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Action Spectra for Light-induced Germination in Dormant Lettuce Seeds

I. Red Region

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Abstract. Fluence response curves for red light-induced germination of thermodormant (TD) seeds of *Lactuca sativa* L. show two regions that differ in their light sensitivity. In the region of high sensitivity, the germination responses differ between seed batches and can be altered by dark storage or far red irradiation. Induction of germination in far red dormant (FRD) seeds requires far higher fluences. Action spectra for induction to 60% germination were determined for these various response types. Spectra for the regions of low sensitivity response are similar for TD and FRD seeds. In comparison, the action spectrum for the highly sensitive response in TD seeds is significantly shifted to longer wavelengths. Analogous differences exist in the action spectra for far red reversal of the red induced germination responses. Germination induction in the low sensitivity region shows repeated red-far red reversibility. Far red reversal of red induction in the high sensitivity region does not saturate even at the highest far red fluences available and requires increased red fluences for subsequent reinduction. A model quantitatively accounting for these observations is presented. It is pointed out that action spectra of processes involving photoreversible pigments with partly overlapping absorption spectra in general are not identical with the absorption spectra of the partners. They should depend upon the degree of phototransformation required to elicit a given physiological response. In the case of induction of lettuce seed germination the observed action spectra can be interpreted as reflecting different requirements for P_{fr} of the various response types. Our results do not necessitate the assumption of spectroscopically different forms of phytochrome in these seeds.

Key words: Action spectra – Dormancy (seeds) – Germination (seeds) – *Lactuca* – Phytochrome.

Introduction

Lactuca sativa L. fruits (called seeds from now on) of the cultivars "Noran" and "May Queen" normally are not light requiring for germination. Dormancy and a subsequent requirement for light may, however, be induced either by prolonged exposure to far red light during the imbibition period, or by high temperature (37° C) (Blaauw-Jansen and Blaauw, 1975). Germination of TD seeds is induced by fluences of red light, about 4 orders of magnitude lower than those required by FRD seeds (Blaauw-Jansen and Blaauw, 1975, 1976a).

In TD May Queen seeds the fluence response curve for red induced germination is complicated, a minimum being evident between a sensitive and a second less sensitive region. This has been ascribed previously to the possibility of two different pigments interfering at the reaction centres (Blaauw-Jansen and Blaauw, 1975).

Using seeds of the cultivar Noran, spectroscopic and other evidence in favour of this two pigment hypothesis has been presented (Blaauw-Jansen and Blaauw, 1976a, 1976b; Blaauw et al., 1976). Notably the action spectrum for induction of germination of FRD seeds was found to be shifted to shorter wavelengths compared with that of TD seeds (Blaauw-Jansen and Blaauw, 1976b).

The present study provides more detailed information on the action spectra of the two response types in thermodormant seeds together with action spectra for the reversal of red induced germination. Comparative studies were made with c.v. May Queen seeds obtained from two different suppliers and with seeds of c.v. Noran.

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Abbreviations: TD = thermodormant; FRD = far red dormant; P = phytochrome; P_r = red absorbing form of P; P_{fr} = far red absorbing form of P

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Materials and Methods

Lactuca sativa L. seeds of c.v. Noran were obtained from Rijk Zwaan's Zaadteelt en Zaadhandel N.V., de Lier, Holland; seeds of c.v. May Queen were obtained from two sources: lot A from the firm Alb. Peterse, Opheusden, Holland and lot B from the firm Hulleman, Utrecht, Holland. Seeds were stored in light tight screw cap jars in a refrigerator at 2° C. For germination tests, lots of 50 seeds were imbibed in 2.5 ml distilled water in 5 cm diameter petri dishes, placed in light tight wooden boxes at 37° C for 48 h (May Queen) or 72 h (Noran) to induce thermodormancy. Far red dormancy was induced by placing seeds under broad spectrum far red light (8.0 $W m^{-2}$) for 48 h. The far red source and its spectral characteristics have been described by Stolwijk (1954).

Irradiation of TD and FRD seeds with light of different wavelengths was done with a Leitz Prado 500 slide projector equiped with appropriate Baird Atomics interference filters. Selection of filters was based on transmittance measurement in a Cary 14 spectrophotometer. A range of intensities was obtained by inserting neutral grey filters (Schott u. Gen., Mainz, W. Germany, type NG) between the seeds and the interference filters. Transmittance of the filters was measured for all actinic wavelengths used.

