Role of Carotenoids in the Phototropic Response of Corn Seedlings[1]

Richard D. Vierstra[2] and Kenneth L. Poff
MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824

ABSTRACT

The herbicide, 4 chloro-5-(methylamino)-2-(α,α,α,-trifluoro-*m*-tolyl)-3 (2H)-pyridazinone (SAN 9789), which blocks the synthesis in higher plants of colored carotenoids but not of flavins, was used to examine the involvement of carotenoids in corn seedling phototropism. It was concluded that "bulk" carotenoids are not the photoreceptor pigment based on the results that increasing concentrations of SAN 9789 (up to 100 micromolar) did not alter the phototropic sensitivity to 380 nanometers light (using geotropism as a control) and did not increase the threshold intensities of fluence response curves for both 380 and 450 nanometers light even though carotenoid content was reduced to 1 to 2% of normal. SAN 9789 treatment, however, did reduce seedling sensitivity toward 450 nanometers light indicating that carotenoids are involved in phototropism. Carotenoids, which are located mainly in the primary leaves, may act in phototropism as an internal screen, enhancing the light intensity gradient across the seedling and thus contributing to the ability of the seedling to perceive light direction. These results indicate that the action spectra for phototropic responses can be significantly affected by the absorbance of screening pigments *in vivo* thus altering its shape from the *in vitro* absorption spectrum of the photoreceptor pigment.

Plants exhibit a wide variety of physiological and morphological responses which are induced by blue light (16). For many years, a carotenoid was believed to be the chromophore responsible for these responses supported primarily on action spectra with maxima at 450 nm and 480 nm, characteristic of β-carotene, and evidence that photoresponsive organisms generally contained large amounts of carotenoids (21). A flavin is now thought to be the chromophore based on a wide range of experimental evidence, most notably that "carotenoid-less" mutants of *Phycomyces* (15) *Neurospora* (18) and *Euglena* (8) display normal light sensitivity and yet, in the case of *Phycomyces*, may have less than 0.004% of the wild type carotenoid content (15).

Obtaining evidence for the role of carotenoids in the blue light response of higher plants (*e.g.* phototropism of cereal coleoptiles) has been hampered by the inability to obtain mutants lacking carotenoids. Bandurski and Galston (1) studied on albino mutant of maize and found that the coleoptiles displayed 50 to 80% of the normal phototropic response to white light with only 0.1% of the normal carotenoid content. A new herbicide, SAN 9789 [(4 chloro-5-methylamino)-2-(α,α,α,trifluoro-*m*-tolyl)-3(2H)-pyridazinone], permits the effective inhibition of carotenoid synthesis *in vivo* (2).

This herbicide interferes with the desaturation of cis phytoene, thus blocking the accumulation of colored carotenoids. Of equal importance is the fact that this herbicide does not appear to alter general metabolism significantly. For example, corn seedlings retain normal light sensitivity for the photomorphogenic responses stimulated by phytochrome (13).

In this paper, we report the effects SAN 9789[3] has on the carotenoid content and the phototropic response of corn seedlings to determine the role, if any, which carotenoids play in such blue light responses.

MATERIALS AND METHODS

Corn seeds (*Zea mays* hybrid MS WFg $\times$ Bear 38 from National Starch and Chemical Co., Decatur, Ill.) were allowed to imbibe overnight in distilled H_2O and sown, embryo up, on Kimpak (Kimberly-Clarke, Neenah, WI) germination paper dampened with distilled H_2O in trays covered with cellophane. If SAN 9789 treated corn seedlings were required, the seeds were allowed to imbibe overnight in a solution of an appropriate concentration of SAN 9789 (trade name, Norflurazon, obtained as an 80% wettable powder from Sandoz Wander, Inc., Homstead, Fla.) and sown on Kimpak dampened with the same SAN 9789 solution. Trays were kept at 22 $\pm$ 1 C for 4 days in complete darkness and exposed to 1 h of 50 μw cm^{-2} red light (630 nm, 30 nm half band width) on the third and fourth nights to inhibit mesocotyl growth. Straight seedlings between 4 and 5 cm long with the primary leaf extending the length of the coleoptile were selected for use.

