Distribution and Effects of Bentazon in Crop Plants and Weeds

H. K. Lichtenthaler and D. Meier

Botanisches Institut der Universität, Kaiserstraße 12, D-7500 Karlsruhe 1,
Bundesrepublik Deutschland

G. Retzlaff and R. Hamm

Landwirtschaftliche Versuchsstation der BASF, Postfach 2 20, D-6703 Limburgerhof,
Bundesrepublik Deutschland

Z. Naturforsch. **37 c,** 889–897 (1982); received July 9, 1982

Bentazon, CO_2-Assimilation, Inhibition of Photosynthesis, Photosystem 2-Herbicide, Shade-Type Adaptation of Chloroplasts

The inhibition of photosynthetic CO_2-assimilation and of the variable chlorophyll fluorescence as well as uptake and transport of ^{14}C-labelled bentazon and the possibilities for a herbicide-induced shade-type modification of the photosynthetic apparatus were investigated in bentazon-sensitive weeds (Galium, Sinapis, Raphanus) and in the tolerant crop plants wheat and maize.

1. In weeds the depression of photosynthetic CO_2-assimilation is irreversible, whereas tolerant plants recover due to the metabolization of the active herbicide.
2. A lower rate of uptake and transport of bentazon associated with its fast metabolization is the reason for the tolerance of crop plants towards bentazon.
3. The transport of [^{14}C]bentazon proceeds in the tracheary elements of the xylem. Uptake and transport of bentazon in the weeds are light dependent.
4. The loss of variable fluorescence (Kautsky effect) in the leaves after root application of bentazon proceeds much faster at high-light than at low light conditions and confirms the light-dependency of the bentazon transport.
5. In the sensitive dicot weeds bentazon not only inhibits photosynthetic electron flow and depresses CO_2-fixation but also induces the formation of shade-type chloroplasts which are less efficient in photosynthetic quantum conversion. This bentazon-induced modification of the photosynthetic apparatus (*e.g.* changes in ultrastructure, pigment ratios, and levels of chlorophyll-proteins) contributes to the effectiveness of bentazon as a herbicide.

Introduction

The photosystem II-herbicide bentazon is known to block the photosynthetic electron transport between the quencher Q and the plastoquinone pool [1]. As a herbicide it is used for selective weed control in cereals, rice and soybeans [2–4]. After application of bentazon the CO_2-assimilation decreases in weeds and crop plants due to the electron transport inhibition [5]. In contrast to weeds the crop plants will recover from bentazon-treatment after a certain time period apparently due to their metabolization capacity for bentazon [6]. The active ingredient bentazon may become hydroxylated and glucosylated to an inactive form and finally will be broken down to CO_2. The sensitivity of the dicot weeds might be caused by a lack of metabolization, but could as well be due to a better uptake and transport of the herbicide.

Like other photosystem II-herbicides [7] (*e.g.* monuron [8], fluometuron [9] and methabenzthiazuron [10]) bentazon can induce a shade-type modification of the photosynthetic apparatus [11], a response which might be different for weeds and crop plants. The efficiency of bentazon as a herbicide also appears to be light dependent [12].

In order to get more information on the differential mode of action of bentazon in sensitive and tolerant plants we studied its distribution within the plants using ^{14}C-labelled bentazon, investigated the light-dependency of bentazon effects and looked for changes in the composition and structure of the photosynthetic apparatus.

Abbreviations: a/b, ratio chlorophyll a to b; a/c, ratio chlorophyll a to β-carotene; $a+b/x+c$, ratio chlorophylls $a+b$ to carotenoids x + c; CPIa and CPI, chlorophyll a-proteins of photosystem I, LHCPs, sum of the light-harvesting chlorophyll a/b-proteins isolated by SDS-PAGE; x/c, ratio xanthophylls to β-carotene; PAGE, polyacrylamide-gel electrophoresis; SDS, sodium dodecylsulphate.

Reprint requests to Prof. Dr. H. K. Lichtenthaler.

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Materials and Methods

Wheat (*Triticum sativum* L. "Diplomat"), and mustard plants (*Sinapis alba* L.) were grown on a soil peat mixture under controlled conditions in a climate chamber (25 °C; HQL-R lamps 20 klux; 55 W · m^{-2}; 70% relative humidity). The CO_2-fixation measurements and the radiolabel experiments (35 mCi/mmol [U-^{14}C]phenyl bentazon) were performed at the 3 leaf stage (wheat, ca. 15–20 d old) and with 10–13 d old mustard seedlings at the primary leaf stage. Radish plants (*Raphanus sativus* L. "Saxa") were grown on a diluted nutrient medium [11] at medium (Tungsram lamps; 3 klux; 6.5 W · m^{-2}), high (Osram HQIE lamps; 20 klux; 55 W · m^{-2}) and low quanta fluence rates (HQIE; 1 klux; 5 W · m^{-2}).