For the irradiations requiring high fluences at low exposure times, use was made of two 4 kW high pressure Xenon arcs ('Xenosol V', Zeiss). In this set-up, the interference filters were cooled by an intense current of air. A 10 cm layer of running tap water, interposed between the condensing lenses and the interference filters removes a large part of the long wave far red. Images of the interference filters were projected on to the samples by means of plano-convex lenses of suitable focal length. Between these lenses and the interference filters, large aperture (80 mm) Compur-type electronically operated camera shutters were interposed. In this way, exposure times between 0.02 s and 30 min could be obtained. They were measured by means of a photodiode connected to an electronic counter. A more detailed description of the equipment will be published elsewhere.

After irradiation, seeds were placed in darkness at 22° C for 48 h after which germination counts were made. Irradiation experiments were replicated four times.

Results

Fluence Response Types

Although fluence response curves for red induced germination of Noran and May Queen B seeds have been published (Blaauw-Jansen and Blaauw, 1975) the inclusion in the present study of c.v. May Queen seeds from a different supplier (A) necessitated a comparative study. Furthermore, it has been suggested that, particularly for far red induced germination of TD seeds a long irradiation time at low light intensity may be more effective than a short irradiation time at high intensity (Blaauw-Jansen and Blaauw, 1976a). Using the Xenon arc system, a number of fluences, obtained with neutral filters, were given at exposure times of 0.02 and 0.3 s. The fluence response curves for red and for far red obtained in this way were compared with those for irradiation times of 3.0 and 6.0 s using the slide projector light source. In these two sets of experiments very similar fluence response curves were obtained (not shown) suggesting that the reciprocity law was satisfied in this range of exposure times. For convenience the slide projector system and an irradiation period of 3 s was mainly used in the following studies.

The response of TD and FRD seeds to 659 nm irradiation is shown in Fig. 1. All three batches of seed behaved almost identical in the FRD state and germinated only after high red fluences (type III response). In contrast, TD seeds responded to very low fluences. In all three varieties a first sensitive region (I) and a second less sensitive region (II) were observed. Response types I and II in May Queen B clearly differed in magnitude from those of the other two seed batches. As reported previously (Blaauw-Jansen and Blaauw, 1976a), far red induces consider-

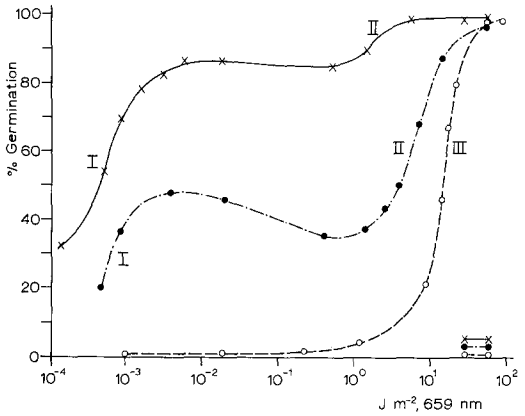

Fig. 1. Energy fluence response types of TD and FRD lettuce seeds. Horizontal lines denote dark controls. Exposure times were 3 s throughout. ×——× TD May Queen A and Noran; ●——● TD May Queen B; ○——○ FRD, all varieties

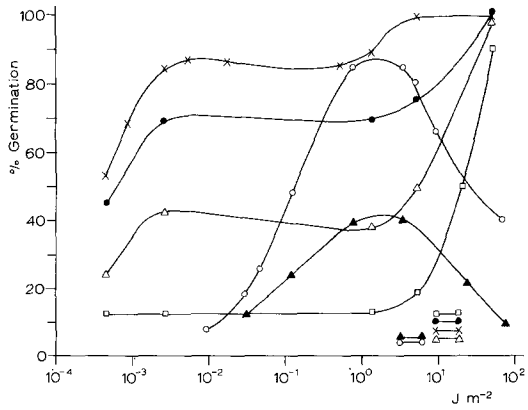

Fig. 2. Effect of dark storage at 22° C of TD May Queen A seeds upon subsequent response to red (659 nm) and far red (731 nm) light. ×——× 0 h storage, then R; ○——○ 0 h storage, then FR; ●——● 24 h storage, then R; △——△ 48 h storage, then R; ▲——▲ 48 h storage, then FR; □——□ 96 h storage, then R