Under a dim green safelight, seedlings, with and without the SAN 9789 treatment, were placed in 50-ml test tubes with 2 cm of the coleoptile projecting through a hole in a cork stopper inserted in the opening of the tube and the roots were immersed in distilled H_2O. The seedlings were placed in a humid Plexiglass chamber for 1 h before and during the 3-h test stimulus (light or gravity). Each experiment consisted of 40 seedlings, 10 germinated with distilled H_2O; and 10 each, germinated with three different concentrations of SAN 9789.

To test for phototropism, the seedlings were exposed unilaterally for 3 h to a quantum flux density of 3.8 $\times$ 10^{-12} E cm^{-2} s^{-1} from either 1.0 μw cm^{-2} 450 nm light (8.5 nm halfband width) or 1.2 μw cm^{-2} 380 nm light (10.4 nm halfband width) from a slide projector in conjunction with a Baird Atomic (Baird Corp., Bedford, MA) interference filter. Fluence response curves for 380 or 450 nm light were determined by varying the light intensity with neutral density filters, holding the presentation time constant. Geotropism was induced by holding the seedlings horizontal for 3 h.

Percentage of carotenoids was calculated from absorption spectra of corn seedlings ($\pm$ SAN 9789). Corn coleoptiles, 0.5 grams

[2] Present address: Dept. of Botany, 139 Binge Hall, University of Wisconsin, Madison, WI 53706.

[3] Abbreviation: SAN 9780, 4 chloro-5-(methylamino)-2-(α,α,α,-trifluoro-*m*-tolyl)-3(2H)-pyridazinone.

fresh weight, including the primary leaves, were homogenized in 0.5 ml H_2O and the absorption spectrum was recorded using a vertical cuvette (7) in conjunction with a single beam spectrophotometer similar to that described by Davis et al. (10) on line with a Hewlett Packard 21MX mini-computer (Hewlett Packard Co., Palo Alto, CA). The A at 481 nm was measured for the seedlings germinated in various concentrations of SAN 9789 and related on a percentage basis to that for seedlings germinated in distilled H_2O.

Absorbance measurements orthogonal to the long axis of a single intact corn seedling at 450 nm and 380 nm were made using the single beam spectrophotometer with the seedling enclosed in a small chanber with aligned entrance and exit slits (1 mm width by 10 mm length). A 14-mm section of the corn seedlings was obtained starting 3 mm from the tip. The section was placed in the chamber parallel to the long axis of the entrance and exit slits' with the slits pressed firmly against the section and care was taken to avoid light leaks around the shoot. The photomultiplier tube current at 450 nm and 380 nm was recorded with the seedling in place and the absorbance was determined from calibration with neutral density filters. Total flavin content was determined by the lumiflavin fluorescence method as described by Jesaitis et al. (14) using FMN as a standard. Light intensity measurements were made using a Kettering (model 65; Laboratory Data Control, Riviera Beach, FL) radiometer.

RESULTS

Corn seedlings germinated in the presence of increasing concentrations of SAN 9789 showed a substantial reduction in the accumulation of colored carotenoids (Fig. 1). The difference spectrum of corn seedlings germinated in distilled H_2O minus seedlings treated with 100 μM SAN 9789 (Fig. 1) exhibits maxima characteristics of the absorption spectrum of β-carotene [i.e. maxima at 481 and 450 nm with prominent shoulders at 423 and 398 nm (9)]. A K_i of 15 μM was determined from a plot of carotenoid content as a function of SAN 9789 concentration (Fig. 2, B). SAN 9789 at 100 μM inhibited 98–99% of the carotenoid accumulation (with respect to that of the control) but did alter significantly flavin content of the seedlings. (Flavin concentration was approximately 4 μM on a gram fresh weight basis for both control and herbicide treated tissue.)

