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A comparative study of the responsivity of *Sinapis alba* L. seedlings to pulsed and continuous irradiation

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Abstract. Anthocyanin formation in 36 h dark grown *Sinapis alba* L. seedlings and inhibition of hypocotyl elongation in 36 h and 54 h dark grown and 54 h and 7 day light grown seedlings in response to continuous red light could be substituted for by hourly 5 min light pulses where the total fluence over the irradiation period is the same. These pulses are partially (36 h) or almost totally (54 h and 7 day) reversible by subsequent far-red (RG 9) light pulses. In contrast to 654 nm light, hourly light pulses with 552 nm, 449 nm and 715 nm can at best only partially substitute for continuous irradiation. These data are discussed with respect to the commonly used models for the phytochrome high irradiance response.

Key words: Anthocyanin formation – High-irradiance response – Phytochrome cycling – and induction – *Sinapis*.

Introduction

The interpretation of action spectra for photomorphogenic responses under prolonged irradiation is still under debate (cf. Mancinelli and Rabino 1978). In etiolated seedlings peaks of photon responsivity can be obtained in the far-red, red, blue and near ultraviolet. There seems to be no doubt that the far-red peak of action can be explained on the basis of phytochrome (Hartmann 1966; Schäfer 1975, 1976; Mancinelli and Rabino 1975; Johnson and Tasker 1979). Whether the blue and near ultra-violet action may be explained on the basis of phytochrome, a separate blue light receptor (cryptochrome) or both photoreceptors is still an open question. The red action is dealt with in the present paper.

Jose and Vince-Prue (1977) published an action spectrum for the inhibition of growth in radish hypocotyls showing peaks of action in the blue, red and far-red wavebands. They attempted to explain this on the basis of a blue light receptor and two different high irradiance responses of phytochrome for the red and far-red spectral regions. Recently, Johnson and Tasker (1979) proposed that phytochrome acts through the multiplicative effect of two components, namely P_{fr} and the cycling rate. Apart from a far-red responsivity, this model also predicts a considerable response to red light. Johnson (1980) suggested that the low responsivity to red light which was observed for some HIRs might be due to the action of two competitive red light responses (Häcker et al. 1964; Hartmann 1967) or to a strong screening by chlorophyll under prolonged red irradiation (Wagner and Mohr 1966; Johnson 1980). In contrast to Johnson's proposals, previous models predicted a very low responsivity and fluence rate dependence under continuous red light (Hartmann 1966; Schäfer 1975, 1976).

We recently published action spectra for the inhibition of hypocotyl growth in both dark-grown and light-grown *Sinapis alba* L. seedlings (Beggs et al. 1980). Whereas dark-grown seedlings showed peaks of action in the blue, red and far-red wavebands, the light grown seedlings were substantially inhibited in their elongation only by the red waveband. This indicated that the red and the far-red responsivity might be due to different primary effects.

Whereas etiolated *Sinapis* seedlings show no significant response to a single red light pulse, white light grown seedlings respond strongly to the same treatment (Beggs et al. 1981). We demonstrated that this responsiveness to light pulses can be developed by a short (6 h) continuous light pretreatment with

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Abbreviations: P_{fr} = far-red absorbing form of phytochrome; SAN 9789 = 4-chloro-5-(methyl-amino)-2-(α,α,α-trifluoro-m-tolyl)-3(2H)-pyridazinone = Norflurazon; HIR = 'High irradiance response'

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blue, red or far-red light, far-red being the most effective. The question arises as to whether the induction response (i.e. the response to light stimuli in a following dark period) also functions under continuous irradiation conditions together with a separate continuous irradiation response, ('high irradiance response') HIR, or whether the continuous light action represents a multiple induction response alone.

To overcome the problem of a possible interplay between two phytochrome actions (viz. induction and HIR) and to obtain the photon effectiveness for the HIR alone one can use a system showing almost no induction response (Hartmann 1967), or one can 'correct' for the contribution of induction (Mohr 1964). Both methods are not without problems. Although a system may not respond to one light pulse without a light pretreatment, it may show it after a relatively short continuous light pretreatment (Beggs et al. 1981). The correction for the contribution of an induction response is even more complex because we do not know the number of light pulses and the dark intervals which should be used.