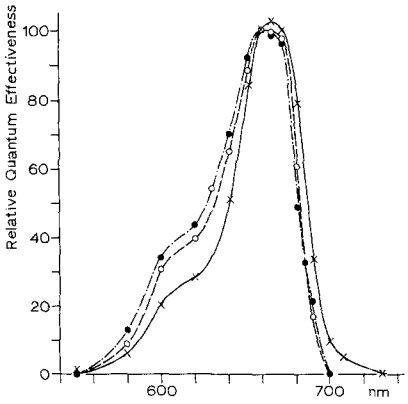

Fig. 3. Action spectra for induction of germination. ×——× Type I response, TD seeds; ●——● Type II response, TD seeds; ○——○ type III response, FRD seeds. Quantum effectiveness for induction of 60% germination was determined in May Queen A (type I, TD), May Queen B (type II, TD and type III, FRD) and Noran (type I, TD and type III, FRD). Response at 659 nm = 100%

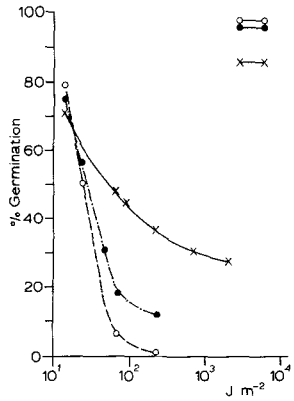

Fig. 4. Reversion of red induced germination by far red (731 nm). ×——× Reversion of type I response, TD seeds. Induction by 1.8×10^{-2} J m^{-2}, 659 nm; ●——● Reversion of type II response, TD seeds. Induction by 63 J m^{-2}, 659 nm; ○——○ Reversion of type III response, FRD seeds. Induction by 63 J m^{-2}, 659 nm. Horizontal lines denote controls (red induction only)

able germination in the high sensitivity TD seeds by itself.

In both TD May Queen A and Noran seeds the magnitude of the region I response may be decreased by dark storage at 22° C following induction of dormancy (Fig. 2). Application of various fluences of far red before exposure to red of TD seeds had a similar effect (not shown). Transition of TD seeds to a far red dormant state is, therefore, not accompanied by a gradual shift of the curves to higher fluences, but rather in a gradual decrease in the magnitude of type I responses.

Action Spectra for Induction of Germination

Log fluence response curves for the three response types over a range of fluences of 6 orders of magnitude at constant exposure of 3 s are shown in Fig. 1. Curves for response type I at other wavelengths were parallel to these within experimental error in the ranges of 10–80% germination. For wavelengths beyond 680 nm, the curves for type II and III responses tended to lower saturation levels with consequent increasing deviations from parallelity for germination percentages in excess of 70. Action spectra were constructed for a standard germination of 60%. They were similar for the type I responses of all three seed varieties. The spectra of type III responses were also the same in the two seed varieties tested, but differed from the type I spectra. Only May Queen B could be used for a determination of the type II spectrum since the amplitude of the germination response in this region was too small in the other varieties due to the high level of germination already reached in their type I responses (see Fig. 1). As Fig. 3 shows, the action spectra for type II and III responses are similar and the differences as shown in Fig. 3 may not be significant. There is, however, a marked difference with the type I spectra. Light of wavelengths shorter than 650 nm proved considerably less efficient for type I than for types II and III. The opposite is observed for wavelengths longer than 670 nm. A most noteworthy feature is the ability of 731 nm light to induce 60% germination in TD seeds (response type I), whereas wavelength longer than 700 nm did not induce germination to this level in FRD seeds and also failed to produce a significant second increase (response type II) in germination of May Queen B seeds. We return to this point and its significance for the construction of action spectra in the discussion.

Far Red Reversal of Red Induced Germination

Blaauw-Jansen and Blaauw (1975, 1976a) have shown that red induced germination of FRD and TD Noran seeds can be reversed by far red light. It appeared appropriate to extend such observations to the various response types and to determine the action spectra.

