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DETERMINATION OF HIT NUMBERS FROM DOSE-RESPONSE CURVES FOR PHYTOCHROME CONTROL OF SEED GERMINATION (LACTUCA SATIVA CV NORAN)

O. H. BLAAUW, G. BLAAUW-JANSEN

Botanisch Laboratorium, Utrecht

O. ELGERSMA

Philips Research Laboratories, Eindhoven

SUMMARY

The dose-response curves for the induction of thermo-dormant (pretreated at 37 °C) *Lactuca* seeds and of far-red dormant (pretreated by long exposure to far-red light) *Lactuca* seeds by red light show a difference in shape. From the shape of the curves it is calculated that for initiation of germination in thermo-dormant seeds at least 2 quanta (λ = 660 nm) must be absorbed per active unit, in far-red dormant seeds at least 7 quanta.

1. INTRODUCTION

Lactuca sativa L. seeds (cv Noran) germinate in darkness as well as in light. However, when the seeds are pretreated by exposure to temperatures above 30 °C (which pretreatment induces thermodormancy) or by exposure to continuous far-red light (which pretreatment induces far-red dormancy) a short red exposure is necessary for germination. *Fig. 1* shows that for thermo-dormant seeds the response threshold for red irradiation is several orders of magnitude lower than for far-red dormant seeds (BLAAUW-JANSEN & BLAAUW 1975). Moreover, the shapes of the dose-response curves are different. In this paper the latter difference is analysed on basis of the concepts of the hit theory. We refer to ZIMMER (1960): "The difference between threshold value and 100 per cent dose was generally regarded as an effect of unavoidable biological variability (scatter of sensibility). According to the "Hit" theory the form of the observed dose-effect curve is due to the fact that absorption of radiation is not a continuous but a quantisized process which follows a statistical principle called after Poisson. The observed effect should appear in a member of a population which has received macroscopically homogeneous irradiation when a minimum number of absorption events (called "hits") have happened to this individual". And: "A very important development led further to the "Target"

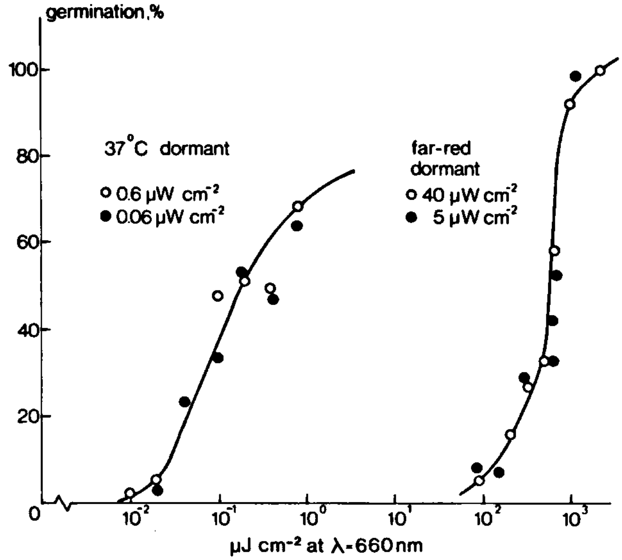

Fig. 1. Dose-response curves for induction of germination of lettuce seeds. Dormancy was induced either by long exposure to far-red light or by exposure to 37°C.

theory. It offered the possibility of calculating from the dose-effect curve a volume, the target, within which the required number of absorption events must occur during irradiation, with given probability".

2. MATERIAL AND METHODS

Dose-response curves for induction of germination of lettuce seeds (cv Noran) were elaborated as described by BLAAUW-JANSEN & BLAAUW (1975). The concepts of the "hit" theory were applied to these dose-response curves as follows:

(This paragraph is written to give non-mathematician readers a rough idea of the statiscical methods applied in this paper. Other readers may proceed with the section "Statistical operations").

