Effect of Biocides on the Development of the Photosynthetic Apparatus of Radish Seedlings Grown under Strong and Weak Light Conditions

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The influence of two biocides (bentazone, triadimefon) on the growth-response of radish seedlings (*Raphanus sativus* L.) was investigated with special emphasis on the development of sun or shade-type chloroplasts.

1. The fungicide triadimefon causes strong-light growth-response and the formation of sun-type chloroplasts as seen from the changed chlorophyll a/b ratio and the carotenoid and prenylquinone composition.
2. The photosystem II herbicide bentazone, in turn, induces a shade-type adaption. It prevents the strong-light induced formation of sun-type chloroplasts, but has little influence on the chloroplast composition in weak light.

Introduction

Depending on environmental factors, the development of chloroplasts from either proplastids or etioplasts will lead to two distinctive types of chloroplasts, which are different in composition, ultrastructure and photosynthetic activity. At low light intensities and in shade-leaves the shade-type chloroplast with high grana stacks, lower Hill-activity rates and a higher level of chlorophyll b is formed. Sun-leaves and plants grown at high light intensities, in turn, develop sun-type chloroplasts with less lamellar material and only few and low grana stacks [1 – 3]. Their higher Hill-activity is correlated with a higher level of prenylquinones, which function as potential photosynthetic electron carriers, and can also be seen in a changed chlorophyll and carotenoid composition. The sun-type chloroplast growth-response can be simulated with blue light [4] and by the application of cytokinins [3, 5]. It is shown in this paper that the application of biocides can shift the growth-responses via the formation of sun-type (+ triadimefon) or the formation of shade-type chloroplasts (+ photosystem II herbicides, *e.g.* bentazone).

Material and Methods

Radish seedlings (*Raphanus sativus* L. var. Saxa Treib) were grown on a modified van der Crone nutrient solution [6]. In the experiments with the fungicide triadimefon the plants were grown for 3 days in the dark (25 °C, 60% relative humidity) and then illuminated with continuous white light of medium intensity (Fluora lamps, 600 $\mu W/cm^2$). Triadimefon (10^{-5} M) was applied to the nutrient solution from onset of germination.

In the experiments with bentazone the plants were grown in the dark for 3 days in a climate chamber at 20 °C and 65% relative humidity at either high light intensity (24 000 or 20 000 lux) or under weak light conditions (1000 lux). The white light source consisted of 20 Osram HQIE lamps (400 W) mounted outside the growth chamber. Bentazone (10^{-4} M) was applied to the nutrient solution just prior to illumination.

The chlorophylls were determined after Ziegler and Egle [7]. The carotenoids were separated by thinlayer chromatography (silicagel plates, solvent system: 70 ml petrolether 50 – 70 °C, 30 ml dioxan, 10 ml isopropanol), eluted with ethanol and measured spectrophotometrically using an extinction coefficient $E_{1cm}^{1\%}$ of 2500. Prenylquinones were determined spectophotometrically (reduction with KBH_4) after separation by thinlayer chromatography [8] or by high-pressure liquid chromatography [9].

The anthocyanin content was determined as described before [10]. The values given in the Tables represent mean values from 3 to 5 separate cultivations.

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Results

Germinating seedlings possess the ability to react with two distinctive growth responses to the different amounts of light available (Fig. 1). The strong-light growth-response of the whole plant (*e. g.* higher dry weight and thickness of the cotyledons, strongly reduced hypocotyl length, increased anthocyanin content of hypocotyls) is correlated with a special morphogenesis of the photosynthetic apparatus as seen by the formation of sun-type chloroplasts. With a lower grana content and often many plastoglobuli their ultrastructure is quite different from that of the shade-type chloroplasts with high grana stacks (Fig. 2). These differences in ultrastructure are associated with differences in the chemical composition of the thylakoids. Good indicators of the sun-type modification of chloroplasts are the changed prenyllipid ratios *e. g.* higher values for chlorophyll a/b, lower values for xanthophylls to carotenes and for chlorophyll a to prenylquinones as compared to shade-type chloroplasts (Table I).

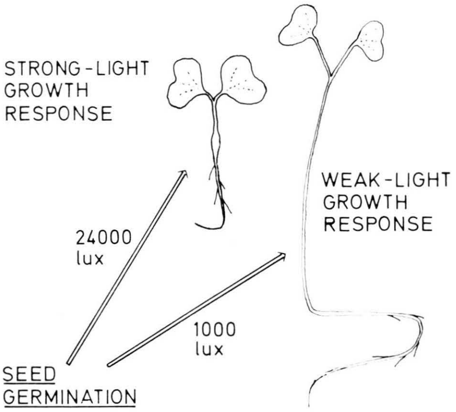

Fig. 1. Development of *Raphanus* seedlings under high and low light growth conditions.

