Change of Chloroplast Ultrastructure in Radish Seedlings under the Influence of the Photosystem II-Herbicide Bentazon

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The influence of the photosystem II-herbicide bentazon on the ultrastructure of chloroplasts of radish seedlings (*Raphanus sativus* L.) was investigated with special emphasis on thylakoid development and grana formation.

Bentazon application (10^{-3} M) induces the formation of broader and higher grana stacks (grana width: 0.5–0.6 µm; greatest frequency: 3–8 thylakoids per granum) than in the control plants (grana width: 0.3 µm; greatest frequency: 2–4 thylakoids per granum). Furthermore, the amount of chloroplast lamellae is enhanced, as are the stacking degree of thylakoids and the grana area. The chloroplasts of bentazon-treated plants appear to be shorter and thicker than in the controls and show all signs of a shade-type adaptation of the photosynthetic apparatus.

Introduction

Depending on the light intensity applied during greening, the development of chloroplasts results in the formation of two distinctive types, which are different in composition, ultrastructure and photosynthetic activity. The *sun-type chloroplast*, being formed at high light intensities, and in sun leaves, contains less lamellar material per chloroplast section, low grana stacks with only a few thylakoids per granum, and exhibits a high starch content and photosynthetic activity. Under weak-light growth conditions and in shade-leaves the *shade-type chloroplast* is formed with high grana stacks, little or no starch and rather low photosynthesis rates [1–3]. The higher Hill-activity of sun-type chloroplasts is correlated with a higher level of photosynthetic electron carriers (prenylquinones and cytochroms), higher values for the ratio chlorophyll a/b and lower ratios for xanthophyll/carotenes (x/c) than in shade-type chloroplasts [1–6].

The strong-light growth response of the photosynthetic apparatus can be simulated with low intensity blue light, while red light at low or higher fluence rate yields shade-type chloroplasts [2, 6, 7]. At medium intensity of white light the formation of sun-type chloroplasts can be induced by application of cytokinins [2, 8, 9] and by the fungicide triadimefon [3].

The group of photosystem II-herbicides (*e. g.* diuron, bentazon, methabenzthiazuron) is known to block the photosynthetic electron transport between the quencher Q and the plastoquinone pool [11–14]. Studies in trypsinated chloroplasts show that there exists a binding area with different specific subreceptor sites for individual photosystem II-inhibitors [15–17]. Photosystem II-herbicides not only inhibit photosynthetic electron transport, but also induce a shade-type adaptation of the photosynthetic apparatus, as seen by lower values for the ratio a/b, higher values for xanthophylls/carotenes (x/c) and a lower prenylquinone content [2, 3, 14, 18, 19]. Bentazon blocks the strong-light induced formation of sun-type chloroplasts and is active in this respect in medium and particularly in high-light intensities (3000 lux and 20000 lux) [3, 10]. At very low-light intensities (1000 lux), when plants already possess shade-type chloroplasts, bentazon has little effect on chloroplast composition [3].

Shade adaptation of the photosynthetic apparatus is associated with the formation of higher grana stacks. The photosystem II-herbicide methabenzthiazuron, in fact, induces an increased ratio of grana to stroma thylakoids [20]. It is shown in this paper that bentazon induces the typical chloroplast ultrastructure of shade-type chloroplasts with higher grana and an increased stacking degree of thylakoids in greening radish seedlings after different days of illumination and bentazon application.

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Materials and Methods

Radish seedlings (*Raphanus sativus* L. var. Saxa Treib) were grown in the dark for 3 days on a 10% modified van der Crone nutrient solution (25 ± 1 °C, 60% rel. humidity) [21]. The etiolated plants were then illuminated with continuous white fluorescent light of medium intensity (Tungsram lamps 65 W; 3000 lux; 6.5 W · m^{-2}). Bentazon (10^{-3}, 10^{-4} M) was applied to the nutrient medium just prior to illumination. In aqueous solutions of neutral pH bentazon dissociates into hydrophilic anions, which are taken up by the plant at a much lower rate than the undissociated lipophilic form obtained at low pH-values [22]. In order to ensure a sufficient uptake of bentazon in all cotyledon cells – with a rather neutral nutrient medium used here –, a concentration of 10^{-3} M bentazon was applied via the roots. The cotyledons did not show a variable fluorescence indicating that the herbicide had reached all cotyledon parts [10]. Some control experiments were performed with 10^{-4} M bentazon with similar results. Lower bentazon concentrations of *e. g.* 10^{-6} M have little effect on the chloroplast composition [10] and were therefore not used in this ultrastructural study.

Segments of radish cotyledons were fixed in buffered (0.1 M cacodylate) 5% glutardialdehyde 4 °C; 3 h (pH 7.4) after 1, 3 and 5 days of illumination and bentazon application. Postfixation was performed in buffered 2% OsO_4 (pH 7.4, 4 °C, 2 h) [23], the segments embedded in a low-viscosity epoxy resin [24] and the ultrathin sections poststained with 10% uranyl acetate and lead citrate [25]. Examination was performed with a Siemens Elmiscop I. For the quantitative determinations series sections were not counted.