One possible experimental approach to this problem is described in the present publication. We wanted to determine whether inhibition by continuous red light can be substituted for by hourly red light pulses of the same total fluence and whether the effect of the red light pulses can be reversed by subsequent far-red light pulses. We also compared the relative effectiveness of continuous and pulsed light of the same total fluence on anthocyanin formation in SAN 9789 treated *Sinapis* seedlings.

Materials and methods

Sinapis alba L. seeds (harvest 1975) were obtained from Asgrow Co. Freiburg-Ebnet, FRG. The growing conditions for young seedlings (Table 1, 2) were the same as those described by Beggs et al. (1980). The seven day old white light-grown plants had been kept for 7 d in a phytochamber at 50–60% relative humidity. To ensure uniform germination, the seeds were sown on chromatography paper irrigated with Hoaglands solution. After 3 d the young seedlings were transferred to plastic pots containing a 1:1 mixture of sand and soil. Out of seven seedlings per plant pot the 5 largest were used for the measurements, the two smallest being discarded before the start of the 24 h irradiation period. At this stage the plants have fully developed cotyledons and small primary leaves. The phytochamber used for these experiments was equipped with two 3 kW OSRAM XQO Xenon arc lamps. Additional irradiation with fluorescent tubes (OSRAM 40W/25, PHILLIPS TL 40 W/18) was given to increase the total photon fluence rate to 273 μmol $m^{-2}s^{-1}$ (in the 400–800 nm waveband).

Monochromatic light was obtained using Schott DAL filters or, for far-red, an RG 9 3 mm colour glass. As light source, a modified Zeiss Ikon Xenosol III equipped with an OSRAM XBO 2.5 kW Xenon arc (for DAL filter irradiation) or a modified Leitz Prado 500 W Universal projector (RG 9 light) was used.

The spectral photon fluence rate was measured as described by Beggs et al. (1980) where further details of the light sources are found. For 36 and 54 h old seedlings, percentage inhibition of hypocotyl growth was determined as described by Beggs et al. (1980).

Hypocotyl lengths were measured for each plant before and at the end of the monochromatic light treatments using dividers. Each experiment is the mean of about 30 plants.

Extraction and determination of anthocyanin was carried out using a slight modification of the method described by Lange et al. (1971). Twenty cotyledon pairs were extracted in 10 ml extraction medium (n-propanol/HCl/H_2O in the ratio of 18:1:81 volumes).

Each value in the tables is the mean of 8–16 (2–4 independent) experiments. The standard error is 4–15% for percent inhibition over 20%.

Results and discussion

Table 1 compares the effects of continuous light, light pulses and the reversion experiment for 54 h dark – and white – light-grown *Sinapis* seedlings. It is obvious that for the dark-grown seedlings the major part of the continuous 654 nm light effect can be substituted for by 654 nm light pulses and this effect can be completely reverted by RG 9 light pulses. Only a small part of the 449 nm continuous light effect and almost none of the continuous 715 nm light effect can be substituted for by hourly 5 min light pulses of the same light quality. Almost the same is true for the white light-grown seedlings. In this case the continuous 654 nm light can be completely substituted for by the 654 nm light pulses.

A straight-forward interpretation of these data would be that the high spectral effectiveness of continuous red light with respect to inhibition of the hypocotyl growth of mustard seedlings is predominantly

Table 1. Inhibition of hypocotyl growth in 54 h old *Sinapis* seedlings by pulses or continuous light treatment. Final measurements were made 24 h after the beginning of the light treatment. The fluence rates were: $N_{715}=120$ μmol $m^{-2}s^{-1}$; $N_{654}=160$ μmol $m^{-2}s^{-1}$; $N_{449}=90$ μmol $m^{-2}s^{-1}$; RG 9=25 μmol $m^{-2}s^{-1}$ ($\lambda<$ 800 nm). For continuous treatments the fluence rates were 1/12 of these values. D=dark-grown, L=light-grown seedlings