The A of single intact seedlings were measured (Table 1) to determine the effect of SAN 9789 on the total A of the seedling (both absorption and scattering). For seedlings germinated with 100 μM SAN 9789 the A at 380 nm was not significantly altered (~9%) but was reduced by 25% at 450 nm (2.44 versus 1.81 A). When expressed on a percentage transmission basis, SAN 9789 treatment increased light transmission by 4.3 and 1.7 times for 450 and 380 nm light, respectively.

The tropic responses of corn seedlings were measured as a function of SAN 9789 concentration (Fig. 2, A). Geotropism was reduced by ~10% at all concentrations of SAN 9789 tested. This effect by 1 μM SAN 9789 on geotropism would indicate that the herbicide does affect other processes in addition to carotenoid synthesis in this variety of corn. The inhibition by SAN 9789 of the phototropic response to 380 nm light was comparable to the inhibition of geotropism at all concentrations tested even though carotenoid content was severely reduced at SAN 9789 concentrations greater than 1 μM. In contrast, the inhibition by SAN 9789 of the phototropic response to 450 nm light was significantly greater than the inhibition of geotropism by SAN 9789. A 25% reduction in phototropism toward 450 nm light was observed as compared to geotropism for seedlings treated with 100 μM SAN 9789.

Phototropic fluence response curves were determined for both control and SAN 9789 (100 μM) treated tissue toward 380 nm or 450 nm light (fluence was varied by altering the light intensity of
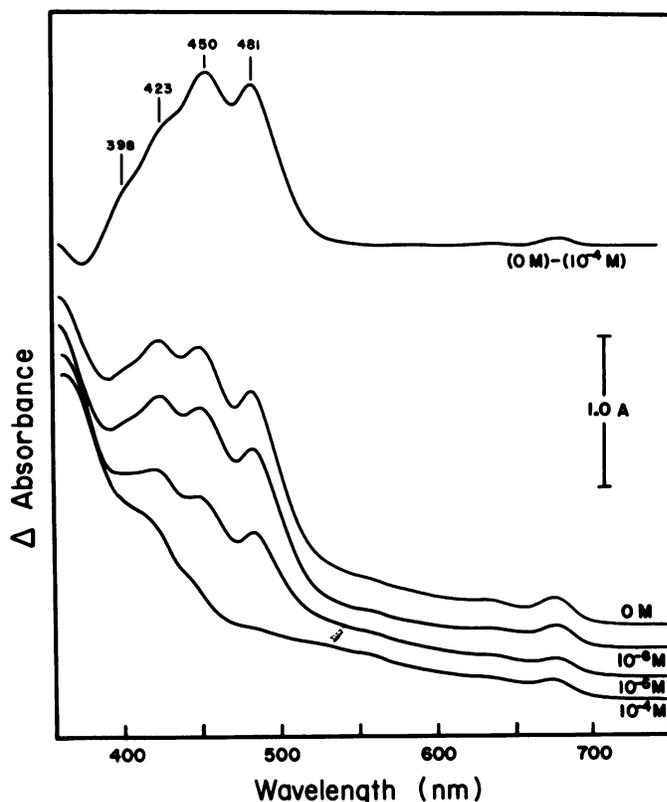

FIG. 1. Absorption spectra of corn seedling germinated with distilled H_2O or increasing concentrations of SAN 9789. The sample consisted of 0.5 g of seedlings tips (including the primary leaf) homogenized in 0.5 ml distilled H_2O. Absorption maxima were determined from the fourth derivative of the difference spectrum.

a constant 3 h presentation time). Both control and SAN 9789 treated seedlings require the same threshold light intensity (approximately 0.1 nw cm^{-2} or a fluence of 4×10^{-12} E cm^{-2} at 450 nm) for phototropic curvature (Fig. 3). At saturating light intensities, the SAN 9789 treated seedlings developed less of a response than control seedlings and, as might be predicted from Figure 2, bent more toward 380 nm light than toward 450 nm light.