The chloroplast size (length and width) was measured on 60 longitudinal chloroplast sections as a minimum for each growth condition. The volume of chloroplasts was calculated from the average length and width, assuming an ellipsoidal body. The number of plastoglobuli per chloroplast was calculated from the total chloroplast volume and the volume of

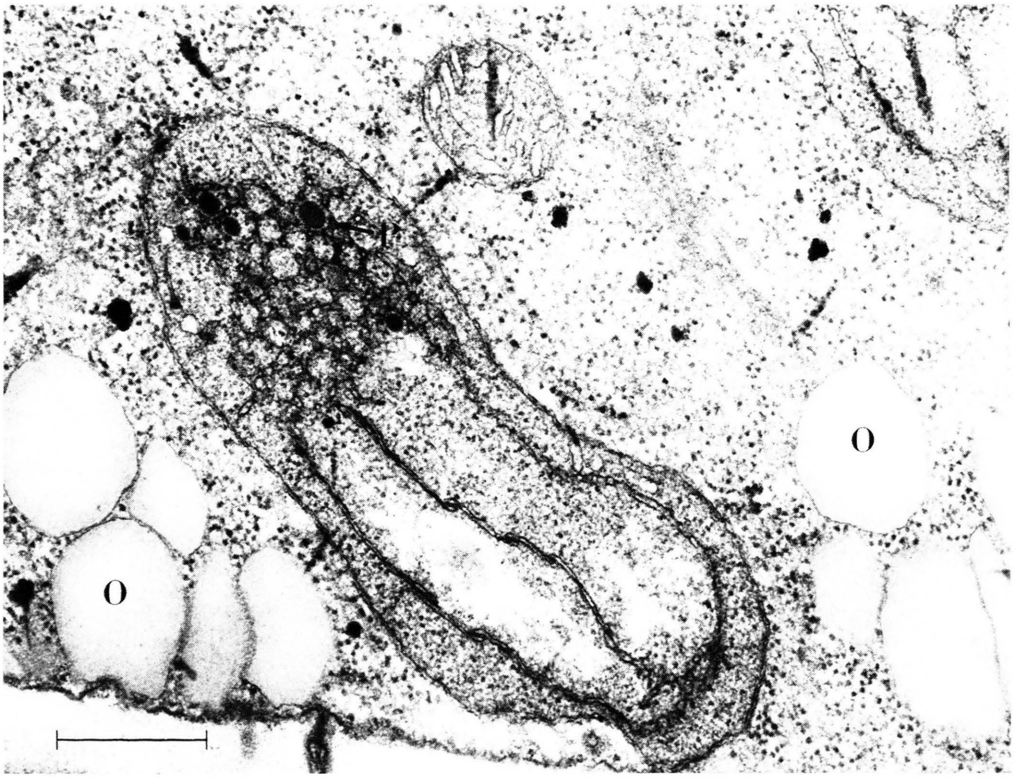

Fig. 1. Etioplast from the cotyledon of 3 d old etiolated radish seedlings. The plastid contains some plastoglobuli (P), in the cytoplasm are seen some oleosomes (O) containing the seed storage lipids (× 40000; bar = 0.5 μm).

longitudinal chloroplast sections (average thickness of 90 nm). The stacking degree of thylakoids was determined by measuring the total length of grana and stroma thylakoids and the length of the stacked regions with a kilometer tracer.

Chlorophylls and carotenoids were extracted with acetone and light petrol. The separation and quantitative determination of the pigments were performed as described before [3].

Results

The yellow cotyledons of 3 d old etiolated radish seedlings contain etioplasts with the typical prolamellar body and with osmiophilic plastoglobuli (Fig. 1). Upon illumination the cotyledons green and exhibit an equal area growth, no matter whether the photosystem II-herbicide bentazon is present or not. The accumulation rate of the thylakoid prenyllipids (chlorophylls, carotenoids, prenylquinones) is decreased, however, by bentazon. The accumulation of chlorophyll a and β-carotene is more reduced than that of chlorophyll b and xanthophylls. This results in lower values for the ratio chlorophyll a/b and higher values for the ratios xanthophylls/β-carotene (x/c) (Table I). These changes in prenyllipid ratios are known as shade adaptation response of the photosynthetic apparatus, which in this case is not induced by a lower light intensity but by treatment with bentazon.

After 1 day of continuous illumination with white light of medium intensity the cotyledons are green. Their chloroplasts possess stroma and grana thylakoids, prolamellar bodies are no longer seen (Figs. 2 and 3). In this development stage the length of chloroplasts of control plants is significantly larger than that of bentazon-treated plants, the width, however, is approximately the same (Table II). With increasing illumination time the chloroplasts of control plants become more elongated, as is visible from increased values for the ratio length to width (Table II). Bentazon-treated plants, on the other hand, contain shorter and thicker chloroplasts.

The greatest difference in chloroplast ultrastructure between both growth conditions is found in the type of grana formation. Chloroplasts of control plants contain a higher number of grana stacks per chloroplast section than bentazon-treated plants. This difference is already seen after 1 d of illumination and becomes intensified thereafter (Table III).