Measurements of CO_2 assimilation

The plants were placed into glass chambers at 20 °C in a constant air stream with 0.04% CO_2 by volume. The plants were illuminated with 40 klux. The amounts of CO_2 assimilated were measured with the conductometric method using a Wösthoff-Ultragas-U3K-CO_2 analyzer.

^{14}C-radioactivity measurements

The shoot and root portions of the [^{14}C]bentazon-treated plants were combusted in a sample oxidizer (IN 4101, Oxymat). The evolved $^{14}CO_2$ was absorbed in an appropriate liquid scintillation cocktail and radioassayed in a SL 30 Intertechnique Scintillation Counter. Evaluation with the Multimat-M_4 calculator according to the ESR method is based on a quench curve [13]. Autoradiography was performed with tried plants and micro-autoradiography (Fig. 4) with freeze-dried plants.

The *variable chlorophyll-fluorescence* (slow signal, minute-range) known as Kautsky effect [14] was measured at room temperature in green 15 min darkened cotyledons with a special apparatus described before [15].

Prenylpigments were extracted in acetone and petrolether and determined spectrophotometrically in diethylether (chlorophylls [16]) and carotenoids in ethanol after thin layer chromatographic separation on silicagel plates (solvent system: 70 ml light petrol 50–70 °C, 30 ml dioxan, 10 ml isopropanol). The chromatographic sequence (with R_f values) is β-carotene (0.85), chlorophyll *a* (0.6), chlorophyll *b* (0.5), lutein + zeaxanthin (0.4), antheraxanthin (0.34), violaxanthin (0.3) and neoxanthin (0.2). When the pigment extract is applied to the silica plate in a pure, waterfree solvent *e.g.* light petrol, chlorophyll *a* and *b* move as single bands, a splitting of the bands and destruction of chlorophylls is avoided.

Leaf segments were fixed in buffered 5% glutardialdehyd (pH 7.4), postfixed in buffered 2% OsO_4 and embedded in epoxy resin as reported [11]. Stacking degree and thylakoid frequency per chloroplast section (total length of thylakoids in μm per 10 μm^2 chloroplast section) were determined by measuring the length of stacked (appressed) and exposed (non-appressed) membranes with a map kilometer tracer.

SDS-polyacrylamide electrophoresis of SDS-digested chloroplasts was performed following the Laemmli method as described [17]. The levels of chlorophyll-proteins were determined from densitometer scans at 663 nm.

Results

Spraying of the leaves of young growing plants with a solution of the herbicide bentazon (4×10^{-3} M corresponding to an average application rate of 1 kg per ha) results in a decreased photosynthetic CO_2-assimilation (Fig. 1). In the sensitive weeds (*Galium*,

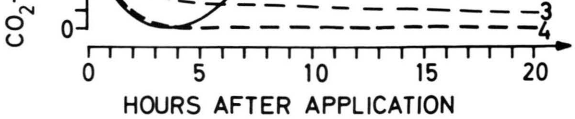

Fig. 1. Inhibition of photosynthetic CO_2-assimilation in crop plants (*Zea mays* L. "INRA corn" and *Triticum aestivum* L. "Kolibri" development stage: 3 leaves) and in weeds (*Galium aperine* L. and *Sinapis alba* L.) after leaf application of the herbicide bentazon (spraying with 4×10^{-3} M solution, which corresponds to an average application rate of 1 kg active ingredient per ha). CO_2-assimilation before treatment = 100%.

Sinapis, Raphanus) the inhibition of CO_2-fixation is irreversible. The tolerant crop plants (*e.g.* wheat, maize), however, recover after several hours from the bentazon-treatment due to a fast metabolization of the inhibitory bentazon [6]. They reach again full photosynthetic CO_2-fixation activity about 20 h after bentazon application. The CO_2-assimilation of the sensitive *Galium* and mustard plants remains zero even after a period longer than 20 h. The high sensitivity of weeds to bentazon could be caused by a higher uptake and lower metabolization rate of bentazon than in the crop plants. In order to prove this possibility equal amounts of ^{14}C-labelled bentazon were applied to one leaf of a wheat and a mustard plant and the distribution of ^{14}C-label studied by autoradiography.