When plants are grown at medium light intensities a chemical substance may either have no influence on plant growth or may induce a strong-light or a weak-light growth-response of the whole plant and of the photosynthetic apparatus (Fig. 3).

Influence of triadimefon

Triadimefon is an efficient fungicide [9], but does not inhibit photosynthesis of the intact leaves or isolated chloroplasts even at concentrations of 10^{-3} M. Upon application of triadimefon radish plants show a typical strong-light growth-response, as is seen from the reduced hypocotyl length, the increased accumulation of chlorophylls and anthocyanins and from other data (Table II). This sun-type growth-response is also seen in the composition of the photosynthetic apparatus with much higher

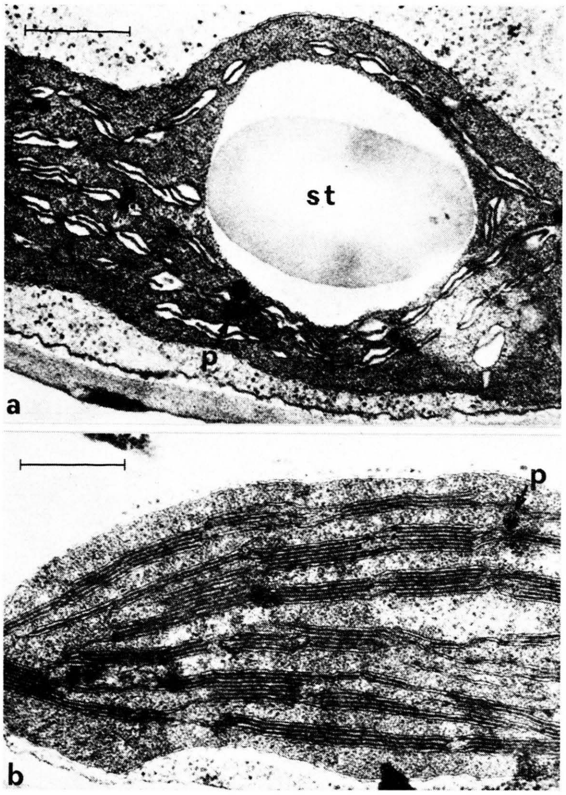

Fig. 2. Chloroplasts from green cotyledons of 6 d old radish seedlings. a) sun-type chloroplast from plants grown at 24 000 lux and; b) shade-type chloroplast from plants grown at 1000 lux; (5% glutaraldehyde + 2% OsO_4, 20 000 × 2, photograph taken by D. Meier 1979; st = starch grain, p = plastoglobuli, marker bar = 0.5 μm).

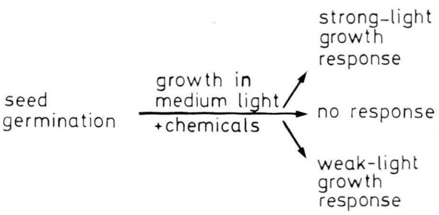

Fig. 3. Assay system for the investigation of the influence of biocides on plant growth.

Table I. Indicators of a strong-light growth-response of the photosynthetic apparatus. With reference to chlorophyll a the sun-type chloroplast contains less chlorophyll b and more prenylquinones and also higher proportion of β-carotene than the shade-type chloroplast. (a, b = chlorophyll a and b, x = xanthophylls, c = β-carotene).

1. less chlorophyll b	higher values for: a/b lower values for:
2. more β-carotene	x/c
more plastoquinone	a/PQ
more phylloquinone K_1	a/K_1
more α-tocoquinone	a/TQ
3. higher Hill-activity	

Table II. Sun-type growth-response of 8 d old *Raphanus* seedlings upon treatment with the fungicide triadimefon. The differences remain even after longer growth periods of 10 or 20 days (content of anthocyanins per 100 hypocotyls, of chlorophylls per 200 cotyledons).

	Control	+ Triadimefon 10^{-5} M
cotyledons (200):		
Fresh weight	5.08 g	6.04 g
dry weight	344 mg	536 mg
water content	93%	91%
hypocotyl length	8.9 ± 0.3 cm	2.1 ± 0.06 cm
hypocotyl diameter	0.9 ± 0.1 cm	1.3 ± 0.10 cm
cotyledo area	1.0 ± 0.04 cm^2	0.94 ± 0.04 cm^2
cotyledo volume	25 μl	25 μl
cotyledo color	light green	dark green
anthocyanins (8 d)	2.250 μg	3.500 μg
chlorophylls (8 d)	6.102 μg	7.765 μg
chlorophylls (10 d)	6.540 μg	12.020 μg

Table III. Content and ratios of chloroplast prenyllipids in 8 d old *Raphanus* cotyledons (μg per 100 plants). (a, b = chlorophyll a and b, x = xanthophylls, c = β-carotene).