Table I. Pigment content and prenyllipd ratios of radish seedlings greened for 2 or 5 d on a bentazon-containing nutrient solution. Mean values of 4 cultivations, standard deviation 5% or less (µg per 50 pairs of cotyledons).

	Control		+ Bentazon, 10^{-3} M	
	2 d	5 d	2 d	5 d
chlorophyll a	2180	2900	1460	1740
chlorophyll b	680	880	540	720
a + b	2860	3780	2000	2460
xanthophylls (x)	240	330	280	280
β-carotene (c)	110	165	70	67
x + c	350	495	350	347
a/b	3.2	3.3	2.7	2.4
x/c	2.2	2.0	4.0	4.1
a + b/x + c	8.2	7.6	5.7	6.8

Table II. Differences in chloroplast size of radish seedlings treated with and without bentazon. The plants were grown for 3 d in the dark and then illuminated for 1, 3 and 5 d. The data (with standard deviation) is based on 60 chloroplast electron micrographs as a minimum for each growth condition.

		Control	+ Bentazon, 10^{-3} M
length (µm) [a]:	1 d	4.29 ± 0.84	3.41 ± 0.64
	3 d	4.46 ± 0.90	3.72 ± 0.57
	5 d	4.44 ± 0.89	3.49 ± 0.58
width (µm):	1 d	1.29 ± 0.26	1.30 ± 0.30
	3 d [a]	1.12 ± 0.21	1.33 ± 0.28
	5 d [a]	1.00 ± 0.31	1.44 ± 0.36
length/width [a]:	1 d	3.50 ± 0.90	2.70 ± 0.70
	3 d	4.10 ± 0.90	3.00 ± 0.80
	5 d	4.70 ± 1.30	2.60 ± 0.80

[a] Error probability 0.1% ($P < 0.001$).

Table III. Differences in the number of grana stacks (with standard deviation) and in the grana width of chloroplasts from radish cotyledons greened for 1, 3 or 5 d without or with addition of bentazon (10^{-3} M). The average area of a median longitudinal ultrathin chloroplast section ranges between 3.5 and 4.5 µm². The data is based on 50 electron micrographs in each case. The differences between controls and bentazon-treated plants are significant. Error probability less than 0.1% ($P < 0.001$).

		Control	+ Bentazon, 10^{-3} M
number of grana stacks: (per 10 µm² chloroplast section area)	1 d	137 ± 29	83 ± 23
	3 d	156 ± 26	59 ± 20
	5 d	168 ± 23	54 ± 13
average grana width (µm):	1 d	0.32	0.42
	3 d	0.31	0.62
	5 d	0.30	0.53
range of grana width (µm):	1 d	0.12 – 1.5	0.12 – 1.4
	3 d	0.12 – 1.2	0.12 – 2.0
	5 d	0.12 – 0.9	0.12 – 1.9

