not only blocks chloroplast differentiation, but also chloroplast division. This also explains the larger average volume of the chloroplasts from the second leaf of sethoxydim-treated plants (Table I). In contrast, the chloroplasts of control plants, which undergo chloroplast division, possess a smaller volume.

Since sethoxydim absorbed by the leaves is also rapidly translocated to the shoot apex, we studied its effect on plastid development in the meristematic regions behind the apex of barley seedlings. In control plants one finds there transitional stages between proplastids and young chloroplasts which we have termed pro-chloroplasts (Fig. 6). They are characterized by vesicles and protubular structures

Table I. Differences in ultrastructural parameters of chloroplasts from 11 day old barley seedlings, which were treated on the 8th day after germination with sethoxydim.

Secondary leaf blade	Controls	+ Sethoxydim
a) middle leaf region:		
thylakoid frequency ++	194	124
stacking degree of thylakoids in %	62	49
ratio of stacked to exposed membrandes	1.6	1.0
average width of grana (μm)	0.61	0.46
volume of chloroplasts (μm³)	51	74
b) younger, lower leaf region:		
thylakoid frequency	170	95
stacking degree of thylakoids in %	62	54
ratio of stacked to exposed membranes	1.6	1.1
average width of grana (μm)	0.58	0.52
volume of chloroplasts (μm³)	27	70

++ μm thylakoid length per 10 μm² median chloroplast section.



and also contain some starch, a few prothylakoids and plastoglobuli; such plastid stages are regular components of chloroplast development in many plants [10–12].

In the sethoxydim-treated plants the meristematic regions next to the shoot apex do not contain any of these transitional stages in chloroplast development. Only proplastid stages are found (Fig. 7). This demonstrates that sethoxydim not only blocks chloroplast replication and the final stages of chloroplast differentiation, but also the early steps of plastid and chloroplast development at the prochloroplast.

Concerning the ultrastructure of mitochondria there appear to be no differences between control and herbicide-treated plants. The process of photosynthesis is also not affected by sethoxydim as has

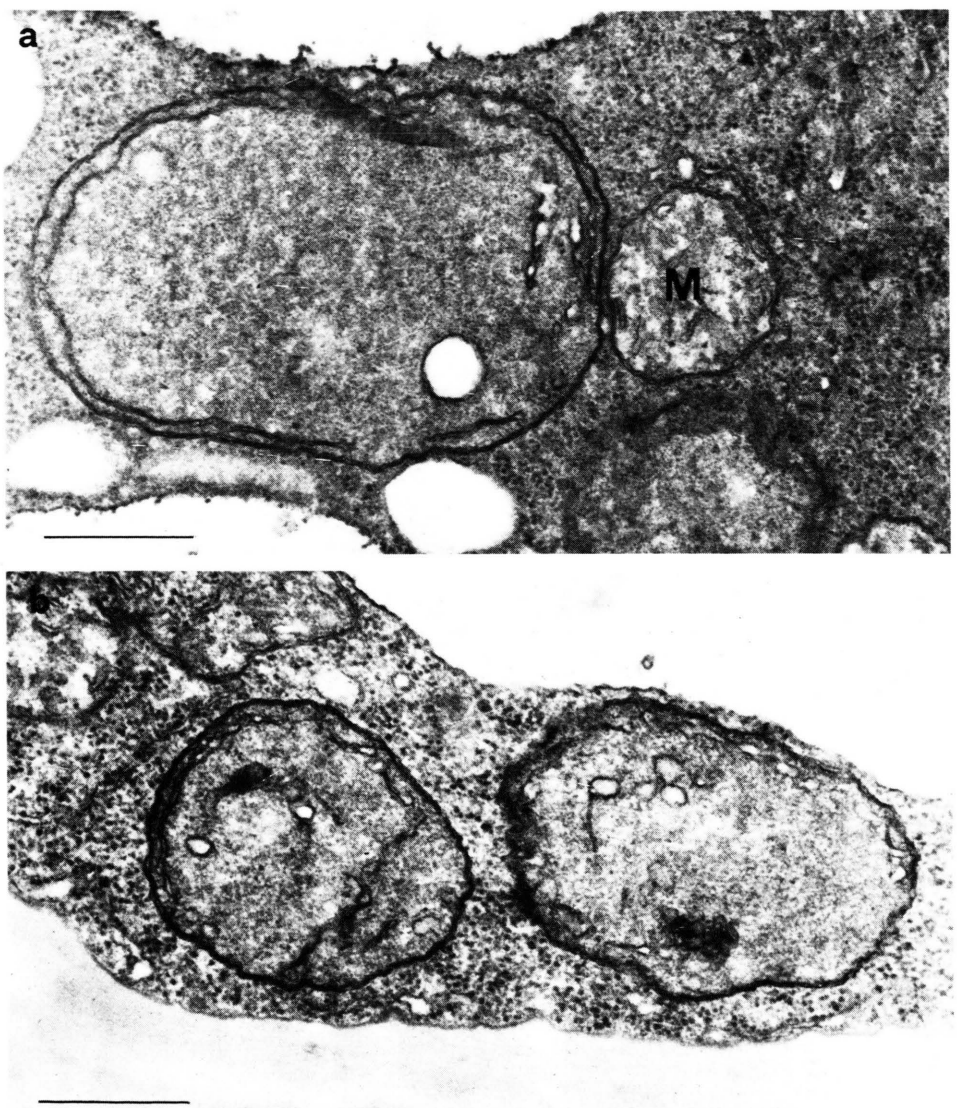


Fig. 7. Proplastids from the shoot meristem of 11 day old barley seedlings three days after sethoxydim treatment. M = mitochondrion; bar = 0.5 μm.

Fig. 6. Developing plastids in the shoot meristem of 1 day old barley seedlings (controls): prochloroplasts with protubular structures, starch and plastoglobuli. G = Golgi apparatus; M = mitochondrion; P = plastoglobuli; PTB = protubular body; bar = 0.5 μm.

been shown by measuring the chlorophyll fluorescence induction kinetics (Kautsky effect) of whole leaves [12]. Our results indicate that chloroplast biogenesis, development and replication are a major target for the herbicide action. Cell multiplication (the tertiary leaf is not formed) and chloroplast multiplication (+development) are inhibited simultaneously by sethoxydim. Since leaf growth and the development and differentiation of cells and chloroplasts are protein-dependent, this points to a possible interaction of sethoxydim in the sensitive gramineous plants with the process of protein formation. Whether the primary mode of action of sethoxydim is in the plastid and/or cytoplasm can only be decided in further investigations.

Acknowledgements

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