Light quality	Treatment	% inhibition	
		D	L
715 nm	continuous	51.9± 4.8	14.2± 3.4
715 nm	24·(5 min N_{715}+55 min dark)	18.4± 7.5	7.8±5.7
715 nm	24·(5 min N_{715}+5 min RG 9 +50 min dark)	16.9±11.2	5.0±4.6
654 nm	continuous	71.2± 1.8	66.8±6.0
654 nm	24·(5 min N_{654}+55 min dark)	50.6± 2.6	66.9±2.7
654 nm	24·(5 min N_{654}+5 min RG 9 +50 min dark)	14.5± 3.17	5.9±0.7
449 nm	continuous	42.8± 4.6	37.0±4.1
449 nm	24·(5 min N_{449}+55 min dark)	24.1± 3.5	15.5±3.9
449 nm	24·(5 min N_{449}+5 min RG 9 +50 min dark)	17.8± 4.2	1.3±3.7
	24·5 min RG 9	18.5± 1.2	10.2±0.66

Table 2. Inhibition of hypocotyl growth and accumulation of anthocyanin in the cotyledons of *Sinapis* seedlings. The seedlings were grown for 36 h in darkness and final measurements made 24 h after beginning of the light treatment (continuous light or hourly light pulses). The seedlings were grown on either Hoagland's solution or on Hoagland's+SAN 9789 ($5 \cdot 10^{-6}$ M). The fluence rates were the same as in Table 1

light treatment	% inhibition		amount of anthocyanin A_{535}	
	Hoagland's	SAN 9789	Hoagland's	SAN 9789
24 h 654 nm light	68.6	66.6	0.417	0.389
5 min pulse N_{654} every hour	66.3	65.5	0.408	0.414
5 min pulse N_{654} reverted by 5 min RG 9 every hour	50.0	52.8	0.346	0.383
5 min RG 9 every hour	24.3	18.0	0.143	0.154

The initial length of the dark controls was about 6 mm and the final length was about 36 mm for both treatments

Table 3. Inhibition of hypocotyl elongation in 7 d old light grown *Sinapis* plants. Final measurements were made 24 h after the beginning of the light treatment. The fluence rates were: $N_{654}=160$ µmol $m^{-2}s^{-1}$; $N_{449}=90$ µmol $m^{-2}s^{-1}$; $N_{552}=110$ µmol $m^{-2}s^{-1}$; WL=273 µmol $m^{-2}s^{-1}$ (400–800 nm) RG 9=25 µmol $m^{-2}s^{-1}$ ($\lambda < 800$ nm)

Light quality	Treatment	Increase in hypocotyl length mm
654 nm	continuous	1.4±0.1
	hourly 5 min pulse	1.1±0.05
	reversion by RG 9 pulse	3.6±0.5
449 nm	continuous	3.0±0.5
	hourly 5 min pulse	2.5±0.3
	reversion by RG 9 pulse	3.9±0.6
552 nm	continuous	1.7
	hourly 5 min pulse	2.1
	reversion by RG 9 pulse	3.5
WL	continuous	1.3±0.2
RG 9	hourly 5 min pulse	3.5±0.4

The initial lengths of the hypocotyls were: 9.5 mm, the final lengths after one 5 min RG 9 light pulse were: 13.2 mm

(dark-grown seedlings) or almost totally (white light-grown seedlings) due to a multiple induction effect of phytochrome. The observed contribution of blue light pulses suggests that this part of the action spectrum may also be masked by an influence of the induction reaction. The hypothesis that the far-red and a (major) part of the blue are due to the HIR whereas the red effectivity, is predominantly due to induction effects is supported by the observation that only the far-red and blue light effectiveness for inhibition of hypocotyl growth are strongly dependent on light pretreatment in the *Sinapis* seedling (Beggs et al. 1980). Table 2 shows that also for 36 h dark grown *Sinapis* seedlings the effect of continuous 654 nm light on hypocotyl growth and anthocyanin formation could be attained using hourly 5 min 654 nm light pulses. In contrast to the 54 h dark- or light-grown *Sinapis* seedlings the effect of the red light pulses could only be partially (about 20%) reverted by RG 9 light pulses. These effects were also obtained with SAN 9789-treated seedlings indicating that the amount of chlorophyll synthesized during the light treatment need not be the dominant factor influencing the relative red/far-red effectiveness (cf. Johnson 1980).