As seen in Fig. 4, germination induced by a high fluence of red light in FRD seeds (all three varieties were tested) could be reversed by light of 731 nm. The same holds for TD seeds (May Queen B) notwithstanding the fact that FR alone can induce considerable germination (see Fig. 2, ○——○). Saturating reversal of the highly sensitive red induced type I

response, however, required very high fluences, as shown for May Queen A. It looks as if in the latter case, germination cannot be reduced to below about 25%, but far red intensities sufficient to test this were not available. Also, repeated red-far red reversal could not be obtained with such seeds (Table 1) unless the red light fluence following far red was raised considerably, viz. by several orders of magnitude (cf. Blaauw-Jansen and Blaauw, 1976a, Fig. 4). On the other hand repeated reversal and induction could readily be obtained with FRD and TD May Queen B seeds. In terms of response types this means that type I responses can be reversed to some extent by far red light but that red reinduction requires high fluences. This suggests that the red induction-far red reversal reaction is accompanied by induction of far red dormancy. Response types II and III proved repeatedly reversible and inducible by far red and red light respectively.

Table 1. Red-far red reversibility of germination

Irradiation sequence[a]	FRD seeds (all varieties) % germ.	TD seeds May Queen B % germ.
R	98	96
R FR	2	11
R FR R	98	91
R FR R FR	3	13
	May Queen A % germ.	
R_L	86	
R_L FR_H	32	
R_L FR_H R_L	36	
R_L FR_H R_L FR_H	28	
R_L FR_H R_L FR_H R_L	28	
R_L FR_H R_H	88	
R_L FR_H R_H FR_H	20	
R_L FR_H R_H FR_H R_H	91	

[a] R = 659 nm, 6.2×10^{1} J m^{-2}
R_L = 659 nm, 1.8×10^{-2} J m^{-2}
R_H = 659 nm, 2.1×10^{2} J m^{-2}
FR = 731 nm, 6.6×10^{2} J m^{-2}
FR_H = 731 nm, 1.8×10^{4} J m^{-2}

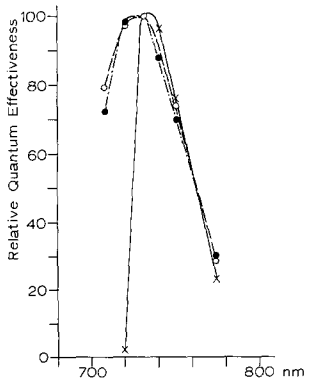

Fig. 5. Action spectra for reversion of germination to 50% of that of red controls. See legend to Fig. 4 for red fluences applied. ×——× Reversion of type I response in TD seeds; ●——● Reversion of type II response in TD seeds; ○——○ Reversion of type III response in FRD seeds

Action spectra for 50% reversal of red induced germination are shown in Fig. 5. FRD (response III) and TD (response II) May Queen B seeds gave very similar action spectra. On the other hand the action spectrum for reversal in TD May Queen A seeds (response I) was appreciably different, the peak being found at a distinctly longer wavelength. More striking still is the sharp drop in the effectiveness towards shorter wavelengths. Light of wavelengths longer than 715 nm proved unable to effect reversal.

Phytochrome Estimations

Phytochrome can be detected in both TD and FRD seeds by differential spectrophotometry. The low values found were in good agreement with those reported earlier in lettuce seeds (Boisard et al., 1968). Within the rather wide limit of error, they were the same for TD and for FRD seeds. Determination of the distribution between P_r and P_{fr} after inductive irradiation proved unreliable due to the low pigment level. The P_{fr} level established in TD seeds for a 60% germination response, moreover, would remain undetectable even with far more sensitive techniques.

Discussion

The response to red light of TD and FRD seeds as observed in this study confirm previous results (Blaauw-Jansen and Blaauw, 1975). In addition, we have now shown that the two types of response to red exposure also exist in TD seeds of two other varieties. Apparently there are differences between seed batches in the magnitude of type I responses which can be altered by either far red irradiation or dark storage of TD seeds.

TD May Queen B seeds proved very similar to FRD seeds in terms of red-far red reversal of germination as well as in action spectra. We therefore conclude that response types II and III are essentially identical and conform to classical phytochrome reactions in their far red reversibility.

In TD seeds, response type I, the situation appears more complicated. The shift to longer wave-

lengths as compared with FRD seeds confirms and extends the results of Blaauw-Jansen and Blaauw (1976b). Further differences were found in red-far red reversibility and in the action spectra for far red reversal of red induced germination.