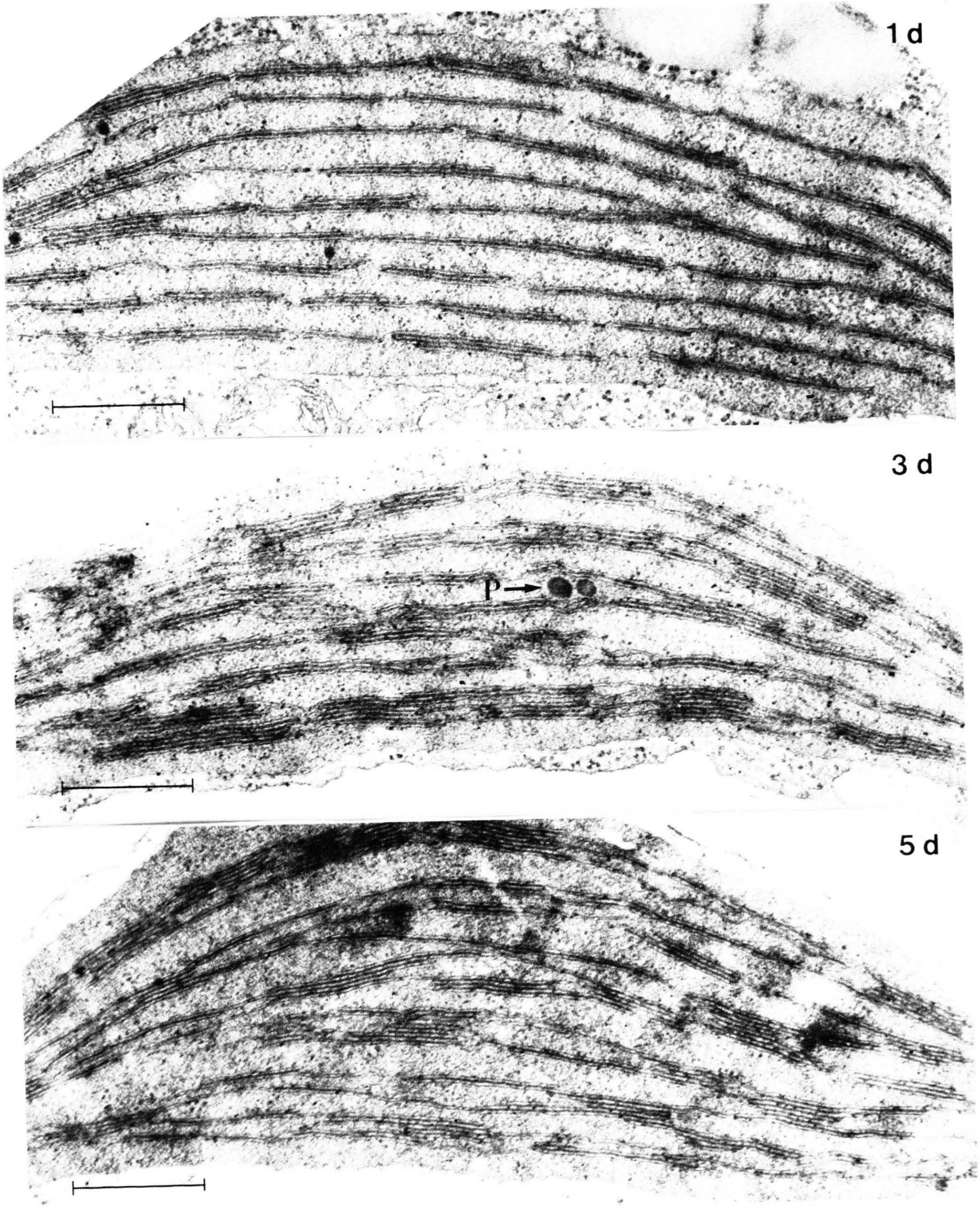

Fig. 2. Ultrastructure of chloroplasts from radish cotyledons after 1, 3 and 5 d of greening. P = plastoglobuli (× 40000; bar = 0.5 μm).