DISCUSSION

From these results, we conclude that "bulk" carotenoids are not the photoreceptor pigment responsible for phototropism in corn seedlings. The percentage response of seedlings treated with increasing concentrations of SAN 9789 for phototropism toward 380 nm light was indistinguishable from that for geotropism even though the accumulation of carotenoids was drastically reduced at SAN 9789 concentrations above 1 μM.

In addition, fluence response curves for seedlings treated with 100 μM SAN 9789 extrapolated to the same threshold intensity indicating that the amount of photoreceptor was not affected by the SAN 9789. If we assume that a prerequisite number of photons must be absorbed by the photoreceptor pigment, and that the amount of excited photoreceptor pigment is the rate-limiting factor for a photoresponse, a significant decrease in photoreceptor pigment content would have resulted in an increase in the threshold intensity. Thus, a reduction in bulk carotenoid by 99% should have increased the threshold intensity by 100 times if they were the photoreceptor pigment.

Because the coleoptiles do contain a trace of colored carotenoids, even at the highest concentrations of SAN 9789 tested (~1 $\times$ 10^{-8} M with 100 μM SAN 9789), it is not possible to rule out all carotenoids completely since the photoreceptor pigment may be
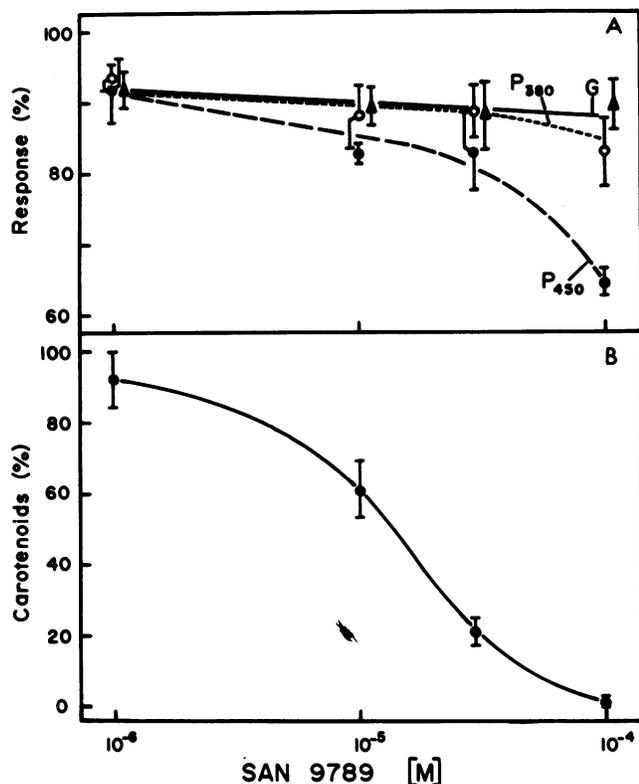
FIG. 2. A, the effect of SAN 9789 on phototropic and geotropic bending of corn seedlings. Geotropic bending (▲) relative to control seedlings germinated in distilled H_2O was measured after 3 h geotropic stimulus. Phototropic bending to 380 nm (○) and 450 nm (●) light relative to control seedlings germinated in distilled H_2O was measured after a 3 h phototropic stimulus. The vertical bars represent ± 1 SE. Each point represents 4 to 6 independent experiments comparing 10 seedlings treated with SAN 9789 to 10 control seedlings. B, the effect of SAN 9789 on carotenoid accumulation in corn seedlings. Carotenoid content of SAN 9789 treated seedlings was determined from absorbance at 481 nm of 0.5 g of homogenized seedling tips and compared to that of control seedlings. Error bars represent ± 1 SD.