The shape of the action spectrum for type I germination resembles that of other phytochrome controlled processes (Smith, 1975). However, this red induction is not reversed completely even by high fluences of far red. Increased sensitivity to red and decreased response to far red following high temperature treatment has also been observed in seeds of other species (e.g. Toole et al., 1955). Taken at face value the present results tend to support the earlier proposal (Blaauw-Jansen and Blaauw, 1976a, b) that different phytochrome species are involved in type I and type III responses. Action spectra alone may not provide conclusive evidence, however. In the case of a photoreversible pigment system with partly overlapping absorption spectra the action spectra should depend upon the degree of phototransformation required to elicit a given physiological response. To illustrate this, we will calculate action spectra to be expected for a number of phototransformation percentages.

Let the total quantity of phytochrome in the photosensitive region be 1. We define the state, F, as the ratio P_{fr}/P_{tot}, and $R=1-F$. Irradiation during a period t of a seed with an initial state F_0 in general will lead to the establishment of a new state, F_t. Assuming that all seeds contain an equal quantity of phytochrome that, moreover, remains constant throughout the experiment, we have:

$$\frac{dF}{dt}=a I_\lambda \varepsilon^r_\lambda \phi^r_\lambda R - a I_\lambda \varepsilon^f_\lambda \phi^f_\lambda F = a I_\lambda [\varepsilon^r_\lambda \phi^r_\lambda - (\varepsilon^r_\lambda \phi^r_\lambda + \varepsilon^f_\lambda \phi^f_\lambda) F],$$

with a representing the product of phytochrome concentration and path length expressed in units that make $R+F=1$. We assume that in vivo as well as in vitro the quantum yields for the two phototransformations are equal:

$$\phi^r_\lambda = \phi^f_\lambda,$$

(Pratt, 1975). Since we are making comparative measurements, we may express our quantum fluxes in reduced units I'_λ so that:

$$\frac{dF}{dt}=I'_\lambda [\varepsilon^r_\lambda - (\varepsilon^r_\lambda + \varepsilon^f_\lambda) F].$$

We then obtain:

$$I'_\lambda = \frac{1}{\varepsilon^r_\lambda + \varepsilon^f_\lambda} \ln \frac{\varepsilon^r_\lambda - (\varepsilon^r_\lambda + \varepsilon^f_\lambda) F_0}{\varepsilon^r_\lambda - (\varepsilon^r_\lambda + \varepsilon^f_\lambda) F_t}.$$

Finally, at constant exposure time, t, the quantum effectiveness for a change from F_0 to F_t relative to a standard wavelength, s, is:

$$W' = \frac{I'_s}{I'_\lambda} = \frac{I_s}{I_\lambda} \quad (\times 100\,\%).$$

Unfortunately, the molar absorption coefficients of phytochrome, ε^r_λ and ε^f_λ are not known with high precision in vivo. We have, therefore, used the values given by Gardner and Briggs (1974) for high MW phytochrome in vitro. Since these data do not yield a phytochrome difference spectrum that exactly duplicates the one observed in vivo, we have shifted, by way of correction, the published absorption spectrum of P_{fr} over 3 nm to longer wavelengths. We assume that in vivo like in vitro, $F_\infty(665)=0.75$ (Pratt, 1975). In addition, some calculations were made with model spectra for P_{fr} differing slightly in the region <700 nm. It appeared reasonable to assume that in dormant seeds $F_0=0$. Figures 6 and 7 give results of such calculations pertaining to induction, Fig. 8 for reversal of the induction provoked by two different fluences of 665 nm, viz. to $F_0=0.75$ and $F_0=0.06$, resp. Calculated action spectra for induction to $F_t \ll 0.01$ are not materially different from the postulated absorption spectra of P_{fr}.