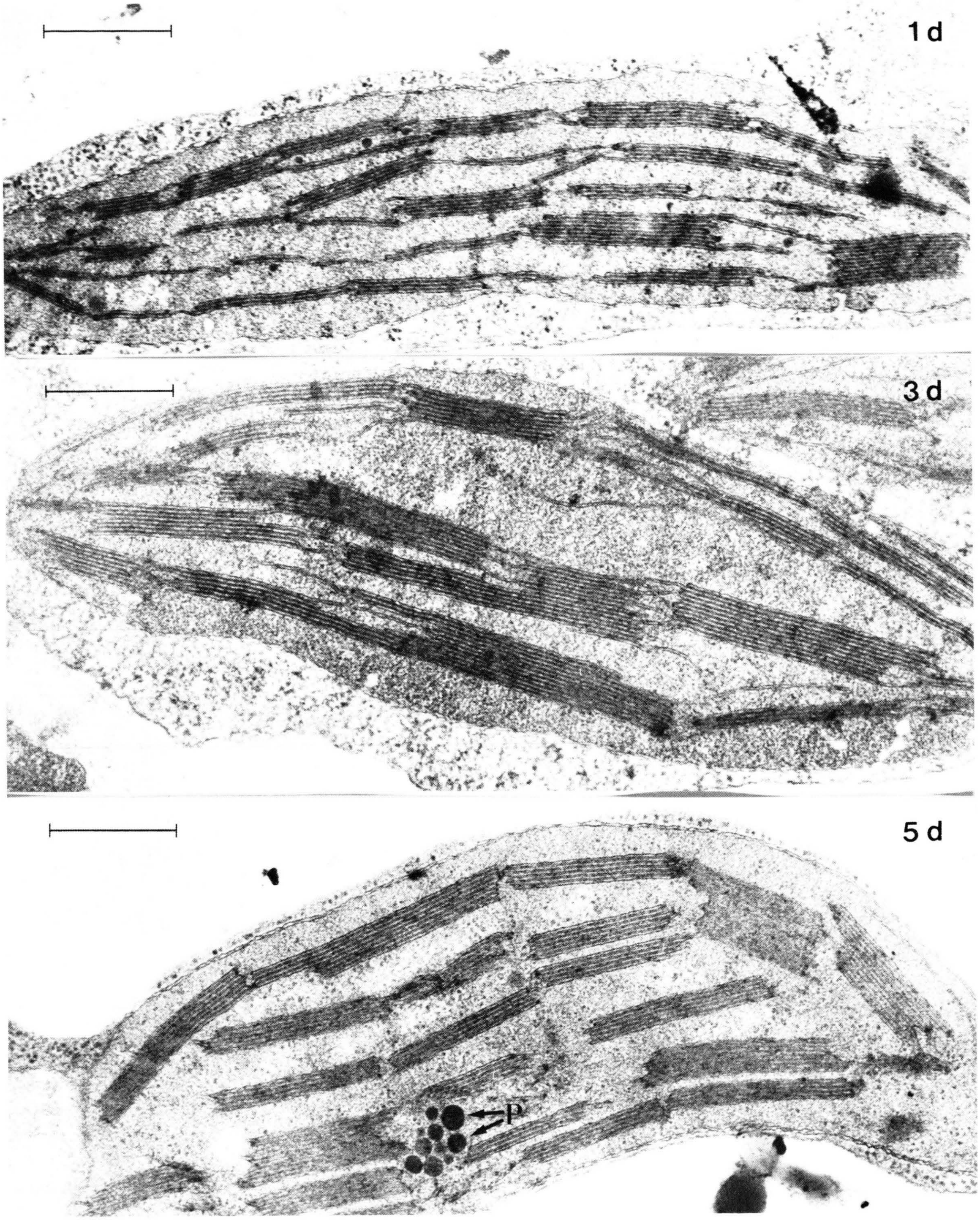

Fig. 3. Ultrastructure of chloroplasts from radish cotyledons of bentazon-treated plants after 1, 3 and 5 d of greening. P = plastoglobuli (× 40 000; bar = 0.5 µm).

The lower number of grana stacks in bentazon-treated plants is associated with much broader grana (greater average width) and higher grana stacks (greater number of thylakoids per granum), as shown in Fig. 2 and 3 and in Table III. The different amounts of thylakoids per granum in control and bentazon-treated plants are shown in Fig. 4. The difference in grana lamellae formation is also seen from the development of the grana area per chloroplast section and from the stacking degree of thylakoids, both of which are more enhanced under the influence of bentazon (Table IV).

The osmiophilic plastoglobuli, the depot for excess chloroplast lipids, are another typical structural chloroplast component. After 1 d of illumination the plastoglobuli are rather small (60–90 nm diameter). They occur with a higher frequency in the chloroplasts of control plants than in bentazon-treated plants (Table V). At longer illumination periods the diameter of plastoglobuli increases. In control plants the plastoglobuli frequency decreases, while that of bentazon-treated plants becomes considerably enhanced. After 5 days of illumination the chloroplasts of bentazon-treated plants contain larger and more plastoglobuli (79) than chloroplasts from control plants (17) (Table IV and V).

Table IV. Differences in the ultrastructure of chloroplasts from radish cotyledons. The 3 d old dark-grown seedlings are illuminated for 1, 3 and 5 days with and without bentazon. Bentazon-treatment increases grana area, the stacking degree of thylakoids and the number of plastoglobuli per chloroplast. The differences found between controls and bentazon-treated plants are significant. Error probability 0.1% ($P < 0.001$).

		Control	+ Bentazon, 10^{-3} M
grana area [a]: (% proportion of total chloroplast section)	1 d	19.4 ± 3.3	24.9 ± 3.6
	3 d	26.9 ± 3.1	32.5 ± 4.2
	5 d	30.0 ± 3.6	40.3 ± 4.9
stacking degree [b] of thylakoids (%):	1 d	54.7 ± 3.2	68.0 ± 2.9
	3 d	57.6 ± 2.7	72.6 ± 3.1
	5 d	62.8 ± 2.6	83.8 ± 4.4
chloroplast volume: (μm^3)	1 d	3.75	3.01
	3 d	2.92	3.36
	5 d	2.32	3.80
number of plastoglobuli per 1 chloroplast:	1 d	38	29
	3 d	20	46
	5 d	17	79

[a] Based on 50 and [b] on 20 chloroplasts.

Chloroplasts from bentazon-treated plants very often show some peripheral vesicles (Fig. 5), which seem to derive from the inner membrane of the chloroplast envelope. In the chloroplast of control plants a peripheral vesicle is seen only occasionally.

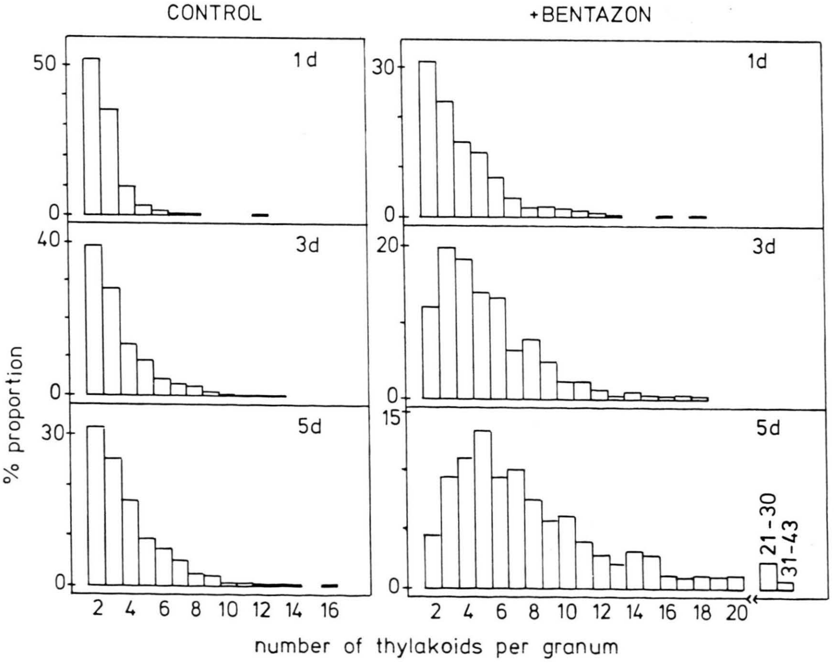

Fig. 4. Differences in thylakoid numbers per granum of chloroplasts from radish cotyledons after 1, 3 and 5 d of greening. The values are based on 50 electron micrographs for each cultivation. 1800 grana (controls) and 900 grana (+ bentazon) were counted as a minimum.