Table I. *Absorbance and Percentage Transmission of Single Intact Corn Seedlings Germinated With or Without 100 μM SAN 9789 at 380 nm and 450 nm*

Absorbance was measured using monochromatic light directed through a 1-mm entrance slit, the intact seedling, a 1-mm exit slit, and then to a photomultiplier tube calibrated using neutral density filters.[a]

Wavelength	Control	100 μM SAN 9789
nm		
380	2.69 ± 0.09 (0.20%)	2.48 ± 0.19 (0.33%)
450	2.44 ± 0.07 (0.36%)	1.81 ± 0.08 (1.54%)

[a] Average of five seedlings ± 1 SD.

required at very low concentrations. A theoretical minimum photoreceptor pigment concentration has been proposed to be 3 × 10^{-7} M for *Phycomyces* (3) and 1 × 10^{-9} M for *Avena* (5). Schmidt *et al.* (19) have shown that compounds that interact with flavins will specifically inhibit phototropism. Those data, in addition to these results, are consistent with the conclusion that a flavin and not a carotenoid is the photoreceptor chromophore for phototropism.

Although we conclude that a carotenoid is not the photoreceptor chromophore, carotenoids appear to be involved in photoreception acting as an internal light filter. For phototropism to occur, the seedling must detect a difference in light absorption between the front and back of the coleoptile. Approximately 90% of the carotenoids of the shoot are located in the center of the coleoptile in the primary leaves. Because the absorbance of carotenoids and flavins overlap at wavelengths between 400 and 500 nm, carotenoids must enhance the light gradient perceived by the seedlings when irradiated with 450 nm light. Any reduction in carotenoid content would allow more light to be transmitted to the backside, collapsing the light gradient and reducing phototropic curvature. SAN 9789 treated seedlings exhibit little reduction of A at 380 nm and hence, no inhibition of their phototropic response toward 380 nm light. In contrast, there is a sizable drop in A at 450 nm and a significant decrease in bending toward 450 nm light. When expressed on a percent transmission basis, 100 μM SAN 9789 treatment increased the coleoptile's light transmission at 450 nm by 430% thus allowing four times more light to reach the coleoptile's shaded side. The difference between phototropism toward 450 nm and 380 nm light and geotropism of seedlings exposed to 100 μM SAN 9789, 25% and 6% respectively, correlate well with the loss of A by such seedlings due to the SAN 9789 treatment, 26% at 450 nm and 9% at 380 nm. The fact that such SAN 9789-treated coleoptiles can still respond phototropically, even with a severe reduction in absorption by carotenoids in the tissue, indicates that scattering is also a major component in the development of a light gradient in corn.

The hypothesis that coleoptiles respond phototropically by detecting a light gradient induced by scattering and internal filters, especially carotenoids, was formulated by Reinart studying *in vitro* reactions of flavins in the presence of β-carotene (17). Evidence that the "filter" theory was valid was obtained from *in vivo* experiments with coleoptiles whose phototropic sensitivity could be signifcantly enhanced by the addition of artificial internal light filters (4, 6). Using a derivation of the Beer-Lambert law (I = I_oe^{-a}), Thimann and Curry (20) developed a model for photoresponses that involves detection of a light gradient. Their equation predicts that such a photoresponse would be logarithmically related to the absorbance of the screening pigment. Thus, a significant decrease in the phototropic response would occur only at substantially reduced carotenoid levels and therefore at high SAN 9789 concentrations which is in agreement with the results reported here (Fig. 2).

Since perception of light direction in corn seedlings involves other pigments in addition to the photoreceptor chromophore, any action spectrum for phototropism will be a function not only of the absorption spectrum of the photoreceptor pigment but also of the shading pigments. In *Phycomyces*, for example, which uses focusing to detect light direction, negative phototropism in response to UV light has been attributed to screening of the photoreceptor pigment by gallic acid (11). In corn seedlings, carotenoids also will have some effect on the action spectrum. Fluence response curves for untreated coleoptiles gave a 450 nm/380 nm ratio of 0.5 for the fluence required to elicit a 20 degree curvature [a similar ratio is reported in *Avena* (12, 20)]. For seedlings treated with 100 μM SAN 9789, this ratio was 0.75 which is in better agreement with the 0.85 370 nm/445 nm A ratio for riboflavin. Therefore, it is plausible that the involvement of bulk carotenoids in phototropism will infer carotenoid-like properties to the action spectrum for phototropism in corn seedlings.