	Control	+ Triadimefon 10^{-5} M
chlorophylls	6102	7765
carotenoids	1294	1205
a/b	3.2	3.5
a+b/x+c	4.7	6.4
x/c	4.7	2.6
phylloquinone K_1	30	85!
plastoquinone-9 (PQ-9)	145	251
α-tocoquinone (TQ)	9	15
α-tocopherol	172	218
a/PQ-9	32	27
a/K_1	155	80
a/TQ	515	450

prenylquinone levels, higher ratios of chlorophyll a/b and lower values for the ratio x/c (Table III). The level of total carotenoids is somewhat lower in the triadimefon-treated plants, the resulting higher value for the ratio chlorophylls to carotenoids (a + b/x + c) is a further indicator of the formation of a sun-type chloroplasts. Like sun-leaves or plants grown at high light intensity, the triadimefon-treated plants possess a distinctly higher proportion of the β-ionone carotenoids (β-carotene + violaxanthin), which is correlated to a lower amount of lutein than in the control plants (Table IV).

Table IV. Change in % composition of carotenoids in *Raphanus* seedlings treated with triadimefon.

Carotenoid	Control		+ Triadimefon 10^{-5} M	
β-carotene	17.5	} 27.2	27.8	} 40.6
violaxanthin	9.7		12.8	
lutein	61.8		48.4	
neoxanthin	11.0		11.0	

Influence of bentazone

It has been shown earlier at medium light intensities that the photosystem II herbicide bentazone, which blocks the electron transport between Q and plastoquinone [12], changes the pigment and quinone composition of chloroplasts, thus indicating a shade-type growth-response of the photosynthetic apparatus [13]. This influence of bentazone has now been studied in further detail at high (24 000 lux) and low light intensities (1000 lux).

The radish plant grown in strong light show the typical signs of strong-light growth-response. As compared to the weak-light controls their cotyledons possess a much higher dry weight and leaf area, which is correlated with a reduced length and a much higher anthocyanin level of the hypocotyl (Table V). In weak light, treatment with bentazone has little or no influence on these parameters. At high light-intensities bentazone reduces cotyledo growth, dry matter production and the accumulation of anthocyanins. This bentazone induced growth of radish seedlings grown at high light intensities is similar to that of radish plants kept at a medium light intensities.

The chlorophyll accumulation of cotyledons in strong-light plants is faster than in weak light. It

Table V. Shade-type growth-response of 8 d old *Raphanus* seedlings (illumination time 5 d) treated with bentazone (10^{-4} M). ( ) = bentazone treated plants.

	24 000 lux strong-light		1000 lux weak-light	
100 cotyledons:				
fresh weigth (g)	7.6	(4.6)	3.4	(3.5)
dry weight (mg)	677	(400)	316	(325)
leaf area (cm^2)	187	(126)	93	(99)
hypocotyl length (cm)	2.0	(3.0)	7.9	(7.7)
hypocotyl fresh weight (g per 100)	4.1	(2.6)	7.9	(6.9)
anthocyanins (μg per 100 hypocotyls)	11837	(5264)	1061	(1068)
chlorophylls (μg per 100 cotyledons)				
illumination time 1 d	1833	(1594)	1307	(1663)
illumination time 3 d	3616	(2972)	2628	(2828)
illumination time 5 d	2624	(2678)	2891	(2743)
chlorophyll (μg per 1 cm^2 cotyledo area)				
illumination time 1 d	37	(29)	29	(38)
illumination time 3 d	29	(28)	40	(35)
illumination time 5 d	14	(21)	31	(28)

Table VI. Content and ratio of prenyllipids in 5 d old *Raphanus* seedlings (μg per 100 plants). ( ) = bentazone treated plants.