The observation that the reversibility by far-red (RG 9) light is low in 36 h and almost 100% in 54 and 168 h *Sinapis* seedlings points to changes in the dynamics of the phytochrome reaction-chain system but needs more experimental data before a discussion of this point is useful.

Table 3 shows that also for 7 d old light-grown *Sinapis* seedlings, continuous light (654, 449 and 552 nm) can be substituted for by hourly light pulses with respect to inhibition of the hypocotyl growth. They show a behaviour almost indentical to the 54 h white light grown plants including the measured action spectrum for the inhibition of the hypocotyl growth (Beggs et al. 1980; Beggs 1980). In contrast to the young seedlings the effect of 654 nm light pulses was stronger than that of continuous 654 nm light.

Conclusion

This paper shows that the effect of continuous 654 nm light on the promotion of anthocyanin synthesis in cotyledons of 36 h dark-grown *Sinapis* seedlings and on the inhibition of the hypocotyl growth of 36–168 h old dark or light grown seedlings could be substituted for by hourly 5 min light pulses. This substitution is 70–90% in young etiolated and 100% and 115% in 54 and 168 h light-grown seedlings. Because continuous irradiation with 449 and 552 nm light can be partially substituted for and 715 nm light *cannot* be substituted for by hourly light pulses, these data strongly support the interpretation that the red peak in action spectra for continuous light responses is predominantly due to a phytochrome induction-response activity (Beggs et al. 1980). This is further supported by the fact that the effect of the light pulses can be reverted by following RG 9 light pulses (Table 1 and 3).

One must therefore conclude that continuous light-response action spectra in photomorphogenesis (c.f. Mancinelli and Rabino 1978) are combinations of an action spectrum for a multiple induction re-

sponse – showing a spectrum which almost parallels the P_r absorption curve –, of a phytochrome HIR-showing a spectrum with a far-red peak, and of a blue light receptor HIR (Fukshansky et al., in prep.). The actual peak heights of the action spectra obtained for continuous irradiation will strongly depend on parameters of the phytochrome dynamics and the length of the irradiation (Gammerman and Fukshanskii 1974, Mancinelli and Walsh 1979). Thus our explanation for red effectiveness under continuous light is completely different from that of Johnson and Tasker (1979). We conclude that the data available are only compatible with a major effect of an *induction* response and not a HIR of phytochrome in the red waveband. It should be mentioned that, apart from the problem of explaining high responsivity towards multiple light pulses, there are also several theoretical shortcomings in the Johnson and Tasker model (1979). The authors have attempted to distinguish between P_{fr} concentration and cycling. Using an *identical* model Gammerman and Fukshanskii (1974) have already shown that P_{fr} is fluence-rate dependent and that the phytochrome dynamics depend strongly on the length of irradiation. Furthermore, the model proposed by Johnson and Tasker (1979) ignores most of the known phytochrome dark reactions.

The model proposed by Schäfer (1975) also ignores dark reversion, P_r destruction (Stone and Pratt 1979) and the slow phase of P_{fr} destruction in white light grown plants (Jabben et al. 1980). Furthermore all models do not consider the interactions between the phytochrome induction reaction, phytochrome HIRs and the blue light receptor. Thus it appears that both additional experimental and theoretical work is required before one can understand the action spectra for continuous irradiation responses. It should be noted however, that by explaining the red responsivity under continuous irradiation by multiple induction effects, one still has to explain why the response shows fluence rate dependence under continuous irradiation. Moreover one has to consider the strong temporal interaction of successive light pulses (Schmidt and Mohr 1981).

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