These calculations can illustrate two points. First, that in general phytochrome action spectra are not necessarily identical with the absorption spectra of the components P_r and P_{fr}, the deviations increasing with the level of P_{fr} (rather: the state F) required to elicit a given physiological response. Second, for fluences involving large changes in F, the log fluence response curves cannot be strictly parallel, particularly in the wavelength regions where the action spectra drop off sharply. This is in agreement with our measurements. As a consequence such action spectra depend, to a larger or smaller extent, upon the reference level of the physiological effect chosen for their construction. For F_t approaching F_0, action spectra for induction and reversion converge to the true absorption spectra of P_r and P_{fr}, resp. If a significant physiological response requires appreciable phototransformations, construction of such spectra presents an experimental problem. On the other hand, accurate action spectra for higher levels of response, such as we have employed in the present study, can afford information about the degree of phototransformation involved that may be hard to obtain by other means. Comparison of Fig. 3 with Figs. 6 and 7 suggests that whereas response type I requires the formation of a very small percentage P_{fr}, types II and III involve much larger phototransformations. This conclusion is further supported by a comparison of Figs. 4 and 8. In order to reverse red induced type I germination, P_{fr} should be reduced to very low levels whereas in the reversion of type III germination a moderate reduction in P_{fr} should suffice. The observed reversion spectra shown in Fig. 5 conform to this model. This interpretation is also in qualitative agreement with the observed ratios of the quantum

Table 2. Quantum requirement ratios, reversion/induction

Calculated	Ratio
induction: $F=0.00\rightarrow 0.1$ reversion: $F=0.10\rightarrow 0.015$	45.0
induction: $F=0.00\rightarrow 0.50$ reversion: $F=0.50\rightarrow 0.10$	2.2
Observed[a]	
type I (May Queen A, Noran)	1.3×10^5
type II (May Queen B)	7.0
type III (all varieties)	1.6

[a] Ratios between fluences for 50% far red reversion of red induced germination and for 50% induction by red, in the three response types resp

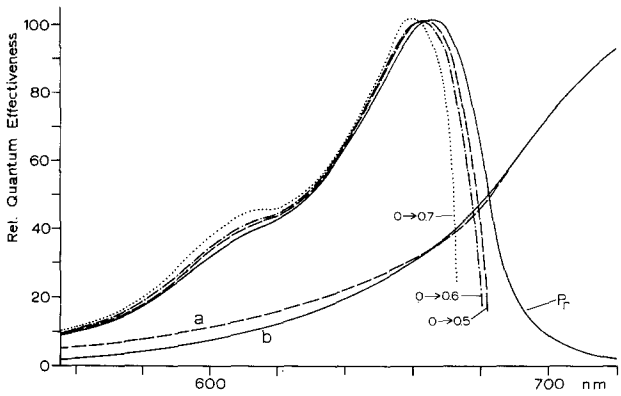

Fig. 7a and b. Calculated action spectra, modified absorption coefficients for P_{fr}. Cf. Fig. 6. a = Original absorption coefficients as in Fig. 6; b = Modified coefficients for P_{fr}

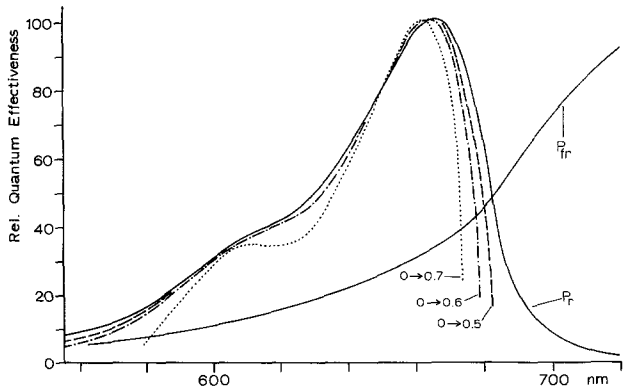

Fig. 6. Calculated action spectra for induction of germination, normalised at peaks. Spectral data for P_r and P_{fr} from literature. Phototransformations from initial state $F=0$ to final values of 0.5, 0.6 and 0.7. The curve marked "P_r" representing the postulated absorption spectrum of P_r is essentially valid for phototransformations up to about $F=0.01$

requirements for induction and reversion. Table 2 give a few examples. If we consider $F_0=0\rightarrow F_t=0.10$ as an example of a "sensitive" induction, about a 50-fold fluence is calculated for a reversal from $F=0.10$ to 0.015. An "insensitive" induction on the other hand, e.g. $F_0=0\rightarrow F_t=0.50$ requires only about half as many quanta as its reversal from $F=0.5$ to 0.1. The ratios depend strongly upon the assumed initial and final values of F of course.