In contrast to the wheat leaf where the [^{14}C]-bentazon label remained almost quantitatively on the application site (2nd leaf), the radioactivity of [^{14}C]bentazon applied to one cotyledo of the mustard plant was exported to the root, epicotyl and the still growing primary leaves (Fig. 2). Only trace amounts of ^{14}C-radioactivity moved into the second cotyledo. By thin layer chromatography of leaf and root extracts it was shown that the transported ^{14}C-label in fact consisted to about 99.5% of the active bentazon. The results of this experiment with ^{14}C-radiolabelled bentazon clearly show that there is a much higher uptake and distribution of bentazon in the mustard plants than in wheat. They furthermore demonstrate that the metabolization of bentazon in *Sinapis* is extremely low.

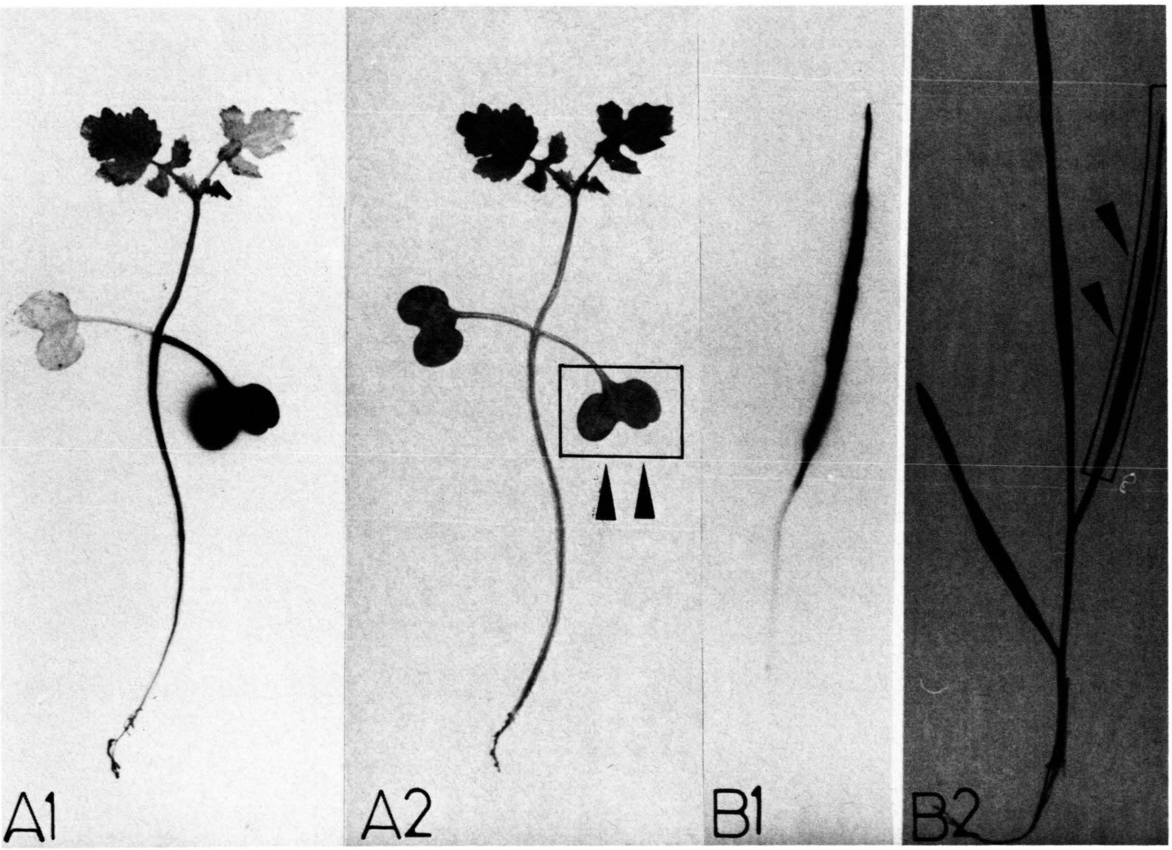

Fig. 2. Uptake and transport of [^{14}C]bentazon A) in the herbicide-sensitive mustard plant and B) in the herbicide-tolerant wheat plant. Leaf application: 10 µg bentazon per leaf (specific radioactivity 35 mCi/mM; radiolabel: [U-^{14}C]phenyl. Duration of labelling experiment 3 d. (0.5 ×). A_1, B_1 Autoradiogram showing the transport of the labelled material. A_2, B_2 Photos of the plants, the treated leaves are indicated.