Thimann and Curry (20) have noted that such an effect will be limited and can not account quantitatively for the carotenoid-like action spectrum. The exact contribution of carotenoids to the measured action spectrum can be evaluated only by measuring a detailed action spectrum in the absence of the carotenoid (in the presence of SAN 9789). Such an action spectrum might lead toward a resolution of the paradox that the action spectrum resembles a carotenoid photoreceptor pigment while other data support a flavin photoreceptor pigment. In addition, such an
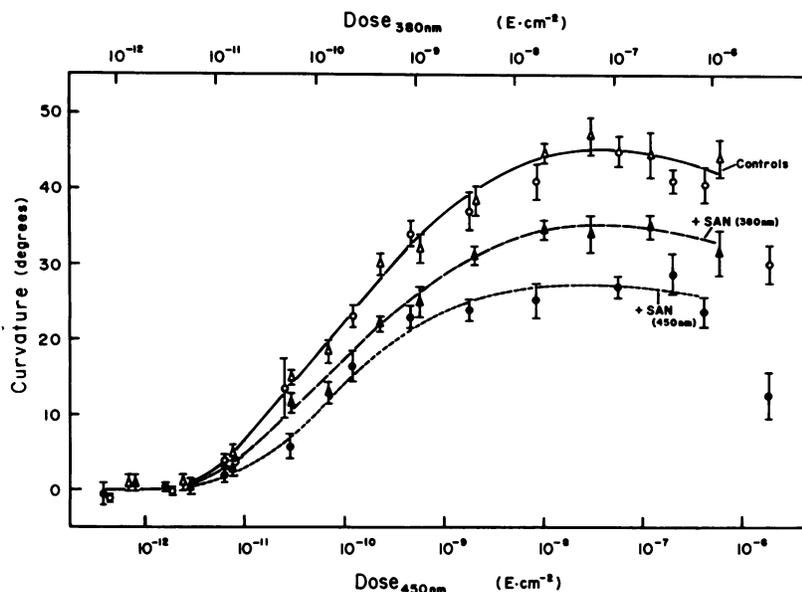
Fig. 3. Fluence response curves for the phototropic response of corn seedlings. (●, ▲), 100 μM SAN 9789; (○, △), distilled H₂O; (▲, △) 380 nm light; (●, ○), 450 nm light. Phototropic bending was measured after 3-h stimulation. Error bars represent ± 1 SE. Each point represents 4 to 6 independent experiments consisting of 10 coleoptiles each. Dose of 380 nm light has been shifted with respect to the dose at 450 nm in order to overlap the fluence response curves of 380 nm light with 450 nm light for seedlings germinated with distilled H₂O.

action spectrum would lead toward the true absorption spectrum of the photoreceptor pigment.

In summary, we provide the evidence that "bulk" carotenoids are not the photoreceptor pigment involved in corn seedling phototropism. These results in connection with other studies involving compounds that affect flavins (19) provide further evidence favoring a flavin as the chromophore. Carotenoids appear to be involved secondarily in phototropism, acting as an internal light screen important in establishing the light gradient required for the perception of light direction. Because of this involvement, the action spectrum for the phototropic response will be a function of carotenoids in addition to the photoreceptor pigment and thus may differ significantly from the absorption spectrum of the photoreceptor pigment.