Suppose that a cross section of σ cm² is irradiated with a dose D (in number of light quanta per cm²). Then the expected number of hits is σD. Now we can make use of the Poisson distribution to describe the probability of the occurrence of 0, 1, 2, 3, etc. hits on surface σ. These probabilities are given by the successive terms of the expansion of the form

$$e^{-\sigma D}\left(1 + \sigma D + \frac{(\sigma D)^2}{2!} + \frac{(\sigma D)^3}{3!} + \ldots\ldots\right)$$

The probability that there should be no hit, 1 hit, 2 hits, 3 hits etc. is therefore

$$e^{-\sigma D},\ \sigma D\, e^{-\sigma D},\ \frac{(\sigma D)^2}{2!} e^{-\sigma D},\ \frac{(\sigma D)^3}{3!} e^{-\sigma D}, \text{ etc}$$

The probability that there should be 1 hit or more, 2 hits or more, 3 hits or more, etc. can be calculated from the above expressions to be respectively

$$1 - e^{-\sigma D}$$
$$1 - (e^{-\sigma D} + \sigma D\, e^{-\sigma D})$$
$$1 - (e^{-\sigma D} + \sigma D\, e^{-\sigma D} + \frac{(\sigma D)^2}{2!} e^{-\sigma D})$$
etc.

Hence these expressions describe the number N out of N_0 irradiated individuals which have experienced 1 hit or more, 2 hits or more, etc. Now curves can be calculated for a range of values of n and σD that give the fraction of individuals which after dose D have received at least n hits. These curves show close similarity in shape to dose-response curves e.g. curves that give the dependence of germination on irradiated dose. By comparison of the experimental with the calculated dose-response curves the hit number (n) for which the agreement is best can be estimated.

3. STATISTICAL OPERATIONS

We can assume the Poisson distribution to describe the probability (p_k) of the occurrence of k hits on cross section σ (k = 0, 1, 2, 3, etc.). This probability is given by

$$p_k = e^{-\sigma D} \frac{(\sigma D)^k}{k!}$$

The probability (P_n) of having at least n hits on the cross section (n = 1, 2, 3, etc.) can then be described by

$$P_n = 1 - \sum_{k=0}^{n-1} p_k = 1 - e^{-\sigma D} \sum_{k=0}^{n-1} \frac{(\sigma D)^k}{k!} \qquad (1)$$

So this expression yields the fraction N/N_0 out of N_0 irradiated individuals, which has experienced n hits or more.

Expression (1) is applied to the fraction of seeds germinating after a dose D of red light. Two extra parameters are introduced:
a_0 = fraction of the seeds germinating without any irradiation
a_i = fraction of the seeds not germinating even after very large light doses.
The fraction of germinating seeds is accordingly

$$P_n = a_0 + (1 - a_i - a_0)\left\{1 - e^{-\sigma D} \sum_{k=0}^{n-1} \frac{(\sigma D)^k}{k!}\right\} \quad (2)$$

For a rough estimation of σ the expression

$$\sigma = \frac{n - {}^1/_3}{D^1/_2} \quad (3)$$

can be used. $D_{1/2}$ = half-value dose.

For n = 1 up to n = 13 the parameters a_0, a_i and σ were varied in order to give a least sqares fitting of the theoretical hit curve (2) to the points of the experimental germination curve. The least sqares sum, defined as

$$\chi^2_{min} = \Sigma \text{ (value of fitted curve − experimental value)}^2$$

can be used for computing the standard error s of the individual points according to the relation

$$s = \sqrt{\frac{\chi^2_{min}}{np\text{-}m}}$$

np-m is the number of degrees of freedom
np is the number of individual points
m is the number of fitted parameters, i.e. 3 (a_0, a_i and σ)

As this standard error s is a measure for the goodness of fit, the hit number n of the theoretical hit curve with the smallest standard error was chosen as the best one. The variation of the hit number caused by 10% increase of the standard error s was chosen as the standard error of the best hit number.

In *table 1* the standard error *s* for different hit numbers for one experiment of far-red exposure and one experiment of heat treatment is shown.