The very large ratios experimentally observed between type I induction and reversal (on the order of 10^{-5}, depending upon the level of germination) is not easily accounted for by our model. The same applies to the difference in sensitivity by a factor of about 4×10^4 between induction of germination in types I and III (Fig. 1). For, assuming an F_t for induction of 60% germination type III of 0.5 (the exact value is rather immaterial) as suggested by the action spectra, this would bring the F_t for 60% germination type I to something on the order of 10^{-4}. Although this may not be impossible in itself, it cannot be reconciled with the observed reversibility by far red of type I induction. The spectroscopic evidence available at present, though derived from phytochrome in solution, indicates that 731 nm light in itself should already establish far higher F values, viz. on the order of 10^{-2}. The discrepancy mentioned above appears to add another example to the list of "phytochrome paradoxes".

Since the spectroscopic phytochrome level was found to be about the same in TD and in FRD seeds, their very different light requirements can hardly be explained from a difference in phytochrome content. Unfortunately, spectrophotometry gives only information on bulk pigment and presumably the physiologically active pigment might represent only a tiny fraction of this.

There appear to be two possible solutions to the problem. The first is that the far red reversion of type I induction in reality may reflect a partial or complete induction of far red dormancy. Such an interpretation is supported by the results, already discussed, of repeated red-far red reversal experiments, shown in Table 1. The second is that the extremely light sensitive type I induction is accompanied by a rapid (time scale of seconds) concentration process of the minute quantity of the P_{fr} formed by the irradiation into a sensitive region of the seeds, thereby locally raising the F value to far above that established in the bulk of the seed. It would appear that concentration factors in the range 10^4-10^5 would be required. These alternatives are at present being further explored.

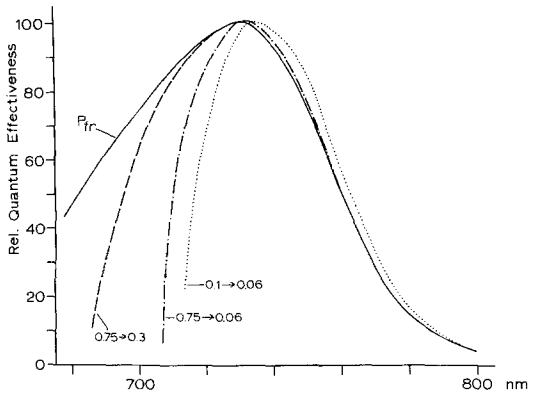

Fig. 8. Calculated action spectra for reversion of red induced germination, for some initial and final states F. Spectral data for P_r and P_{fr} from literature

Apparently there exists a discrepancy between observed action spectra for germination induction and the model spectra shown in Fig. 6 in the region around 610 nm. The observed spectra for response type III lie considerably above those for type I in this region, whereas the reverse seems to hold for the spectra calculated from literature data. It may be remarked, however, that the shape of the calculated spectra depends rather strongly upon the assumptions about the absorption coefficients of P_r and P_{fr}. A comparison of Figs. 6 and 7 shows that a relatively minor adjustment of the absorption coefficients postulated for P_{fr} in this region suffices to yield action spectra that conform more closely to the observed sequence. Since neither the precise absorption coefficients of phytochrome, the quantum yields of the phototransformations or the photostationary states under saturating red or far red irradiation are known with high precision in vivo, we feel that the discrepancy between observed and calculated spectra in this region should not be of immediate concern.

Our observations throw little light on the nature of the differences between TD and FRD seeds. The largely different P_{fr} requirements for a standard germination response in these two dormancy types may imply that induction of visible signs of germination in lettuce seeds is under the control of processes, two of which may under appropriate conditions be linked to phytochrome and which are acting in series. The first being extremely sensitive to P_{fr}, the second much less so, induction of dormancy could consist of making one or the other decisive for germination. High temperature treatment is suggested to result in a bypass of the second, less sensitive process possibly by activating an essential metabolic step otherwise under phytochrome control. Prolonged exposure to far red light on the other hand would direct an increasingly larger fraction of the reactions through the insensitive system. There are indications that the latter process may be of the nature of a high irradiance reaction (Rollin and Maignan, 1966; Rollin et al., 1970).

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