The transport of [^{14}C]bentazon within the mustard plants proceeds in the xylem elements of the cotyledo, petiole and stem bundles as shown in Figs. 3 and 4.

The uptake and transport of [^{14}C]bentazon is light-dependent in the sensitive mustard plant but not in the tolerant wheat as was shown in an additional experiment (Table I). 24 h after bentazon application in the light the radioactivity in the shoot was about 8 times higher in the mustard than in wheat plants. It further increased in the mustard from 24 to 72 h after ^{14}C application but decreased in the wheat plant during the same time. In the mustard plants kept in the dark much less ^{14}C-activity was found in the shoot (hypocotyl, epicotyl + primary leaves) and root portions than in the light. The wheat plants, in turn, exported in the dark and in the light only little [^{14}C]bentazon from the application site (2nd leaf). In a supplementary experiment we could show that in wheat and maize [^{14}C]bentazon was continuously metabolized and respired to $^{14}CO_2$. The results of Table I also indicate that the basipetal transport of [^{14}C]bentazon is low and negligible in both plant species, wheat and mustard.

That the uptake of bentazon *via* the root and its transport to the leaves are also light dependent was shown with the dicot radish seedlings by means of *in vivo* chlorophyll fluorescence. Radish plants are as sensitive to the herbicide bentazon as mustard plants. The fully developed cotyledons of control plants cultivated under high-light (HL) or low-light (LL) growth conditions show, upon illumination after a 15 min dark period, the typical variable fluorescence pattern known as Kautsky effect (Fig. 5). After an initial high fluorescence yield, the maximum fluorescence decreases with beginning

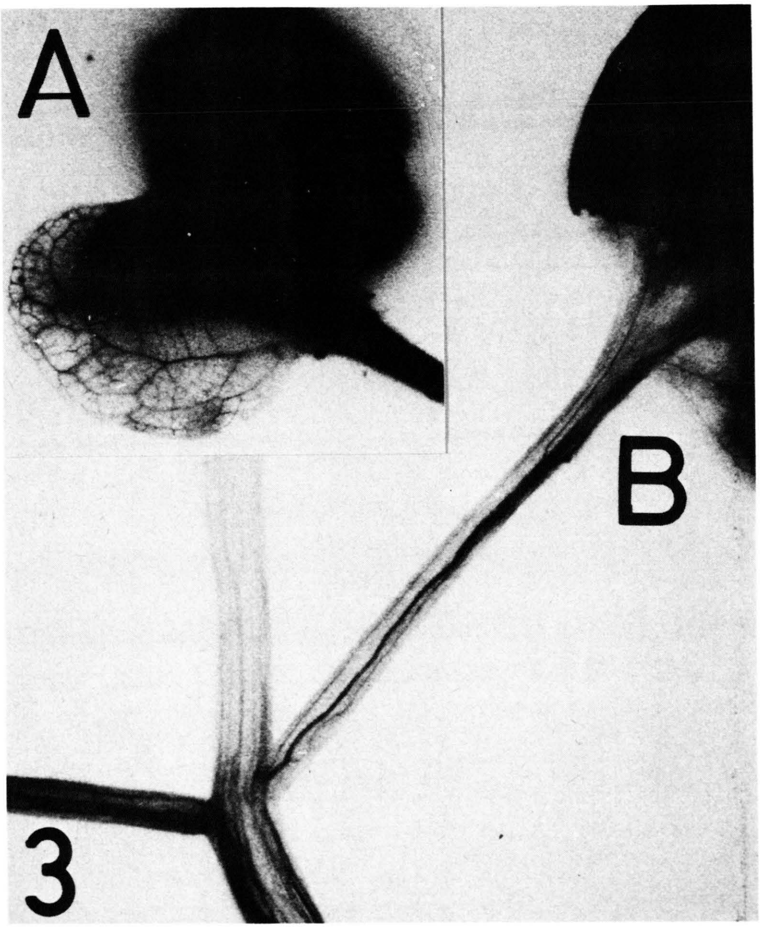

Fig. 3. Autoradiograms of *Sinapis alba* showing the transport of [^{14}C]bentazon in the xylem elements A) of the leaf and B) of the petiole and stem bundles. [^{14}C]bentazon application in the upper leaf area, amounts as in Fig. 2. (Magnification: A 2.6× and B 3.5×).