Table 1.

	Mean standard error s (in %)											
hit number	1	2	3	4	5	6	7	8	9	10	11	12
far red exposure					4.8	4.0	3.8	3.7	3.7	3.7	3.8	4.0
heat treatment	6.2	6.7	7.7									

From this table it may be concluded that the best hit number for this far-red exposure treatment is 9 ± 3 and for this heat treatment experiment 1 ± 1. In *figs. 2* and *3* the experimental points and the least sqares fitted theoretical hit curve with the best hit number are shown for these two experiments. From the figure it is obvious that there is no systematical deviation of the points from the curve, the length of the bar indicating the mean standard *s*.

In this way the best hit numbers for 14 dose-response curves for far-red dormant seeds and 10 dose-response curves for thermo-dormant seeds were com-

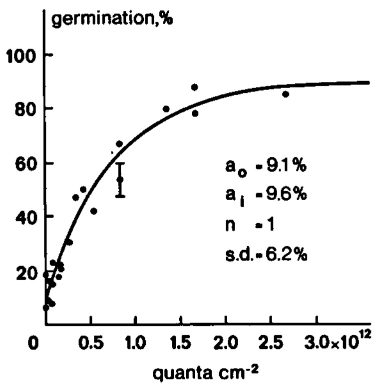

Fig. 2. Theoretical 1-hit curve with the experimental points derived from an experiment on the relation of red light dose and the germination percentage of thermodormant seeds. $\sigma = 1.8 \times 10^{-12}$ cm^2.

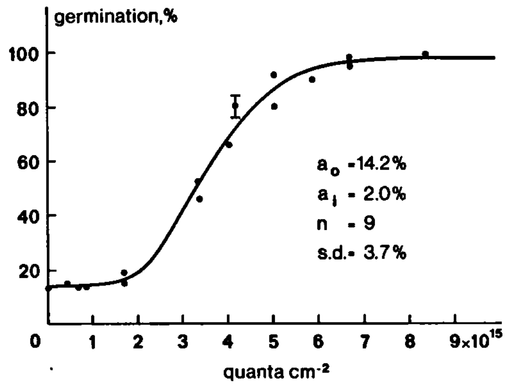

Fig. 3. Theoretical 9-hit curve with the experimental points derived from an experiment on the relation of red light dose and the germination percentage of far-red dormant seeds. $\sigma = 2.8 \times 10^{-15}$ cm^2.

puted. The standard error *s* was varying from 0.8% to 6.9% for the far-red exposure experiments and from 2.9% to 12.9% for the heat-treatment experiments. 2 Experiments with a mean standard error (3.8 and 6.2% respectively) are shown as typical in *table 1* and *figs. 2* and *3*. The calculations were performed on the Philips Time Sharing Computer System at Rijswijk.

4. RESULTS

The hit numbers which give the theoretical curves with the best fit to the experimental dose-response curves vary for far-red dormant seeds between 5 and 10 with a mean of 6.7 ± 0.4, and for thermo-dormant seeds between 1 and 4 with a mean of 1.6 ± 0.4. *Table 2* shows these values with the mean of the parameters.

Table 2.

Dormancy induced by	Hit number	σ in cm^2	a_0 in %	a_i in %	s.d. in %
far-red exposure	6.7 ± 0.4	2.8×10^{-15}	5	1	3.6
37° treatment	1.6 ± 0.4	4.5×10^{-12}	12	20	6.8

5. DISCUSSION

The action spectrum of the germination of thermo-dormant lettuce seeds is shifted to larger wavelengths in comparison with the action spectrum of the germination of far-red dormant lettuce seeds (BLAAUW-JANSEN & BLAAUW 1976a). Moreover the threshold dose of red light for induction of germination of far-red dormant seeds is about 10^4 as large as the threshold dose for thermo-dormant seeds (BLAAUW-JANSEN & BLAAUW 1975). It was concluded that light-induced germination of thermo-dormant seeds and of far-red dormant seeds is mediated by different phytochrome species.