Table V. Size and frequency of plastoglobuli in chloroplasts of radish cotyledons in control seedlings and in plants treated with bentazon. After 3 d dark growth the seedlings were illuminated for 1, 3 and 5 days. The data is based on 100 median longitudinal chloroplast sections.

	Illumination time					
	1 d		3 d		5 d	
	control	+ bentazon	control	+ bentazon	control	+ bentazon
plastoglobuli size: (diameter in nm)	50 – 90	50 – 90	50 – 90	50 – 100	50 – 150	50 – 250
greatest frequency (diameter in nm):	60	60	65	65	80	70
average number of plastoglobuli per 100 chloroplast sections:	397	292	240	470	227	746
classification of 100 chloroplast sections by increasing number of plastoglobuli:						
0 plastoglobuli	19	12	19	15	15	9
1 – 2	22	38	43	30	50	16
3 – 4	23	29	21	18	22	19
5 – 6	12	11	12	16	11	18
7 – 8	9	6	2	4	1	13
9 – 10	9	3	2	6	1	4
11 – 15	4	1	1	4		7
16 – 20	2			3		5
21 – 30				4		8
31 – 40						1

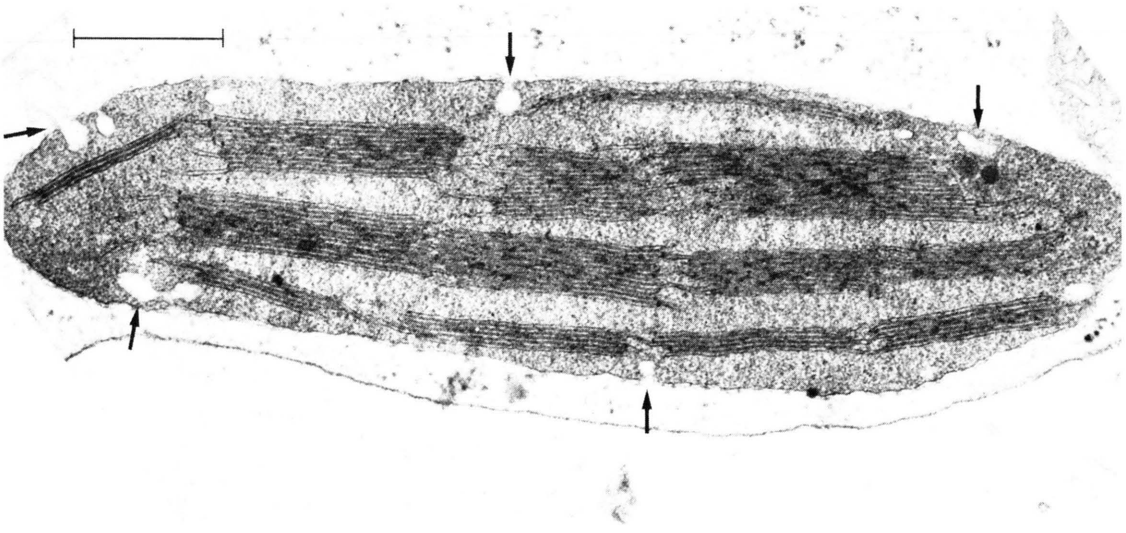

Fig. 5. Chloroplast from the cotyledon of 8 d old radish plants after 5 d of bentazon treatment. Arrows indicate peripheral vesicles (× 40 000; bar = 0.5 μm).

Discussion

The photosystem II-herbicide bentazon not only blocks the photosynthetic electron transport [13] but also induces the formation of shade-type chloroplasts. The latter are characterized by a different thylakoid prenyllipid composition, as seen by lower values for the ratio chlorophyll a/b and higher values for the ratios xanthophylls/β-carotene and chlorophyll a/prenylquinones [2, 4]. In plants grown at medium light quanta fluence rate (3000 lux) the bentazon-induced changed prenyllipid ratios, described here, are obtained through a reduced accumulation rate particularly for chlorophyll a and β-

carotene. The level of lutein and chlorophyll b, which are preferentially located in the light-harvesting chlorophyll a/b-protein complex (CPII) [4, 26, 27] is affected by bentazon treatment to a lower degree. At a high light quanta fluence rate (*e. g.* 20000 lux) bentazon blocks the strong-light induced formation of sun-type chloroplasts, the chloroplast prenyllipid composition then resembles that of the weak light control (1000 lux) [3].