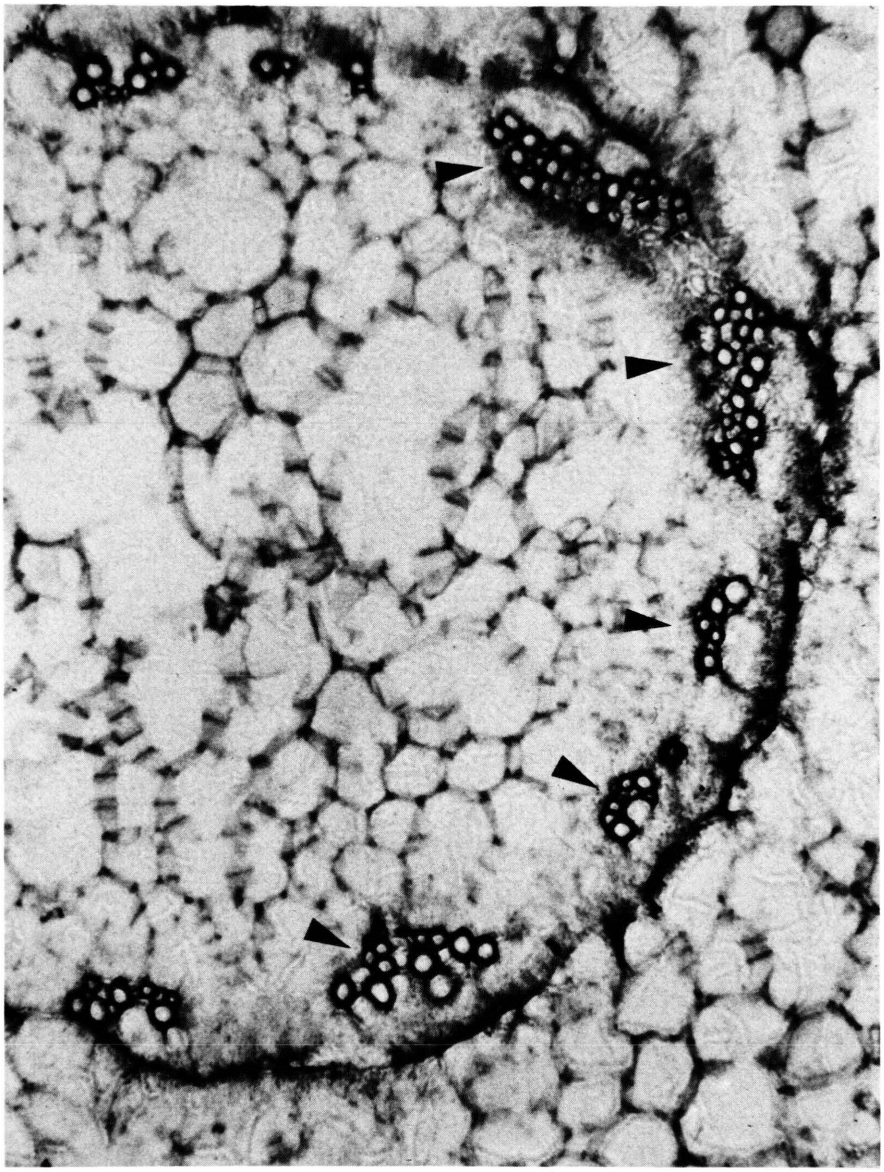

Fig. 4. Freeze-dried epicotyl section of the mustard seedling together with its autoradiogram after leaf application of [^{14}C]bentazon (conditions as in Fig. 2). The bentazon transport proceeds in the xylem elements (▸) which are deep black (autoradiogram). The cell walls of the tissue section are faint black from staining with methylene blue. (Magnification 150 ×.)

photosynthesis to a steady state level of the fluorescence (fs), which is slightly higher than the ground fluorescence fo seen in the initial fast fluorescence rise signal [18], which is not shown here. Maximum fluorescence and steady state fluorescence are higher in the LL-leaf while the fluorescence decrease (fd) proceeds faster in the HL-leaf (Fig. 5). Though there are certain differences between HL and LL-leaves, due to the presence of either HL or LL chloroplasts with different photosynthetic capacity [18], the general fluorescence pattern is the same. The ratio of fluorescence decrease to steady state fluorescence, defined here as variable fluorescence *vF*, is a measure of photosynthetic quantum conver-

Table I. Uptake and transport of leaf-applicated [^{14}C]bentazon in sensitive and in tolerant plants in the light (20 klx; 55 W · m^{-2}) and in the dark. ^{14}C-activity and application site of bentazon as given in Fig. 2. Age of seedlings 10 d (mustard) and 18 d (wheat).