In this paper another argument for this view is presented: the dose-response curve for the thermo-dormant seeds corresponds with a 2-hit curve, for the far-red dormant seeds with a 7-hit curve. This might be interpreted as follows: in thermo-dormant seeds the phytochrome molecules or units of phytochrome molecules must be hit by at least 2, in far-red dormant seeds by at least 7 quanta to initiate a reaction.

Our experiments do not permit a choice between the following two possibilities: the reactive unit consists of a number of n molecules each of which has to be hit at least once, or the unit consists of 1 molecule that has to be hit at least n times in succession.

The surface σ entering in equation (1) and calculated in *table 2* need not to be identical with the surface of the irradiated unit. σ is the product of a real surface S with the probability p of the effectiveness of a hit anywhere on the surface S. Not knowing the action probability we are not able to find the true area of the irradiated unit which can even consist of a number of discrete subareas.

WASSINK & BOUMAN (1947) plotted dose-response curves for the phototropic reaction of *Phycomyces* (% curved against log energy) from data of BLAAUW (1909). Assuming a similar line of approach as we adopted for the germination of seeds they demonstrated that phototropism in *Phycomyces* at threshold intensities might be initiated by the effective absorption of one light quantum in the unicellular sporiangiophore. The slope of the dose-response curve for the phototropic curvature (% curved against log energy) of *Avena* coleoptiles as estimated from data of BLAAUW (1909) appeared to be much steeper than the above mentioned curve for *Phycomyces*. The much more simultaneous reaction of the separate coleoptiles was considered to be due to the large number of cells (and of quanta) contributing to the reaction of one coleoptile. In lettuce seeds, though multicellular, germination occurs when two or seven light quanta per reactive unit are absorbed (this paper). It might be concluded, considering the line of thought of Wassink & Bouman, that only a very limited number of initial processes is controlling the germination of a seed, and that the perception of light by lettuce seeds occurs in a very limited number of cells.

RAVEN & SHROPSHIRE (1975) elaborated fluence-response curves for red-light induction of chlorophyll-a accumulation in pea seedlings. Pre-irradiation with otherwise inactive doses of red or red + far-red light resulted in a ca 10^4

fold increase of the threshold red-dose and a 3 fold increase in slope of the dose-response curve (cf. the corresponding shift and increase in slope of the dose-response curve for red-induced seed germination, BLAAUW-JANSEN & BLAAUW 1975), By the pre-irradiation far-red almost completely lost its inductive capacity if applied in small doses. A model by RAVEN & SPRUIT (1973) involving migration of phytochrome to active sites by the preirradiation was used to explain the shift in threshold values.

Migration of phytochrome molecules as an explanation of our results is presumably excluded as this is considered by Raven & Spruit to be a time-requiring process. The modification from "thermo-dormant" to "far-red" dormant phytochrome however is an instantaneous one performed by a dose of far-red light of ca $10^4 \mu J\ cm^{-2}$ (BLAAUW-JANSEN & BLAAUW 1976b).

The increase in slope of the dose-response curve was attributed by Raven & Shropshire either to the redistribution of phytochrome or to an increase in quantum efficiency or quamtum yield of the phytochrome reactions. In our view the increase in slope is due to the change from a 2-hit process to a more-hit process. This change is necessarily accompanied by an increase of the threshold dose, but the experimentally found increase of the threshold dose is much too large to be explained in this manner.

A similar increase in slope of dose-response curves was observed for the phytochrome-mediated inhibition of the growth of etiolated *Avena*-mesocotyls (BLAAUW et al. 1968). This inhibition occurs in two steps: the first step is produced and saturated by $10^{-3} - 10^{-1}\ \mu J\ cm^{-2}$ (660 nm); the red dose has to be increased 10^3 à 10^4 fold to induce the second step of the growth inhibition. This difference in threshold doses too is accompanied by an increase in slope of the respective dose-response curves.

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