Another typical feature of shade-type chloroplasts is their greater number of thylakoids per granum, which results in higher grana stacks and a higher stacking degree of thylakoids as compared to sun-type chloroplasts [1, 2, 28]. Bentazon application, in fact, induces the formation of higher grana stacks and a greater stacking degree of thylakoids, which is correlated with a lower number of grana stacks per chloroplast section, as is shown in this investigation. In addition to this, bentazon treatment results in a considerable broadening of the grana to almost double the width of the controls (Table III). Higher grana stacks and/or stacking degree have also been reported using other photosystem II-herbicides, *e. g.* methabenzthiazuron in wheat [20], monuron in beans [29], fluometuron in velvet leaf (Abutilon) [30]. More thylakoid discs per lamella stack of Euglena chloroplasts have been described under the influence of DCMU [31]. A broadening of grana stacks, as shown here for bentazon, has also been reported for monuron [29]. That shade-type chloroplasts possess higher grana stacks than sun-type chloroplasts has been observed by many investigators; less attention has, however, been paid to the simultaneous broadening of the grana. This broadening effect has been described for *Antirrhinum* chloroplasts [32] and is found to some degree in cotyledon chloroplasts of low-light radish plants, as compared to the high-light plants [3].

The results of this investigation clearly show that a higher degree of thylakoid stacking, as induced by bentazon, is associated with a higher level of chlorophyll b (on a chlorophyll a basis) and a concomitant decrease in the chlorophyll a/b ratio. The higher grana stacks in shade-type chloroplasts are also accompanied by a higher chlorophyll b level and lower chlorophyll a/b ratios as compared to the sun-type chloroplasts [1, 2]. "Red-light-chloroplasts" of barley seedlings also possess lower a/b ratios and higher grana stacks than "blue-light-chloroplasts" [7]. These observations demonstrate the great importance of chlorophyll b for thylakoid stacking and grana formation. Further evidence for this view is given by mutant work. Grana-rich barley mutants possess low chlorophyll a/b ratios (2−2.4), while grana-deficient mutants exhibit high a/b ratios (> 5.2) [33]. Since within the thylakoids chlorophyll b is primarly bound to the light-harvesting chlorophyll a/b-protein complex (LHCP, CPII) [26, 27, 34, 35], it is evident that the amount of CPII-accumulation determines the degree of thylakoid stacking as well as the width and height of grana stacks.

The greater increase in the number and in the size of plastoglobuli in bentazon-treated plants on the 3rd and 5th days of illumination, as compared to the controls could be due to a herbicide-induced impaired accumulation of thylakoid lipids and thylakoid proteins.

The number of plastoglobuli per chloroplast of the radish cotyledons, which is investigated here, is rather small in both controls and bentazon-treated plants, as compared to chloroplasts of fully developed, dark-green spinach leaves, which may possess 800 to 1500 plastoglobuli in 1 chloroplast [36]. It has been shown in spinach [36, 37] and Ficus leaves [38] that only few small plastoglobuli show up during the thylakoid synthesis and multiplication phase. When the thylakoid synthesis phase is finished, excess chloroplast lipid material is formed and deposited in the osmiophilic plastoglobuli, the size and number of which are enlarged with increasing age of leaves [36−38]. This inverse correlation between thylakoid formation and plastoglobuli accumulation also occurs in *Raphanus* chloroplasts. Upon illumination of the etiolated plants thylakoid accumulation proceeds faster and yields more lamellae and fewer plastoglobuli in bentazon-treated plants within 24 h than in the controls. More and larger plastoglobuli are also formed during chloroplast senescence and thylakoid breakdown [38, 39]. After 3 days of bentazon treatment the radish cotyledons were still green, signs of a beginning chlorophyll breakdown are, however, seen after 5 days. The larger increase in size and number of plastoglobuli of the bentazon-treated radish plants observed here, may therefore in part be due to chloroplast senescence.

On the other hand sun leaves possess more chloroplasts in their cells than shade leaves, as has been found by many authors *cf.* [40]. High-light *Sinapis* plants contain 3 · 5 times more chloroplasts per pallisade cell than weak-light plants [41]. Since bentazon induces a shade-type growth response [3, 10], one can expect that it affects chloroplast multiplication. The lower number of plastoglobuli in control chloroplasts could thus be due to a higher chloroplast multiplication than in bentazon-treated plants. The correlation of a higher chlorophyll level with a lower thylakoid content per chloroplast of control plants can also be explained by the assumption that cotyledon cells of controls contain more chloroplasts than the bentazon-treated plants.

The appearance of some peripheral vesicles under the influence of bentazon in the radish seedlings (a C_3-plant) seems to be a stress phenomenon. Peripheral vesicles (or peripheral reticulum) are typical structural elements of C_4-plants [42]. Laetsch [42] states that these are normally not found in chloroplasts of C_3-plants expect for stress situations. In more recent work peripheral vesicles, which may be connected by narrow tubules, have been observed in several C_3-plants; for literature see [43].

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[1] N. K. Boardman, O. Björkman, J. M. Anderson, D. J. Goodchild, and S. W. Thorm, Proc. 3rd Intern. Congress on Photosynthesis, (M. Avron, ed.), p. 1809, Elsevier, Amsterdam 1974.

[2] H. K. Lichtenthaler and C. Buschmann, Chloroplast Development, (G. Akoyunoglou *et al.*, eds.), p. 801, Elsevier Biomedical Press, Amsterdam 1978.