	% of applicated ^{14}C-activity							
	Sensitive plant (mustard)				tolerant plant (wheat)			
	Shoot		Root		Shoot		Root	
	24 h	72 h	24 h	72 h	24 h	72 h	24 h	72 h
light	23.1	25.1	1.0	1.0	2.9	1.8	1	1
dark	6.3	7.0	1	1	2.6	1.8	1	1

Table II. Prenylpigment content of 8 d old radish seedlings (μg per 50 pairs of cotyledons). After 3 days dark growth, the plants greened for 5 days (3 klx = 6.5 W · m^{-2}) on a bentazon-containing nutrient medium. Mean values from 4 to 6 cultivations with standard deviation of 5% or less. The differences between controls and treated plants are significant ($P < 0.005$).

	Control	10^{-4} M Bentazon	Control	10^{-3} M Bentazon
pigment levels:				
chlorophylls (*a* + *b*)	3545	2675	3780	2460
carotenoids (x + c)	567	448	495	347
pigment ratios:				
a/b	**3.3**	2.4	**3.3**	2.4
a/c	16.8	**24.3**	17.6	**26.0**
a + b/x + c	**6.3**	6.0	**7.6**	6.8
x/c	2.5	**4.7**	2.0	**4.1**

sion. It is higher in HL ($vF = 2.5$) than in LL-leaves ($vF = 2.0$) and directly related to photosynthetic CO$_2$-fixation.

By direct application of bentazon to the leaf the variable part of the fluorescence disappears within 20 min and the fluorescence remains on the initial high value due to the block of the photosynthetic electron transport chain by bentazon [11]. By root application of bentazon (*via* the nutrient medium) bentazon reaches the cotyledons of the HL-plants grown at high fluence rates (55 W · m^{-2}) much faster than that of LL-plants cultivated at 7 W · m^{-2}. 4 h after bentazon treatment the variable fluorescence of HL-leaves is very low (0.3), indicating an almost fully inhibited photosynthesis, while in LL-leaves a similar situation is only reached about 20 h after bentazon application (Fig. 5).

In HL-leaves of bentazon-treated plants the variable part of the fluorescence is completely lost after 20 h and the fluorescence signal is much smaller because of a considerable photooxidative chlorophyll destruction which does not occur in the plants kept at LL-condition.

In order to show that these results are really light-dependent and not due to a possible faster transport system of HL-plants, bentazon was applied via roots in a parallel experiment to LL-plants at HL-conditions and to HL-plants at LL-growth conditions with the same effect. The LL-plants kept under HL-growth conditions had lost their variable fluorescence and photosynthetic capacity much faster, indicating the light-dependency of bentazon uptake and transport.

After application of bentazon to the roots of wheat plants the variable fluorescence changed only little after 24 h indicating that only little bentazon was transported to the wheat leaves.

Bentazon not only blocks photosynthesis in the already developed leaves of green plants but in addition to this modifies the morphology and composition of the photosynthetic apparatus by inducing the formation of shade-type chloroplasts. This is best being seen during the light-induced development of chloroplasts upon illumination of etiolated plants. When 3 d old etiolated radish seedlings are allowed to green on a bentazon-containing nutrient medium they show chlorophyll, carotenoid and thylakoid accumulation for several days as long as there is reserve material in the storage cotyledons.

The bentazon-treated plants not only accumulate less prenylpigments than the photosynthetically fully active control plants, but also a lower proportion of chlorophyll *a* and β-carotene and a higher percentage of xanthophylls and chlorophyll *b*. This is visualized by lower values for chlorophyll *a*/*b* and increased values for the ratio of chlorophyll *a*/*β*-carotene (a/c) and xanthophylls/*β*-carotene (x/c) (Table II). The reason for these changes in prenylpigment composition of thylakoids is the bentazon-induced formation of a higher proportion of light-harvesting chlorophyll *a*/*b*-xanthophyll-proteins (LHCPs) than in the control plants which possess a higher percentage of the photosystem I chlorophyll *α*/*β*-carotene proteins CPI and CPIa (Table III). The LHCPs of radish chloroplasts are known to contain low a/b ratios (1.1 − 1.3) and *β*-carotene only in trace amounts, while CPIa + CPI exhibit high a/b ratios (8 − 15) with primarily *β*-carotene as carotenoid [20]. The higher proportion of LHCPs in the chloroplasts of the bentazon-plants is associated with a different ultrastructure than in controls (Fig. 6, Table III). Broader grana, a higher stacking degree and a higher ratio of appressed to non-appressed membranes (stroma thylakoids + grana end membranes) in the chloroplasts of bentazon-treated plants as compared to control plants are further indications for the formation of shade-type chloroplasts. Similar differences have been found between LL and HL-chloroplasts of different plants

Table III. Differences in the ultrastructure and in the level of chlorophyll-proteins (LHCPs and CPIa + CPI) of chloroplasts from cotyledons of 6 d old radish seedlings grown with and without root-application of bentazon 10^{-3} M. Illumination time and exposure to bentazon 72 h. The differences are significant in all parameters ($P < 0.001$).