LITERATURE CITED

1. BANDURSKI RS, AW GALSTON 1951 Phototropic sensitivity of coleoptile of albino corn. Maize. Genet. Coop. Newsletter 25:5

2. BARTELS PG, C MCCULLOUGH 1972 A new inhibitor of carotenoid synthesis in higher plants: 4-chloro-5-(dimethylamino)-2-α,α,α,(tri-fluoro-*m*-tolyl)-3(2H)-pyridazinone (Sandoz 6706). Biochem Biophys Res Commun 48: 16–22

3. BERGMAN K, PV BURKE, E CERDA-OLMEDO, CN DAVID, M DELBRÜCK, KW FOSTER, EW GOODELL, M HEISENBERG, G MEISSNER, M ZALOKAR, DS DENNISON, W SHROPSHIRE 1969 *Phycomyces.* Bacteriol Rev 33:99–137

4. BRAUNER L 1955 Über die Funktion de Spitzenzone beim Phototropismus der *Avena*-koleoptiles Z Bot 43:467–498

5. BRIGGS WR 1964 Phototropism in higher plants *In* A Geise, ed, Photophysiology Vol. 1. Academic Press, New York, pp 223–271

6. BUNNING E, J DORN, G SCHNEIDERHOHN, J THORNING 1953 Zur Funktion von

7. BUTLER WL 1972 Absorption spectroscopy of biological materials. Methods Enzymol 24B:3–25

8. CHECCUCCI A, G COLOMBETTI, R FERRARA, F LENCI 1976 Action spectra for photoaccumulation of green and colorless *Euglena*: evidence for identification of receptor pigments. Photochem Photobiol 23:51–54

9. DAVIES BH 1976 Analysis of carotenoid pigments. *In* TW Goodwin, ed, Chemistry and Biochemistry of plant pigments Vol. I Academic Press, London, pp 655–679

10. DAVIS KA, Y HATEFI, KL POFF, WL BUTLER 1973 The *b*-type cytochromes of bovine heart mitochondria: absorption spectra, enzymatic properties, and distribution in the electron transfer complexes. Biochim Biophys Acta 325:341–356

11. DELBRÜCK M, W SHROPSHIRE 1960 Action and transmission spectra of *Phycomyces.* Plant Physiol 35:194–203

12. EVERETT M, KV THIMANN 1968 Second positive phototropism in *Avena* coleoptiles. Plant Physiol 43:1786–1792

13. JABBEN M, G DEITZER 1979 Effects of the herbicide SAN 9789 on photomorphogenic responses. Plant Physiol 63:481–485

14. JESAITES AJ, P HENERS, R HERTEL, WR BRIGGS 1977 Characterization of a membrane fraction containing a *b*-type cytochrome. Plant Physiol 59:941–947

15. PRESTI D, WJ HSU, M DELBRÜCK 1977 Phototropsim in *Phycomyces* mutants lacking β-Carotene. Photochem Photobiol 26:403–405

16. PRESTI D, M DELBRÜCK 1978 Photoreceptors for biosynthesis, energy storage and vision. Plant Cell Environment 1:81–100

17. REINART J 1953 Uber die Wirkung von Riboflavin und Carotin beim Phototropismus von *Avena*—koleoptilen und bei anderen Pflanzlichen Lichtreizreaktionen. Z Bot 41:103–122

18. SARGENT ML, WR BRIGGS 1967 The effect of light on a circadian rhythm of condidiation in *Neurospora.* Plant Physiol 42:1504–1510

19. SCHMIDT W, J HART, P FILNER, KL POFF 1977 Specific inhibition of phototropism in corn seedlings. Plant Physiol 60:736–738

20. THIMANN KV, GM CURRY 1980 Phototropsim and phototaxis *In* M Flory, HS Mason, eds, Comparative Biochemistry Vol 1 Academic Press, New York, pp 243–309

21. WALD G, HG DUBUY 1936 Pigments of the oat coleoptile. Science 84:247

Lactoflavin und Carotin beim Phototropismus und bei lichtbedingten Wachstumsbeeinflussungen. Ber Dtsch Bot Ges 66:333–340