	20 000 lux strong-light control		1000 lux weak-light control	
chlorophylls	4853 μg	(2513)	2990 μg	(3044)
carotenoids	1240	(589)	574	(564)
a/b	3.7	(2.6)	2.9	(2.5)
x/c	3.6	(4.4)	4.2	(4.0)
plastoquinone-9	354	(106)	164	(141)
phylloquinone K$_1$	35	(12)	15	(15)
α-tocoquinone	10	(5)	5	(4)
α-tocopherol	266	(167)	113	(123)
a/PQ-9	11	(17)	14	(15)
a/K$_1$	109	(155)	148	(143)
a/α-TQ	381	(360)	444	(584)
a/α-T	15	(11)	20	(18)

goes through a high maximum on the third day of illumination and reaches a lower, more stable value on the fifth day (Table V). At 24 000 lux bentazone reduces the chlorophyll accumulation rate but yields the same chlorophyll content as in the controls on the fifth day. In the weak-light controls, in turn, bentazone initially induces an enhanced chlorophyll formation ("greening effect") with a maximum level on the third day of illumination. It is remarkable that the chlorophyll accumulation pattern in the bentazone-treated plants is nearly the same, no matter whether the plants are grown at high or at low light quanta fluence rate. When referred to the cotyledon area, there is little difference in weak-light in the chlorophyll content of 8 d old controls and treated plants. Under strong-light conditions, however, the chlorophyll level per cotyledon area is considerably higher in the bentazone-treated plants wich possess less leaf area than the controls (Table V). This gives the plants that darker green appearance which has often been observed after application of photosystem II herbicides.

In strong light, synthesis and accumulation of chlorophylls, carotenoids and prenylquinones is much higher than in weak-light (Table VI). This is due to the higher rate of photosynthesis. In addition to this, there are specific differences in the prenyllipid ratios (a/b; x/c; a/prenylquinones) which indicate the presence of sun and shade-type chloroplasts in the plants grown at 20 000 and 1000 lux respectively.

Bentazone application not only suppresses the strong-light induced increased formation of chlorophylls and other prenyllipids but also prevents the specific change in the prenyllipid ratios which are characteristic of a sun-type chloroplast. It is of interest in this respect that the accumulation of chlorophyll b is much less affected by bentazone than that of chlorophyll a. That bentazone particularly prevents the strong-light induced modification of the photosynthetic apparatus but has little influence in weak light can also be seen in the carotenoid composition (Table VII). It corresponds in bentazone-plants of both light conditions to that of weak-light controls with a higher proportion of lutein than β-

Table VII. % Composition of carotenoids in 6 d old *Raphanus* seedlings. The strong-light plants exhibit a higher proportion of β-ionone carotenoids (β-carotene + violaxanthin) and a lower level of lutein.

	20 000 lux strong-light control		1000 lux weak-light control	
β-carotene	21.3	45.9	19.2	38.0
violaxanthin	24.4		18.8	
lutein	43.7		51.6	
neoxanthin	10.5		10.4	
	+ bentazone 10^{-4} M		+ bentazone 10^{-4} M	
β-carotene	18.5	39.9	19.8	38.3
violaxanthin	21.4		18.5	
lutein	53.3		53.2	
neoxanthin	6.8		8.5	

ionone carotenoids. The chloroplasts from bentazone-treated strong-light plants possess, like the weak-light controls, more and higher grana stacks than the strong-light controls [14].

Though bentazone is a good photosystem II herbicide, the photosynthetic electron transport is inhibited only to about 90% under both light conditions as measured by the variable fluorescence [12] of the intact leaf. This indicates that in bentazone-treated plants under both light conditions growth and the formation of chloroplasts mainly proceeds at the expense of the storage material.

Discussion

The results of this investigation show that the application of biocides can result in a strong-light or weak-light growth-response of the whole plant which is correlated with the formation of sun-type and shade-type chloroplasts respectively. The strong-light growth-response is induced by triadimefon, by cytokinins (kinetin, benzylaminopurin) [3] and also by blue light [4] as compared to red light. Shade adaptation is caused by bentazone and is also found by the application of other photosystem II herbicides *e. g.* methabenzthiazuron [13, 15, 16], simazin [17] and diuron. Evidently the plants ability to react readily to a changed environment by a strong or weak-light growth-response plays a great part in the regulation of plant growth.

At high light quanta fluence rates the sun-type chloroplasts, which allows a higher photosynthetic light quanta conversion, is of advantage for the plant. The formation of sun-type chloroplasts is possibly related to the increased cytokinin levels in strong and blue light [3, 18]. Triadimefon apparently decreases the formation of gibberellic acids [19] which may induce the sun-type growth-response. The physiological meaning of the shade adaption response to photosystem II herbicides is not yet clear. The increased formation of chloroplast lamellae and grana stacks in bentazone-treated plants can be interpreted as part of an inactivation mechanism for the herbicide through providing more binding sites within a chloroplast. In any case the data indicate that photosystem II herbicides not only block photosynthetic electron flow but exhibit additional effects on plant metabolism. Whether these are caused by interference with endogeneous phytohormone levels or are due to the reduced formation of soluble sugars as a result of the greatly inhibited photosynthesis, must be a matter of further research.

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