	Control	+ Bentazon 10^{-3} M
grana width (μm) [a]	0.31	0.62
stacking degree [b]	58 ± 2.7	73 ± 3.1
ratio of appressed to non-appressed membranes [b]	1.4 ± 0.2	2.7 ± 0.4
ratio of LHCPs to CPIa + CPI [c]	1.0	1.4
average number of thylakoids per granum [a]	3.5	5.7
total length of thylakoids (μm) per 10 μm² chloroplast section [b]	174 ± 22	258 ± 19

[a] Based on 50 median chloroplast micrographs.

[b] Based on 20 median chloroplast sections.

[c] Mean of 6 SDS-PAGE runs of SDS-digested chloroplasts from 2 independent cultivations.

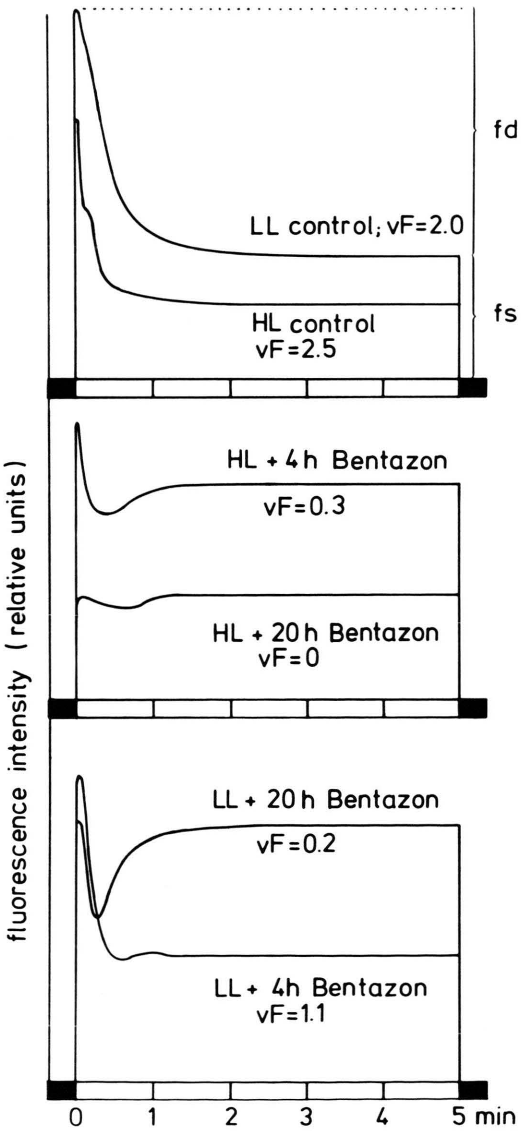

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Fig. 5. Light-dependent loss of the variable fluorescence (*vF*) in green *Raphanus* plants as induced by treatment with the photosystem 2-herbicide bentazon under high-light (HL) and low-light (LL) growth condition. The 6 d old green HL and LL-plants were incubated (roots) in a nutrient solution containing bentazon (10^{-3} M, pH 6) and then further kept under HL (20 klux; 55 W · m^{-2}) or LL growth conditions (2 klux; 7 W · m^{-2}). The variable fluorescence (*vF*, Kautsky effect), an indicator of photosynthetic quantum conversion, is defined here by the ratio of fluorescence decrease *fd* to the steady state fluorescence *fs* ($vF = fd/fs$).