[3] H. K. Lichtenthaler, Z. Naturforsch. **34 c,** 936 (1979).

[4] H. K. Lichtenthaler, Advances in the Biochemistry and Physiology of Plant Lipids (L.-A. Appelqvist and C. Liljenberg, eds.), p. 57, Elsevier, Amsterdam 1979.

[5] A. Wild, Ber. Deutsch. Bot. Ges. **92,** 341 (1979).

[6] A. Wild, W. Rühle, and H. Grahl, Environmental and Biological Control of Photosynthesis, (R. Marcelle, ed.), p. 115, Dr. W. Junk, The Hague 1975.

[7] C. Buschmann, D. Meier, H. K. Kleudgen, and H. K. Lichtenthaler, Photochem. Photobiol. **27,** 195 (1978).

[8] C. Buschmann and H. K. Lichtenthaler, Z. Naturforsch. **32 c,** 798 (1977).

[9] C. Buschmann and H. K. Lichtenthaler, Advances in the Biochemistry and Physiology of Plant Lipids, (L.-A. Appelqvist and C. Liljenberg, eds.), p. 145, Elsevier, Amsterdam 1979.

[10] H. K. Lichtenthaler, G. Burkhard, K. H. Grumbach, and D. Meier, Photosynthesis Research **1,** 29–44 (1980).

[11] F. M. Ashton and A. S. Crafts, Mode of Action of Herbicides, P. 504, J. Wiley and Sons, New York 1973.

[12] J. R. Corbett, The Biochemical Mode of Action of Pesticides, p. 330, Academic Press, New York 1974.

[13] K. Pfister, C. Buschmann, and H. K. Lichtenthaler, Proc. 3rd Intern. Congr. on Photosynthesis (M. Avron, ed.), p. 675, Elsevier, Amsterdam 1974.

[14] C. Fedtke, Naturwissenschaften **61,** 272 (1974).

[15] K. Pfister and C. J. Arntzen, Z. Naturforsch. **34 c,** 996 (1979).

[16] G. Renger, Z. Naturforsch. **34 c,** 1010 (1979).

[17] A. Trebst, Z. Naturforsch. **34 c,** 986 (1979).

[18] H. K. Kleudgen, Pestic. Biochem. Physiol. **9,** 57 (1978).

[19] C. Fedtke, Z. Naturforsch. **34 c,** 932 (1979).

[20] C. Fedtke, G. Deichgräber, and E. Schnepf, Biochem. Physiol. Pflanzen **17,** 307 (1977).

[21] L. Verbeek and H. K. Lichtenthaler, Z. Pflanzenphysiol. **70,** 245 (1973).

[22] G. Retzlaff, J. L. Hilton, and J. B. St. John, Z. Naturforsch. **34 c,** 944 (1979).

[23] G. E. Palade, J. Exp. Med. **95,** 285 (1952).

[24] A. R. Spurr, J. Ultrastruct. Res. **26,** 31 (1969).

[25] E. S. Reynolds, J. Cell Biol. **17,** 208 (1963).

[26] J. P. Thornber, Ann. Rev. Plant Physiol. **26,** 127 (1975).

[27] D. Siefermann-Harms and H. Ninnemann, FEBS Letters **104,** 71 (1979).

[28] T. Guillot-Salomon, C. Tuquet, M. de Lubac, M.-F. Hallais, and M. Signol, Cytobiologie **17,** 442 (1978).

[29] S. Klein and J. Neumann, Plant Cell Physiology **7,** 115 (1966).

[30] W. P. Wergin and J. R. Potter, Pesticide Biochem. Physiol. **5,** 265 (1975).

[31] J. A. Schiff, M. H. Zeldin, and J. Rubman, Plant Physiol. **42,** 1716 (1975).

[32] B. Abel, Naturwissenschaften **43,** 136 (1956).

[33] N. C. Nielsen, R. M. Smillie, K. W. Henningsen, D. v. Wettstein, and C. French, Plant Physiol. **63,** 174 (1979).

[34] J. M. Anderson, J. C. Waldron, and S. W. Thorne, FEBS Letters **92,** 227 (1978).

[35] J. P. Markwell, J. P. Thornber, and R. T. Boggs, Proc. Natl. Acad. Sci. USA **76,** 1233 (1979).

[36] H. K. Lichtenthaler, Protoplasma **68,** 65 (1969).

[37] H. K. Lichtenthaler, Endeavour **27,** 144 (1968).

[38] H. K. Lichtenthaler and H. Weinert, Z. Naturforsch. **25 b,** 619 (1970).

[39] H. K. Lichtenthaler, Protoplasma **68,** 315 (1969).

[40] T. Butterfass, Patterns of Chloroplast Reproduction, pp. 92–97, Springer-Verlag, Wien 1979.

[41] A. Wild and G. Wolf, Z. Pflanzenphysiol. (submitted).

[42] W. M. Laetsch, Ann. Rev. Plant Physiol. **25,** 27 (1974).

[43] J. B. Harris, Cytobios **21,** 151 (1978).