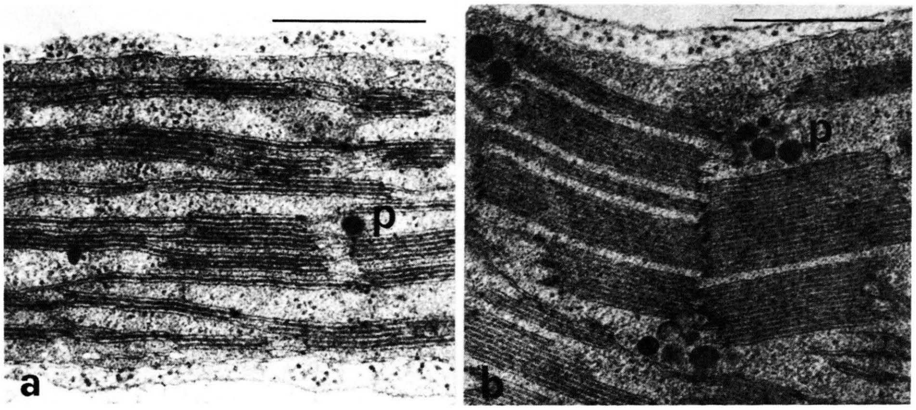

Fig. 6. Ultrastructure of chloroplasts from cotyledons of 6 d old radish seedlings grown at 3 klx (6.5 W · m^{-2}). a) controls, b) grown in presence of bentazon (root application 10^{-3} M). p = plastoglobuli. (5% glutardialdehyde + 2% OsO_4; bar = 0.5 µm).

[18, 19]. In similar experiments we applied bentazon to wheat and maize plants; after 3 or more days we could, however, not detect any significant changes in pigment composition (a/b or x/c ratio) or in the ultrastructure of chloroplasts.

Discussion

The results of this comparative investigation show that the different sensibility of weed and crop plants to bentazon are at least partly due to an increased uptake and transport of bentazon from its application site on one leaf to the other parts of the plant. This high export of ^{14}C-labelled bentazon proceeding in the tracheary elements of the xylem goes predominantly to the upper parts of the sensitive mustard plant and is light dependent. This fast distribution of non-metabolized bentazon to the young growing leaves causes a fast inhibition of the photosynthetic electron transport in these leaves. We have also observed a similar fast transport of bentazon in other dicot plants (*Galium*, *Raphanus*) and this seems to be a general property of bentazon-sensitive weeds. Since no detectable metabolization of the active herbicide ingredient occurs in the weeds within the duration of the experiment (24 h), the inhibition of CO_2-assimilation is persistent in these plants. This is in contrast to the crop plants wheat and maize.

The partial initial inhibition of CO_2-assimilation in bentazon-sprayed whole wheat and maize plants indicates that leaf-applicated bentazon also in crop plants quickly reaches the binding sites in the chloroplasts of the treated leaves and blocks photosynthetic electron flow. Because of metabolization of the active ingredient the degree of photosynthesis inhibition is, however, lower than in the sensitive weeds and only lasts for a few hours. In addition to this the results of the distribution of ^{14}C-labelled bentazon from the applicated leaf to the other leaves indicate that bentazon transport in the crop plant wheat is much lower than in weeds and is not light-dependent. The major part of bentazon thus remaining in the leaf cells of the application site, will be metabolized and broken down to $^{14}CO_2$ as found in this investigation.

The rate of bentazon transport from the roots to the leaves is also light-dependent as is seen from the variable fluorescence which disappears much faster in the radish plants kept at high-light conditions than in plants grown at low light-intensities. The little decrease of variable fluorescence of wheat plants after root application demonstrates that the herbicide uptake and its acropetal transport to the leaves is very low in crop plants. The higher uptake and transport rate of the herbicide from the root to the shoot in weed than in the crop plant are the reason for the efficiency of bentazon as a soil herbicide.

The induction of a shade-type adaptation of the photosynthetic apparatus is a further effect of bentazon that contributes to its efficiency as a herbicide. The formation of shade-type chloroplasts in developing leaves proceeds in the sensitive weeds under continuous presence of bentazon as seen from the changed pigment ratios (a/b; a/c; x/c), the different chloroplast ultrastructure with broader grana and a higher stacking degree and also by the increased ration of the light-harvesting chorophyll *a*/*b*-proteins LHCPs to the chlorophyll *a*-proteins of photosystem I (LHCPs/CPIa + CPI). In the crop plants this shade-type adaptation does not take place because of the low transport and the high metabolization rates of bentazon. Chloroplasts of shade or low-light leaves exhibit at light saturation lower photosynthetic CO_2-fixation rates on a chlorophyll or leaf area basis than sun leaves and leaves from high-light plants which possess a better photosynthetic quantum conversion [18, 19]. This bentazon-induced modification of the photosynthetic apparatus of growing leaves to the less efficient shade-type chloroplast enhances the effect of bentazon as a herbicide since it further decreases the photosynthetic rates of weeds in the field where the applied bentazon amounts may not be sufficient for a full block of the photosynthetic electron transport.

Acknowledgements

Part of this work was supported by a grant from the Deutsche Forschungsgemeinschaft. We wish to thank Mrs. G. Kuhn and Mrs. U. Prenzel for excellent assistance and Mrs. U. Widdecke for valuable help during the preparation of the manuscript.

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